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## **Building sustainable cropping systems by combining scientific and empirical knowledge. The example of soil-born disease control on vegetable sheltered crops**

Marc Tchamitchian, Mireille Navarrete, Frederique F. Bressoud, Joel Fauriel,  
Laure Pares

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in Europe**

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## **PREFACE**

Dear Friend,

on behalf of the Organising Committee I am pleased to welcome you at the ISHS “1<sup>st</sup> Symposium on Horticulture in Europe”.

The symposium is held under the aegis of national and regional European horticultural societies and research Institutes such as APH (Portuguese Horticultural Association), DGG (German Society for Horticultural Science), GSHS (Greek Society for Horticultural Science), INRA (Institute National Recherche Agronomique), PSHS (Polish Society for Horticultural Science), IOH (Institute of Horticulture), SECH (Spanish Society for Horticultural Science), BNL-SHS (Benelux Society for Horticultural Science), SOI (Italian Society for Horticultural Science), NJF (Nordic Association of Agricultural Scientists), with the local organization of ALVA (Austria).

It has been a great effort to but we hope you will appreciate the programme as well as your staying in Vienna.

In the next three days you will have the opportunity to share your time with Scientists coming from more than 30 Countries, mostly European ones, and you could choice among 7 plenary lectures concerning some of the most outstanding themes of world and European horticulture, 48 oral presentations (8 for each of the themes) and 9 workshops on very specific topics. Moreover, 300 posters will be presented during the symposium.

We wish all participants will benefit from this great and deep exchange of knowledge and experience on different areas of horticulture, to let new ideas and new projects grow and develop into advanced techniques and innovation for the benefit of European horticulturalists and consumers.

The scientific success of the symposium depends on participants, but it is also the result of the work and the cooperation of many people, public institutions and private enterprises that gave us their invaluable support. In particular we wish to thank the Mayor Governor of Vienna, the Vienna Convention Bureau and the Federal Ministry of Agriculture, Forestry, Environment and Water Management of Austria, together with DGG, INRA, SOI and the colleagues of ALVA. We are particularly indebted with Dr. Gerhard Bedlan, DI Wolfgang Palme, Mag. Astrid Plenk, and anyone who cooperated with us to make this event possible and pleasant for each and everyone of you.

Enjoy the Symposium!

Prof. Paolo Inglese  
Chair of the Organising Committee

## WELCOME ADDRESS

The local organising committee of the 1<sup>st</sup> Symposium on Horticulture in Europe is very pleased hosting SHE2008 from today here in Vienna. We are looking forward to welcoming as many colleagues as possible and thus to give a strong impulse to the fact that horticulture is still very much alive in Europe and that it is an important factor for our society and our economy. We also want to give researchers studying all aspects of horticulture an opportunity to exchange knowledge, information, ideas and techniques. The symposium will cover all research areas relevant to horticulture in Europe, e. g. plant physiology, plant genetics, plant-environment relationships, plant quality, plant health, economics and technical engineering.

Vienna is hot & cold, loud & soft, expected & unexpected. This is no contradiction - Vienna is a city of many facets. Electronic DJs are a part of Vienna as much as the Vienna State Opera. Trendy clubs, "young" galleries, modern architecture and stylish shops are as much Vienna as the concert halls, the large museums, the splendid buildings and the nostalgic shops.

Walk in the footsteps of the Habsburgs, visit the splendid baroque Schönbrunn and Belvedere Palaces, here you can live and experience art, listen to music and dive deeply into the worlds of sound. Welcome to the world's music capital! More famous composers have lived here than in any other city.

There is hardly a city in the world that boasts as much green as Vienna - even in the city centre. Vienna's outskirts are especially attractive: romantic landscapes and picturesque villages invite you for a day's outing.

And the historic centre of Vienna has been added to UNESCO's World Cultural Heritage list. Thus, St. Stephen's Cathedral, the Imperial Palace and many other historic buildings are now recognized as being among the 700 outstanding cultural and natural monuments of mankind.

I wish to thank all members of the local organising committee for their excellent work organising this symposium, all the sponsors and the amicable teamwork of the organising committee. All together guarantees a successful and fruitful event as well as a pleasant stay.

Welcome in Vienna

Dr. Gerhard Bedlan  
Chair of the Local Organising Committee

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\* = Author who is presenting the lecture or the poster

# **Plenary Session**

## **HORTICULTURE IN EUROPE: FROM HISTORY TO INNOVATION**

# Horticulture in Europe: From History to Innovation

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While few would deny that today's highly specialised, intensive horticulture travels in step with the market, its historical roots have grown robust over the last sixty years in soils that have offered challenges, and the means to meet them, more dramatic in nature than all the others of the past two thousand years. It is thus worthwhile to take a brief look at key moments in this trajectory and highlight their significance

Perhaps the most notable have been the signal developments marking the goods and services industry since World War II. The range and performance of new tools that have issued from such areas as mechanical engineering, chemical manufacturing, biology, information technology and communications have been little short of prodigious. Yet, despite these advances and the effects they have brought to our work in field and laboratory, the fruit industry remains the only farm sector that has still to mechanise all of its crop management operations, harvesting for the fresh market being but one good example.

Impressive too are the new tools that research in such fields as physiology and chemistry, genetics and biotechnology, have provided in all areas of the fruit industry. Spurred initially by efforts in the public sector and more recently by inputs from the private, these innovations have brought significant changes in field testing and orchard management and greatly enhanced the expertise of growers and the quality of their crops. The biggest beneficiaries of these inputs have been the advanced economies, which despite their climatic disadvantages have upgraded their production capacity to the point where they have become exporters of both fruit and state-of-the-art technology. In effect, these R&D efforts have led to an authentic 'green revolution' in certain areas such that once traditional garden-cum-orchard operations have been transformed into veritable vehicles of industrial-scale fruit and vegetable production.

The engines driving these developments have been breeding in association with biotechnology, propagation, the nursery sector with its plant certification, soil management practices linked to fertility, nutrients and water, plant protection with new biocides, tree growth control and dwarfing stocks, and enhanced crop quality in field, storage and shelf-life.

Another novelty that has taken root is the awareness that by its intensive nature the fruit industry must be eco-compatible and, hence, sustainable. The first fruits of this imperative were the principles embodied in the protocols of integrated and organic production, codes which have led to a more proactive approach to emerging trends throughout the supply chain. Nowhere perhaps do we see this better than in the industry's response to the demands of the market and consumers for safe and healthy foods and the greater sense of stewardship towards the environment and agro-ecosystems by growers themselves, especially in redressing such issues as safeguarding soil potential, reducing external input, and not-renewable energetic resources.

Noteworthy too in this march of historical development has been the leadership of the European Union. It has taken, and is taking, a guiding role in informing the ethical and socio-economic principles with which technological innovation can be harnessed to promote and enhance collective expectations and well-being. These efforts are perhaps most visible today in the policy agenda driving research priorities in biodiversity, biotechnology and GMOs, precision technologies, the life sciences, food safety, health and nutraceuticals.

In effect, the EU has championed collaborative, interdisciplinary research through partnerships in both public-private funding and integrated projects aimed at boosting the competitive edge and value-added of the end-users in the global arena. Enormous strides have been made in methodology, especially in terms of molecular biology, bio-information technology and nanotechnology, and in computer-based tools for monitoring physical and biological processes in field, greenhouse and laboratory, including on-line, real-time extension services that are giving providing growers greater proactive capabilities in regard to plant protection and scheduling fertigation and harvesting for higher crop quality.

One effect of an increasingly global marketplace is to put the spotlight on the issue of crop quality in relation to health and price. Quality in this connection is to be seen not simply in a fruit's sensory properties but more broadly in terms of healthfulness, residue-free levels, certification and traceability, parameters that

now have to be factored into price. This is why research too has been focusing more intently on all the issues concerning pre- and post-harvest, packaging, transport, marketing, logistics and the related services demanded by the big supermarket chains, which control over 70% of European market share today.

This is in fact the background against which the EU and its CMO reforms are re-ordering the map of Europe's most suitable orchard districts and reorganising the ways the supply chain is managed by promoting producer organisations and membership therein. These efforts are intended to encourage the EU's producing countries to regain their specific profiles and to safeguard the future of their traditional crops through the awarding of IGP, DOP and other EU quality seals.

The overall intent of these policy measures is to encourage EU producers, both in the southern countries with their climate advantages and in the colder, disadvantaged northern ones, to make the best use of their natural and technological resources to gain a greater edge in competing for share in the marketplace with premium-quality, typical local niche-market produce, Norwegian cherries being just one of many examples. A closely related aim is to help protect fruit from the wild market swings that beleaguer such commodities as grains, oil seed and pulses and that are largely the making of speculation driven by globalisation and the strategies of the big multinational corporations.

# **Lectures and Posters of Theme 1**

**HEALTH ASPECTS: FROM VEGETABLES TO HORTICULTURAL  
THERAPY AND FROM ORGANIC TO FUNCTIONAL FOOD**

## Fruits, vegetables, phytochemicals and human health: past and future

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**Fruit and vegetables take part in food balance.** F&V are low energy dense foods, and generally eaten in place of high-energy dense, fatty and sugary foods. Substantial evidence indicates that optimal diets with a high intake of F&V, whole grains as the main form of carbohydrates, nonhydrogenated unsaturated fats as the predominant form of dietary fat, and adequate polyunsaturated fatty acids can offer significant protection against chronic disease. There is epidemiological and experimental evidence confirming that the consumption of F&V may reduce the risk of several cancers, mainly stomach and lung cancers. In addition, a daily intake of F&V in an adequate quantity (400-500 g per day) is recommended to reduce the risk of cardiovascular diseases, stroke and high blood pressure.

**Fruit and vegetables may protect against chronic diseases because they are important sources of a wide range of bioactive substances, such as fibres, vitamins and minerals.** Health protective could be attributed to the additive and synergistic effects of these diverse bioactive substances. Dietary fibre allows moving potentially harmful substances through the intestinal tract and lower blood cholesterol levels. It has been recently shown that fibre from fruit was associated with lower blood pressure. F&V are the unique source of vitamin C that with vitamin E are powerful antioxidants. Vitamin B9 (folic acid) one of the most common B-vitamins present in green vegetables is reported to significantly reduce the risk of neural tube birth defects in newborns and may contribute to the prevention of heart diseases. Vitamin A provided by provitaminic carotenoids ( $\alpha$  and  $\beta$ -carotene and  $\beta$ -cryptoxanthin) maintains eye health and its deficiency also impairs immunity resulting in an impaired ability to counteract infectious diseases.

**Fruits and vegetables also provide numerous substances known as phytochemicals.** F&V contain over 5000 bioactive phytochemicals, which are structurally diverse and are classified into four main groups: terpenoids, phenolic compounds, nitrogen-containing alkaloids and sulphur-containing compounds. Many more phytochemicals continue to be discovered. It is estimated that more than 100 different phytochemicals are present in one serving of fruit or vegetables. Phytochemicals are considered as non-essential micronutrients. Since 1980s', numerous studies are dedicated to isolate a great number of these phytochemicals, to analyze them in food products and to specify their disease-preventing properties. A recent meta-analysis on flavonoid, a sub-class of polyphenols, revealed an inverse association between flavonoid intake and fatal or nonfatal coronary heart disease (Arts & Hollman, *Am J Clin Nutr*, 2005). Recently, it has been reported that high dietary intakes of plant lignans (another group of phenolic compounds) and high exposure to enterolignans were associated with reduced risks of postmenopausal breast cancer in a Western population that does not consume a diet rich in soy (Teyssier et al, *J Natl Cancer Inst* 2007). In addition, epidemiological studies indicate that human exposure to isothiocyanates and indoles through cruciferous vegetable consumption may decrease cancer risk (Higdon et al, *Pharmacol Res* 2007). Other studies provide some evidence that an increased consumption of lutein and zeaxanthin with a diet rich in green vegetables is associated with a lowered risk for age-related macular degeneration, a disease with increasing incidence in the elderly (Stahl, *Dev Ophthalmol* 2005).

**Phytochemicals display diverse functions involved in health benefits.** Many phytochemicals present in F&V have been identified as antioxidants and a considerable body of literature supports their capacity to counteract oxidative stress through direct scavenging of free radicals. Carotenoids are then able to scavenge free radicals (Krinsky and Yeum, *BBRC* 2003) such as singlet molecular oxygen ( $^1O_2$ ) and peroxy radicals, and protect cellular systems from oxidation. The most potent antioxidant among various carotenoids is lycopene. The antioxidant capacity of flavonoids has been largely reported in numerous *in vitro* and *ex vivo* systems. Nevertheless, with the recent advances in the field of metabolism and absorption, it is clear that numerous phytochemicals are poorly absorbed and for some of them extensively metabolized by the intestine, liver and through the actions of the colonic microflora (Holst & Williamson *Nat Prod Rep.* 2004; Manach et al, *Am J Clin Nutr* 2005; Rao & Rao, *Pharmacol Res* 2007). Thus, antiradical effect is supposed improbable and it is recently hypothesised that the bioactivity of phytochemicals or their metabolites could be related to their ability to modulate some cell signalling pathways and gene expression, and their ability to react with certain receptors. Scientists are currently using new "omic" tools to specify the role of phytochemicals



and are considering genetic differences that can modulate nutrient bioavailability and the response of target tissues related to disease risk. Thus, diet and nutrition advice in the future will be increasingly personalized.

**In general, if there is sound evidence of some protective effect of a frequent consumption of F&V, there is no convincing evidence for dietary supplements. Improving the quality of F&V is a challenge and an opportunity for a healthy and sustainable diet.**

## Why and how managing the phenol content in horticultural crops?

TREUTTER DIETER

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Phenolics compounds occur widespread in plants and are a biological important and chemical diverse group of secondary metabolites. In contrast to former times, these compounds are no longer judged as waste products or as evolutionary remnants without current function, nor as mere metabolic end products that are toxic to the plant and are therefore stored away in vacuoles. Moreover, they possess a wide range of biological activities. One of them is their contribution to human health which made them prominent in the past 10 years. They are also beneficial for the plant itself as physiological active compounds, as stress protecting agents, as attractants or as feeding deterrents, and, in general, by their significant role in plant resistance.

Defence-related metabolites can be divided into two groups: “preformed” and “induced” compounds. The “induced” compounds are synthesised by plants in response to physical injury, infection, or stress. They may also be constitutively synthesised but, additionally, their biosynthesis is often enhanced under the influence of several types of stress. Or they may occur only after infection or several types of stress as so-called phytoalexins. The “preformed” phenolics are innate compounds which are synthesised during the normal development of plant tissue. These preformed metabolites are often stored at strategically important sites where they may play a signalling and/or a direct role in defence. The relation between the localised accumulation of flavonoids and their activity is plausible with regard to the accumulation of UV-absorbing flavonoids in epidermal tissues. The site of accumulation seems to clearly indicate a beneficial function. In other cases, a proof, however, is much more difficult to be carried out.

With the consciousness of the beneficial effects of phenolics in protecting plants from pests and pathogens or for human health, one may create the idea to actively stimulate their biosynthesis and accumulation. Simmonds (2003) stated that at least “in theory we could be creating a world of plants richer in flavonoids”. It is often described that pathogens induce the biosynthesis of resistance related metabolites but also non-pathogenic strains are capable to elicit secondary metabolism. Furthermore, the phenylpropanoid and flavonoid synthesis varies and is induced by ecological factors such as UV-light, hydric stress, temperature or ozone.

Beside these factors it is the general supply of nutrients which can indirectly modify the secondary metabolism via controlling growth and differentiation processes. Therefore, the directed induction of both phenol biosynthesis and accumulation may be limited by substrate availability, this means carbon supply and energy, and by ontogenetic constraints.

These aspects will be reviewed with respect to the following topics: breeding, use of cultivars, cultivation techniques (environment, light, fertilisation), induction / elicitation (biotic and abiotic elicitors), alteration of biosynthesis (modification of enzyme activity), gene technology.

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## **Induction of glucosinolate accumulation by targeted pre- and postharvest applications**

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Inverse associations between vegetable intake and chronic diseases, such as different types of cancer and cardiovascular disease, have been demonstrated in numerous epidemiological studies.

Phytochemicals such as glucosinolates have been indicated to be responsible for this observed protective effect. Glucosinolates are a group of secondary plant metabolites found exclusively in plants of the order Capparales, including horticultural important crop plants of the Brassicaceae family. Hydrolyzed products of glucosinolates are known to confer health-promoting effects due to their assumed anti-carcinogenic properties.

However, the overall vegetable consumption in the industrialized nations of Northern Europe and North America is relatively low, and is also well under internationally recommended amounts, i.e. approx. 375 g vegetables per day as advocated by many international health bodies. Therefore, one way of increasing the consumption of health-promoting phytochemicals in the diet would be by increasing their levels in the vegetables themselves.

Application of pre- and postharvest elicitors can trigger distinct changes in the plant's secondary metabolism. Thus, targeted pre- and postharvest elicitor treatments may be used to obtain vegetables enriched with glucosinolates for sale as fresh market products or used as raw material for functional foods and supplements, thereby promoting higher consumption of these health-promoting substances.

Specific pre- and postharvest elicitor treatments, e.g. low or high temperature treatments, ultraviolet and gamma irradiation, altered gas composition or application of signaling molecules, enhance glucosinolate concentration.

Therefore, aims of this presentation are (1) to present an overview of the types and effects of pre-postharvest applications on glucosinolate concentration and composition in vegetables and (2) to discuss the implications of this in terms of targeted pre- and postharvest management.

## **Broccoli at different head development stages - a chance to increase phytochemicals for different customers**

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### ***Introduction***

Broccoli, a cabbage vegetable enjoying increasing popularity in Germany, is rich in vitamin C (ascorbic acid) and phytochemicals like carotenoids, chlorophylls, flavonoids and glucosinolates that have health-

promoting properties and influence product quality. Health-promoting compounds may be affected by numerous preharvest factors such the developmental stage at which a vegetable is harvested. The objectives of this investigation were (1) to determine the ontogenetic variation during head development on the content of quality-determining compounds in broccoli and (2) to clarify whether compound contents rise or fall during head development and whether similar trends are observed between broccoli cultivars.

### **Material and Methods**

Three broccoli cultivars, spear broccoli 'Emperor', crown broccoli 'Marathon', and violet broccoli 'Viola' were grown in the field and harvested during head ontogeny in three different years. According to the BBCH scale (uniform coding of phenologically similar growth stages of plant species) by Meier (1997), we classified the harvested heads in five development stages: 1, start of head development; 2 and 3, mini broccoli; 4, fully developed heads (usual commercial stage); and 5, over maturity stage (first individual flowers visible). The carotenoid (lutein,  $\beta$ -carotene), chlorophyll (chlorophyll a, b), flavonol (quercetin, kaempferol), ascorbic acid, and glucosinolate contents were analyzed by HPLC (Krumbein et al. 2005, 2007).

### **Results and Discussion**

Heads of over maturity stage (first individual flowers visible) had the highest contents of carotenoids, chlorophylls, flavonols and ascorbic acid. However, the genotype fundamentally determined the quantity and course of the increase. The total glucosinolate content in the broccoli florets as an average of the three cultivars was 50% higher in the first development stage (start of head development) than in the fully developed heads (commercial stage) mainly due to a decrease of the indole glucosinolates glucobrassicin and neoglucobrassicin. The highest contents of the alkyl glucosinolates were mainly due to glucoraphanin and were found in the first development stage followed by a decrease until the second or third development stage; both of which are used as mini products by broccoli growers. Mini broccoli as a new trend for marketing vegetables has also lower contents of flavonols than the commercial stage, which indicates a reduction in healthy potential.

Thus, we conclude that for production of glucosinolate-rich broccoli, younger, less developed heads are preferable to fully developed ones. However, when wanting a high antioxidative potential, i.e. relatively high carotenoid, chlorophyll, flavonol and ascorbic acid contents are desirable, fully developed heads should be harvested for consumers. Harvesting broccoli heads of over maturity stage should use as raw material e.g. for development of new functional foods.

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## **Anthocyanins from *Prunus mahaleb* L. fruits**

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*Prunus mahaleb* L. is traditionally used as rootstock for sweet and sour cherry culture. It produces small stone fruits, highly pigmented, that have no importance for crop production, due to their astringent and sour taste. These fruits have never been characterized for their chemical composition. Currently, research interest

is particularly focused on the phenolic composition of fruits and vegetables in order to assess the potential of unknown or not traditionally-used plants as sources of biofunctional compounds. To this purpose, we report here the extraction of anthocyanins from the fruits of *Prunus mahaleb* and their characterization by means of diode array detection (DAD) and mass spectrometry (MS) coupled to high-performance liquid chromatography (HPLC).

'Mahaleb cherry' fruits have been picked up at full ripeness from a local selection orchard. The pigments were extracted from the fruits with acetone and chloroform as reported by Rodriguez-Saona and Worlstad (2001). This extraction method has the advantage of producing an extract with no lipophilic contaminants and with no pigment degradation, due to the absence of a concentration step. Preliminary results from HPLC/DAD/MS analysis indicated that 'Mahaleb cherry' fruits were extremely rich in anthocyanins. The HPLC chromatogram showed three peaks corresponding to four different cyanidin derivatives.

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## **Bioactive components in fruits of different apple varieties-effect of cultivar, ripeness and storage conditions**

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Consumption of fruit and vegetables has been shown to be effective in the prevention of chronic diseases. The benefits are often attributed to the high content of foods. Apples are commonly eaten in central Europe during the whole growth period and are large contributors of secondary plant metabolites.

The present investigation was undertaken to determine the antioxidant content in selected apple varieties, depending on cultivar, ripeness and different terms of postharvest storage (4 and 12 weeks after harvest in cold and CA storage, 1 year after harvest under controlled atmosphere). An additional aim was to establish a relationship between the antioxidative capacity and the content of secondary plant components.

For sampling, fruits were harvested from definite positions of the tree for each variety and location. Optimal harvest date was determined by Streif-Index. The antioxidants in apple fruits differed between variety and cultivation methods. Ripe harvested fruit had higher ascorbic acid content and also higher antioxidative capacity in comparison to unripe and overripe harvested fruit (in the lipophilic extract as well as in hydrophilic extract). Correlation studies showed that the total phenols (Folin value) contributed strongest to the TEAC antioxidant value of apple fruits while the contribution of ascorbic acid seemed to be low. This was true for harvested as well as for stored apple fruit ( $r^2 = 0.6$ ). The antioxidative capacity (TEAC value) of 'Jonagold' fruit increased strongly after storing for one year under CA conditions. The reason for this effect is still unknown. The high content of secondary metabolites in some varieties, especially in new developed varieties, and the related antioxidative capacity indicated that the content of secondary metabolites can be enhanced by breeding. These fruits with high contents of phenolic substances may impart health benefits when consumed and should be regarded as a valuable source of antioxidants.

## Effect of variety, cultivation and storage conditions on the apple allergen Mal d 1

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The prevalence of allergies is increasing throughout the world. Today up to 70% of birch-pollen-allergic patients may be food sensitive, most frequently to apples. The parallel appearance of birch and food allergy can be explained by cross-reactive IgE. The major birch pollen allergen Bet v 1 and the in Central Europe most important apple allergen Mal d 1 share allergenic epitopes leading to these IgE cross-reactivities. Mal d 1 has been identified as a 18 kD protein, present in pulp and peel of apple fruits. Both Bet v 1 and Mal d 1 belong to the so called pathogenesis-related (PR) 10 proteins, a family of proteins that are induced by pathogens, wounding, or certain environmental stresses.

The allergenic composition of most apple cultivars has not yet been fully characterized. Data on the IgE binding potency of Mal d 1 in vitro and in vivo showed that this allergen is influenced by cultivar, degree of maturity and storage conditions of the fruits. The amount of Mal d 1 depending on these factors and the effect on human health has not been determined so far. The aim of the study was to quantify Mal d 1 content of the fruits depending on cultivation method (organic and integrated production), cultivar and different terms of postharvest storage. Fruits of different apple cultivars (*Malus domestica*) were cultivated at the Research Stations Klein-Altendorf, Bonn University and Bavendorf, Hohenheim University, Germany. Optimal harvest date was determined by the Streif-Index. Fruits were harvested from definite positions of the tree for each variety and location.

Protein extracts were prepared from the apple fruits after removing the core according to Björkstén et al. 1980. Mal d 1 content was quantified by a Sandwich-Enzyme-linked Immunosorbent Assay.

Apple cultivars differed considerably in their Mal d 1 content. Highest amounts of Mal d 1 were determined in 'Gala' and 'Rubens' fruits. The lowest content was found in the cultivars 'Braeburn' and 'Elstar'. During storage the Mal d 1 content increased significantly compared to the Mal d 1 content at harvest date.

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## Ellagitannin content in raspberry and blackberry cultivars grown in Trentino (Italy)

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Berries are amongst the richest sources of phenolic compounds in the human diet. Some of the phenolic compounds found in berries are present in many other fruits and vegetables, while others (e.g. ellagitannins) are specific to some species of berries. In red raspberries and blackberries ellagitannins are the main class of phenolic compounds and, therefore, this type of fruit represents one of the richest sources of ellagitannins in the human diet. In view of increasing consumer attention to nutrition, the antioxidant content of berries and, in particular, their phenolic profile ought to be promoted as an important trait for breeding programmes. Therefore, screening programmes should be established which clarify qualitative and quantitative genotypic differences in species in relation to berry antioxidant composition.

This study provides a picture of the variability of the ellagitannins in 36 important *Rubus* genotypes: 21 raspberry and 15 blackberry cultivars. The cultivars investigated were chosen in order to have a balanced number of primocanes and floricanes of different origin and commercial importance. Acid hydrolysis of the ellagitannins in methanol was carried out in order to quantify all the major reaction products (ellagic acid, methyl-sanguisorboate, methyl-gallate and an unknown ellagic acid derivate) and estimate their mean degree of polymerisation. The ratio between maximal and minimal ellagitannin concentrations recorded among red raspberry cultivars did not exceed 1.7, indicating relatively limited variability among the cultivars investigated, while this variability was much higher in blackberries reaching a ratio of 4.4 between the cultivars. The mean degree of polymerisation was between 1.61-1.86 in raspberries and between 1.59 - 1.92 in blackberries, thus confirming the presence of a mixture of monomers and low oligomers in *Rubus* ellagitannins. The data supports the hypothesis of a genetic determinant of ellagitannins content and profile in the different genotypes.

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## **Developing Human Capital through Horticulture**

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Global warming, environmental pollution and the search for an alternate planet for future human habitation have focused increasing attention on the importance of green plants for human survival. Plants have been part of human nature, civilisation and culture since the beginning of recorded time, however, while plants are embedded in society, physically and socially, few of us are conscious of their immediate importance. While most people in society suffer this “plant blindness” those focused on plant science have expanded their research beyond the traditional boundaries of plant biology, genetics and cultivation to explore the less obvious interactions between people and plants.

In developed economies, less encumbered with food production for survival, a further realisation has emerged that an understanding of the psychological, physiological and social responses of people to plants could be a very valuable tool in improving the physical and mental health of individuals and communities, particularly in urban areas. Consequently, “Human Issues” in horticulture has received considerable recent attention. The term Horticulture has been redefined and expanded. Horticulture has moved beyond the garden and commercial holdings to embrace topics such as Horticultural Therapy, Therapeutic Horticulture, Social Horticulture and the use of horticulture for the development of human capital. The specific benefits of using horticulture as a medium for the development of human capital are reported in this paper. Human capital in this context is not focused solely on improving the economic output of individuals but rather on their inalienable or personal traits.

Over a number of years the horticulture staff at University College Dublin (UCD) has provided an outreach programme linking faculty, postgraduate and undergraduate students with communities of people with developmental disabilities. One programme, now in its 6th year hosts the practical component of a two year National Foundation Certificate in Horticulture. Students who participate in this course come from a single disability support service provider and have a mild to moderate learning disability. Typically, students complete the practical elements of this vocational horticulture course by attending the glasshouse and field research facilities at University College Dublin. Acquisition of horticulture skills is achieved through the students working on daily horticulture tasks, assisting on research projects and attending relevant undergraduate practical sessions. While this is a primary learning outcome for this course, the students were observed to have expanded their social skills, particularly, in relation to independent travel and their ability to work with other people.

In 2004 a unique educational course, the National University of Ireland undergraduate Certificate in Citizenship and Advocacy (CCA) was established at UCD. This course developed from an initiative by the Centre for Disability Studies and evolved organically from a group of interested individuals within the university. The CCA programme was delivered by an interdisciplinary team of academic, administrative, technical staff and students representing Disability Studies, Horticulture, Computer Science and Communications disciplines. One of the modules in the CCA programme was titled "Horticulture and Environment". This featured plant biology, plant propagation, plant cultivation and interaction with urban trees as a platform to enable students to utilize the skills gained on the computer, communications and advocacy modules. The horticulture module proved to be a safe, non-threatening environment which facilitated individual and group learning. A number of graduates from the Foundation Certificate in Horticulture were joined by other students from around Ireland on the CCA course. Students attended college two days per week during the academic year and were fully registered UCD students. While students had a wide range of developmental disabilities, the focus of the CCA was on developing the students' abilities and capacity and love of learning. The learning outcomes for the CCA were targeted to develop "life skills" including self-advocacy, communications and social skills. The horticulture module provided one platform in this course for the development of the student's human capital. To date two groups of 20-24 students have successfully graduated from this certificate course. The experiences of students and tutors on these courses are reported in a qualitative fashion.

## **Small urban gardens for the elderly in Italy**

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### ***Introduction***

With ongoing world-wide urbanization, small urban gardens (SUGs) have become an important reality for a wide range of purposes including a source of food and ornamentals as well as recreation (Groening, 2005), with the main users represented by the elderly. Different from home gardens, these are community gardens not attached to the home and may be found on a land of few to hundreds square meters that belongs to public bodies or to private landowners. Politicians, professionals and others engaged in urban land use need to consider such reality. The aim of this research was to study the situation of SUGs in Italy.

### ***Materials and methods***

In 2001, we carried out a survey throughout Italy by sending a questionnaire to all municipal administrations of Province towns and some other municipalities. Data from the questionnaire were also supported by interviews with municipality technicians and representatives of elderly associations.

### ***Results and discussion***

Among the municipalities that answered our questionnaire, 111 have SUGs, but the most (100) are in Northern Italy, above all in the Emilia Romagna Region (Table 1). Most of gardens were developed starting from 1975 with an increasing rate in the following 3 decades: indeed, in many regions SUGs exist from less than 10 years. The number of gardens per town (from few to thousands) and the size (from few to hundreds square meters, in most cases 30-70 m<sup>2</sup>) vary with the town, independent of the region and seem to be mainly affected by the municipality guidance, which in turn is the result of size and arrangement of urban areas, land availability, governors' sensitiveness, presence of complementary/alternative services and facilities for citizens, water availability/saving aspects (water is often scarce in Southern Italy) (Table 1). Anyway, more and more town-planning schemes provide for small garden areas to meet the increasing citizens' demand.

Table 1. The spread and size of small urban gardens (SUGs) in Italy.

Italian areas	Regions	Municipalities with SUGs	SUGs per region	Frequency (%) of SUGs per class of area (m <sup>2</sup> )				
				10-30	31-50	51-70	71-100	>100
North	E. Romagna	77	13774	15	37	32	3	13
	Friuli	1	50	0	0	0	0	100
	Liguria	1	74	0	0	0	100	0
	Lombardia	12	1919	31	31	19	19	0
	Piemonte	6	645	0	40	20	20	20
	Veneto	3	726	43	29	14	14	0
Centre	Marche	6	974	0	57	28	15	0
	Toscana	1	55	0	0	100	0	0
	Umbria	3	460	0	0	0	67	33
South	Campania	1	32	0	0	0	0	100
<i>Italy total / weighed average</i>		<i>111</i>	<i>18709</i>	<i>16</i>	<i>36</i>	<i>28</i>	<i>8</i>	<i>12</i>

Each garden is generally allotted to one person, so the higher the number of gardens the higher the people involved. Generally, there is a list with the highest ranking for elderly resident in the surroundings and non-landowner, but other economical and social aspects can be taken into account (yearly income, disability, loneliness, etc.). In most municipalities the allotment and use of a garden follows a regulation for administrative aspects (ranking in the list of applicants, contract conditions, duration, lease rate, consumption rates, insurance) and technical aspects (way of use, keeping, fencing, use of chemicals and water), while agromonomical aspects (rotation, use of crop residues, rational fertilisation, etc.) are often neglected.

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## Phenolics and anthocyanins in strawberry

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Phenolic compounds are plant secondary metabolites with a large variability in their structure and occurrence that can be divided into subgroups including anthocyanidins, flavonols, flavones, flavanols, flavanones, chalcones, dihydrochalcones and dihydroflavonols. Very simple phenolics such as hydroxybenzoic acids as well as large polymers such as condensed and hydrolysable tannins have relevance both in plant resistance reactions and in the quality of plant-derived food. The defence-related flavonoids can be divided into two groups: “preformed” and “induced” compounds. The “induced” compounds are synthesised by plants in response to physical injury, infection, or stress and may occur constitutively in plants or they may occur as so-called phytoalexins. The “preformed” flavonoids are innate compounds that are synthesised during the normal development of plant tissue and they may be involved in several host-pathogen interactions. Small differences in the chemical structure of defence-related compounds lead to a change in the defence-capacity against pathogens (LANGCAKE ET AL. 1979; JEANDET ET AL. 2002; PEZET und PONT 1988). Probably different compounds may induce various reactions in human. So it is important to comprise the exact chemical structure of polyphenolics for estimating and comparing the anti-microbial and health effect after consumption. Strawberries involve various compounds associated to benzoic acids, hydroxy cinnamic acids, flavonols, flavanols, anthocyanins and combinations from different groups. Some compounds are associated with resistance against fungal pathogens on leaves (YAMAMOTO ET AL. 2000), against rooting pathogens (OKASHA 1967) or postharvest pathogens on fruits (ZHANG ET AL. 2006).

In this study the secondary compounds of the strawberry cultivar „Elsanta“ were investigated by HPLC- (high pressure liquid chromatography) and mass-spectroscopy at different degree of ripeness as foundation for comprising the dynamics of secondary metabolism and possible interaction between several compounds and fungal pathogens like *Botrytis cinerea*.



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## Seasonality of salicin and phenolic glycoside contents in the bark of three *Salix* species

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## Introduction

Herbal medicine products are dietary supplements that people take to improve their health. Many herbs have been used for a long time for claimed health benefits. In recent years herbal medicine gained more and more interest, even within the scientific community. To date the extraction, identification, and quantitative determination of biologically active phenolic compounds (such as salicylates like triandrin and salicin) from *Salix* bark and usage in ready medicines are of significant importance. Certain phenolic glycosides of *Salix* bark, particularly salicin and their esters like tremulacin or salicortin, have been shown to relieve rheumatic disturbances, infections, and headache. These glycosides have also non-inflammable, temperature-reducing, and pain-alleviating effects. Adverse effects, like a negative influence on the aggregation of the thrombocytes or local lesions of the gastric mucosa, caused by the synthetic medicine product acetylsalicylic acid (Aspirin) are not reported for salicin.

## Material & Methods

Due to the health promoting effects of salicin and their esters, the object of the study was to identify clones of *Salix* species which contain high phenolic glycoside contents in the bark for possible later recovery of certain phenolic compounds. We analyzed the phenolic glycoside contents of three willow species: *Salix daphnoides*, *Salix purpurea*, and *Salix pentandra*. Osier stacks of different willow clones were collected north-east of Germany and north-west of Poland in April and May 2006 and stock planted in Zepernick. Bark samples of clones were taken in March 2007 and lyophilised for phenol glycoside extraction and HPLC analysis. According to the chemical profiles seven independent clones of *S. daphnoides* and *S. purpurea* as well as four clones of *S. pentandra* with high phenolic glycoside contents were picked for further studies. The relative proportion of the characteristic secondary metabolites present in willow species and the changes within the beginning vegetation period were determined.

## Results

Clones of *S. daphnoides* showed the highest mean salicin and phenolic glycoside contents followed by *S. pupurea* and *S. pentandra*. Every *Salix* species had a characteristic phenol glycoside profile. The major salicylate of *S. daphnoides* and *S. pupurea* were determined as salicortin, whereas main compound of *S. pentandra* was 2'-O-acetylsalicortin. Phenol glycosides identified in willow species were the salicylates salicin, salicortin, 2'-O-acetylsalicin, 2'-O-acetylsalicortin, and tremulacin. Other phenolic glycosides were syrengin, catechin, ampelopsin, vimalin, purpurein, and narengenin-5-glucoside. According to our study the content of the secondary metabolites decreases from March to June 2007 and further from June to July 2007. In addition it could be shown that the seasonal variability of the secondary metabolites content in willow bark was larger than the inter- and intra-specific variation. Further research about the seasonality of phenolic glycoside contents of willow bark in autumn and winter is ongoing.

## Discussion

The fabrication of willow bark extracts is very expensive. Due to this the use of willow species and clones with highest amounts of salicylates is obligatory. Furthermore, we have to select individual clones from which maximum harvests per hectare can be gained, e. g. with fast growth performance. Beside the bark other plant organs of willow like the leaves contain phenolic glycosides. Usage of these parts for phenolic glycosid production could be considered. There are other factors which influence secondary metabolite content in willow bark such as the climate, the nutrient supply of the plant, the time of day and gender. All parameters need to be considered for effective commercial recovery of phenol glycosides.

## Antimicrobial and antifungal activity of different extracts from *Melia azedarach* L. on phytopathogenic bacteria and fungi of agro-food interest

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## Introduction

Bacteria and fungal contamination can negatively affect growth of in vitro cultured plant tissues and even cause large losses of plants, increasing the production costs of commercial laboratories. Alternative treatments to antibiotics and antifungal products have been found but sometimes the high costs did not allows their use in plant mass propagation. Over the last twenty years, a more responsible attention to environment preservation has increased interest for natural compounds to be used. Most studies reported the biocide effect of extracts from different tissues of *Azadirachta indica* and other species belonging to the family of *Meliaceae*, mainly related to the tetraterpenoid azadirachtin and to other terpenoids and limonoids. *Melia azedarach* L. is an ornamental tree that grows in mediterranean gardens and parks. It is generally propagated by seeds and cuttings, and can be also easily multiplied *in vitro* from nodal explants of seedlings and mature plants and from root and leaf explants. These findings have suggested the present research; an aqueous extract from callus and leaves of *Melia azedarach* L. was studied as potential antimicrobial agents for 24 selected phytopathogenic bacteria (*Pseudomonas* spp., *Sphingomonas* spp., *Kocuria* spp., *Bacillus* spp., *Pectobacterium* spp., *Xanthomonas* spp.) and compared with a commercial azadirachtin (Supelco); preliminary studies were also performed against 36 strains of different food yeast species (*Candida* spp., *Hanseniaspora* spp., *Schizosaccharomyces* spp., *Zigosaccharomyces* spp., *Pichia anomala*, *Saccharomyces* spp., *Trichosporon*, *S' Codes Ludwigii*).

## Materials and Methods

Aqueous extracts from callus (48g/10ml H<sub>2</sub>O) and leaves (40g/100 ml H<sub>2</sub>O) of in vivo grown plants and an ethanolic leaf-extract (20g/100ml EtOH) were tested. The effect of an azadirachtin solution (10 mg/500µl

ethanol) was also checked, as the major neem metabolite with well-known biocide activity. Experiments were performed in vitro by using an agar diffusion assay; Plate Count Agar (Merck) for bacteria and Malt Extract Agar for fungi was seeded with late exponential cultures of the test organisms (final population  $10^6$ /ml) and allowed to set in 90 mm petri dishes. Three 7-mm-diam wells were cut from the medium and filled with 50  $\mu$ l of the ethanol or aqueous extract and the azadirachtin solution; distilled water (pH 4,5), a 40% ethanol solution and absolute ethanol were used as controls for the leaf extract and azadirachtin.

Plates were incubated aerobically for 24-48 h at the temperature of the test strains. The diameter of the inhibition zone (reduced growth/no growth), including the diameter of the well, was taken as a measure of the bacteriostatic/bactericidal activity for each extract and azadirachtin, and reported as the mean diameter  $\pm$  standard error (SE) for triplicate tests.

### **Results and Discussion**

Nineteen of the 24 bacteria tested were strongly inhibited by the leaf-extract (inhibition zone >16mm). The extract from callus exhibited a great bacteriostatic effect towards *B. circulans*, *B. subtilis* and *K. kristinae*. The solution of azadirachtin showed a weak bactericidal effect against *Bacillus* spp., *Pseudomonas* spp. and in few cases a bacteriostatic effect. In regard to yeasts, the aqueous extract showed a significant biocide effect on *Pichia anomala* and *S' codes ludwigii* which represent contaminant yeasts in the foods. The strong antimicrobial activity of the aqueous leaf-extract from *Melia azedarach* is of great interest in respect to extracts in organic solvents that might be phytotoxic to plant tissues and human health. These extracts could be taken into account also on the application of food preservation and oenology, considering that yeasts showing inhibition for *M. azedarach* extract result resistant to other antimicrobial compounds. Moreover, these results suggest the presence of natural compounds different from azadirachtin in the extracts that need to be identified.

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## **Development of a new horticultural therapy evaluation method through color reflection**

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### **Introduction**

In Japan it is very important to make the society as much comfortable as possible for the elderly to spend because Japan is an aging society like the ones in European countries or more than those.

The horticultural activity is said to improve effectively the QOL of the elderly. However there is not much research that proved that definitely. Brain wave monitoring and blood tests, POMS etc. are used for the measurement of the effects of gardening activity. However these methods are undesirable for the elderly because there is a considerable mental and physical burden in using them. Even if we try to make measurements within a short time there are a few mental and physical burdens that will affect the pleasure that the horticultural activity brings. We developed the "color evaluation method" which is an evaluation method that uses color reflection.

We evaluated the horticultural activity of several object persons for the purpose of inspecting the effectiveness of the color evaluation method while comparing it with other psychological and physiological investigation methods.

### **Methods**

1. Object persons: we divided them in three groups:

- a) Elderly people who did horticultural activities;
- b) Students who did horticultural activities;
- c) Students who attended a normal lecture without doing on horticultural activities.

2. Horticultural activities: Gardening activity of about 1 hour.

3. Survey item: Color evaluation method, psychological test (POMS, ABS) and the measurement of the heart rate. We did not use POMS for the elderly because of the burden caused.

4. Survey method: We carried out each measurement before and after the horticultural activities or lecture. Each person was showed a sample of 12 colors and s/he had to choose the color that most matched his/her emotional state at that time.

### ***Results and Discussion***

Before activity the object persons chose different colors but it was observed that they tended to choose warm colors after the horticultural activity. This tendency was more remarkable with the students than with the elderly. The object persons who did not work on horticultural activity (i.e. the students who attended a normal lecture) choose cool colors by the time the other object persons finished horticultural activity. These tendencies strikingly resembled the results of POMS and ABS which express psychological condition: when an object person choose warm colors, the psychological test showed a comfortable emotional state and when an object person choose cool colors, the psychological test showed an uncomfortable emotional state. In addition, high correlation between the color evaluation method and the other methods was observed in the measurement results of the heart rate although it was thought the there would be comparatively few burdens when the measurements were made.

From these results, the color evaluation method can easily and simply grasp the change of emotional state during horticultural activity. We also think that the color evaluation method is very effective as an evaluation method which does not cause any mental or physical burdens to the elderly who were hard to be evaluated before. As our future work, we intend to continue investigating more cases and we want to study horticultural activities and evaluation methods suitable for the elderly.

## **Storage temperature effects on the antioxidant profile in tomato fruit**

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Ripening is an aspect of development that is unique to fruit and is initiated after seed maturation has completed. During this final stage, fruits undergo a complex series of physiological and biochemical alterations involving tissue softening and textural changes, in some cases the conversion of chloroplasts into carotenoid-accumulating chromoplasts, flavour development and alteration of sugar and acid levels. Fruit harvest does not complete the ripening process, but ripening stage at harvest and storage conditions are assumed to have an impact on fruit development during further off-vine ripening.

Trading and consumption of domestic fruits are generally quite prompt. Unfavourable market conditions as well as the necessity to store the product after purchase can lead to the consumption of fruits several days old. Therefore knowledge about quality changes during storage is quite important and forces the research in this field.

In the current study tomato fruits (*Solanum lycopersicum* L.) of two cultivars ('Caransa', 'Favorita') were stored at 12 C and 26 C for 16 days. The analyses included secondary compounds that highly influence quality and provide nutritional quality. Ascorbic acid, carotenoids and tocopherols were analysed with HPLC after 2, 4, 8, 11 and 16 days of storage.

Both cultivars differed strongly in their response to the storage temperature: They did not show any changes of ascorbic acid, beta-carotene and lycopene at 12 C. However, storage at 26 C lead to massive increases of ascorbic acid (+34%), beta-carotene (+47%) and lycopene (+108%) in fruits of 'Caransa', whereas these compounds were less affected in fruits of 'Favorita'. In cultivar 'Caransa', lutein concentration decreased at 12 °C, whereas it showed a highly significant increase at 26 C after 8 days of storage. In 'Favorita', there was a highly significant tocopherol and lutein increase only after 2 days of storage at 26°C. An important of the study showed that the storage temperature (26°C) decreased the total tocopherol content and changed the composition to a lower contribution of  $\delta$ - and  $\gamma$ -tocopherol. The data support the hypothesis that temperature may play a specific role in the regulation of  $\gamma$ -tocopherol methyl-transferase and lycopene cyclase in fruit tissue.

## **The Effect of Two Different Growing systems (Integrated Pest Management and Organic Cropping System) on Strawberry Fruit Health Components and Quality**

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### ***Introduction***

Public strawberry breeding Italian programs, started more than 40 years ago, released several varieties of commercial importance for northern areas (Po Valley). Now particular attention is given to improve fruit taste (good balance of sweetness and acidity, flavour and juiciness) and more recently, to enhancement of the antioxidant compounds content. In fact fruits and vegetables contain many antioxidant components of different nature and in very variable amounts. Their consumption has been primarily associated with lower incidence and mortality rates due to degenerative diseases such as cancer and heart disease. The phytochemicals responsible for the antioxidant capacity, can largely be attributed mainly to the phenolics, anthocyanins and flavonoid compounds. Strawberry fruit is considered a source of many bioactive phytochemicals. It contains ellagic acid, a natural phenolic, considered a dietary antimutagen and anticarcinogen; ascorbic acid and other polyphenols, which provide protection against harmful free radicals and skin ageing. However, little information is available on the effect of environmental factors and growing systems on the scavenging capacity of strawberry. Only a preliminary investigation on the effect of two different growing systems (Integrated Pest Management and Organic Culture), on healthy compounds and quality traits in fruits of some strawberry varieties, was done by other authors and the results were advantaged to organic strawberries.

### ***Materials and Methods***

The present study has been carried out, for three years (2005-2007), to know the effect of two different growing systems (Integrated Pest Management and Organic Culture), on strawberry fruit health components and quality traits of three Italian varieties (Alba, Onda and Queen Elisa).

These cultivars were grown in experimental field located in Cesena (Po Valley area), in two different growing systems. Cold stored plants were planted in open field, according to four replications randomized block design. During the fruiting season, fruits yield, average berry weight, earliness index were registered. Twenty ripe fruits from each cultivar were used for quality determinations (skin colour, firmness, skin resistance, soluble solids content, titratable acidity, nitrates content) and the analysis of health – promoting properties and components (antioxidant capacity, polyphenols, ellagic acid and ascorbic acid content).

### ***Results***

The organic growing system gave lower fruit yield, but the fruits had the highest sugar content, vitamin C and antioxidant capacity. These results could be used to improve market value of the strawberry produced by organic culture.

# Phenolic and antioxidant activity of wild and cultivated Chicory cultivar growing in South Salento (Apulia, Italy)

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## Introduction

Knowledge of the biochemical composition of wild and/or cultivated species can provide useful information about their possible use as food or as potential sources of biologically active metabolites. Many epidemiological studies have pointed out the links between diet and some diseases, as well as the protective effects of eating fruit and vegetables. It has recently been shown that in addition to vitamins and  $\beta$ -carotene, some dietary plant compounds such as phenols contribute to protection from free radicals. The latter, which cause lipid peroxidation, may lead to several chronic diseases. For these reasons, there is growing interest in food composition, particularly in those compounds which may confer “nutraceutical” properties on foods. *Cichorium intybus* L. is an important crop widely used in human nutrition and is also well-known as an important medicinal herb, having been used in folk medicine for liver disorders, gallstones and inflammations of the urinary tract since 17th century.

The aim of this study was to determine the bioactive constituents (total phenols, caffeic acid derivatives) and the antioxidant activity (AA) of extracts obtained from three *Cichorium* species cultivated in the Salento area (Southern Puglia, Italy).

## Materials and Methods

The varieties used were *C. intybus* wild, *C. intybus* cv. *Catalogna* “cicoria Galatina”, and *C. endivia* cv. “riccia”, supplied by the Botanical Garden of Salento University. The extracts were prepared, purified and analyzed as reported by Negro et al. (*Italus Hortus* 13(2) 2006: 749-751). Antioxidant activity (AA) was determined both as  $\beta$ -carotene oxidation protection and DPPH quenching.

## Results

The results obtained show that the total phenol content was 0.45, 0.48 and 0.81 mg/ml, corresponding to about 2, 1.2 and 1.1 mg/g of fresh weight (FW), for *C. intybus* wild, *C. intybus* cv “cicoria Galatina”, and *C. endivia* cv. “riccia”, respectively. The caffeic acid derivative content ranged from 0.14 to 0.39 mg/ml corresponding to 0.36 and 0.99 mg/g FW. The antioxidant activity of the extracts ranged between 63.8% (*C. intybus* wild) and 81.7% (*C. endivia* cv. Riccia) when determined as  $\beta$ -carotene protection, and between 61.8% (*C. intybus* cv “Galatina”) and 78.1% (*C. intybus* wild) when determined as DPPH quenching.

These results support the hypothesis that a diet rich in vegetables can reduce oxidative stress and its effects. Thus, they may help increase the consumption of various edible plants with potentially beneficial effects on health.

Table 1 – Total Phenols, Caffeic acid derivatives (mg/g of fresh weight) and antioxidant activity (%) of *Cichoria* spp. extracts.

Sample	Total Phenols	Caffeic acid derivatives	Antioxidant Activity (%)	
			$\beta$ -carotene protection	DPPH quenching
<i>C. intybus</i> wild	2.01 $\pm$ 0.28	0.99 $\pm$ 0.08	81.7 $\pm$ 0.8	78.1 $\pm$ 1.4
<i>C. intybus</i> “Galatina”	1.19 $\pm$ 0.20	0.36 $\pm$ 0.09	67.7 $\pm$ 0.9	61.8 $\pm$ 1.1
<i>C. endivia</i> “riccia”	1.13 $\pm$ 0.18	0.39 $\pm$ 0.08	63.8 $\pm$ 1.1	62.2 $\pm$ 0.9

## Fire blight resistance in apple: Influence of polyphenols and salicylic acid

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Fireblight is a devastating bacterial disease, *Erwinia amylovora* (*Ea*) and infects most members of the *Maloideae* such as pear and apple. Natural resistance exists in some wild apple species, such as *Malus robusta*, while commercial apple cultivars are highly susceptible to fireblight. To elucidate the functional basis of resistance molecular and biochemical investigations have been performed with *M. robusta* and *M. domestica* cv. Idared. Secondary metabolites such as polyphenols and in particular flavonoids have been shown to be important in defense against bacterial pathogens. The gene expression patterns of flavonol synthase (FLS), chalcon synthetase (CHS), chalcone isomerase (CHI), dihydroflavonol reductase (DFR), flavonol synthase (FLS), phenylalanine ammonia-lyase (PAL), anthocyanidin synthase (ANS), Anthocyanidinreductase (ANR), Flavonoid 3-O-glucosyltransferase (F3GT) were studied. The results reveal a general stable expression pattern in the resistant *M. robusta* after inoculation with the pathogens, whereas in the susceptible cultivar “Idared” severe up- and down regulation of genes has been observed. The signal molecule, salicylic acid has been analyzed, as SA molecules are triggering in plants different defense mechanisms such as hypersensitive response and systemic acquired resistance. In *M. robusta* the SA-level is five times higher compared to susceptible “Idared”. In addition we developed an in situ quantitative PCR-assay to monitor the bacterial density of *Ea*. After three days of infection, the bacterial density in *M. robusta* is lower compared to “Idared”. Therefore we conclude that constitutive defense compounds are the basis of fireblight resistance, where polyphenols might have some contribution, but are not the sole source.

## Variation of tocopherols content in Italian olive drupes during ripening stage

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Traditionally, the olive tree (*Olea europaea* L.) is grown mainly in the Mediterranean area, but the benefits of olive products on human health have been widely recognized and spread throughout the world. Olive drupes can be either processed as table olives or milled to produce olive oil. According to varieties, some of them are cultivated specifically for table consumption while the majority is used for oil extraction. Tocopherols are antioxidant compounds that play a key role in conferring nutritional value to olive drupes. Tocopherol biosynthesis takes place on the inner membrane of chloroplasts and chromoplasts. The four tocopherols (alfa, beta, gamma and delta forms), differ from one another by number and position of methyl groups in the phenolic part of the chromane ring. The multifunctional roles of these compounds are related to their preventive action against reactive oxygen species (ROS) in biological systems. In the past, alfa-tocopherol was considered as the isomer exhibiting the highest biological activity. Nevertheless, recent studies suggest that the other vitamin E isoforms have also important roles in the human organism. For example, gamma-tocopherol has considered as a cancer chemo preventive agent and as a potent and effective agent in the prevention of cerebral infarction induced by middle cerebral artery occlusion. Thus, the knowledge of tocopherol profile is essential for estimating the potential antioxidant and the biological

activities of a specific food. In this context, the aim of present study was to estimate the tocopherols profile in drupes of ten Italian olive cultivars (Buscionetto, Casaliva, Carolea, Frangivento, Gaggiolo, Gnagnaro, Semidana, Sinopolese, Taggiasca and Verdello). During drupe development, several structural changes and chemical transformations occur that affect the different components of fruits. On this basis, tocopherols were monitored at different fruit ripening stages. The obtained results demonstrate that tocopherol profile is not constant depending on both cultivar and fruit ripening stage.

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## **Omega-3/omega-6 fatty acids ratio in olive oils from Italian olive varieties**

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Omega-3 and omega-6 fatty acids are essential fatty acids which cannot be synthesized by the body. For this reason must be obtained from food. Omega-3 and omega-6 fatty acids play a crucial role in brain function as well as normal growth and development. Therefore, in order to propose extra virgin olive oils particularly rich in ALA and/or characterised by a lower omega-6 - omega-3 ratio, in the present work we report the results of an investigation aiming at assess the content of ALA, Linoleic acid and Linoleic-Linolenic ratio in Italian single cultivar olive oils. The virgin olive oils were produced in Calabria, from 100 different cultivars. The olive samples were collected from the CRA National Germoplasm Collection at Mirto Crosia (CS, Italy). Fatty acids methyl esters were determined according the Official methods of analysis stated by the EU Regulations (Reg. CEE 2568/91, Reg. CE 796/2002, Reg. CE 1989/2003).

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# Organoleptic and nutritional attributes of the major apple cultivars grown in Romania

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## Introduction

Generally, the nutritional and organoleptic attributes of fruit besides the commercial ones are paramount for choosing the fruit cultivars. With this view, these attributes for the apple species are taken into account especially in case of newly bred or introduced varieties which have enriched the range of varieties propagated for the new orchards establishment.

Since 2005, at the Research Institute for Fruit Growing Pitesti – Maracineni and Research Station for Fruit Growing Voinești have been conducted studies on the tasting quality and biochemical components (antioxidants, mineral substance) in some Romanian bred cvs.: Generos, Rebra, Rustic, Ardelean, Auriu de Bistrita, Romus 4, Romus 5, Colmar, Aura, versus the foreign cvs.: Liberty, Sir Prize, Enterprise, Goldrush, Florina, Braeburn assessed for their adaptability.

This paper shows the results recorded in the two research places, the most representatively for the apple culture and introducing the new cultivars in Romania.

## Results

*Flesh texture and firmness (kgf/cm<sup>2</sup>)* At the ripening time, the flesh firmness varied from 5.1 (Romus 3, summer cv. and Romus 4, autumn cv.) up to 8.6 and 9.1 (Jonathan and Florina cvs.). The cultivars created at Fruit Research Station Voinești, Pionier, Frumos de Voinești, Iris had a lower fruit firmness (4-6) and Ciprian and Voinea a higher one (7.5-8.5 kgf/cm<sup>2</sup>). Compared to other newly introduced cultivars from Europe and studied under our conditions (Braeburn, Ariwa, Goldrush), a higher firmness and a denser texture which make them to have a longer shelf life in the cold storage.

*Fruit taste*, as a result of sugar / acidity balance is variable: 13.3-15.9 (Romus 3, Idared, Jonathan, Granny Smith) with higher acidity and less sugar and 44.5-81 (Delicios de Voinești, Starkrimson) with sweet unbalanced fruit. A balanced taste had the following cvs.: Frumos de Voinești, Prima, Ardelean, Golden Delicious, Goldspur, Jonagold. The apples harvested at Voinești Station recorded a ratio of sugar/acidity of 16-18 (Florina, Generos, Jonathan) and 21 (Golden Delicious) highly acid taste.

*Acid ascorbic content (vitamin C)*, mg % fresh fruit is also variable related to variety; it was higher with Idared (14.0), Jonathan (13.0), Granny Smith (11.3), James Grieve (10.2). Half of this amount was found in Delicios de Voinești, Delia, Ardelean, Frumos de Voinești, Romus 3, Goldspur, Jonagold. For the other cultivars there were moderate values.

*Antocians content* is found in the epidermic cells and in 2-3 layers of hypoderm being genetically controlled. Its values are getting higher in the red apples. In yellow apples (Sir Prize, Goldrush, Baujade) was 0 and for others: 2.19 mg% (Ardelean), 2.95 mg% (Florina). A positive exception was the columnar cultivars Colmar with red fruits which recorded 13.10 mg%, 4-5 times more than Jonathan and Florina cvs.

## Conclusions

The fruit quality is a complex feature related to several factors like the genetic, environmental, biotic ones but sometimes to the subjective evaluation as well. The studies showed that according to the years and place of investigation, the behaviour of cultivars is differently within the species limits.

The range of varieties enabled us to make a classification and arrangement of the cultivars according to the firmness (at harvesting and marketing time), sugar (acidity ratio, taste, as well as tannins, antioxidants (vitamin C,  $\beta$  caroten, antocianic pigments) and minerals. It was noticed that the commercial look is not always in concordance with the inner quality of flesh and the genetic resistant cultivars have had the same response like the other ones, respectively some of them behave well some others do not.

From the quality view point it is necessary to have a compromise between the grower and consumer to achieve the yield and market needs.

# Environmental friendly fertilising products in organic tomato growing

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The aim of the 3 years experience is to elaborate new and in organic farming applicable, environmental friendly fertilizing products and methods of vegetables. Further aim is to investigate the possible effect of immunity increasing conditioner, which can increase the resistance against fungi diseases.

The different combinations of fertilizing products are used during organic field tomato growing. The combinations of the following products were used in the treatments: AVA (glass- structured non- crystal mineral fertilizer), AS-4/20 (bacterium fertilizer), AS-Melasz (sugar industry waste product), AS-BTF (certain metabolites of *Arthrobotrys oligospora*, *A. conoidus*, *Paecilomyces funosoreus*, *P. lilacinus*, *Verticillium chlamyosporium*, and *Mycothecium verrucaria*), AS-Arrest (*Talaromyces flavus*), NOVOSIL (natural plant extract, mixture of tri- terpenol acids), AS-Plex (*Azotobacter vinelandii*, *A. bejiernicki*, *A. croococcum*), AS-BAG (*Trichoderma harzianum*, *Trichoderma lignorum*, *Gliocladium virens*, *Bacillus subtilis*), AS-BR (*Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanil* and *Bacillus polymyxa*), AS-B.sub (*Bacillus subtilis*)

Table 1: The used combinations and treatments

Treatments	Investigated products	Use
1. Untreated control	-	
2. Standard ecological technology	Biomit, Bioplasma, Hungavit U, Dipel, Ré-zoxiklorid 50 WP	Plant treatment
3. Trifender WP	Trichoderma asperellum (technical agent)	Soil treatment
4. Trifender	Trichoderma asperellum (prepared product)	Soil treatment
5. Trifender WP + ecological, farming methods	Trichoderma asperellum	Soil treatment
6. Trifender + ecological, farming methods	Trichoderma asperellum	Soil treatment
7. AS I. combination	AS-4/20, AS-Melasz, AS-BTF, AS-Arrest	Soil treatment
	NOVOSIL, AS-Plex, AS-Melasz, AS-Arrest	Plant treatment
8. AS II. combination	AS-4/20, AS-Melasz, AS-BAG	Soil treatment
	AS-Plex, AS-Melasz, AS-BAG, AS-BR	Plant treatment
9. AS III. combination	AVA, AS-4/20, AS-Melasz, AS-BAG	Soil treatment
	AS-4/20, AS-Melasz, AS-BR	Plant treatment
10. AS IV. combination	AVA, AS-4/20, AS-Melasz, AS-B.sub	Soil treatment
	AS-4/20, AS-Melasz, AS-B.sub, AS-BR	Plant treatment

The experiment is carried out on converted organic experimental field of the Department of Ecological and Sustainable Farming Systems in Central Hungary, near Budapest in Soroksár. Soil type is humic sandy soil. Climate condition is dry continental. Every treatment is carried out in six repetitions on 25 m<sup>2</sup> plots. The rate of growing of plants, yield, state of health and inner quality of crop is measured. Measuring method is visual survey to assess the health condition of plant and fruit.

The experiment showed that AS product combinations prolong flowering and result slower tomato initiation. The extent of Late Blight (*Phytophthora infestans*) was significantly lower in both years in case of ecological farming methods, “Trifender + ecological farming methods” and “Trifender WP + ecological farming methods” combinations. In the micro plot experiment the “Trifender WP + ecological farming methods” treatment resulted significantly higher fruit numbers than that of the control plot. Regarding to yield measurement the “Trifender WP + ecological farming methods” treatment had significantly better results than that of the control.

# Protection of table grapes and possible residues levels - A Research Project from Austria

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## Introduction

Table grape production in Austria is getting more interesting. Professional cultivation of table grapes in cooler climate regions recently started maybe as a consequence of the separation from the wine contingent. Since the market gets sufficient fruit nearly all year round from other countries with high quality at relatively favourable prices, however with a certain amount of residues. Austrian table grapes could also have a chance in this market niche if high quality can be supplied.

Austrian growers prefer cultivation of fungi-tolerant varieties, however plant protection agents have to be used to achieve a high quality crop to a certain extent. Concerning plant protection it seems essential to find out what procedure allows a production with less or no residues. On the other side the growers will convince the customers about the quality of their grapes and offer products free from residues.

## Material and Methods

In different trials variable application possibilities were tested in biological as well as in low chemical form. The disease assessments were done visually. Residue analyses were made by gas chromatography – mass spectrometry to separate, identify and quantify the components, finally to discuss and to recommend a proper form of application.

table1: plant protection procedures

variant	cultivar	treatment
1	Gutedel	end of flowering period application with spiroxamine (0,8l/ha), benthialicarb/folpet (2 kg/ha), mepanipirim (1,2kg/ha), no other applications
2	Gutedel	customary biological application plan
3	Gutedel	customary integrated application plan
4	Gutedel	application after blossom with boscalid (1,2kg/ha), spiroxamine (0,8l/ha), benthialicarb/folpet, more applications with biological products
5	Aron	applications with sulphur and myco - sin
6	Aron	no application after blossom

## Results and Discussion

The results of the residue analyses have shown that the samples of variants 1,2 and 6 were free of pesticides. Application after flowering period with active components caused residues in a quantity of 0,034 mg/kg mepanipirim and 0,011mg/kg pyrimethanil, also when the components were not directly applied on the culture, possibly caused by driftage from neighbouring cultures. Similarly, the cultivar Aron, treated only with biological agents showed residues of 0,027 mg/kg mepanipirim. Nevertheless the legal maxima level of pesticide residues was not reached and is much higher.

Visually, all variants were of satisfying quality as far as *botrytis cinerea* and *plasmopara viticola* infestations are concerned. The assessment of *oidium tuckeri* showed some statistical differences, both within the variant with treatment and between the five variants with treatments and the variant 6 (with no application after flowering period).

## Relevant information

552 Verordnung: Änderung der Schädlingsbekämpfungsmittel-Höchstwertverordnung, Bundesgesetzblatt, 3. September 2003, S. 3425-3441

# Phenolic compounds and antioxidant activity in fruits of peach and nectarine

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## *Introduction*

Epidemiological studies have shown that consumption of fruit and vegetables have health benefits against chronic diseases including cardiovascular disease and certain kinds of cancer because of their antioxidant components. Antioxidants, which can neutralize free radicals, may be of central importance in the prevention of these diseases. The aim of the present work is to determine the amount of antioxidants in peach and nectarines fruits (in particular phenolic compounds and anthocyanins) and antioxidant activity and to correlate the antioxidants content with physical- chemical characteristics of fruits.

## *Materials and methods*

The study has been carried out on 20 peach and nectarine cultivars conserved in the National Fruit Tree Germplasm Centre at CRA – Centro di Ricerca per la Frutticoltura in Rome. For each genotype, fruits were picked at the same time and, later on, were separated in three groups according to the ripening degree. For each fruit, flesh firmness and soluble solids content have been determined. The flesh and the peel were frozen separately and kept at -80 °C until the analysis. The total phenolic content was quantified with Folin-Ciocalteu method (Swain and Hillis, 1959). The anthocyanins were analyzed with the spectrophotometry at 520 nm and expressed as cyanidin chloride. DPPH radical (2,2-diphenil-1-picrylhydrazyl) has been used to test the antioxidant activity; wich was measured by the decrease in absorbance at 513 nm. Individual phenolic constituents, using a high-performance liquid chromatograph with a photodiode detector (HPLC-DAD) method, were identified and quantified.

## *Results*

The present work has shown that phenolic compounds and anthocyanins are more present in the skin than in the flesh. No statistical difference was observed, as antioxidant activity, in the flesh and the skin. The white flesh peach cv Iris Rosso has the highest content of phenolic compounds in the skin, while in the yellow flesh peach cv Maeba Top they are in the flesh. A high anthocyanins content was observed in the skin of yellow flesh nectarine cv Weinberger and in the flesh of the yellow flesh nectarine cv Maria Carla. The antioxidant activity is more consistant in the skin of the white flesh nectarine cv Caldesi 2000. Antioxidant capacity resulted strictly correlated with anthocyanins and phenolic compounds. HPLC analysis showed that the main compound of phenols is the chlorogenic acid.

## Testing of different seed treatment materials on seed borne bacterial disease of tomato and pepper

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In ecological farming systems farmers can not use chemicals against pests. In ecological plant protection the aim is to prevent diseases; if it is not possible the use of allowed materials are permitted. EU decree No. 2092/91 deals especially with ecological plant cultivation, regulates reproduction and usage of seed and propagation material (EC Council Regulation on Organic Agriculture article 6, No. 2092/91). According to EU decree No. 1452/2003 (14 August, 2003) the use of ecological propagation material is obligatory in organic farming. The application of synthetic dressing powders for protecting propagation materials is not permitted, however high quality healthy seeds are essential for successful organic farming. Until now there

have not been enough effective and environmental friendly materials for seed treatment in organic farming. For this reason it is very important to find such substances which can provide a good alternative for seed protection. The aim of our study is to find new, environmental- friendly, permitted materials against seed borne bacterial strains of tomato and pepper, which don't ruin germination capacity. These diseases can cause serious losses in yield, so finding appropriate inhibitors has a great importance. In Hungary bacterial canker (*Clavibacter michiganensis subsp. michiganensis*) of tomato cause high yield losses in tomato (*Lycopersicon esculentum*) production since 1960, while bacterial speck (*Pseudomonas syringae pv. tomato*) is just sparse problem. Bacterial spot (*Xanthomonas campestris pv. vesicatoria*) of pepper is one of the most important diseases of field produced pepper (mainly in moist weather). In Hungarian conventional farming kasugamycin agent chemicals are generally used against these pathogens for seed dressing. In ecological farming just maximum 1,5% Sodium-hydroxide (NaOH) is allowed to use against these diseases; however it is a disinfectant, so currently there is no seed dressing material is allowed in ecological farming in Hungary. Different materials were tested against these bacterial strains for seed treatment in this study. *In vitro* trials have shown that vinegar, cider vinegar, red wine vinegar and white wine vinegar have inhibiting effect against the causative agent of bacterial canker (*Clavibacter michiganensis subsp. michiganensis*), bacterial speck (*Pseudomonas syringae pv. tomato*) of tomato. These materials also have inhibiting impact on the causative agent of bacterial spot of pepper (*Xanthomonas campestris pv. vesicatoria*). The bacterial strains were more sensitive to acidic than alkaline circumstances. The lowest examined concentration (0,5 %) of vinegars had also bactericide impact. In alkaline materials it is necessary to use higher concentration of at least pH 13 (1,5% NaOH), but it had not impact in all case. We can conclude that all examined vinegar types could be useful in biological plant protection systems against bacterial diseases of tomato and pepper. Among examined essential oils cinnamon oil proved to be the most effective, but all oils decreased germination ability. In low concentrations (0,1%, 0,5%, 1%) the examined volatile oils had no inhibition effect except in case of *Xanthomonas campestris pv. vesicatoria* B. 01771 with use of cinnamon oil. The peppermint essential oil was not effective except in 100% concentration against *Pseudomonas syringae pv. tomato* B 01277 strain. Thyme and savory teas were effective against *Pseudomonas syringae pv. tomato*. Other examined materials had insufficient bactericide impact (sucrose, NaCl, ethanol, valerian extract, peppermint tea). Germination test has shown that examined vinegar types do not decrease germination ability.

We plan to test further materials and carry out *in vivo* experiments on the fields with the most effective ones. Using different combination of materials and treatments it might give better effects against bacterial canker (*Clavibacter michiganensis subsp. michiganensis*), bacterial speck (*Pseudomonas syringae pv. tomato*) of tomato and bacterial spot of pepper (*Xanthomonas campestris pv. vesicatoria*). We would like to extend our examinations for certain fungi, too. (Focused on economical aspects and germination ability view we will try to use the lowest effective concentrations.)

## **Climate influences on fruit development and health beneficial components in strawberries**

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Strawberry is one of the fruits getting most attention in relation to fruit quality and particularly its content of beneficial components for human health. The content of antioxidants, vitamins and other substances proven to be of importance is generally high compared to many other fruits. Combined with a high level of consumption both as fresh and processed fruits all over Europe places the strawberry in a very important

position. In addition, strawberries are produced in more diverse conditions from North to South than probably any other fruit. However, very little information is available on the effects of preharvest growing conditions on fruit quality in general, and less on health beneficial components. The genetic differences have a dominant impact on all fruit quality components and are difficult to separate from effects of growing conditions. With the ambition to generate some of this basic information a trans-European experiment has been initiated by a working group within the frame of the EU-Cost network Euroberry 863. Strawberries are grown at 7 locations from Trondheim and Copenhagen in the north and south of Scandinavia to Germany and Switzerland in Central Europe to Ancona and Zagreb in the south of Europe. In Switzerland plants are also grown in two altitudes at 500 m and 1100 m above sea level. The cultivar 'Elsanta' is grown in all locations in combination with a local cultivar optimal for the region: in North 'Korona' and 'Clery' in the South, while all cultivars are grown in Germany. A common practise for growing techniques has been described as well as logging procedures for temperatures in soil, canopy and 2 m height and irradiation (PAR). Time of flowering of individual flowers is registered for detailed calculation of temperature sums from flowering to harvesting of fruits. Fruits are harvested and sub-samples collected according to a common protocol. They will be chemical analysed at Geisenheim and Ancona. First results are reported on general fruit development, beneficial components and the relationships to cultivars and climatic growing conditions.

## **Nutritional Value of “Ready to Eat” Endive during Storage as Influenced by Conventional, Integrate and Organic Farming**

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### ***Introduction and methodology***

In this study we assessed the effect of different growing protocols on the quality and shelf-life of ready-to-eat endive (*Cichorium endivia* L. var. *crispa*). Plants were grown at the experimental station of Portici,(NA) on a sandy soil. Before sowing, 110 kg N ha<sup>-1</sup> N were applied using three different fertilization protocols: organic farming (OF, Bioilsa®, N = 12%), conventional farming (CF, ammonium nitrate N = 34,5%) and integrated fertilization management (IF, in even contributions of ammonium nitrate and Bioilsa®). Planting density was 10 plants m<sup>-2</sup>. The crop cycle lasted from the 14th of January to 26th of April 2007. The experimental design was a randomized block with three replications. At harvest, fresh produce was husked, washed and dried under air-flow, packaged into plastic boxes and closed in permeable bags. Fresh weight loss, moisture content, antioxidant capacity and nitrate contents were measured on the fresh produce and after 3, 5 and 10 days of storage at 4 °C.

### ***Results and Discussion***

Integrated and conventional fertilization managements gave the maximum yield (3.6 kg m<sup>-2</sup>) whereas the lowest yield (2.0 kg m<sup>-2</sup>) was obtained under organic farming. Concurrently, the leaf area was reduced from 4.46 (IF and CF) to 2.98 (OF) dm<sup>2</sup> plant<sup>-1</sup>. According to Worthington (1998), organic fertilizers have a slower N release compared to the one used in conventional farming, which may lead to a reduced plant development.

The visual quality did not change during the first five days of storage, whereas an evident decay was observed at the end of the storage period (10 days). For all treatments, a significant fresh weight loss was measured. Similarly, no differences in dry matter content were attributable to the growing protocols, but the moisture content was reduced during the storage period from 94.3 to 93.9%.

The different farming protocols did not affect the leaf nitrate accumulation (750, 790 and 815 mg kg FW<sup>-1</sup>, respectively for OF, CF and IF). Nevertheless, nitrate contents were far below the limits imposed by the European Union for other crops. The nitrate content was reduced of 37% relatively to the initial value during the storage period.

The leaf hydrophilic antioxidant capacity (HAC) was higher in organic farming compared to both conventional and integrated farming (0.27, 0.18 and 0.20 mmol Ascorbic Acid eq 100 g FW<sup>-1</sup>, respectively). The

higher nitrogen availability in conventional and integrated cropping protocols could have reduced the synthesis of ascorbic acid with a consequent decrease of the antioxidant capacity (Brandt and Molgaard (2001). A linear decay of the HAC was observed for all treatments during storage, mainly attributable to vitamin C degradation (Lee and Kader, 2000), which was enhanced by the air-flow drying procedure (Asami et al., 2003). The lipophilic antioxidant capacity (LAC) did not differ among cropping treatments (mean value 1.27 mmol Trolox eq 100 g FW<sup>-1</sup>) and respect to the storage period (De Pascale et al., 2006).

### ***Acknowledgements***

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## **Ascorbic acid content in early potato tubers in field cultivation with covers applied**

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### ***Introduction***

Potato is an important source of ascorbic acid (vitamin C) in human nutrition. Consumption of 200 g of potatoes per day is providing 50% of the current recommended daily amount. Vitamin C as an antioxidant nutrient may trap free radicals and thereby reduce the oxidation that initiates or mediates the development of heart disease, cancer and other chronic disease. Ascorbic acid concentration in tubers influences degree and rate of enzymatic browning of potatoes because it is a naturally occurring inhibitor of this proces (Kolasa 1993, Almeida & Nogueira 1995, Leszczyński 2000). The ascorbic acid content in potato tubers depends on genetic factors of the cultivar and tuber maturity, but it may change as an effect of environmental and agronomical factors (Mondy & Munshi 1993, Hamouz et al. 1999).

### ***Material and methods***

The effect of cover type (perforated polyethylene foil with 100 holes per 1 m<sup>2</sup>, polypropylene fibre Pegas Agro 17UV) and the date of its removal (2 and 3 weeks after plant emergence) on the ascorbic acid content in early potato tubers was investigated. The field experiment was established in the splitblock method with a control object without covering. The 8-week seed potatoes presprouted of Aksamitka and Cykada cultivars were planted on 9-16 April and harvested 60 and 75 days after planting, respectively. For laboratory studies, 50 tubers of different size, according to the proportional participation in the yield of each treatment were taken. The ascorbic acid content estimated the Tillmans method. The results of the experiment were analysed statistically by means of analysis of variance. The analysis of the results was conducted using the orthogonal contrast to compare the control object without covering with the remaining objects. The significance of differences was verified using the Tukey's test at P=0.05.

## Results and conclusion

The content of ascorbic acid in tubers ranged from 11.01 to 12.69 mg 100 g<sup>-1</sup> FM after 60 days from planting and from 12.16 to 14.49 mg 100 g<sup>-1</sup> FM after 75 days from planting, in relation to the cultivar and potato cultivation way. The forcing of plants vegetation by covering resulted in a slight increase in the ascorbic acid content in tubers, especially at a very early date of potato harvest. At this method of potato cultivation, the ascorbic acid content in tubers after 60 days from planting was higher by 0.45 mg 100 g<sup>-1</sup> FM on average in the three-year period and after 75 days from planting by 0.18 mg 100 g<sup>-1</sup> FM than that in the cultivation with no covering. The type of cover (perforated foil or polypropylene fibre) and the length of plant covering did not significantly affect the ascorbic acid content in potato tubers. A little higher profitable effect of perforated foil use on ascorbic acid content in tubers was obtained only in a very early date of potato harvest. Ascorbic acid content in tubers to a higher degree depended on the cultivar and weather conditions in the potato vegetation period than on the potato cultivation way. The tubers of Cykada cultivar contained more ascorbic acid than Aksamitka one. Elongation of the covering period to 3 weeks after plants emergence resulted in a little more increase in the ascorbic acid content in tubers only in the very early date of potato harvest. In both dates of potato harvest the ascorbic acid content in tubers of Cykada cultivar was higher by 0.36 mg 100 g<sup>-1</sup> FM on average in the three-year period. The higher temperature in the vegetation period of potato increased the ascorbic acid content in the tubers.

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## Secondary plant ingredients of *Parthenium hysterophorus* L. and their possible impact on human health in Ethiopia

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## Introduction

*Parthenium hysterophorus* L. (Parthenium) is a species in the family Asteraceae. It is native to South America and the Gulf of Mexico. Parthenium is one of the worst weeds in countries where it has been introduced. After its introduction to Ethiopia it developed to an aggressive weed and spread over the whole country within a few years. It causes direct losses to the grazing industry and is a human health hazard, causing allergic rhinitis and contact dermatitis. Human health impact is often associated with secondary plant metabolites found in Parthenium such as parthenin, a sesquiterpene lactone.

## Material and methods

Parthenium was sampled from different geographic areas in Ethiopia, India, and Taiwan. Additionally, plants were cultivated in Ethiopia and Berlin under regular greenhouse conditions and under water stress. We assume that the secondary metabolite profile of Parthenium will change in quantity and quality depend-



ing on the water stress conditions. We extracted phenolic acids and parthenin from taken samples and analyzed extracts by HPLC.

Furthermore, we interviewed 64 Ethiopian farmers in heavy infested regions in order to find out the health impact of Parthenium.

## **Results**

Surprisingly, parthenin is not the only dominant secondary plant compound as it is often cited. We analyzed the plant material for specific phenolic acid contents. The phenolic acid content of parthenium was very high in comparison to other plant species. Two major phenolic acids, beside minor compounds such as vanillic acid and caffeic acid, were present in Parthenium. One of the major acids was identified as chlorogenic acid and the second, a cinnamic acid derivate, was identified as isochlorogenic acid.

The results showed that the phenolic acid content depends on plant compartment and the growth location. In flowers we found the major amount on phenolic acids. The concentration of phenolic acids varied strongly depending on growth location.

The interview of 64 farmers (age between 19 and 44 years) in four highly infested areas in Ethiopia revealed that all of them had health problems in different nuances. Most frequently they responded to contact with Parthenium with light allergic symptoms like hay fever. Some farmers suffered from severe health problems like contact dermatitis, fever, prickle of the skin over whole body, cracks on hand balms, and asthmatic problems.

Currently, we are evaluating the allergic potential of Parthenium extracts in selected mouse models at the Paul-Ehrlich Institute in Langen. Our first results indicate that parthenin has a high allergic potential.

## **Study concerning the content in trans- resveratrol at the grape and wine variety**

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## **Introduction**

Resveratrol (3,4,5'- trihydroxystilbene) is a natural phytoalexin produced by a wide variety of plants such as grapes (*Vitis Vinifera*), peanuts (*Arachis hypogaea*) and malberries as a response to the stress (termical and hydric) injury, ultraviolet (UV), irradiation, fungal (*Botrytis cinerea*) infection and it exists in cis- and trans- forms. Several clinical studies have demonstrated that phenolic compounds such as resveratrol (especially trans-resveratrol) are responsible for the health benefits of red wine. This substance is synthesized by several plants in response to adverse conditions such as environmental stress or fungal infection (*Botrytis cinerea*) – Ramona Capruci - 2005. In the future, this determination will be used to extract the resveratrol and to use it in medical area. In 1992, Siemann and Creasy, have reported that resveratrol could also occur, in grape products and particularly in wine is thought to be responsible, at least in part, for the protective effects of wine against coronary heart disease (Philippe Jandet and c. - 1995). Growing evidence suggest that resveratrol plays a role in the prevention of human pathological processes, such as inflammation, atherosclerosis and carcinogenesis (Lucia A. Stivala and c. - 2001).

## **Material and methods**

This study implicates the detection of the trans - resveratrol from grapes and red wine variety Cabernet Sauvignon and Merlot from Banu Maracine viticultural center by HPLC, and also, a comparative evaluation in order to establish the synthesis potential of grape variety. Cabernet Sauvignon and Merlot grapes were used

for analysis during the 2007 - studying year. In order to identify the trans resveratrol by HPLC it were used 50 grape berries and the resveratrol was extract with ethyl acetate. The ethyl acetate was removed in vacuum (40° C). The residue obtained was redissolved in 5 ml 99,9% methanol, followed by dry evaporation. After the dilution with 5 ml 50% acetonytril, the sample was analyzed by HPLC, using C<sub>18</sub>10U, special for phenolic substances (250 mm x 4,6 mm) and acetonytril and water as aluant at 1 ml/min flow. Trans-resveratrol at 307 nm absorbance.

### **Results and discussion**

For Cabernet Sauvignon grapes, maximum level was recorded during ripeness with 25,3 µg/g resveratrol in Banu Maracine compared with 26,6 µg/g for variety Merlot. This phenomen was interfered by higher terminal conditions in during the 2007 - studying year, and also by a longer period of sun lighting in Banu Maracine viticultural center. At maturity phizyologic contents in resveratrol were diminished (1,4 µg/g for Cabernet Sauvignon and 2,3 µg/g for Merlot in Banu Maracine. The Merlot and Cabernet Sauvignon red wine obtained in 2007 - studying year in Banu Maracine contained high levels of trans-resveratrol (3,34 mg/l for

### **Conclusions**

The content in trans-resveratrol, from grapes the variety Cabernet Sauvignon and Merlot by Banu Maracine viticultural centre fallowed a descendant curve during the ripening process with maximum level in pre-ripenees. This phenomen can be the explanation for the pathologenic attach of *Botrytis cinerea* during maturing process of grapes.

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## **Yielding and biological value of some cultivars of *Cucurbita pepo* L.**

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### **Introduction**

Nowadays the most popular as vegetables are two forms of *Cucurbita pepo*: zucchini and summer squash which are cultivated for their immature fruits. The rest cultivars of this species harvested when fruits are fully developed or reach physiological maturity have minor importance and are cultivated mainly as animal feed as well as for seed yield and oil production. But pumpkin is a tasty and valuable vegetable, distinguished by dietetic characteristics. It can be consumed raw, boiled, fried, stewed, pickled, or processed as sauces, jams, sweetmeats, juice, seed oil.

The aim of this study was to estimate yielding and nutritional value of some cultivars of *Cucurbita pepo*: 'Miranda' and 'Junona' (both cultivars belong to oilseed pumpkin) 'Pyza' (spaghetti type) and 'Danka'. The control was 'Melonowa Żółta' cultivar belonging to species of *Cucurbita maxima*.

### **Material and methods**

The experiment was conducted in 2004-2005 in Horticultural Experimental Station on fine sandy clay soil. Seeds were sown on plots in spacing 1x1 m in the second decade of May. Nitrogen was supplied in a split dose 200 kg N·ha<sup>-1</sup> (100+100). During harvest done on 20 September there were estimated total and market-

able yield of fruits. In samples of fruits there were analysed content of macronutrients: P, K, Mg, Ca, vitamin C, carotenoids, total and reducing sugars, dietary fiber, total acidity and pH.

## Results

Among estimated cultivars the highest yield was obtained from 'Danka' and 'Miranda'. The marketable yield of *Cucurbita pepo* cultivars were twice or three times lower than from 'Melonowa Żółta'. On average 'Danka' produced about 4.19 matured fruits per plant, when 'Pyza' 3.87, 'Miranda' 2.40, 'Junona' 2.54, and 'Melonowa Żółta' 1.53.

'Junona' had significant higher level of nitrates ( $745 \text{ mg}\cdot\text{kg}^{-1}$ ), in fruits of the rest tested cultivars of *C. pepo* content of nitrates was lower than in 'Melonowa Żółta' ( $570 \text{ mg}\cdot\text{kg}^{-1}$ ).

Cultivars 'Danka' and 'Junona' had higher or similar to 'Melonowa Żółta' (8.51%) level of dry matter in fruit (9.92-8.01%), while for Miranda and Pyza only 4.48 and 5.25%, respectively. The content of total and reducing sugars in tested cultivars was lower than in 'Melonowa Żółta' cv., except 'Danka' cv. which had higher content of total sugars than 'Melonowa Żółta'.

The higher level of vitamin C was in fruits 'Danka' ( $20.68 \text{ mg}\cdot 100\text{g}^{-1}$  f.m.), 'Melonowa Żółta' ( $16.11 \text{ mg}\cdot 100\text{g}^{-1}$  f.m.) and 'Pyza' ( $11.96 \text{ mg}\cdot 100\text{g}^{-1}$  f.m.), lower in 'Miranda' ( $5.65 \text{ mg}\cdot 100\text{g}^{-1}$  f.m.) and 'Junona' ( $5.75 \text{ mg}\cdot 100\text{g}^{-1}$  f.m.). The level of carotenoids in cultivars belonging to *C. pepo* varied from 3.32 to  $2.61 \text{ mg}\cdot 100\text{g}^{-1}$  f.m. while in 'Melonowa Żółta' was  $5.13 \text{ mg}\cdot 100\text{g}^{-1}$  f.m.

The fruit of 'Melonowa Żółta' that belongs to the species *C. maxima* contained significantly greater amounts of starch, pectins, NDF (neutral dietary fiber) and hemicellulose as compared to the fruit of the cultivars belonging to the species *C. pepo*.

## Occurrence and potential role of Phosphoenolpyruvate carboxykinase in the metabolism of organic acids in the flesh of gooseberries (*Ribes grossularia* L.)

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## Introduction

Previous studies showed that, in several fruit species (blueberry, blackberry, raspberry, red currents, tomato and grape), phosphoenolpyruvate carboxykinase (PEPCK) might function in the catabolism of organic acids. The present study, carried out in 2006 in central Italy, investigated the occurrence and potential role of PEPCK in the flesh of gooseberries (*Ribes grossularia* L. – cv. White Smith).

## Methods

Abundance of PEPCK was determined at different stages of their development. In addition, changes in the amounts of citrate, malate, glucose, fructose and sucrose in the flesh were determined. The abundance of PEPCK was determined by using western blot analysis and activity measurements.

## Results

All soluble sugars (glucose, fructose and sucrose) increased remarkably from 60-70 days AFB to harvest (110 days AFB), when the considered soluble sugars showed similar concentrations (around  $160 \mu\text{mol g}^{-1}$  f.w.). Malate and citrate increased up to 70-80 days AFB (malate up to about  $70 \mu\text{mol g}^{-1}$  f.w. and citrate up to about  $80 \mu\text{mol g}^{-1}$  f.w.); afterwards the content of malate decreased progressively, whereas that of citrate remained constant up to about 85 days AFB and then decreased. The abundance of PEPCK increased during ripening and resulted most abundant when there was the reduction of the malate and citrate contents.

## ***Discussion and conclusion***

The results are consistent with PEPCK playing a role in the catabolism of malate and citrate in the flesh of gooseberries during ripening.

## **Control of the metabolism of the oxalic acid and its interactions with calcium (Ca) in fruits of *Actinidia deliciosa* ‘Hayward’**

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## ***Introduction***

The calcium (Ca) has a central role in metabolic processes involved in ripening and post-harvest life of fruits. In *Actinidia*, the Ca seems have a positive influence on flesh firmness and storability of fruits, even though not always a positive correlation between total Ca content and quality and storability of fruits has been found. The knowledge of the control of the Ca content and metabolism in the fruits is limited. In *Actinidia*, of the total amount of Ca contained in the berries a large part is present as insoluble calcium oxalate (CaAOX), that has an influence also on the organoleptic and nutritional characteristics of the fruits. The fact that the fraction of Ca in the form of insoluble oxalate can be very high and variable could explain the not constant correlation between the total Ca content of the fruits and their quality and storability.

## ***Methods***

This research, carried out in 2006 in central Italy, investigated the quantitative variations of total Ca, soluble oxalic acid (AOX) and CaAOX in kiwifruits during their development and ripening and their partitioning in the different fruit tissues (epidermis, outer and inner pericarp and core), in relation to different environmental factors (light/shading) and source-sink relationships (fruit sink strength and assimilate availability modified using growth regulators, such as CPPU and 2,4-D, and girdling). Nine weeks after full bloom (AFB), in epidermis and inner pericarp (IP) the highest amounts of total oxalic acid were observed.

## ***Results***

Nine weeks after full bloom (AFB), in epidermis and inner pericarp (IP) the highest amounts of total oxalic acid were observed. Intermediate values were registered in the outer pericarp (OP) and the lowest ones were observed in the core. At harvest, in all the tissues the concentrations of total oxalic acid were lower than those observed 9 weeks AFB. Also the amounts per fruit (mg fruit<sup>-1</sup>) were lower. This indicates a net decrease of total oxalic acid in the fruits, which was mainly due to the decrease of the soluble fraction of oxalic acid.

## ***Discussion and conclusion***

The results showed changes in the content of oxalic acid (both soluble and insoluble) in kiwifruits related to the development stage and applied treatments (light/shading and source-sink relationships), suggesting the possibility that Ca could be obtained from the degradation of CaAOX. Such amount, from preliminary estimates, should be low but its metabolic role could be significant.

## ***Acknowledgments***

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# Comparison of antioxidant parameters in fruits of a range of apricot (*Prunus armeniaca* L.) cultivars

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Epidemiological studies suggest that the rate of fresh fruit consumption is conversely proportional to the risk of degenerative diseases. Since active oxygen species are involved in the aetiology of several diseases, beneficial health effects are attributed to the antioxidant compounds present in fruits and vegetables. Redox characterization of fruits and quantification of the individual compounds may contribute to more specialised fruit consumption and to the establishment of cultivars possessing enhanced functional properties.

In the present study several redox parameters involving ferric reducing ability of plasma (FRAP), total phenolics content (TPC), 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical-scavenging activity, total radical scavenging capacity measured in a photochemiluminescence (PC) assay and vitamin C content were determined in fruit juice and after hydrophilic and lipophilic extractions.

Apricot fruit has different types of antioxidants, i.e. the lipophilic carotenoids as well as the hydrophilic vitamin C and a wide range of phenolics. Carotenoid content of apricot fruit is the most investigated antioxidant fraction that shows close correlation with fruit flesh colour. A great variability was observed when testing the antioxidant capacity of twenty-nine different cultivars. FRAP values ranged from 0.47 to 10.35 mmol ascorbic acid/L, while TPC from 0.99 to 20.57 mmol gallic acid/L. In general, a correspondence was obtained between the antioxidant capacity (FRAP and TPC) and ripening time with early ripening cultivars showing the lowest antioxidant capacities. In case of all tested cultivars possessing various levels of antioxidant capacity, gradually increasing FRAP and TPC values were detected during the ripening process of fruit reaching their maximum levels at the stage of full ripeness.

Correspondences between the measured redox parameters were also very different. The strongest correlation occurred between FRAP and DPPH radical-scavenging activity, but DPPH radical-scavenging activity and TPC, FRAP and TPC were also closely correlated. Vitamin C content showed also close correlations with the FRAP and TPC values and with the DPPH radical scavenging activity. Correlations were much lower between total radical scavenging activity (PC) and any other redox parameter indicating that this technique measures another aspect of antioxidant capacity. However, this might be quite interesting since this assay indicates the eliminating efficiency of a physiologically active oxygen form, the hydroxyl radical.

Hydrophilic antioxidant capacity represented 50-80 % of the total antioxidant power of apricot fruit pointing to the fact that light skin and flesh coloured fruits with lower carotenoid content may have also possess considerable antioxidant capacity due to their hydrophilic fraction of antioxidant molecules, mainly vitamin C and several phenolics.

Our analyses revealed correlations between several widely used redox parameters, and detected a great variability in the antioxidant value of different apricot species, which provides a feasible possibility to design crosses in functional breeding programmes.

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## Comparative antioxidant characterization of stone fruits grown in Hungary

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Due to their antioxidant molecules, fresh fruits are important constituents of a healthy diet. Several environmental challenges as well as many chronic diseases impose an oxidative burden, which may be alleviated by the increased consumption of natural antioxidants. Among these, ascorbic acid, anthocyanins and other flavonoids, carotenoids are considered to have the most favourable health effects. Conventional breeding or genetic modifications seem to provide efficient tools to enhance the antioxidant value of the consumed fruits. Initially, both of these strategies require the determination of variability in this trait.

As part of a comprehensive analysis, in the present study two widely used redox parameters involving ferric reducing ability of plasma (FRAP) and total phenolics content (TPC) were determined in fruit juice and after hydrophilic or lipophilic extractions. Four sour (*Prunus cerasus* L.) and 10 sweet cherry (*Prunus avium* L.) as well as 7 Japanese plum (*Prunus salicina* Lindl.) cultivars and 6 cherry plum (*Prunus cerasifera* Ehrh.) accessions were tested.

Cultivar averaged mean values of FRAP results were the highest in sour cherry, followed by Japanese plum, sweet cherry, while cherry plum reached the lowest values. Variations between species were the highest in case of sour cherry, the lowest value was obtained for 'Érdi bőtermő', while the greatest value was measured in 'Újfehértói fürtös'. Sweet cherry cultivars showed much lowered antioxidant capacity, only three of them could reach the FRAP levels measured in 'Érdi bőtermő', the worst performing sour cherry cultivar.

As concerns the cultivar-averaged values of TPC, Japanese plum ranked first, although sour cherry had very similar total phenolics content. These were followed by sweet cherry, which also accumulated considerable amounts of phenolics, while cherry plum had again the lowest values. Considerable diversity was shown between Japanese plum cultivars. Some cultivars possessed nearly three times higher TPC than others. Sweet cherry cultivars were also different in their phenolics content, while sour cherries proved to have uniformly high TPC values. One red coloured cherry plum genotype showed much higher FRAP and TPC values than other yellow coloured accessions putatively due to its relatively high anthocyanin content.

Correlation between the FRAP and TPC values were generally close ( $r=0,855$ ). The correlation was slightly higher in sweet and sour cherry, while in cherry plum and Japanese plum it was somewhat lower. Hydrophilic antioxidant capacity represented 85-90 % of the total antioxidant power of fruits of cherries and plums indicating that water-soluble compounds (vitamin C and mainly polyphenolics) are the main antioxidant compounds in these stone fruits.

Our analyses revealed a great genetic diversity among cultivars in terms of their fruit antioxidant capacity. Close correlation occurred between the widely used redox parameters, FRAP and TPC. The most significant antioxidant molecules in sour and sweet cherries as well as in cherry plums and Japanese plums contribute to the hydrophilic antioxidant capacity. Our results may be utilized in breeding programmes to establish value-added cultivars.

This work was funded by the NKTH-OTKA K68921 grant.

## **Lectures and Posters of Theme 2**

**BIOTECHNOLOGY: FROM SOCIAL ISSUES TO "-OMICS" IN  
EUROPEAN HORTICULTURE**

## **A role for Metabolomics in marker assisted breeding for crop compositional traits**

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Tomato represents an important source of fiber and nutrients in the human diet and is a central model for the study of fruit biology. To identify components of fruit metabolic composition, here we have phenotyped tomato introgression lines (ILs) containing chromosome segments of a wild species in the genetic background of a cultivated variety. For this purpose we used an established GC-MS metabolite profiling method alongside measurement of traditional morphological traits used for generations of crop breeding. Using this high-diversity population, we identified 889 quantitative fruit metabolic loci and 326 loci that modify yield-associated traits. The mapping analysis indicates that at least 50% of the metabolic loci are associated with quantitative trait loci (QTLs) that modify whole-plant yield-associated traits. We generate a cartographic network based on correlation analysis that reveals whole-plant phenotype associated and independent metabolic associations, including links with metabolites of nutritional and organoleptic importance. Further experiments based on lines heterozygous for the wild species introgression have also been profiled allowing a comprehensive survey of the mode of inheritance of these QTLs. We currently expand the analysis to include studying the metabolic interactions between leaf and fruit and fruit and seed. The results of these genomic surveys illustrate the power of genome-wide metabolic profiling and detailed morphological analysis for uncovering traits with potential for crop breeding

## **Molecular biology for improving production and quality in horticulture**

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Molecular biology methods can be used in horticultural research for different aims. On the one hand, the knowledge about DNA sequences from many different organisms allows the identification, differentiation and detection of beneficial and pathogenic bacteria and fungi. Examples are the screening of large numbers of root endophytic fungi for their phylogenetic position or the development of a specific tool to identify *Rhizoctonia* isolates which are the causal agents for a disease of lettuce. On the other hand, the RNA accumulation patterns are indicators for specific physiological stages of a plant. This can help to elucidate the molecular basis of particular processes as e.g. the interaction between phosphate nutrition and carbohydrate metabolism in tomato. In addition, screening of such patterns results in the identification of genes which might play a role in certain phenomena. The analysis of the mycorrhiza-induced resistance, which could be important for biocontrol of plant-diseases, will be presented as example. The tripartite interaction between *Medicago truncatula*, the root-pathogen *Aphanomyces euteiches* and the arbuscular mycorrhiza fungus *Glomus mosseae* was chosen as model. The AM fungus significantly reduced disease symptoms and spread of the pathogen. Screening of an array carrying fragments of 16.000 genes of *M. truncatula* resulted in the identification of approximately 100 genes which could function in mycorrhiza-induced resistance. These genes are potential markers in future breeding programs which are aimed to the generation of new cultivars which are highly responsive to the application of biocontrol techniques.



## Gene expression analyses during ripening of apricot (*Prunus armeniaca*) fruit, using a peach microarray

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Transcriptional hierarchy during development provides information for thousands of genes, as well expression data for many genes of unknown function. High-throughput methodologies for comprehensive transcriptome analyses may lead to further elucidation of fruit ripening. The use of microarrays as a tool in comparative genomics can be useful for gene identification in species with limited or unavailable genetic resources. Considering the high degree of sequence conservation within the Rosaceae family and, in particular, among the *Prunus* species we employed the first available peach microarray ( $\mu$ PEACH1.0) for the investigation of the transition from immature (6 weeks before maturation) to fully-ripe stage in apricot fruit (*Prunus armeniaca* cv. 'Goldrich'), a species belonging to the Prunoideae sub-family. Data analysis indicated that among significant genes 203 and 189 were up- and down-regulated, respectively, while 122 were not affected during the transition from the immature to the mature stage. Regarding their biological process many genes were implicated into responses in abiotic or biotic stimuli and to signal transduction, while regarding their molecular function a considerable number of genes were annotated as transcription factors. Genes implicated in cell wall metabolism were both up and down-regulated; interestingly, some heat shock proteins, probably involved in the pectin depolymerisation, were induced in apricot fruit during the transition from the immature to mature stage. Transcription of genes encoding proteins with antioxidant activities were down-regulated during this transition. Overall, the unraveling of the mechanisms underlying the ripening process in the stone fruits, through the identification of genes differentially expressed during apricot ripening and their correlation with traits of agronomic interest are discussed.

## X-omic approach of tomato fruit quality

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The organoleptic quality of tomato fruit is a complex characteristic involving a set of components such as fruit size, flavour, aroma, texture. Our research program on the bases of fruit quality is focused on genetic polymorphism evaluation and QTL characterization for these traits. We have first mapped QTL controlling quality traits in several populations and introgressed quality trait QTL by marker-assisted selection into elite lines. This introgression allowed the production of Near Isogenic Lines (NILs), that were characterised at several levels (quality components, metabolome, proteome and transcriptome) We have developed a proteomic analysis of accessions and Near Isogenic Lines (NILs) to identify candidate proteins involved in the genetic variation of fruit quality. The analysis of total proteins allowed the quantification of more than 1000 spots. Proteins showing either genetic or developmental variations were identified by MALDI-TOF and/or nano-LC-MS/MS. The proteome was first characterized at 6 stages along fruit development of two varieties and then at two stages in 20 accessions. Many spots showing differential expression were related to oxidative stress, protein turn-over and cell wall synthesis or degradation. Screening for proteins showing variation of quantity in Near Isogenic lines (NILs) revealed some spots varying according to the allele at the QTL or

according to the genetic background. The percentage of variation at protein level was close to the percentage of varying genes pointed out by transcriptome approach. Transcriptome and proteome analysis provided complementary results that will be discussed.

## **Inhibition of prolyl 4 hydroxylases delays senescence in cut carnation flowers through the suppression of climacteric ethylene production**

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Carnation flower senescence has been studied extensively in relation to ethylene production and perception. However, developmental changes in the cell wall and particularly in the extracellular matrix glycoproteins related to petal senescence have not been studied extensively as in fruit ripening model plants such as tomato. Considering that 4-hydroxyproline-rich glycoproteins accounting for as much as 10-20% of the dry weight of their cell walls, their importance in cell wall-associated developmental programs such as senescence is evident. Regulation of 4-hydroxyproline formation, the site of protein O-glycosylation in the plant secretory pathway, through regulation of prolyl 4 hydroxylase (P4H) activity might lead to changes in the developmental program of petal senescence. Therefore, we cloned and characterized two full-length carnation P4H cDNAs and tested P4H activity inhibitors as an initial step towards understanding their molecular role in senescence progression. Pyridine-2,4-dicarboxylate, a 2-oxoglutarate analogue, inhibited the climacteric increase in ethylene production and as a result delayed senescence. This inhibition was accompanied by the suppression of expression of carnation petal ACC synthase and ACC oxidase as well as the upregulation of ethylene receptors. These alterations in the expression of ethylene biosynthetic and ethylene perception genes can explain the suppression in ethylene production. The effect of pyridine-2,4-dicarboxylate on the enzymatic activity of ACC oxidase was also investigated.

## **Fruit trees transformation: methodology and application**

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Fruit crops are an especially suitable target for improvement through direct gene manipulations because of the genetic limitations associated with high heterozygosity and polyploidy which hamper the conventional breeding programs. The development of reliable regeneration systems from somatic tissues is a prerequisite for the application of gene transfer techniques to improvement of woody species. High regeneration frequencies of commercial apple, pear, plum and sour cherry cultivars as well as pear and apple dwarf clonal rootstock have been achieved in our laboratory. Efficient transformation methods for these cultivars were also developed by usage different transformation protocols and selective agents. Positive selection based on phosphomannoseisomerase genes (pNOV35S-GFP, Syngenta) was used for production of marker free transgenic plum trees, that very important for improve its attractively for consumers. Herbicide resistant fruit rootstock is a new way conferring selectivity and enhancing fruit crop safety and production. The *bar* gene cloned in have been used in our research for obtaining phosphinotricine-resistant apple and pear clonal rootstocks. For fruit taste improvement of important temperate horticultural crops apple and pear, the gene of supersweet protein thaumatin II from *Th. danielli* has been transferred to apple and pear cultivars by usage

agrobacterial strain CBE21 and vector pBI121thau based on the coding sequence cloned in Unilever. For improving resistance for one of the most serious disease of stone fruits - sharka or plum pox, vector constructs based on coat protein gene in sense, antisense orientation and RNAi phenomena generation were created. Transgenic plum plants expressed coat protein was obtained on hygromycin based selection. Since 2000 year most of resulted transgenic plants are testing in field conditions.

## Genetic control of stature in ornamental plants

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The application of growth retardants is a common commercial practice to inhibit stem elongation of ornamental species to produce compact plants, suitable for growing in pots. However, the cost of chemical growth retardants and the general concern regarding applications of agrochemicals of any kind, leads to the search for alternative ways of controlling plant stature. The effects of growth retardants are similar to those found in gibberellin-deficient mutants. The GA biosynthesis pathway has been established and most genes encoding GA-biosynthetic enzymes, including the GA 2-oxidase (*GA2ox*) genes, which encode GA-deactivating enzymes, have been identified (Hedden and Phillips, 2000). It has been shown that in many plants, such as rice and wheat, it is possible to control plant height by modifying *GA2ox1* expression (Sakamoto *et al.*, 2003; Hedden and Phillips, 2000). Using this approach, experiments are being undertaken to control the stature of ornamental species. In addition, studies are in progress to investigate the feasibility of tissue-specific expression of a *GA2ox1* gene.

Seven gene constructs have been prepared. The runner bean (*Phaseolus coccineus*) GA 2-oxidase gene (*PcGA2ox1*) combined with the cauliflower mosaic virus 35S promoter, which drives high levels of expression in all plant tissues, and with three other promoters selected from AtGeneExpress microarray databases for their expression profile, has been cloned into pLARS120 and BJ40 binary vectors, respectively. In addition, promoter-GUS ( $\beta$ -glucuronidase) constructs have been made in order to confirm the expression pattern of each promoter in *Solanum nigrum*, *Nicotiana sylvestris*, *Chrysanthemum morifolium* and *Petunia hybrida*. All constructs contained the kanamycin resistance (*nptII*) selectable marker gene, and were introduced into *Agrobacterium tumefaciens* strain LBA4404, which was used for the transformation of *S. nigrum* and *N. sylvestris* leaf explants and pedicel explants of *D. grandiflora*. *A. tumefaciens* strain GV3101 carrying the same constructs was used for the floral dip transformation of *A. thaliana*.

Transgenic plants of *S. nigrum*, *N. sylvestris* and *A. thaliana* have been regenerated and their molecular and phenotypic analysis is in progress. Experiments to transform chrysanthemum and petunia with 35S::*PcGA2ox1* are also underway.

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## Development of Tobacco Plants for the Affordable Production of a Cancer Vaccine

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Production of pharmaceuticals in plants is up to 50 times more cost effective than in fermenters through microorganisms. Plant derived drugs are an interesting alternative for developing countries where people cannot afford sufficient medical treatment.

Therefore it makes sense to use this approach for synthesis of vaccines. Vaccines can be delivered as antigens either for peripheral or oral immunization.

Transformation of plants with genes carrying selected antigens of the respective pathogen allows producing immunogenic proteins on the field with high yields. However, transformation of the nucleus leads to transgenic pollen which is spread to the environment.

Our solution to this ecological problem consists in the alternative to transform the chloroplasts. In contrast to the nucleus tobacco plastids are not contained in the pollen, and thus can not be dispersed in the vicinity. Our goal is the production of an antigen vaccine against Human Papilloma Virus (HPV), which causes cervical cancer prestages in nearly 2% of all women in Germany.

In order to synthesize the antigenic L1 epitope from HPV 16 we constructed a chloroplast transformation vector containing a synthetic expression cassette: For increased transcription it contains two different promoters, and for enhanced translation it carries a 5' motif from the T7 phage G10L sequence. Further the L1 gene was N-terminally fused to the sequence codons for the first 14 amino acids of GFP (green fluorescent protein, Ye et al. 2001) which confers an increased translation. On transcriptional level this construct was followed by a selection cassette containing the *aadA* gene.

Regenerating callis carrying this construction were selected on a modified MS medium containing spectinomycin. Positive transformants were identified by PCR and Southern analysis. Further Western blot analysis demonstrated successful expression of the L1 protein. This recombinant protein was identified with conformation specific antibodies which only bind when L1 proteins formed virus like capsomers.

By our work, plant transformants were shown to be a reasonable alternative for production of vaccines. Due to these results the next step to proof the immunogenicity has good prospects.

## Process Engineering as a Means of Regulating the Microclimate in a Photoautotrophic In Vitro Culture

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In conventional in vitro cultures, different problems have to be faced. Physiological abnormalities as hyperhydricity and problems with photosynthesis may lead to a reduced quality of micropropagated explants. Addition of sugar to the nutrition medium results in high infection risks.

A photoautotrophic cultivation in a closed-cycle temporary immersion system (TIS) may be a means of overcoming the aforementioned problems. By regulating the humidity and providing high CO<sub>2</sub>-concentrations, the plants will be offered ideal conditions for in vitro propagation and rooting. An automated

addition of CO<sub>2</sub> according to the plants' requirements will reduce CO<sub>2</sub> consumption, resulting in reduction of needed resources. Omitting an additional carbon source will limit contamination, facilitating an upscaling to larger vessels with more explants of higher quality. The use of TIS may lead to an improvement in the quality of the explants. A flexible application of light management affects positively the explants' quality as well as production costs.

At our institute, a system is currently being developed and tested for the micropropagation and rooting of apple shoots (*Malus x domestica* cv. 'Hosteiner Cox').

The micropropagation system was designed at our institute, combining a forced, closed-loop ventilation with a temporary immersion system. It provides the possibility of adding CO<sub>2</sub> according to the plants' consumption. Therefore, the CO<sub>2</sub>-concentration is monitored constantly via an infra-red sensor and regulated to a high level (1500 μmol mol<sup>-1</sup>) enabling high photosynthetic activity. The advantages of a closed-loop system over a conventional CO<sub>2</sub>-enrichment in an open system are the following: the CO<sub>2</sub>-consumption is clearly reduced and thereby the costs of cultivation. In addition, reduced CO<sub>2</sub>-emissions are environmentally friendly. The net photosynthetic rate can (P<sub>n</sub>) be directly deduced from the monitored CO<sub>2</sub>-uptake, like in a leaf cuvette. The TIS is based on the principle of a twin-flask system, but the plants are separately inserted into a customized polycarbonate tray, which allows for a total submersion of the shoots without flotation. No supporting materials like vermiculite, rockwool or nets are needed, so that the plants' roots can grow unhindered and a future ex vitro transfer is eased. The immersion cycles with sugar-free medium can be managed flexibly in length and repetitions, the nutrition reservoir can be detached and replaced, making a cultivation throughout different phases possible. The humidity is monitored and can be reduced by a bottom-cooling system. The cooling is also responsible for the temperature regulation in vitro. An adaptive light management can be applied, measuring the photosynthetically available radiation (PAR) constantly and providing for a sufficient photosynthetic photon flux (PPF).

Apple shoots supplied with sugar-free MS medium containing indole-3-butyric acid (IBA, 1,5 μM) were successfully rooted in this system. It has been shown that the PPF plays a crucial part in the success of the rooting. Further tests will be conducted concerning the efficiency of the system and other fields of application.

## **Effects of boron and salinity on red raspberry in vitro**

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Raspberry (*Rubus idaeus* L.) explants were cultured in vitro under three levels of NaCl salinity (0, 5 and 10 mM) and two Boron concentrations (0,5 and 5 ppm) on a modified MS medium. Cultures were grown in vitro for 20 days before evaluation. Salinity and high-B concentrations diminished mean shoot length and fresh and dry weight of the explants. Furthermore leaf chlorophyll and leaf fluorescence were also reduced in the high salt and boron treatment. The absorption of boron, chloride and sodium was enhanced by increased concentrations of NaCl and boron in the medium. Explants nutrient status changed compared to the control. Finally the relative growth rate and a salinity tolerance coefficient reduced significantly at the maximum values of NaCl and Boron.

# Regulation of gene expression in roots of cabbage varieties differing in root hair growth under P-deficiency

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Different phosphorus efficiency in Ethiopian mustard (*Brassica carinata*) was attributed to longer root hairs in the more P-efficient cultivar 'Bale' (Eticha and Schenk, 2001). The presented study aims to identify differences in the gene expression of cabbage varieties under P-deficiency.

Seeds of *Brassica carinata* cultivars 'Bale' and 'Bacho' were germinated for five days and then transferred to a P-free nutrient solution. After five days of cultivation under controlled environmental conditions lateral root tips of 1 cm length were harvested for RNA extraction. To determine differences in the gene expression under P-deficient conditions in both cultivars a Suppression Subtractive Hybridization (SSH) was carried out. This method resulted in a subtracted cDNA library with differentially expressed genes. The resulting gene sequences were verified for differential expression by semi-quantitative RT-PCR with gene-specific primers and specified by corresponding homologous sequences in databases. Another approach was the use of whole genome microarrays of *Arabidopsis thaliana*. The taxonomic relationship enabled the hybridization of *Brassica carinata* cDNA to sequences of this completely studied plant.

Differentially expressed sequences revealed genes involved in cell wall synthesis and cell extension that were upregulated in the P-efficient 'Bale' compared to the more inefficient cultivar 'Bacho' during P deprivation. Another group of genes have a function in the ethylene biosynthesis process that were more expressed in 'Bale'. Sequences with a higher expression in 'Bacho' had similarities to cell wall proteins and to genes that are responsible for determining the epidermal cell pattern. The effect of these genes on root hair elongation under P-deficiency is discussed.

## References

Eticha D. and Schenk M.K. 2001 Phosphorus efficiency of cabbage varieties. In: Horst et al. (Eds.) Plant Nutrition - Food Security and sustainability of agro-ecosystems through basic and applied research. 542-543

## Pollen characteristics of intergeneric crossings in the family of the *Ranunculaceae*

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*Ranunculus asiaticus* L. and *Anemone coronaria* L. (Ranunculaceae) are interesting cut flowers. There is a large variation in leaves, flower shape and flower colour within these species. Intergeneric hybridization might therefore result in the creation of new phenotypes with original combinations of these forms and colours. Spontaneous intergeneric crossings between *Ranunculus asiaticus* and *Anemone coronaria* are not reported and little research effort is given to controlled crossings.

Crossing barriers occur frequently when intergeneric crosses are attempted. A pre-fertilization barrier for these species is linked to a non corresponding flowering time. Therefore a study of pollen viability and pollen storage was undertaken.

Plants were grown in the greenhouse from September till May and pollen characteristics were studied. The average pollen diameter was for *Ranunculus* and *Anemone* respectively 0.028 mm and 0.031 mm. Pollen can be binucleate or trinucleate. Binucleate pollen grows easier than trinucleate pollen in culture conditions. Both genera used in our experiment had binucleate pollen.

A frequently used method to assess pollen viability is in vitro germination. Sixteen germination media were tested for three *Anemone* and two *Ranunculus* cultivars. Germination rates as well as pollen tube lengths were measured. For *Anemone* a general pollen germination medium, containing 100 mg/l H<sub>3</sub>BO<sub>3</sub>, 700 mg/l Ca/(NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O, 200 mg/l MgSO<sub>4</sub>.7H<sub>2</sub>O, 100 mg/l KNO<sub>3</sub>, 100 g/l sugar, 150 g/l PEG 6000 and 0.5 g/l MES (pH 6.0), resulted in the highest germination rate (22.6 % ± 7.3 %) and the longest pollen tube (132 µm ± 12 µm) for the three cultivars studied. The two cultivars of *Ranunculus* reacted different to the pollen germination media. One of the cultivars had the highest germination rate on the same medium as *Anemone* (23 % ± 3 %), the other cultivar preferred a medium with higher sugar content (200 g/l) and lower boron content (10 mg/l H<sub>3</sub>BO<sub>3</sub>) (pH 5.8) (22.7 % ± 3 %). The length of the pollen tube growth was for *Ranunculus* the best in 62 mg/l H<sub>3</sub>BO<sub>3</sub>, 236 mg/l Ca(NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O and 100 g/l sugar (pH 5.8) (717 µm ± 183 µm).

The growth of the pollen tube in vivo was also assured by an aniline blue staining method in order to study the occurrence of prezygotic barriers. In most cases of the crossings between *Ranunculus* and *Anemone* (and vice versa) the pollen tube did not reach the ovules. In order to overcome this barrier, in vivo as well as in vitro techniques will be further optimised.

Pollen viability during storage was studied in the following temperature conditions: 4°C, -20°C, -80°C and a direct immersion in liquid nitrogen (-196°C). Viability was tested 0h, 24h, 2 weeks, one month, 3 months and 6 months after the start of the treatment with fluorescein diacetate (FDA). Crossability barriers imposed by different flowering periods of the parents might be effectively overcome through pollen storage. As the pollen storage protocol was not working properly (6 months of storage resulted in a drastic loss of viability (< 20%)), further research to synchronise the flowering period is needed.

## **Histological and molecular analysis in self-incompatible and inter-compatibles mandarins**

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Citrus species produce parthenocarpic fruits and seedlessness is a very important feature of mandarins to get valuable fruit for fresh consumption. This trait is often achieved by using self-incompatible genotypes. However seeds are commonly found when a self-incompatible cultivar is not cultivated in solid block, and pollination with sexually compatible cultivar occurs. The rise of this problem in mandarins, with the recent introduction of new cultivar, has prompted the study of the pollen-pistil incompatibility in *Citrus* species. In contrast to what is known in other fruit tree species, very little is known on the self-incompatibility reaction in *Citrus*. It is assumed to be of gametophytic control, with pollen tube arrest after the pollen tubes have grown some distance through the pistil, and genetically controlled by the *S*-locus, which in other species encodes for glycoproteins showing ribonuclease (S-RNases) activity, regulated by several enzymes such as TGase.

In order to investigate the pollen tube behaviour in different controlled pollination of mandarins we sequentially examined, along two weeks after pollination, the number of pollen tubes at various pistil levels and also TGase activity in the style. Besides, we evaluated intervarietal compatibility in seedless cultivar by observing pollen tube behaviour 12 days after controlled pollinations. In the incompatible combinations different inhibition sites were observed: stigma, style and even ovary appear to be involved in the incompatibility mechanism of different genotypes. Finally, a molecular approach has been used in order to identify differential expressed sequences in a self-compatible and in self incompatible Clementine varieties. Follow-

ing a cDNA-AFLP analysis, so far 100 differentially expressed transcript derived fragments have been isolated. Results are discussed in terms of the characterisation of the incompatibility reaction in *Citrus* species.

## Haploid technology in fruit tree breeding

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World fruit production amounted to over 524 million metric tons in 2006 (FAOSTAT, Database). Fruit crop breeding uses either conventional methods (hybridisation and selection) and biotechnological ones, employing embryo culture, regeneration from protoplasts, somatic hybridization, in vitro mutant selection, genetic transformation and haploid production.

The most important application of pollen biotechnology in breeding and genetic studies derives from the ability to obtain haploids and doubled haploids.

Haploid plants are sporophytes carrying the gametic chromosome number ( $n$  instead of  $2n$ ), and doubled haploids (DHs) are haploids that underwent, spontaneous or induced, chromosome duplication. The interest of breeders in haploids and DHs, lies in the possibility of shortening the time needed to produce homozygous lines compared to conventional breeding. Haploid-diploidization through gametic embryogenesis allows single-step development of complete homozygous lines from heterozygous parents. In a conventional breeding programme, a pure line is developed after several generations of selfing. In the case of fruit crops, characterized by a long reproductive cycle, a high degree of heterozygosity, large size, and, sometimes, self-incompatibility, there is no way to obtain haploidization through conventional methods.

Haploid plants arouse interest in the fields of genetic and developmental studies, as well as for plant breeding. In fact they have a potential use in mutation research, selection, genetic analysis, production of inbred lines required to utilize hybrid vigor (heterosis), and genetic transformation. Moreover, new superior cultivars produced via gametic embryogenesis (above all through pollen embryogenesis) have been reported for several genotypes, and doubled haploids are being routinely used in breeding programs for new cultivar development in many crops.

Several are the methods available to obtain haploids and DHs in fruit trees, inducing regeneration from the male gamete (pollen embryogenesis) or from the female gamete (gynogenesis).

Considerable research has been carried out since the 1970s to obtain haploids for fruit tree breeding through gametic embryogenesis, but they were not always successful (Ochatt & Zhang, 1996; Germanà, 2006). However, the increasing number of recent reports regarding haploid research in fruit trees, shows the great interest in this useful breeding tool, and encourages us to look with optimism at its future applications in these important crops.

The current status of research on haploid and doubled haploid production in the main fruit crops is reported.



# **The development of SNPs (Single Nucleotide Polymorphism) markers in *Fragaria vesca* and *Rubus idaeus* and their transferability between the two species**

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Single Nucleotide polymorphisms (SNPs) are widespread and distributed throughout the genome of most species. Because of their abundance and relative ease with which they are detected from sequence data, these polymorphisms could be used as simple genetic markers. Withal there is also great potential for the use of SNPs in the detection of association between allelic forms of a gene and phenotypes, especially for common disease that have multifactorial genetics. For these reasons this markers would prove very useful in the indirect marker assisted selection (MAS) exercised during plant breeding. To date, however, only few SNPs were described in a published work based on quantitative identification of strawberry and raspberry presence, in food products. Identification of SNPs could lead to the development of markers useful for genotyping and for determination of genetic diversity among and between *Fragaria* and *Rubus* varieties. These research works focuses on SNPs discovery on different DNA sequences of *Fragaria vesca* and *Rubus idaeus* varieties and on transferability of developed markers between the two genera. Amplifiable DNA was recoverable from strawberry and raspberry leaves and a set of SNP markers was started to be developed. To achieve this objective published SCAR primers, developed on *Fragaria*, were used to amplify and sequencing specific region of strawberry genes. At the same time a new set of primers was designed on conserved region of enzyme sequences involved in the specific biochemical pathways and used to amplify and sequencing raspberry DNA. The polymorphisms found in *Fragaria* will be investigated in *Rubus* and vice versa and used for varietal identification. Moreover, technological improvements, make the use of these SNPs attractive for high-throughput screening in the study of genetic diversity, in the marker-assisted selection (MAS) and in food traceability.

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## **Proteome anslsysis of wild and cultivated *Cynara cardunculus* L. sl.**

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*Cynara cardunculus* L. belongs to the family Asteraceae (Compositae), and includes three subspecies: cultivated cardoon (var. *altilis* DC), globe artichoke (var. *scolymus* L.) and wild cardoon (var. *sylvestris* (Lamk) Fiori). Molecular (Lanteri et al., 2004a, Acquadro et al., 2005), cytogenetic and isozyme (Rottenberg et al., 1996) studies have confirmed that wild cardoon is the ancestor of both cultivated forms, as suggested by Rottenberg and Zohary (1996).

As wild cardoon is fully cross compatible with the globe artichoke and with the cultivated cardoon, the objective of the present paper is to assess the proteomic relationships among wild, cultivated artichoke and cardoon. To the best of current knowledge, the present study represents the first description of the use of proteome to define differentiation among wild and cultivated *Cynara cardunculus* L. sl..

MS/MS (MS/MS ion search and *de novo* sequencing) in combination with similarity searches allowed successful identification of 60 spots from wild, cultivated artichoke and cardoon. The identified proteins were involved in glycolysis, stress response, other essential metabolisms and cell structures.

The remarkable level of proteomic variation detected in wild cardoon confirms the notion that the wild form is the ancestor of both cultivated ones.

Furthermore, proteome data have provided an accurate assessment of the amount and distribution of protein variation in cultivated cardoon, and they supply information important for a scientific approach to the conservation and utilization in breeding programs.

## **Immundiagnostic method to detect viruses in *Agaricus* species**

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Champignon (white button mushroom) production is a prevailing division of the vegetable production sector. In the course of mushroom cultivation a large number of pests and infective agents, among those also viruses may endanger the yield. Earlier the La France Isometric Virus caused relevant losses, and lately the Mushroom Virus X (MVX) is the major virus incurring losses. The unequivocal detection of virus diseases is problematic, because the symptoms are often similar to those caused by errors in cultivation technology. The detection of the MVX-infection present an extreme difficulty, because up to now it couldn't be identified which one of the 23 double-stranded RNA (dsRNA) species, occurring in variable number and size in MVX diseased mushrooms, is causally connected with disease development. This is why the polymerase chain reaction (PCR) based specific and sensitive methods are still not available for reliable MVX detection. The aim of our experiments was to introduce a sensitive, simple and reliable method, which will allow us to detect all dsRNAs present in a single step. The dsRNA-immunoblot method used in our experiments is based upon the application of monoclonal antibodies, which specifically recognise dsRNAs, independent of their sequence and nucleotide composition. We showed, that by immunoblotting dsRNAs can be detected directly in unfractionated nucleic acid extracts of champignon, without chromatographic purification on CF11 cellulose. It was found, that even healthy, symptom-free mushroom hybrids collected from different sources may differ in their dsRNA-pattern. In addition, in MVX diseased reference samples as well as in some "suspicious" samples we were able to detect dsRNAs not present in any of the healthy mushrooms. The occurrence of dsRNA species in wild *Agaricus* species was also investigated. We found that dsRNAs, which might be of viral origin, are present in *A. romagnesii*, *A. squamuliferus* and in *A. vaporarius*.

## **Formation of embryo like structures on *Pelargonium***

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### ***Introduction***

*Pelargonium* is one of the most important ornamental crops. Many cultivars are propagated vegetatively by cuttings. Somatic embryogenesis as an in vitro propagation system could be an alternative. The induction of somatic embryogenesis was based on Hutchinson and Saxena (1996), who induced somatic embryos on hypocotyl explants of seed propagated cultivars.

### ***Material and methods***

Explants were cultured on a MS-medium containing 10 µM of the cytokinin Thidiazuron (TDZ) for an induction phase of one week followed by an expression phase of three to four weeks on a medium without

TDZ. Flower buds, peduncles and petioles were used as explant types. Surface sterilization was done using ethanol (3 min) and sodium hypochlorite solution (0,75 %, 6 min).

### **Results and discussion**

Different genotypes of *Pelargonium x hortorum*, *Pelargonium grandiflorum* and *Pelargonium crispum* were tested. The frequency of formed somatic embryo like structures depends on the genotype. Somatic embryogenesis was induced successfully on flower buds, peduncles and petioles of vegetatively propagated cultivars. Varieties tested in this study differed in their response of the explant type. For example formation of embryo like structures occurred in *P. x hortorum* 'Meloblue' on flower bud and peduncle explants, but not on petioles. Petiole explants of *Pelargonium crispum* 'Angeleyes Bicolor' formed embryo like structures.

Expression phase normally was conducted on agar solidified medium in petri dishes. The transfer from the induction medium to a temporary immersion system (RITA) increased the multiplication rate.

### **Literature**

Hutchinson, M.J. & P.K. Saxena, 1996: Acetylsalicylic acid enhances and synchronizes thidiazuron-induced somatic embryogenesis in geranium (*Pelargonium x hortorum* Bailey) tissue culture. *Plant Cell Reports* 15, 512-515.

## **Priming onion seed lots differing in the level of ageing for improved germination and emergence**

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The effect of osmopriming of onion (*Allium cepa* L.) seeds on the percentage of normal (germination capacity) and abnormal seedlings development and the mean germination time (MGT), were studied. Eight seed lots of two cultivars Sochaczewska and Rawska harvested in 2002, 2003, 2004 and 2005, were used. These lots differing in initial seed quality, were subjected to priming treatments. Seeds were osmoconditioned at 15°C in 0,1 MPa polyethylene glycol 6000 (PEG) and potassium nitrate (KNO<sub>3</sub>) solutions for 1, 2, 3, 4 and 5 days, in the dark. After the priming treatment four replicates of 50 seeds were uniformly placed in 150 mm petri dishes on the moistened filter paper. Germination capacity and percentage of abnormal seedlings were recorded 12 days after imbibition and seedlings were classified using ISTA (International Seed Testing Association) guidelines. MGT of onion seeds was calculated according to Pieper method – seeds were assessed daily and the number of germinants, as evidenced by radicle emergence, was recorded. Untreated seeds of each lot were used as the control. All germination tests were carried out in an incubator with forced air circulation at 15°C.

This study showed that the osmoconditioning was the most effective for increasing the speed of germination and the number of normal seedlings, and decreased the percentage of abnormal seedlings, particularly in lots harvested in 2004. The obtained results demonstrate that, in comparison with untreated control seeds:

- 1) the kind of conditioning solution and the duration of seed treatment, were significantly effective in reducing the incidence of the number of seeds that developed into abnormal seedlings, as well as the mean germination time. However, priming carry on for three days gave the best results. Both tested kinds of osmosolutions in the same way influenced the germination of treated seeds,
- 2) the presowing treatments did not allow the germination capacity to be enhanced for most of the tested seed lots, independently on the used solutions and the duration of priming.

## Cloning and molecular characterization of double-stranded RNA of two Beet Cryptic Viruses (BCV1 and -2)

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Beet cryptic viruses belong to *Cryptovirus* genus of *Partitiviridae* family, and have small, segmented double-stranded RNA (dsRNA) genomes with segments of 1-2 kbp. Cryptic viruses are widespread in *Beta vulgaris* subspecies and wild-type sugar beets (*Beta maritima*, *Beta macrocarpa*) and may be present in almost every member of the subspecies/cultivar without being detected. Cryptic viruses persist within the host throughout its life without causing any symptoms.

Up to now three different *Beet Cryptic Viruses* (BCV1, -2, -3) were identified. They can be distinguished serologically and on the basis of the molecular weights of their genomic RNAs. The aim of our work was to characterize the genomic dsRNA of *Beet Cryptic Virus* 1 and 2 by cDNA-cloning and sequence analysis. The genome of BCVs is composed of two dsRNA segments, one encodes the coat protein (CP), the other the viral RNA-dependent RNA-polymerase (RdRp). After cDNA-cloning of BCV1 the sequence of the 2008 bp dsRNA1 was determined. Computer analysis of the sequence predicted a single open reading frame (ORF) encoding the putative RdRp (616 AA, 72.5 kDa). In case of BCV2 the putative RdRp is encoded by the larger genomic segment (1575 bp) and has a MW of about 54.2 kDa. Six of eight conserved polypeptide motifs that are considered as markers of dsRNA viral RdRps were identified in both putative RdRp molecules. According to multiple RdRp alignments, BCV1 and BCV2 belong to clearly different groups of cryptic viruses. BCV1 RdRp shows highest homology to *Vicia Cryptic Virus*, *White Clover Cryptic Virus* 1 and to some fungal viruses of the *Partitiviridae* family, whereas BCV2 resembles a group of cryptic viruses, which, to our present knowledge, occur only in plants (*Raphanus sativus Cryptic Virus* 2, *Pinus sylvestris partitivirus*, *Beet Cryptic Virus* 3, *Pepper cryptic virus* 1, *Pyrus pyrifolia partitivirus*). Sequence comparisons of coat proteins will also be presented and the evolutionary relevance of the findings will be discussed.

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## Genetic, environmental and molecular components affecting pollen viability and sexual incompatibility in olive tree (*Olea europaea* L.)

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In plant species, self-incompatibility (SI) is a natural, widespread mechanism that prevents self-fertilization and promotes out-breeding. For horticultural purposes understanding the mechanisms that affect SI can help us to optimize fruit-set through proper orchard design and cultivars' choice. This, in olive tree (*Olea europaea* L.) farms is expected to improve crop yield as well as crop sustainability. In the framework of the present effort we studied, for two consecutive years, floral biology, self compatibility and cross pollination requirements in four olive tree cultivars. The varieties were the oil producing 'Koroneiki' and 'Mastoidis'

and the table olive cvs. 'Kalamata' and 'Amygdalolia' which are all originating from Greece and the field trials were implemented in the "Olive Germplasm Collection of Subtropical Plants and Olive Trees Institute of Chania". *In vivo* pollen tube growth in the pistil was employed as parameter to determine sexual incompatibility. In parallel, parthenocarpy was also determined following controlled self- and cross-pollinations. Different levels of self- and cross- incompatibility have been detected. Fruit set in cv. 'Koroneiki' was higher after free pollination (8.97%), followed by cross pollination with pollen of 'Kalamata', 'Amygdalolia' and 'Mastoidis' and self pollination. In the case of 'Mastoidis', the fruit set percentages were 4.24% (free pollination), 2.59% ('Koroneiki'), 2.2% ('Kalamata'), 2.07% ('Amygdalolia') and 1.83% after self pollination. The fruit set trend was similar for 'Kalamata' at 3.18% (free pollination), followed by 'Mastoidis', 'Amygdalolia' and self pollination. Increased fruit set percentages through free and cross pollination were recorded for 'Amygdalolia', as well. The incidence of shotberries was highest after self pollination and lowest after free pollination for all of the studied cultivars and the rate -% shotberries after self-pollination / % shotberries after free pollination- was up to 20 for 'Kalamata' and 'Amygdalolia', while it was significantly lower for the two oil cultivars ranging between 1.4-4.4. We also assessed pollen viability and *in vitro* pollen germination as affected by the different genotypes of the donor tree as well as by pre-incubation (10, 20, 30 and 40°C) and incubation temperatures (15, 20, 25 and 30°C). The highest pollen viability was recorded in 'Amygdalolia' (89.1%) followed by 'Kalamata' and 'Mastoidis' while 'Koroneiki' had the lowest pollen viability. The optimum incubation temperature for pollen germination was 25°C for 'Koroneiki', 'Mastoidis' and 'Kalamata' while for 'Amygdalolia' pollen, 25°C and 30°C incubation treatments were similarly effective. Pollen germination was drastically reduced through pre-incubation at 30°C and diminished in the case of 40°C treatment. Further, a first attempt is under way to study some of the molecular components of the SI mechanism in olive. For that, presently deposited DNA sequences from other plant species are employed in order to design a strategy that would allow us to clone olive tree genes with a putative function in SI and preliminary results of the bioinformatics study are presented.

## Use of molecular markers linked to QTLs for fire blight resistance in pear breeding

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Pear breeding is a long-term process due to the long juvenile period and the absence of consistent criteria for early phenotypic selection. Therefore, marker-assisted selection (MAS) could be a promising tool to improve the efficiency of pear breeding programs. Fire blight (*Erwinia amylovora*) resistance in pear is known as a quantitative trait and cultivars are differently susceptible to the disease. The objective of this study is to identify reliable SSR markers linked to the two main quantitative trait loci (QTLs) for fire blight resistance identified by Dondini et al. (2004) in the pear cultivar "Harrow Sweet" and to verify the consistency of the two QTLs in a second background. The regions of the two QTLs under study are on the top part of linkage groups 2 and the bottom part of linkage group 4. These regions have been enriched with simple sequence repeat (SSRs) markers developed for pear and apple. SSR markers closely linked to the QTLs have then been used to identify the different combinations of the Harrow Sweet QTLs alleles in the progeny plants of a second segregating population (Harrow Sweet x Verdi progeny). Artificial inoculation with *E. amylovora* will be conducted on representative sets of progeny plants carrying different combinations of the Harrow Sweet QTLs alleles. This will enable us to validate the fire blight QTLs in a different background. The utility of our molecular markers in MAS to streamline pear breeding will be discussed. (Dondini et al., 2004, *Molecular Breeding* 14:407-418).

## Biotechnological approaches to control the expression of potential allergens in small fruits

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Regular consumption of fruits has a positive influence on human health by disease prevention. However parallel to dietetic benefits, IgE-mediated fruit allergies represent an increasing health risk in the Northern hemisphere. In apple, so far four major allergens have been reported (<http://www.allergen.org>), including Mal d 1 (PRP-10), homologous to Bet v 1 with a molecular mass of 17.5 kD, Mal d 2 (PRP-5), a thaumatin-like protein (TLP), with a molecular mass of 23 kD, Mal d 3 (PRP-14) a lipid transfer protein (LTP) with a molecular mass of 9 kD and Mal d 4 (profilin), homologous to Bet v 2 with a molecular mass of 14 kD (Marzban et al. 2005a, Herndl et al. 2007).

Small fruits like blueberry, raspberry and strawberry may have a huge impact on human health due to their high content of antioxidants and unknown biofactors, which play a role in ageing, cancer and infection prevention. Reports on allergenicity of small fruits such as strawberry, raspberry, blackberry and blueberry are still rare (Marzban et al. 2005b). Strawberries are under investigation and strong evidence for the existing Bet v 6, Bet v 1 and Mal d 3 homologous proteins. Allergic reactions were also reported in contact to raspberry and blueberry.

Here we present an approach using RNAi technology to control the expression of Mal d 3 homologous proteins in *Vaccinium* species. To this purpose adventitious regeneration from leaf discs of *V. corymbosum* and *V. cylindraceum* was established and *Agrobacterium* mediated transformation optimized to transfer the Mal d 3 sequences.

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## Studies on ABA sensitivity and drought tolerance in *Arabidopsis* and tomato

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A particularly important trait in horticultural practice and breeding new plant varieties is tolerance against drought. The physiological background of this trait is very complex. We have found that an *Arabidopsis* mutant line with improved drought tolerance carried a lesion in the *CBP20* gene, which is a member of the nCBC complex (Papp et al., 2004, *Plant Mol. Biol.* 55: 679–686). The *cbp20* mutant displays only mild pleiotropic morphological abnormalities, but elevated sensitivity to abscisic acid (ABA). Apparently this later property leads to fast stomatal closure and thus improved drought tolerance.

We study the physiological consequences of improved water retention in the *cbp20* and other ABA oversensitive mutants, with special emphasis on changes of integrity of photosynthetic apparatus and competitive fitness coupled with drought hardiness. We found that faster ABA responses of stomata do not impair pho-

tosynthetic capacity under normal conditions. When exposed to drought the *cbp20* mutation helped to maintain the integrity of the photosynthetic system demonstrated by fluorescence kinetic analysis. Surprisingly we found that restricted gas exchange in *cbp20* did not confer drought tolerance in the presence of competitors for water. We observed the same effect when we tested other ABA oversensitive mutants, so these data reveal a new aspect of ecophysiology of water use in plants.

With the aim of testing whether the regulation of nCBC function is conserved in crop plants, in particularly in the horticultural model plant tomato, we have created a cDNA construct for silencing the tomato homolog of the Arabidopsis CBP20 gene and adapted an efficient Agrobacterium based plant transformation method. With an efficient gene silencing strategy we can possibly achieve higher ABA sensitivity and better water use in transgenic tomato lines. As our method is based on creating a loss-of-function mutation, selection of transgene free mutants might be feasible by biotechnological methods in the future.

## **New digital techniques for yield prediction of fruit crops**

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Yield predictions on a European scale are a pre-requisite for effective planning, fruit quality assessment (fruit number and fruit size) as well as food chain (storage and transport capacities) and traceability of the commodity.

Traditional yield predictions like the Winter model rely on empirical data and skilled personal, mostly extension service. They assess fruit trees in the same orchards every year for their respective fruit numbers for each cultivar after June drop. Fruit counts are taken for every other tree in a row from two angles on both sides, which makes it time-consuming. This takes 2-3 people almost one week for each fruit growing region.

Three new approaches commenced in the last three years. The approach presented here is an approach using image analysis systems to distinguish between fruit and leaves of a tree by shape and size at a time when fruit are still green. Digital photos are taken from either side of a tree using references for size and dimensions. Correlations for 2006 and 2007 are presented between predicted and actual yields of cv. 'Braeburn', 'Gala' and 'Elstar' apple trees. Different thinning techniques, including the new mechanical thinning, were employed for these experiments to achieve fruit trees with different magnitudes of cropping to test the techniques and their accuracy.

## **Response of transgenic mannitol-producing tobacco plants to water deficit**

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### ***Introduction***

Plants have evolved a range of anatomical and morphological structures and physiological and biochemical mechanisms to cope with drought. One common mechanism is the accumulation of compatible solutes, low-molecular-weight compounds that are non-toxic at high concentrations. Among a variety of organic molecules, polyols, may act as osmolytes in osmotic adjustment, facilitating retention of water in the cytoplasm and allowing ion sequestration in the vacuole or apoplast. Sorbitol and mannitol are widely distributed in the plant kingdom and mannitol seems to contribute to osmotic adjustment in olive under water deficit (Xiloyannis et al., 1999). Yet, better survival and/or performance of salt- or water-stressed transgenic plants com-

pared to wild types do not seem to be attributable to osmotic protection by mannitol (Karakas et al., 1997; Abebe et al., 2003). Other indirect mechanisms may be involved, and mannitol accumulation in the chloroplasts of transgenic tobacco plants seems to provide oxidative stress protection by supplementing endogenous radical scavenging mechanisms (Shen et al., 1997). It has also been suggested that polyols are strong water-structure formers and this may explain their function as effective stabilizers of the hydration shell of native proteins (protection against heating, freezing, and drying) and potential as stabilizing/protecting agents at both molecular and whole-cell level (Galinski, 1993). In this study, we used transgenic mannitol-producing and wild-type tobacco plants to test whether mannitol is directly or indirectly involved in plant responses to water deficit.

### ***Materials and Methods***

Twenty rooted cuttings, 10 transformed (+mtID) and 10 untransformed (-mtID), were fully irrigated [100% evapotranspiration (ET)] or received 25% ET. After 40 days of deficit, 25% ET plants were re-watered to control levels. Mannitol contents were determined by gas chromatography to verify the successful introduction of the mtID gene. Plant growth was estimated by measuring the cumulative length of all stems (TSL) present at each sampling date in every plant by digital image analysis. After 30 days of deficit irrigation, leaf drop was estimated and expressed as a percentage of the total number of fully expanded leaves. Osmotic potential at full turgor (with an Osmette A 5002 osmometer) and relative water content (RWC) were measured twice a week. Mannitol-1-phosphate dehydrogenase (M1PDH) activity was determined on mature leaves of +mtID plants.

### ***Results and Conclusions***

Water deficit reduced RWC of both plant types after 22 days, TSL of -mtID 25% ET already after 11 days, whereas that of +mtID 25% ET only after 34 days. After 30 days of deficit, a greater percentage of mature foliage was retained by +mtID 25% ET compared to -mtID 25% ET. Osmotic potential was lower in the 25% ET plants on day-14 contributing to the relatively high RWC and the reduction in TSL of the -mtID 25% ET plants. Osmotic potential showed an increasing trend with the progress of deficit, indicating that osmotic adjustment did not play a significant role in protecting plants from dehydration. Mannitol-1-phosphate dehydrogenase activity of +mtID plants was not affected by water deficit. The presence of mannitol in transgenic tobacco seems to provide some tolerance to water deficit, especially in terms of delaying growth reductions. However, the amount of mannitol produced by +mtID plants did not allow for significant osmotic protection, whereas it may provide a specific system for protecting cells from free radical-induced damage.

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## **A wholistic approach in understanding oak drought stress response**

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Drought periods due to climate changes, as being forecasted for the upcoming decades, will demand specific drought tolerating strategies of trees due to their long life cycle. Selecting trees with the potential to more easily adapt to the changing environmental conditions would be reasonable, especially for afforestation purposes where sustainable forest management is a major issue. Thus, the final goal of this project is to develop easy applicable functional markers for drought resistant oaks from natural populations. These can support plant selection to enhance drought resistance of oaks (*quercus* spp.).

In order to identify informative markers, four approaches are combined. Based on a two-year glasshouse experiment, five year old clonal plants originating from tissue culture are subjected to controlled drought stress conditions in a closed environment. During this time, in order to get a 'full picture' of the response to long-term drought stress on the "whole-plant level", i) the transcriptome (cDNA-Microarray) ii) the proteome iii) physiological parameters such as proline and carbohydrate content as well as iv) phenotypic parameters are monitored and plant material is sampled in regular intervals. During the two years of the experiment, three treatments have been applied: 1) control: soil kept moist (vol. soil moisture > 30%), 2) drought stress: cycles of increasing water stress from 20% to <10% vol. soil moisture for two growing seasons and 3) drought stress in the 1st year but "control conditions" in the 2nd year.

During the first growing season not only a delay in leaf development could be observed in the drought stressed plants but also a significant reduction in growth ( $p < 0.05$ ) as compared to control plants. This observation was underpinned by the physiological results showing significantly higher ( $p < 0.05$ ) sugar and polyol concentrations in the drought stressed plants at each sampling time compared to the controls. Furthermore, a significant accumulation of carbohydrates ( $p < 0.05$ ) took place in the stressed plants with persisting drought treatment.

In the second year, the plants of both drought stress treatments started earlier with leaf development than the controls. However, while DS-2 resumed growth DS-1 almost stopped growing and did not show a 2nd flush. Microarray and proteome analyses are still running.

## **Impact-Analytcs: implementation of Bioelectric Recognition biosensors to enhance absolute food safety on a country wide scale**

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The recent series of food scares has heightened consumer's awareness of food and its production. Previously the majority of consumers rarely questioned the safety of food; for most it was inconceivable that food was not safe. What is more, as changes in legislation along with the increase of consumer's interest, became apparent, the opportunity arose for novel analytical technologies to provide assurance along the whole supply chain from the farm to retailer's shelf.

The already existing food quality control laboratories use conventional techniques, providing a limited range of analyses. For that reason they are unable to cover the needs of the domestic market, thus the offer to demand ratio is extremely low.

Impact-Analytics is a model food quality control laboratory under establishment in the German market. The company will provide services for the detection of pesticide residues in food samples as well as microbiological testing.

Impact-Analytics plans to use the exceptionally advanced Bioelectric Recognition Assay (BERA) technology of cellular biosensors. The biosensor is a nanogel containing tens of thousands of mammalian cells that are specifically reacting to pesticides or any other molecule to customized fashion. The technique uses cells with modified cellular membrane surface (MIME cells), by the artificial insertion of molecules (receptors) in such way that they react selectively with analytes under determination. Binding of the analytes (e.g. pesticides) to the receptors causes a change in membrane structure and consequently a change in the membrane's electric potential. This change is detected by using a microelectronic circuit, connected to a computer, and its results are presented with the help of special software. The competitive advantages of the cellular biosensory technologies are:

- Test results delivery. BERA allows for test results in 24h, while the competitive laboratories require 2-6 days to deliver their results.
- Cost. Very low compared to conventional methods.
- Capacity. There is possibility for multiple sample analyses which will allow thousands of tests per day, while only tens are conducted by the competition.

Impact-Analytics will provide services for ingredient suppliers, food processing plants, retail and distribution companies, food service operations and trade associations.

## **In vitro micropropagation of *Adenium* selections by axillary branching**

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Plants belonging to the *Adenium* ("Desert Rose") genus (Apocynaceae), are spectacular evergreen succulent shrubs or small trees native to tropical and subtropical eastern and southern Africa and Arabia. They are characterized by the presence of pachycaul stems as well as by the occurrence of a swollen caudex at the basal part of the stem. As these striking features are combined with large and beautiful flowers often present over a long period, *Adenium* have long been considered as high-value succulents well-suited both for landscaping horticulture in tropical regions as well as for indoor gardening in temperate regions. Despite intense breeding and selection efforts made in Thailand and Taiwan during the last decades, *Adenium* have just entered mainstream floriculture in Europe. At the moment, most material is still produced in Asia and the availability of large amount of high-quality propagation material is a prerequisite before *Adenium* may be routinely grown by regular nurseries and bought as pot plants by non-collectors in Europe.

Development of a highly efficient in vitro micropropagation method may therefore be useful for ensuring quick and true-to-type propagation of highly attractive hybrid selections while avoiding phytosanitary problems commonly associated with large-scale grafting propagation.

### ***Results and conclusions***

Seeds from different selections of *Adenium* (*Adenium obesum*, *Adenium somalense*, Thai hybrid lines) were surface-sterilized and germinated in vitro on modified Murashige and Skoog (MS) medium. Efficient induction of axillary bud development (multiplication rate >5) was observed when shoots were subcultured every 4 weeks on the same medium supplemented with high cytokinin levels (multiplication rate > 5).

Ninety percent of these shoots readily regenerated roots on MS medium supplemented with indole-3-butyric acid. Rooted shoots were easily acclimated under standard greenhouse conditions (100 % survival rate) and the obtained plants are currently being observed for their growth and development in vivo.

In conclusion, an efficient propagation method, with a potential for commercial true-to-type production of elite *Adenium* hybrid selections, is available in our laboratory.

## Quality in peach fruit: the locus *f* in two peach populations

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The role of locus *f* is crucial to determine quality in peach since it controls two simple traits: melting/non-melting flesh (MF/NMF) and freestone/clingstone (F/C). Monet (1989) first proposed the combination of three different alleles (F, f, f1) to explain the major phenotypes observed (FMF, CMF and CNMF). Recently, this locus has been linked to the endopolygalacturonase gene (endoPG) by means of Gene Target Markers (GTM; Peace et al., 2005). The endoPG gene codes for an enzyme that depolymerizes pectin during late stages of softening, determining the melting flesh phenotype. The absence of this event characterizes the non-melting flesh peaches. A different flesh type is the stony hard (Hd), due to the lack of ethylene burst that inhibits the expression of the enzymes (endoPG, etc) positively regulated by the hormone. The stony hard trait is epistatic to the melting/non melting flesh traits (Haji et al., 2005).

The present work was carried out as an international partnership with the aim of comparing the locus *f* region in different species of *Prunus* and *Malus*.

The locus *f* was located in the distal part of linkage group G4 in two different peach maps (P x F, OH x Y). Both these progenies segregate for the freestone/clingstone trait and the OH x Y progeny also for stony hard trait. Two GTMs (Peace et al., 2005) were mapped in both populations and were found to co-segregate with the locus *f*, as expected. Several microsatellite markers, located in G4 in the *Prunus* reference map (T x E) via BIN analysis, were analyzed in our populations to saturate the region of interest and to identify anchor loci in T x E. A comparative analysis of the target region revealed an almost complete synteny and colinearity among the three maps. Due to the epistatic effect of the stony hard trait in the OH x Y progeny only three phenotypic classes were expected to be present (FMF, CMF, CHd – Clingstone/Stony hard flesh). Instead, an FHd (Freestone/Stony hard flesh) class was also found. The two traits show an independent segregation ( $\chi^2 = 2.2$  df3), as they are not located in the same linkage group (freestone in G4, stony hard unlinked). Likely, an explanation of this evidence could be the presence in the locus *f* of different copies of endoPG gene with dissimilar sensitivity to ethylene.

## Developing a VIGS vector for cucumber (*Cucumis sativus* L.)

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Aim of the presented project is to develop a powerful reverse genetic tool for the *Cucurbitaceae* plant family. With the help of posttranscriptional gene silencing, particular genes can be knocked down, enabling us to reveal information about their functions. However, the genetic transformation of cucurbitaceous plants is difficult and time-consuming, and the transient method of agroinfiltration isn't effective either. We are developing a VIGS (virus induced gene silencing) vector from the SH strain of CGMMV (cucumber green

mottle mosaic tobamovirus), which causes weak systemic symptoms on cucumber, musk melon and on further cucurbits.

As the first step to develop the vector we are producing a full-length virulent clone of CGMMV in the pUC18 vector, driven by a viral T7 promoter. In order to be able to use this clone as a VIGS vector, we duplicate the promoter region of the viral coat protein gene, and design a cloning site after the promoter. Into this site, silencing constructions can be built in. Infection of the plants with the recombinant virus triggers RNA-silencing against the complement target mRNA.

The functionality of the vector has to be proved using the Pds (phytoene desaturase) gene as reporter. To this end we isolated a ~600bp mRNA fragment from cucumber, homolog to the Pds gene described from watermelon (*Citrullus lanatus* L.).

# **Lectures and Posters of Theme 3**

## **ENVIRONMENTAL CONSTRAINTS AND CLIMATE CHANGE IN EUROPE**

## The causes and the extent of climate change - past and present

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The global temperature increase over the last 150 years is calculated to be 0,7°C and about 1,8°C to 2°C in the more sensitive Alpine area. Precipitation trends are more inhomogeneous in time and space, but there is a tendency for increase in the North of Europe and decrease in the South and of wetter winters and dryer summers. Enhanced frequencies of heavy precipitation events are observed, but there are also indications of an increase of frequency and length of dry spells in some parts of Europe. The onset and the length of spring and summer, as defined by the phenological stages of indicator plants, have changed by more than one week in the last decade, as compared to the 30-year period before.

The Fourth Assessment Report (FAR) of the IPCC (2007) sums up present day knowledge on future climate change: For a low emissions scenario (B1) the best estimate is that temperatures will rise by 1,8°C (the likely range is 1.1 to 2.9°C) compared to the year 2000 to the end of the century, and for a high emission scenario (A1F1) the corresponding value is 4°C (the likely range is 2,4 to 6.4°C). Warming is expected to be greatest over land and at most high northern latitudes and least over the Southern Ocean and parts of the Northern Atlantic. Snow cover is projected to further decrease and permafrost to thaw to even greater depths. It is very likely that the frequency of hot extremes, heat waves and heavy precipitation events will continue to increase. The track of extra-tropical storms is projected to move northwards with consequences for wind, precipitation and temperature distributions over Europe. Precipitation is very likely to increase in high-latitudes of the northern hemisphere and likely to decrease in the subtropics, matching patterns observed in recent trends.

There are still uncertainties due to lack of scientific understanding of processes, lack of data and limits to spatial resolution, and a number of potentially important feed back mechanisms that – on the whole - tend to enhance global warming, are not yet taken account of in most climate models. In spite of these caveats comparisons of calculated and observed climate change justify high confidence in Global Climate Models (GCM).

Global climate change must be downscaled to regional and local scales for impact and adaptation studies, as the local manifestations of climate change can significantly deviate from those seen by global models with spatial resolutions of some 150 km. Thus temperature increase in the Alpine area will continue to significantly exceed that of central Europe, with especially large increases in summer. The frequency of tropical days in summer e.g. is expected to double in Austria within 50 years. Among the consequences of temperature rise in Alpine areas are reduced snow cover in valleys in winter with increase of frost hazard, earlier snow melt leading to reduced and earlier moistening of the ground and stronger warming due to reduced albedo. This results in earlier and enhanced drying of the ground. In the long run, when glaciers have melted in the Alps, availability of water for irrigation will also be reduced. Enhanced photosynthesis due to higher CO<sub>2</sub> concentrations and increased evapotranspiration in consequence of temperature rise lead to increased need for water while availability of water decreases. This leads to enhanced water stress. In areas of reduced yearly precipitation or increased heavy precipitation events leading to more surface run-off, water stress is further enhanced.

In the last 650.000 years carbon dioxide concentrations in the atmosphere fluctuated between about 180 ppm and 280 ppm in cycles of the order of 100.000 years. Over the last 150 years, however, the carbon dioxide concentration rose to 380 ppm and continues to rise by about 2 ppm per year. The bulk of this rapid increase is considered to be a consequence of the burning of fossil fuels by man. Annual fossil CO<sub>2</sub> emissions averaged 7.2 Gt C per year in 2000-2005. This has disrupted the near equilibrium between carbon input into the atmosphere and carbon depletion into the ocean and uptake by the biosphere and soil. Other Green House Gas emissions have also risen significantly through human activities, including agriculture. A cut back of emissions of 20% by 2020 in industrialized nations and of at least 50% globally by 2050 is necessary to stabilize Green House Gas concentrations at some 450 to 550 ppm CO<sub>2</sub>e, limiting temperature rise to 2°C to 3°C. Consequences of such a temperature increase are considered to be still manageable, whereas higher values are expected to lead to significant economic, social and political disruptions.

# **Modelling apple flowering time as a tool for exploring the effects of global warming on the completion of chilling and heat requirements in French and Belgian cropping areas**

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## ***Introduction***

In France, since the end of the 1980s, a trend toward an advance in flowering time was highlighted by long-term chronological series of flowering dates recorded in various conditions of temperature (mean advance by 7-8 d). By using modelling tools, the study aimed to understand how global warming in Europe since the same period has had an impact on apple flowering time.

## ***Material and method***

Dates of onset of flowering time (stage F1) for 'Golden Delicious' apple, collected in a national database, were selected for three cropping areas from North-West to South-East of France. A sequential model composed of a chilling sub-model and a heat sub-model was considered. The input data were consisted of 81 F1 dates (3 areas x 27 years from 1976 to 2002). A user-oriented software package, called 'Pollenoscope', automatically optimised the parameters of combinations tested between seven chilling and three heat temperature functions, respectively considered in the chilling and heat sub-models. This was achieved by maximizing the values of the coefficient of determination between the observed and simulated flowering dates.

## ***Results and conclusion***

The study provided comparative information for assessing the respective interests of temperature functions commonly used for the modelling of flowering time in temperate trees, and rarely compared one with the other. Three selected models explained 82-86 % of the flowering variability observed. The fitness of their parameters for an accurate simulation of the F1 date for 'Golden Delicious' apple was validated using their respective values to simulate F1 dates in the case of flowering data (location x year) not used in the first step of parameter optimisation. The validation step was achieved from 55 contrasting data (31 in France and 24 in Belgium) and showed promising results. Moreover, at the different locations, all three models were used to simulate the time-course changes in the duration of the chilling effect. This original output of our modelling work highlighted similar trends toward a longer duration of the chilling effect (3-5 days since the end of the 1980s). Consequently, it suggested that the mean duration of the heat effect has decreased (10-13 d) to explain the observed trend toward an advance in flowering time. Hence, our results support the idea that global warming exerted two opposing effects simultaneously in Europe since the end of the 1980s: a slower mean rate of completion for the chilling requirement and a higher mean rate of completion for the heat requirement. A more marked effect of global warming on the completion of the heat requirement may have resulted from more pronounced warming from January to April, corresponding to the active growth phase of floral primordia, than from October to January corresponding to the dormancy breaking phase.

# Olive flowering monitored in a large area of Italy and local climatic trends

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## Introduction

Phenological observations of the anthesis phases were used to study olive (*Olea europaea* L.) flowering in the Mediterranean area. The aim of this research was to study the relationships between climatic trends, climatic changes in the Mediterranean region and the biological behaviour of the olive as a possible bio-indicator species in order to study its potential to adapt to future climatic scenarios.

## Materials and methods

Pollen emission from the olive anther is the result of all phenological stages of flowering, which can thus be monitored by remote instrumentation. Therefore, aerobiological investigations can be used as a tool for studying the floral phenology of different anemophilous plants. Phenological data regarding the daily pollen concentrations (pollen/m<sup>3</sup>) in the anthesis phenophase were recorded in some olive areas of southern Italy from 1999 to 2005 and in a central Italian area from 1982 until present using a pollen monitoring methodology (volumetric pollen trap).

Aerobiological monitoring was carried out weekly using a pollen trap, developed from the Hirst trap (1952), VPPS 2000 Lanzoni model. The sampling instrument is made up of two parts: a lower fixed part, and an upper mobile part which has a small winged portion that positions the trap with the slit opening facing into the wind. The "mouth", through which the airborne material enters, allows a constant air flow volume (10 l/min) and when particles enter they stick to a weekly drum that moves at a rate of 2 mm/hr. By knowing the volume of air that enters, the concentration of particles/m<sup>3</sup> can be expressed hourly, daily or weekly (Fornaciari et al. 2005; Orlandi et al. 2005).

## Results and conclusions

The data obtained give indirect evidence of the flowering phenophase that occurs in the olive groves and considering that arboreal species are very sensitive to climatic changes they can be used to show climatic variations. In particular, the rhythm of the phenological phases emerged as directly dependent on the meteorological trend of the spring forcing temperature which in the last years of the series (2002-2006) generally indicates the possible start of a cooling trend to be confirmed by further studies.

The olive as a typical Mediterranean species is sensitive to low temperatures and resistant to water shortage. In this manner, the northern and the southern limits of olive cultivation in the Mediterranean basin are conditioned by low temperature and low rainfall. From a climatic point of view, the suitable area for olive cultivation could be enlarged due to the changes in temperature and precipitation patterns, considering particularly a possible phenomenon of increasing temperature. In this way, a spatial increase and a northward shift of the potential olive cultivation area is expected under the condition of climate warming.

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# Cherry leaf roll virus - an emerging disease in Finland?

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## Introduction

More than one fifth of the Finnish forest is birch forests. In total, the genus *Betula* represents the most common group of deciduous trees, which comprises an important raw material in the mechanical and chemical forest industry (Peltola, 2006).

Virus-related symptoms such as vein banding, leaf roll, chlorosis and subsequent necrosis on birch leaves were increasingly recorded throughout Finland since 2002. In a survey throughout the country symptoms on birch were especially distinct during the dry summer of 2006 (Jalkanen et al. 2007). They are abundant in the country and affected trees often exhibited reduced leaf size in combination with dieback of twigs and branches. Symptomatic foliage has so far been found on *Betula pendula* (silver birch), *B. pubescens* (downy birch), *B. nana* (dwarf birch), *B. pubescens* var. *czerepanovii* (mountain birch) and var. *appressa* (Kiilopää birch), respectively.

## Methods and Results

During the year of 2006/2007, samples were taken from fifteen trees, all *B. pubescens*, in Rovaniemi showing distinct symptoms to study verifiability of the virus during the seasons. To gain insight of the distribution of CLRV, 76 trees exhibiting characteristic symptoms (27 *B. pendula*, 33 *B. pubescens*, 6 *B. pubescens* var. *czerepanovii*, 5 *B. pubescens* var. *appressa* and 4 *B. nana*) have been sampled randomly all over Finland, including four trees from a 100-year-old birch stand used for seed collection.

Young leaves, buds and catkins of diseased shoots were tested by a CLRV specific IC-RT-PCR. The virus was found in all trees of the Rovaniemi samples. Virus detection was reliable during the vegetation period between May and September, while CLRV was not detectable in winter.

Sampled trees from southern as well as the boreal area of central and northern Finland revealed numerous CLRV infections, including *B. pubescens* and *B. pendula* saplings originating from the 100-year-old birch stand. Amplified CLRV fragments from certain downy and silver birch trees were confirmed by sequencing. Altogether 57% of tested trees turned out to be infected, hereof 58% of *B. pubescens* and 52% of *B. pendula*. Furthermore, CLRV was detected in several mountain and singular dwarf birches. This is the first time that these species have been confirmed to be hosts for this virus. Nor has CLRV been recorded earlier in northern Finland or on *B. pubescens* in entire country.

## Conclusions

Referring to the rate at which symptoms have spread within the last few years, CLRV might become a serious problem in northern birch forest ecosystems and for the local forest industry, due to reduced tree vitality and wood quality. Therefore, rapid efforts are needed to conduct detailed studies on distribution, ways of spreading as well as biological and economic importance of CLRV among birch species and possible other hosts.

It has yet to be revealed, why CLRV is spreading quickly and widely in Finland and whether increasing summer temperatures may be responsible for a higher susceptibility to CLRV. The means of viral spread is of special interest because CLRV has emerged in a few years time in a large geographical area, occurs in several birch species and on very distinct site types far from each other.

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## Fluxes of carbon in italian orchards

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To be ecologically sustainable, productive tree ecosystems should be managed in order to enhance their potential to fix atmospheric carbon (C). We report data from a coordinated national project where C fluxes were determined using similar methods in 2005 and 2006. The multisite study included mature plantings of wine grape and apple in Northern Italy, olive in Central Italy, and peach and orange in Southern Italy. The orchard locations differed for rainfall (from 450 to 833 mm) and average yearly temperature (from 12 to 17 °C). Objectives included the quantification of the total (NPP) or above ground net primary productivity (ANPP) and its allocation, of the total soil ( $R_s$ ) and root respiration ( $R_a$ ) and of the net ecosystem productivity (NEP). ANPP was estimated by measuring the biomass of abscised leaves, harvested fruits, shoot lengths and estimating tree framework biomass increase using allometric relationships. Carbon concentration of subsamples were determined (from 42 % to 51 % DW) and used to convert biomass data into amounts of carbon. ANPP of trees varied from 4.2 to 5.1 t C/(ha\*year). Grassed alleys contributed to ANPP by an additional average value of 1.6 t C/(ha\*year). Total tree NPP was estimated in the apple orchard only, by coupling ANPP data to measures of CO<sub>2</sub> exchange of the whole canopy using a custom-built open system mounted for periods of around one week per each month. Data indicate that only 16 % of NPP was allocated below ground. In general, fruit represented the most important sink for carbon. Soil respiration was measured at monthly intervals on days representative of the period on a number of soil positions in the apple, olive and citrus orchards. Cumulative yearly  $R_s$  fluxes varied from 0.5 to 1 kg C m<sup>-2</sup>. Soil respiration depended on soil temperature only when soil moisture was above a threshold level and was highest in the olive orchard soil that was maintained free from weeds by tillage.  $R_a$  was also estimated by measuring respiration flux also in plots where the presence of tree roots was avoided (by trenching) and average about 33 % of  $R_s$ . NEP in the vineyard, measured by net ecosystem CO<sub>2</sub> exchanges with eddy covariance technique, was equal to 8.2 t C/ha; NEP in the apple orchard, estimated by subtracting heterotrophic soil respiration to NPP, amounted to 6.8 t C/ha. Overall, data indicate that significant fluxes of C occurs in italian orchards and that they represent a net sink for atmospheric C.

## Ozone impacts on lettuce growth and photosynthesis

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Tropospheric ozone is a common gaseous pollutant that affects large areas of the earth's land mass. Lettuce (*Lactuca sativa* L.) is a crop generally classified as sensitive to ozone and of particular significance in Europe.

*Lactuca sativa* L. cvs. Paris Island and Grenada were exposed in controlled environment chambers to either charcoal/Purafil □-filtered air (CFA) or CFA plus ozone (75 nmol mol<sup>-1</sup> or 100 nmol mol<sup>-1</sup> between 8:00 and 16:00 h). 'Sensitivity' to ozone was gauged in terms of visible foliar injury and effects on relative growth, biomass, stomatal conductance and the capacity for carbon assimilation.

Plants exposed to ozone exhibited reduced growth rates and decreased biomass accumulation after 24 and 40 d. Both genotypes were sensitive to ozone in terms of effects on shoot dry weight; environmentally-relevant ozone exposure resulting in a 25% reduction shoot weight. In contrast to generally-observed patterns of change in dry matter partitioning driven by ozone, no significant change in root: shoot investment was observed in the present study.

Diagnostic gas exchange measurements revealed an O<sub>3</sub>-induced decline in the light- and CO<sub>2</sub>-saturated rate of CO<sub>2</sub> assimilation (A<sub>max</sub>). This was accompanied by a parallel reduction in Rubisco activity (V<sub>cmax</sub>), and the maximum capacity for RuBP regeneration (J<sub>max</sub>). No changes were observed in the relative stomatal limitation (RSL) of CO<sub>2</sub> assimilation in ozone-treated plants. Furthermore, no significant change was found in the quantum yield of CO<sub>2</sub> assimilation, suggesting effects on the capacity for CO<sub>2</sub> assimilation were attributable to shifts in carboxylation efficiency rather than maximal PSII photochemical efficiency or PSII-mediated electron flow.

## Dynamics of UV-B radiation mediated changes in secondary plant metabolism of vegetables

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The increasing exposures of plants to UV-B radiation by air pollution-induced ozone depletion causes stress protective mechanisms in plants. This protective stress response triggers distinct changes in the secondary metabolism of fruit and vegetables resulting in an accumulation of phytochemicals, e.g. phenols, carotenoids, glucosinolates and hydrocolloids. This accumulation of different phytochemicals is a desired item in terms of their health-promoting effects on humans. Members of the Brassicaceae family such as *Brassica juncea* L., *Tropaeolum majus* L. (nasturtium) and *Brassica* sprouts are of special interest because these plants are a rich source of phytochemicals which have been positively linked to several health-promoting properties. The aim of our investigations was to study the effect of UV-B radiation on secondary plant metabolism, i.e. on the subsequent phytochemical content and composition in order to contribute to a better understanding of environmental stress mediated dynamics in health-promoting plant compounds.

A short-term UV-B application was conducted on different *Brassica* spp. (*B. juncea*, *Tropaeolum majus*, *Brassica* sprouts) at a fully developed stage. Plants were subjected to UV-B radiation (UV dosage: 8.2 J m<sup>-2</sup>) for 60, 90 or 120 min using an UV-B fluorescence light source (FL 20SE, 305-310 nm). After an adaptation time of 2 and 22 h the phytochemical composition, i.e. phenols, carotenoids, glucosinolates and pectins were determined.

It was found that UV-B had a pronounced effect on the synthesis of the health promoting substances studied; however, plant reaction differed depending on the morphological compartment, duration of UV exposure and adaptation time. These results will be also discussed in terms of a targeted stress application in pre- and postharvest for increasing bioactive health promoting substances in horticultural food products.

# **Hailnet composition and structure affects light transmission, light spectrum, photosynthesis, vegetative growth and colouration plus non-destructive measurements**

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The incidence of hailstorms during the vegetation period is on the increase, possibly due to climate change. Hence, fruit crops are increasingly grown under hailnets, which adversely affect vegetative and reproductive growth primarily due to light deprivation. Recently, coloured, “photo-selective” hailnets came onto the European market, which allegedly improve photosynthesis and yield. The objective of this joint project was to examine these coloured hailnets and study their plant physiology effects at Klein-Altendorf nr Bonn, Germany.

White, black, red and green hailnets comprised double twisted longitudinal (parallel to the tree rows) and single transverse high density polyethylene (HDP) fibres of 288 µm to 356 µm diameter, irrespective of hailnet colour.

Black and green-black hail nets contained double black longitudinal fibres. White-translucent, grey or red hail nets contained double longitudinal translucent or red fibres.

Visible or photosynthetically active radiation (PAR; 400-700 nm) was reduced by white (by 5.7%), red-white (12.8 %), green (13.6 %) or red-black (16.8%) hail nets 50 cm underneath the net. Double black, thick fibres in the green-black hail net may cause their lower UV and visible light transmission relative to white hail nets with their translucent fibres and larger mesh size. The use of reflective cloth on the grass alleys could overcome the light losses. Overall, the apparent visual colour of a hail net was not indicative of its light transmission.

Spectral analysis showed that coloured hail nets transmitted more NIR than PAR/visible light with a UV peak at 375 nm. Light transmission increased by 3% above 500 nm (green) in green and by 2-5% above 570 nm (orange-red) in red hail nets, affecting neither the red (666 nm) : far red (730 nm) (R:FR) ratio nor the phytochrome system.

The mesh size, i.e. the distance in between the fibres, varied from translucent (white) hail nets with the largest mesh size of 3 x 9 mm, followed by 3.9 x 6.9 with green-white, 3.3 x 7.7 mm with red-white, 3.5 x 6.5 with green-black nets, 2.8 x 6.9 mm with grey as well as red-black and black hail nets both with the smallest mesh of 2.5-3 x 6.5 mm; these large variations in mesh size between hail nets predominantly influenced their light transmission, which was also affected by the proportion of translucent or black fibres in a hail net.

A simple test is proposed to estimate the geometric light transmission without a magnifying glass based on measuring mesh size with a ruler and correcting for fibre strength and proportion of translucent or black fibres.

Fruit colouration of the poorly coloured apple cv. ‘Pinova’ followed this geometric light transmission, while that of the late-ripening, well-coloured cv. ‘Fuji Kiku 8’ was sufficient and unaffected by hail net colour; fruit yields of the young apple tress were unaffected by net colour.

Black hail nets appear suitable for single-coloured green, or bi-coloured apple varieties with good colouration, or those otherwise susceptible to sunburn in Southern Europe. Crystal hail nets (with their translucent fibres, widest mesh size and largest light transmission), or grey hail nets (with twin translucent longitudinal, single black 0.32 mm transverse fibres and 2.5-3.5 mm x 6-8 mm mesh) appear suitable for apple crops in NW Europe with sunlight deficiency and without risk of sunburn. Red-white nets appear unsuitable due to the greater shading than the translucent fibres and for landscape reasons; their alleged photo-selective effects as reported from Southern countries are interpreted to be due to reversal of photo-inhibition under high light intensities and heat in these regions.

Labelling of hail nets with tear and Langley values for UV durability is also suggested.

Blanke, M.M, 2007: Farbige Hagelnetze: Ihre Netzstruktur sowie Licht- und UV-Durchlässigkeit bestimmen die Ausfärbung der Apfelfrüchte (with English summary and sub-titles). Erwerbs-Obstbau 49 (4), 00-00 (in print).

# Grafting enhances salt tolerance of melon plants

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## Introduction

The decreasing availability of fresh water has become a problem in arid and semi-arid regions, so the use of secondary quality water (marginal water), such as treated sewage effluent and saline water, for irrigation is increasing. Marginal water often contains relatively high concentrations of saline elements, such as sodium (Na) and boron (B). Long-term irrigation with this water could increase the Na and B concentrations in the soil, and may limit the sustainability of agriculture. Grafting vegetables, including cucurbits, is a common practice in many countries. As with other vegetables, the main purpose of employing this technology in melons is to control soil-borne diseases. In addition to disease resistance, grafted plants may have improved tolerance to environmental stresses such as high soil salinity and low soil temperatures. Therefore, the objective of the present study was to determine the effects of effluent salinity and boron on Na and B uptake, growth and yield of grafted and non-grafted melon, *Cucumis melo* L., plants.

## Materials and Methods

Non-grafted melon plants (cv. Arava) and melon plants grafted onto the commercial *Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne rootstock 'TZ-148' were grown in the field and in pots in a heated greenhouse and were irrigated with fresh water, effluent, or saline water containing various Na and B concentrations. Na concentration was determined by a flame spectrophotometer and boron concentration was determined by an inductively coupled plasma emission spectroscope. In order to determine the differences in the selectivity of the root systems to boron absorption, seedlings of the melon and pumpkin were grown in pots in the greenhouse and irrigated with fresh water containing various boron concentrations. Thirty days after planting and immediately after irrigation, the stems of the plants were cut 3 cm above the surface of the growth medium and the xylem sap exudates from the cut stem were collected.

## Results and Discussion

Foliar injury caused by boron was more severe in the non-grafted than in the grafted plants. Increased sodium and boron concentrations in the irrigation water resulted in increased Na and B concentrations in the plant tissue. The concentrations of all the saline elements (Ca, Na, and Cl) in the leaf, stem, and fruit tissues were higher in the non-grafted than in the grafted plants. The greatest difference between the non-grafted and the grafted plants was in the Na concentration, which was one order of magnitude lower in the grafted plant tissues than in the non-grafted ones. The lower Na and B concentrations in grafted plants is possibly due to the higher selectivity and lower Na and B absorption by the pumpkin root system. Indeed, the xylem sap exudates from non-grafted plants contained more Na and B than grafted plants. Growth and yield of non-grafted plants were more sensitive than grafted plants to high Na and B levels. These findings for lower Na and B concentrations in the greenhouse experiment are consistent with those for the plant tissues in the field experiment, and underscore the high selectivity of the pumpkin roots against Na and B absorption.

## Conclusions

It can be concluded that grafted melons irrigated with saline and effluent tolerate excess Na and B. These may enable the expansion of marginal water use.

## Possibilities for unpolluted crop production in heavy metal contaminated areas

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Plants are very good indicators for the presence of some pollutants in soil and its physical condition. Some of them can be entitled as hyper-accumulators for some heavy metals, without showing any change in the appearance. In general, plants that are produced on contaminated soils have positive correlation of heavy metals concentration found in their tissues and the same in the soils were they grow.

The objective of this study is to evaluate the effect of AGAT 25K (inactivated bacteria *Pseudomonas aureofaciens* used as biostimulator) on yield and heavy metals contamination level on some vegetables produced in the region of Veles, which is highly contaminated area in Republic of Macedonia. The initial examination on this biostimulator gives promising results with some vegetable crops. Application of AGAT 25K resulted in retarded uptake of heavy metals from the soil and improved the yield, as well. As a control, the same vegetables varieties were grown in uncontaminated area and the experimental crop field was divided in two parts: treated and untreated crops. The results are presented and discussed in this study.

## The Influence of Climatic Changes on Quality of Wines Obtained From The Romanian Varieties of Grapes

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The global heating phenomenon has an influence upon the Viticulture and Vinification. The beginning of our century is for sure warmer than the beginning of last century and this climatic heating produced changes in the quality of wines obtained in different viticol areas.

In Romania, the climate heating changed the quality of wines obtained from the Romanian varieties of grapes and their geographical spreading. So, one of the well known Romanian variety of grapes used for the quality of white wines, named White Feteasca, restricted its growing area comparing with the last century. During the last years this variety is less and less cultivated in the Southern vineyards of the country, where the annual medium temperature is over 11<sup>0</sup> C. In these conditions, in certain years the wines obtained from the variety White Feteasca has a low acidity, without freshness and harmony. Cultivated in the Moldova and Transylvania vineyards, when the climate is coldly, this variety given us in each years wines with a good quality, with a lot of personality, freshness, with a fine aromas, specific, very appreciated by the authorized tasters. In the warmly Southern vineyards of the country, these qualities of the wines can not be put in evidence in all the years.

The heating phenomenon have had also influence on the changing of the white wines quality and composition of the varieties: Zghihara de Husi, Plavaie and especially Galbena de Odobesti. The alcoholic degree of these white wines had increased with

1 % vol., comparatively with the white wines obtained with 50 years ago, because the sugars content in grapes is higher.

Also for the black grapes varieties used for obtaining red wines, the climatic changes were favorable. The most well know variety named Feteasca Neagra extended its growing area in the vineyards of the Sub-

Carpathians hills and in the Southern part of Moldavia. The Feteasca Neagra wines are very colored, harmonious and fullness. From the quality point of view generally speaking the Feteasca Neagra, is higher than Merlot or even Cabernet Sauvignon wines. Therefore this is the most wanted variety for cultivate in the red wine vineyards from Romania.

The climatic change influence on the Romanian viticulture is revealed in the oldest Romanian vineyards, over 2000 years- Dragasani vineyard. This is situated in the Southern of the Carpathians mountains, among the river Olt, with a length of 60 km, was famous for its white wines, agreeable, suppleness and aromatic. The varieties for the red wines, have entered in the vineyard only after the *Phylloxera* attack at the end of the XIX century, but in the last decades it's extended very much. Presently the grapes varieties for the white wines are cultivated in the third Northern part of the vineyards closely to the Carpathians mountains, the Southern part is used mostly for obtaining red wines especially local creating –Novac and Negru de Dragasani. These changes regarding the types of wines obtained in the Dragasani vineyard and their quality are the consequences of the climatic changes, which had encouraged the varieties for the red wines.

Also in the Dragasani vineyard the climat heating determined qualitative changes at the famous variety from the vineyard- Tamaioasa Romaneasca. Lot of time, from this variety were obtained white, aromatic and lightly wines. In the last decades, from this variety are obtained natural and sweet white wines, very appreciated and wanted.

## **Plant damages in zoo exhibits**

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Plants are used in zoological gardens for the landscape of the visitor-areas as well as the animal-enclosures. Indoor-plants, ornament-plants, trees and grasses are put in differently situations to landscape-design, around animal-enclosures and to shape natural situations and to increase the visitor-attractiveness of a zoo through an individual landscape. Especially in the enclosures plants are damaged by the individual location-situation many times as examinations show at the example of the Berlin Zoological Garden.

Indoor plants, conventionally evergreen ornament-plants become in the pot, in the field or as cut-green decoratively started. Many plants suffer from inadequate light - and temperature-conditions and are damaged mechanically by birds and by contaminated phytotoxic excrements. Many plants therefore show chlorosis and necrosis as well as mechanical leaf-damages. Pathogens occur latently, can be controlled by the use of antagonists in the framework of the horticultural care in their population-development however. Nevertheless, plants must be replaced frequently, about an aesthetically appealing enclosure-formation the visitor, to present. The growth-conditions are only caused in big free-hangars to optimize.

In the filed there are usually bigger animals and these are held in bigger trimming-densities in addition. Many animals nourish themselves vegetarian, so that - provided efficient precautions don't prevent this - plants in enclosures of the animals eating becomes. In addition, they damage plants at the trunk and at the roots during their body-care, through their playfulness and with the search after food. To it, ground-compressions and destructions of the ground-vegetation are caused by their movement-activities in dependence on weight and foot-construction. The ground-burdens through the entry of urine or excrement are however rather low.

With the future landscape of animal-enclosures, an optimized construction of the enclosures can therefore take place to the protection of the growth-conditions of the plants. Therefore, subterranean construction ground-compressions and efficient precautions must prevent damages at trunk and branches through the animals. An optimized location of plants in the enclosure must the impairments through excrement as well prevents the growth of the shaped vegetation like the technical optimization of the interior-climate and the individual composition of the ground-substratum secures. Only on this way, the natural formation of artifi-

cial habitats is realizable under local realities. Operators of zoological gardens require these compartment-information to the economic protection their usually extravagance landscape.

The F+E-project is promoted by EFRE

## **Reduced water use in the management of containerised bedding plants: effects on ornamental properties, growth and physiology - Petunia case study**

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Bedding plants are popular landscape features in public parks and private gardens, as well as in containers and hanging baskets in town centres. Most popular bedding species such as *Petunia x hybrida* or *Impatiens walleriana* are, however, identified as ‘water-demanding’. With decreasing summer rainfall and increasing temperature, there is pressure on both amateur and professional gardeners to minimise water input. Consequently, bedding plants are often replaced in the landscape with apparently less-water demanding species. In addition to affecting the market for bedding plant nurseries, these alternative species rely on the foliage for impact rather than flowers, and are often generally less popular with the public.

Using *Petunia x hybrida* cv. Hurrah white grown in peat-based compost in containers we set out to: 1. determine to what extent reducing the amount of applied water after transplanting into containers affected plants’ physiology and aesthetic quality in terms of flowering and growth; 2. examine if there was any difference in quality of the plants when they were watered on an *ad hoc* basis rather than daily at regular intervals and 3. examine if there was any difference in plant quality if the plants were watered in the morning or evening.

There was a proportional decrease in leaf stomatal conductance and stem xylem water potential as soil moisture content decreased, but there was little measurable difference in the appearance of plants (height, flower longevity and number) Even when plants were watered to maintain 25% of container capacity, quality was generally good, although flower size was fractionally smaller than other treatments. *Ad hoc* watering with the total watering amounts comparable to 25% container capacity decreased flower size and number, but not flower longevity. Employing regulated deficit irrigation based on transpirational water loss (as opposed to set automatic watering) lent further opportunities for water saving, without compromising the plants’ ornamental properties. Time of day when water was applied had little impact, but over-watering dramatically reduced plant quality.



## **Monitoring and evaluation of environmental factors incidence on biodiversity variability in wine- growing**

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Viticulture represents a traditional occupation with deep roots in the culture and civilisation of the Romanian people, with significant role in the economy and agriculture of Romania.

The present modern viticulture in Romania, characterised through a high specialisation of the viticulture and vine regional production is the result of the complex relationships established by different genetic resources with the climatic aptitudes of different viticultural areas, the adjustment of the biosystems graft x rootstock, the viticultural and oenological techniques.

In the present world context of alarming signals coming from international organisations (F.A.O., F.I.D.A., O.I.V.) concerning obvious climatic changes and their long-term forecast, on one hand, and significant losses of genetic resources, on the other, intensified and complex approaches of research studies are required in the following fields: establishing the mechanisms to adjust vegetal bodies to such climatic changes; identifying technical solutions (choosing the most resistant cultivars against unfavourable ecological factors).

In order to record and to evaluate in a complex way the ecological factors evolution, proportional to the biological and ecological requirements of the grapevine, we have taken into account the recording of some restrictive climate index, with great impact on the bio-productive and quality behavior of the grapevine. In this respect, there were processed and analyzed climatic data not only as general climatic indexes, but also as synthetic ones.

With a view to the impact of certain parameters of thermal stress during the vegetative rest and also in order to slate precisely the biological potential of adjustment and endurance of the grape vine to the action, we've accomplished a series research to: the evaluation of the ecological resources in proportion to ecological and biological needs of the grape vine; to quantize and establish the temporal sequence of the occurrence of critical temperature; the adjustment reactions and response of the grape vine to different thermal conditions and establishes technological management for diminishing the effects generated by stress thermic.

The components of the viticulture biocenosis, microorganisms and especially the yeasts with spontaneous microflora, are not stationary elements of the regional viticulture ecosystem; they present o great dynamicity in the interchange with the biotope, this dynamics have been amplified also by the fact that the yeasts join with its main source of nutrition (grapes). In order to establish which are these dynamics and changes within the viticulture ecosystem, the results obtained in this sense are referring to: the influence of the viticulture biotope and of the viticulture biocenosis over the microorganisms implied in the fermentation processes of the must of grapes; the frequency quantification of the species distributions, within the yeast conveyer, specific to the studied regional ecosystem and the identification of the factors that control these distributions.

Along the time, the climatic changes within the Romanian viticulture agro-ecosystem have determined the homogeneity perturbation of the biocenosis conditions. Thus it have been disturbed the normal proceed of the biological, physiological and biochemical processes that take place within the plant, with repercussions on the evolution and dynamics of the biological diversity ( the emergence or disappearance of some local – traditional varieties), on the productivity, on the quality and specificity of the wine and viticulture products.

In conclusion, biodiversity represents a very important source and an alternative for adjusting Romanian cultivated grapevine varieties to disturbing factors (abiotic and biotic factors) within the viticulture ecosystem and a must for making a durable viticulture, therefore the scientific research studies on its evaluation, preservation and use are widely known at national scale.

# The influence of the varied hydric regime on the bioproductive parameters of Grapevine

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## *Introduction*

Grapevine cultivation on certain area with superior productive results requires the continuous appreciation of the ecological favourableness of the space used for this purpose, as well as the identification and application of the most appropriate counter measures against variable factors within the system. Given the fact that counterattack possibilities against climatic changes and against the effect of hydric deficient periods are limited, in order to reduce the major negative result on viticultural production, research studies monitoring grapevine reactions to water deficit are required, with view to finding methods to stimulate the physiological and biochemical mechanism increasing the tolerance of plants to water deficit.

## *Material and Methods*

In accordance with the research topic proposed, the observations and determinations were focused on: monitoring climatic factors (mostly hydric supply) for the evaluation of the favourableness of the study years (by using individual, binary and urinary climatic indicators); studying the influence of the varied hydric supply regime characteristic to the study years on bioproductive parameters. The effect of varied humidity conditions on the studied varieties was evaluated through the analysis of the indicators: biological (leaf and shoot growing, bud fertility, grape growing); physiological (the intensity of transpiration and photosynthesis, stomata conductance, sapflow velocity) biochemical (change of the content of sugar, organic acids, anthocyanins). In order to reach the targets, experiences was focused on the influence of the varied hydric regime on bioproductive parameters and determinations were made for Feteasca alba, Riesling italian, Sauvignon, Fetească neagră, Cabernet Sauvignon and Merlot varieties, during period 1997-2007.

## *Results and discussion*

During the years characterised by hydric deficit regime (2000, 2002 and 2003) was determined strongly and distinguishably significant reductions of the leaf surface; more severe reductions of the foliar surface were recorded in case of Riesling italian, Cabernet Sauvignon and Merlot varieties, fact that may indicate their higher sensitivity to water suboptimal conditions. The suboptimal hydric regime determined a significant fertility reduction reflected in the low values of the relative fertility coefficient recorded, namely 0.67 in Riesling Italian, 0.94 in Sauvignon, 0.48 in Fetească albă, 0.42 in Fetească neagră; 0.65 in Merlot and 1.2 in Cabernet Sauvignon; As result of the varied conditions of hydric supply, the weight of grapes during harvesting time showed higher variations in case of Riesling italian (44.8 %), Cabernet Sauvignon (34.29%) and Merlot (31.8%)

The regressions between the transpiration intensity and the stomatal conductance shows a good correlation between the two factors to the limit of approximately 34 °C, up to which, besides the reduction of the stomatal conductance, a proportional drop of the photosynthesis intensity also takes place. Between 34 - 38 °C, the conductance drops more than transpiration, which maintains itself at higher levels. For temperatures between 38 to 41°C, the variation of the two factors under study goes in tandem, big differences being noticed between determinations (significant ups and downs). The moderate hydric deficit has positive effects on the quality of red wine grapes, as it determines the accumulation of a large quantity of anthocyanins; under conditions of high water deficit, characteristic to the year 2000, the content of anthocyanins dropped, as result of the inhibition of the biosynthesis process due to conditions of suboptimal humidity.

## *Conclusions*

Every variety interacted with the environment conditions, expressing their productive potential in a specific way. The diversity of the hydric supply water regime during the research period was found in the variations of the biochemical and productive indexes studied.

# Influence of irrigation on nut traits, production and photosynthetic activity of hazelnut cultivar 'Tonda di Giffoni'

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## Introduction

Hazelnuts are important dry fruits in the world and the most important producers are Turkey, Italy, Spain and USA. This species is considered sensitive to water stress (Girona et al., 1994). Irrigation is then an appropriate technique to improve growth and productivity, principally in young orchards and when the plants are cultivated in areas with total annual rainfall not exceeding 700 mm and with a dry period in July and August. These conditions are common in the south of Italy, like in Campania region, that is the first producer area of hazelnut in the Country. Drip irrigation based on water balance can help to obtain a good ratio between production and vegetative growth, as previously observed on cv. Tonda Gentile Romana and for some Spanish cultivars grown in Catalonia (Bignami et al., 2005; Girona et al., 1994). Furthermore, it can offer the opportunity to optimise water use and to reduce the cost of irrigation.

## Materials and Methods

The present trial, supported by the project FRU.MED. MIPAAF (D.M. 212/7303/05), has been carried out in 2006 in Caserta province on five-year old plants of the local cv. Tonda di Giffoni trained to free vase and spaced 5 x 5m. Four treatments were applied: an un-irrigated control and three different water levels, corresponding respectively to the restitution of 50, 75, 100 % ETM by means of a drip irrigation system. The treatments were arranged in a complete randomized block design, with two replications, and 4 plants per each plot were used for the experimental observations and measurements. ETM was calculated using the FAO method performed by Blaney and Criddle. Yield per plant, nut traits and defected nuts, oil content in the kernel and leaf assimilation rates were measured. Experimental data were analyzed by means of the analysis of variance, and the treatments were compared by L.S.D. ( $p=0.05$ ).

## Results and Discussion

The plant yield increased at the highest irrigation volumes. Nut fall at ripening was more concentrated in the un-irrigated control. Nut and kernel weight and size were greater in irrigated than in control plants, with the highest values observed in 100 % ETM treatment. The percentage of empty and defected nuts was higher in condition of limited water availability. The oil content in the kernel of un-irrigated control and 50 % ETM restitution was lightly higher than in the other treatments. Leaf assimilation rates during the summer showed low differences among treatments, with more uniform values during the day at 100 % ETM.

The results obtained in our study confirm that drip irrigation system improves vegetative growth, productivity and technological traits of the nuts in comparison with un-irrigated control, in accordance with previous studies (Bignami et al., 1997). In conclusion, water supply of 75 % and 100 % ETM could be valid irrigation levels for young hazelnut cv. Tonda di Giffoni in areas characterized by insufficient rainfalls during the year.

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## Isotopic evidences and constrains in atmosphere-soil-plant system

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In the continuum Atmosphere-Soil-Plant (ASP) the water presents variable isotopic composition. The precipitations, as the main water input in plants via soil, present spatio-temporal natural variations of the isotopic content of oxygen and hydrogen, which are correlated strong with the air mean temperature at the ground level and humidity. These variations find again in water of plants, via soil.

This paper is focused on the determination of the deuterium isotopic signature of the leaves water for plants with short lifespan, particularly on lettuce and tomatoes, growing in greenhouse, either directly in the soil or in containers without drainage and evaporation from soil, and irrigated in continuous and pulse chase techniques with water having three controlled deuterium contents corresponding to the means and extreme values of natural variations in precipitations.

For the water extracted from leaves of the tomatoes (*Lycopersicon esculentum* Mill.) and the lettuce (*Lactuca sativa* LL) grown in soil within greenhouse the deuterium content have an increase trend during plants growing in time and in suite of samples irrigation water- rachis- leaflets for tomato and irrigation water - entire inner leaves – entire outer leaves for lettuce the deuterium content is increasing.

For lettuce grown in containers and irrigated with water of constant isotopic concentration, the mesophyll water of outer leaves was highly enriched in deuterium compared with than its mid-vein water and irrigation water, but was not significantly different of the value of inner leaves water. The ratios of the concentration of the *chlorophyll a* to *chlorophyll b* modified for the different deuterium concentration of the irrigation water.

A pulse chase technique has been used to determine the response time of the plants at the isotopic signal from the soil water, which is greater than we expected for a short lifespan plant. The alternation of the isotopic concentration revealed the modification of the ratio of *chlorophyll a* to *chlorophyll b*. All results lead to hypothesis that deuterium content in the plants leaves play an important role no only in the evapotranspiration but also in photosynthetic process. The plants adaptation to high or low isotopic ratios made by adjusting the ratio *Chl a*: *Chl b*.

The isotopic composition in the water from plants is variable due to the isotopic input of precipitation, the isotopic fractionation during the transport and interface processes of system, and the plants ability to use the hydrogen in photosynthesis, respiration and biosynthesis processes.

## Study of climatical variability influence on several quality parameters of black grapes varieties for high quality wines

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Being a traditional occupation in our country, Romanian viticulture has already established the most favourable areas in order to obtain high quality wines characterized by good organoleptical and compositional parameters ready to satisfy even the most exquisite taste.

The high specialization of every great viticultural region of Romania for different grape varieties and wines is due to a right and complex appreciation of numerous factors like: climatical conditions (long time period

observations), pedological factors, viticultural techniques etc, and most of all, the adaptability of every grape variety to environmental conditions of different areas.

In order to reach the objectives, we developed our experiment in a well- known viticultural center from southern part of Romania, region with excellent climatical conditions collocated with edaphic favorability, where – during time- awarded high quality red and white wines were obtained. The quality of wines appreciated in our country and also abroad since the beginning of XX century attests the high favorable degree for vine growing in this area.

The grapes variety, the soil and the viticultural techniques are considered constant factors, the differences year to year between grapes quality parameters being caused by climatical conditions typical for every viticultural studied year.

The researches had been developed during 2005 and 2006 viticultural years and concerned the climatical variability and the biological response of grapevine to this factor. We studied grape varieties that are well known all over the world and cultivated in this center for almost a century: Cabernet Sauvignon and Merlot.

The analysis of climatical availability of studied viticultural center was made using data from Hydrological and Meteorological National Institute.

During those two viticultural years we studied climatical data and compared with 50 years media values calculated for this center.

Using climatical indicators (individual, binary and trinary) we have monitorised the climatic conditions: their duration, intensity and frequency, especially during ripeness and maturation period. Our determination and observation focused also on evaluation of biochemical and productive indexes of grapes.

The global climatical changes and, especially, the untypical phenomena for an area or for a time of the year are not in concordance with biological needs of grapevine. So, we try to establish the influence degree that climatical factors have over the accumulation of main chemical compounds of grape berry that are important for obtaining high quality wines. Also for both viticultural years, the amounts of precipitations – during ripeness and maturation - were higher than media values, the temperatures recorded for these periods of time make the difference.

We followed the evolution of main quality grapes parameters of Cabernet Sauvignon and Merlot varieties: glucides content, acidity, anthocyanins and the chromatic structure of total anthocyanins.

The variation of climatical conditions determined the changes of quality and productive parameters of grapes.

Untypical viticultural year can determined not only quantitative losses of crop, but also the quality is affected by decreases of sugar content and anthocyanins values and increases of total acidity, elements that define the tehnological potential of grape varieties and the possibility of obtaining high quality wines.

## **Evaluating drought adaptation in olive trees grown under different irrigation regimes through high resolution stem growth analysis**

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The long-term effect of different irrigation regimes on several water status indicators was studied in order to assess water use efficiency and drought adaptive strategy in *Olea europea* cv. Nocellara del Belice.

The study was conducted on sixteen-years-old grafted plants, grown under different irrigation regimes for fourteen years in an experimental site plantation in southern Italy. Irrigation regimes were: a non-irrigated control (T0) and three treatments that received seasonal water amount equivalent to 33 and 66% of  $ET_C$  from the beginning of pit hardening to early fruit veraison (respectively T33 and T66), and 100% of  $ET_C$  throughout the irrigation season (T100).

During 2006, plants were continuously monitored by automatic point dendrometers measuring stem radius variation and whole-plant water use was determined using a xylem sap flow method (compensation heat-pulse technique). Additional ecophysiological parameters, such as stomatal conductance and water potentials were periodically measured, as well as vegetative development. There was a considerable degree of agreement between daily transpiration deduced from heat-pulse velocity and that determined by calibration using the water balance technique. Stem increment showed wide temporal fluctuations throughout the season without significant differences between treatments, probably stimulated by precipitation events, and dropped in early fall. Stem radial increment and sap flux of T0 plants did not differ consistently from irrigated ones. However, plants growing under rainfed conditions showed a little increase in maximum daily shrinkage during drought periods compared to irrigated ones. Significant seasonal indentations were also observed for rate of  $\Delta R$  and MDS, following climatic instability, and peaks were relatively shallower in T0 than T66 and T100 trees. By contrast, no marked differences were recorded between irrigated treatments. Whole plant water use was comparable between treatment antipodes (T0 and T100), while water relations (particularly from midsummer and thereafter) and vegetative development were a function of water available to plants. The limitation to water use in olive trees lies in the conductance pathway from soil to leaves to air, with contributions of canopy or meteorological factors and soil water availability.

These results support the idea that rainfed trees showed more conservative water use strategies than irrigated trees, and that deficit-irrigated trees acclimate somewhat functionally and structurally to partial watering. On the basis of these results, we can argue that both sap flow rates and stem radius fluctuations represent valuable parameters to automatically assess long-term adaptive strategy to irrigation regimes in olive trees.

## **The estimation of winter hardiness of peach trees on Pumiselect rootstock in the conditions of Poland**

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### ***Introduction***

The small production of peaches in Poland results from the lack of optimal climatic conditions for the cultivation of this species. The major factor limiting the cultivation of peach trees is the minimal temperature in the winter and spring frost. In the winter, the stem of peach tree, its branches and shoots are destroyed at  $-25^{\circ}\text{C}$ , flower buds of peach are frozen at  $-20^{\circ}\text{C}$ . Another problem with the intensification of peach cultivation is too strong growth of the trees, due to the lack of the dwarf rootstock for this species. Peach trees are budded on seedling rootstock from *Persica vulgaris* var. *Mandshurica* and planted at wide planting distance. For that reason, in many countries all over the world, the experiments with the dwarf rootstock for peach trees are carried out. a lot of research is conducted with *Prunus bassey* and *Prunus Pumila*.

The rootstock Pumiselect is a clonal of the *Prunus pumila* rootstock for peach and apricot, selected by Prof. F. Jacob in Geisenheim Institute. The preliminary results suggest that Pumiselect belongs to dwarf or semi dwarf rootstocks. The vegetative growth of peach trees on this rootstock is 65% smaller than those budded on Nemequard, and apricot trees are 50% smaller than those on seedling rootstock. The peach and apricot trees on rootstock Pumiselect start bearing in the 2<sup>nd</sup>-3<sup>rd</sup> year after planting and have about 50% higher yield efficiency, than those budded on Nemaquard. Pumiselect rootstock propagates readily from hardwood and softwood cuttings, stool beds or tissue culture

The aim of the present study was to estimate the level of damage of vegetative and generative parts of peach trees depending on the rootstock and spacing of planting.

## **Material and methods**

The experiment was founded after the winters 2005/2006 and 2006/2007 on 'Redhaven' and 'Inka cvs. young peach trees, budded on Pumiselect and *Persica vulgaris* var. *Mandshurica*. The trees were planted at 4 x 3.0 m (seedling rootstock) and 4 x 1-1.5 m (Pumiselect). During the winter 2005/2006, the temperature fell to  $-25^{\circ}\text{C}$  and in the next winter, the temperature was  $-20^{\circ}\text{C}$ . In order to estimate the extent of frost damage, Hołubowicz's method was used. At the beginning of the vegetation one - year old shoots of peach trees were kept in room temperature, in water for 14 days. Then the grade of damage on the longitudinal section of shoots of each cultivar was described according to the five-grade scale, where 1 indicated 'no damage' and 5 'totally dead tissue'. In the early spring of 2006 on the longitudinal section of shoots of each cultivar the percentage of frozen shoots was counted on the bases of the difference between the total length of healthy shoots and the total length of frozen shoots. In the spring of 2007, 100 flowers from each tree were taken in order to estimate the level of flower buds damage.

## **Results**

The rootstock, age of planting material and spacing of planting did not have the influence on the sensitivity of peach shoots to frost. According to 1-5 scale, one - year - old shoots of peach showed the damage of frost on the level 3.4-4.1. However, one - year - shoots of peach on Pumiselect were frozen in 20.8 % whereas with peach shoots on seedling rootstock in 11.3 %. The peach trees on Pumiselect rootstock planted as one - year - old material were more destroyed by frost ('Inka' 22.0 %, 'Redhaven' 19.7 %) than those planted as two-year - old trees ('Inka' 10.5 %, 'Redhaven' 12.4 %).

After the winter 2006/2007, the damage of bud flowers was very high (92.1-100%) and was independent of the estimated factors. However, the tendency to higher resistance to frost was observed in the case of trees planted on rootstock Pumiselect as two-year old planting material.

## **Risk analysis of the establishment of the Western Flower Thrips (*Frankliniella occidentalis*) under outdoor conditions in Austria as a result of the climatic change**

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## **Introduction**

The Western Flower Thrips (WFT, *Frankliniella occidentalis*) originates from the southwest of North America. In the last 30 years it has spread worldwide in greenhouses, where it lives on ornamentals and vegetable plants. It is considered here as a key pest.

Published data of laboratory experiments at low constant temperatures show that its adults can survive at a temperature of  $+5^{\circ}\text{C}$  for only 26 days. Presumed that its survival at lower temperatures is even more critical, this factor alone would nearly exclude survival in outdoor conditions of Southern Europe, where its survival is documented. In this study it was assumed therefore that overwintering is successful also if the wintertime is interrupted by warmer periods where development of WFT is still possible. Such development at a low level could take place at temperatures of  $15^{\circ}\text{C}$  or somewhat lower: at this temperature which lies above the threshold of development, oviposition is well documented. No map of the present outdoor distribution of WFT in Europe was available, but numerous publications about damage on typical outdoor cultures such as wine or nectarines by WFT show that the most northerly outdoor distribution is situated in the Italian province of Emilia Romagna. At such a location the mean daily maximum temperatures during winter range between  $7^{\circ}$  and  $8^{\circ}\text{C}$ , and daily maximum temperatures higher than  $15^{\circ}\text{C}$  occur in intervals of 16,3 days.

These winter conditions are thought to be similar to the temperature conditions that allow overwintering of WFT. According to regional scenarios for the alpine regions, based on the results of the GCM ECHAM5 A1B and A2 runs, winter conditions that presently occur in the Emilia Romagna could prevail in the warmest regions of Austria at the middle of this century. Accordingly, escape of WFT from greenhouses and outdoor overwintering in Austria are assumed to be very likely after that time. In that case numerous ornamentals, vegetables, fruit crops and wine would be damaged by this new key pest.

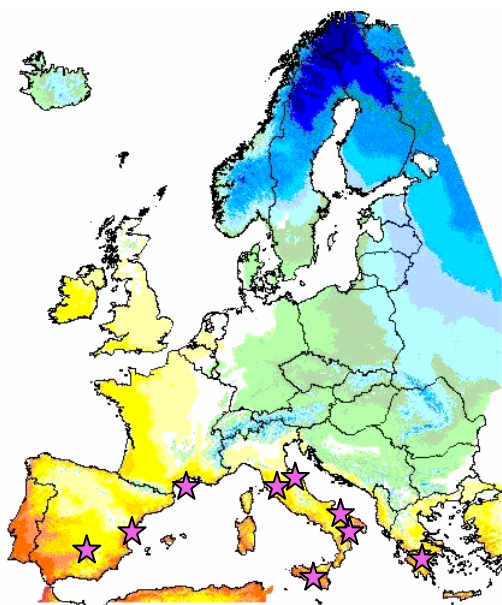


Figure: In southern countries Western Flower Thrips (*Frankliniella occidentalis*) is able to damage outdoor crops like nectarines and wine. It is presumed, that at such locations (marked by stars) this pest overwinters under outdoor conditions. The background shows mean monthly temperatures of January (IIASA 2006).

## **An assembled project to evaluate past and future impacts of global warming on agronomic traits in fruit tree crops, exploiting a phenological database and optimizing an experimental network**

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A relatively strong increase in air temperature has been observed in France since the end of the 1980s regardless of the fruit tree cropping areas. As phenology is mainly influenced by temperature, changes in flowering phenology timing could have impacts on fruit production, because of the indirect influences of flowering timing on spring frost risk, pollination success and fruit set efficiency. In France, long-term phenological series obtained from a national database devoted to fruit species and wine (called 'PhénoClim') have revealed clear biological responses to global warming since the end of the 1980s. Surprisingly, quite similar trends toward an advance in flowering time have been observed in the main deciduous fruit species (apple, pear, apricot, cherry, plum) as well as in olive tree. At present, the flowering advance is of no great matter at the level of global fruit production, whereas some regional negative impacts have been recently observed (in 2001 and 2007) such as discordant and lengthy flowering times (negative impact on pollination). Also, changes in growth fruit timing could modify varietal orders in maturity time and disturb the economic organization based on specific varietal panels by cropping region.

With these observations and impacts in mind, a network of partners (Inra, Ctifl and climatology Associations) assembled experience, data and experimental tools to constitute a national project supported by the French Agency for Rural and Agriculture Development. The aim was to inform the profession by a dedi-



cated internet site (called 'arviclim') providing data and advices related to the biological effects and agromomic impacts of global warming. Practically, the project was composed of three interactive tasks including fruit trees and wine: (i) exploitation and incrementation of the 'PhénoClim' database, harmonization of phenological observations (appropriate traits, cultivars and locations), optimization of partner experimental tools (orchard network, climatic rooms), agromomic impacts (spring frost, bud drop,...), (ii) modelling and forecasting using climatic scenarios, (iii) diffusion and advices (website). Owing to the large scope of the project, only task (i) is presented for main fruit tree species.

## **In-situ visualization of water flow in seedling using neutron radiography**

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In future years water may become a limiting factor in horticulture production in many countries. Breeding of plants with improved drought tolerance may help to partially overcome this challenge. Conventional construction of plants with improved water uptake and transport performance such as grafting of high yield shoots on water effective roots may also be a solution. For both breeding and grafting a better understanding of water transport phenomena is essential. However effective and non-destructive methods to in-situ study water transport are missing.

Conventional methods providing information on water transport in plants either offer a macroscopic image only or give a detailed picture of a very small region of the plant. In contrast, 3D imaging with neutrons and X-rays provides both high resolution and a relatively large field of view. Using cold neutron tomography water flow images were successfully obtained in tomato seedlings by applying a D2O tracer technique. The water flow image will help to understand grafting process of tomato seedlings, and varies problems related to water stress of plants.

In experiments performed at HMI's (Berlin) new neutron tomography instrument CONRAD, tomato seedlings were supplied with D2O from some time and neutron radiograms of the plants were taken at regular time intervals. Due to different attenuation coefficients of D2O and H2O for cold neutrons, these two liquids can be clearly distinguished in the radiographic images. An automated device for exchange of liquid tracers and a climatic chamber to control temperature and humidity around the plant were developed. These devices guaranteed for reliable and reproducible results.

From observing the D2O level in the stem at different times after the exchange process, it was possible to deduce the velocity of water uptake in the seedling. The technique of chlorophyll fluorescence imaging was used to prove that neither the cold neutrons nor the exchange of H2O by D2O does affect plants' physiology. Consequently, neutron radiography provides reliable information on the water transport processes in plants.

## **GIS aided Agro-ecological zoning of Republic of Macedonia**

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Sustainable agricultural development requires a systematic attempt towards the planning of land use activities in the most suitable way. Agro-ecological zoning (AEZ) is one of the most significant approaches for agricultural developmental planning because survival and failure of particular land use or farming system in a given region heavily relies on careful assessment of agro-climatic resources. This approach is used to categorize agro-climatically uniform geographical areas for agricultural developmental planning and other interventions. Modern tools such as satellite remote sensing and Geographical Information System (GIS) have been providing newer dimensions to effectively monitor and manage land resources in an integrated way for agro-ecological characterization. This paper tries to demonstrate incorporation of new tools to extend applicability of Agro-ecological zoning in Republic of Macedonia.

For the purpose of this study long term (30 years) monthly maximum and minimum temperatures has been collected. Digital elevation model with 80 m grid in a national Gauss Kruger projection has been employed. The empirical relations thus developed were used to utilize inherent spatial quality of digital elevation model in GIS environment for depicting spatial variation in normal annual mean temperatures as well as annual rainfall condition over the whole country, which was further on used to compute the spatial PET and moisture index (MI). With overlaying of the moisture index (MI) map and reclassified CORINE land cover an output map indicating the Agro climatic zones was created.

Agro edaphic zoning for this study was done by incorporating several factor such as: soil texture map to which four textural classes were assigned to each soil type respectfully, terrain parameter as slope map derived from the Digital elevation model (DEM) classified in five classes (Lambert, J.J. et al 2002) and soil map that has been drafted in digital format in a scale 1:100 000. All agro edaphic layers and climatic layer described above were integrated or overlaid to derive different unique agro-ecological cells. These agro-ecological cells were further combined to arrive at agro-ecological zones based on their potential to support agriculture and different vegetation patterns.

## **The impact of the atmospheric noxes from Craiova town on some physiological parameters at annual flower species**

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Studies on the effects of air pollution of some urban and industrial areas on local crops and vegetables suggested that atmospheric pollutants concentration could reduce the growth and yield of crops. The physiological and biochemical responses of plants to air pollution demonstrated a reduction in chlorophyll content and inhibition of photosynthesis.

The annual flower species, which have an abundant blooming, of long duration, with a special decorative effect, occupy an important place in the assortment of the flower species used in the landscapes.

The objective of this research was to determine the influence of the atmospheric pollutants (SO<sub>2</sub>, NO<sub>2</sub>, NH<sub>3</sub>, powders) on some physiological parameters (total pigments and the respiration intensity) at three annual flower species *Petunia hybrida*, *Tagetes erecta*, *Ageratum mexicanum*. The plants were grown in six urban areas with different pollution grades.

It was established that NO<sub>2</sub> and powders were determined a decrease of the content in total pigments and the respiration intensity was decreased with the increase of the content in SO<sub>2</sub>, NO<sub>2</sub> and powders.

# Revealing the drought stress vulnerability of asparagus plants

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## Introduction

The predicted climate change in Europe will be accompanied by a change of seasonal distribution of precipitation (IPCC 2001). With a diminution of estival precipitation the requirements of water supply for asparagus crops will arise. In respect of an environmentally and economically sound irrigation management the plant water relations under water shortage have to be understood in a better way. A literature survey indicates that asparagus crop obligates an optimal water supply even if this species is considered drought tolerant. In order to avoid plant damages during cultivation and a subsequent reduce in spear-yield of the following season, it is important to reveal the vulnerability of *Asparagus officinalis* L. to soil drought.

## Materials and Methods

Well-watered one-year old *Asparagus officinalis* L. plants with fully developed shoots were desiccated in 15 l pots by irrigation stop and evapotranspiration in a greenhouse. While the desiccation course the predawn xylem water potential ( $\psi_{xyl}$ ), using a pressure bomb by SCHOLANDER, the hydraulic conductivity of shoots, applying a method according to Sperry et al. (1988), and the porometric gas exchange of twigs, measured at midday in a portable gas exchange system (GFS-3000, WALZ, Germany), were determined. The soil matric potential ( $\psi_m$ ) was monitored as suction pressure (-kPa) with tensiometers and data logged continuously.

## Results and Discussion

The combined recording of changes in predawn water status and hydraulic conductivity of shoots, as well as limitations in the photosynthetic gas exchange of twigs displayed the onset of water deficiency within the plant while soil drought gradually increased. Thresholds for drought stress of these hydraulic parameters were performed relating to  $\psi_m$  and predawn  $\psi_{xyl}$ , respectively. The parallel monitored changes of  $\psi_{xyl}$  and  $\psi_m$  at predawn demonstrated an unimpaired plant water status through decreasing soil moisture until a sudden debasement of plant water status at a  $\psi_x$ -level about -60 kPa. The absence of an early impact of decreasing  $\psi_m$  indicates a strategy of drought tolerance. In vulnerability curves, illustrating the relationship between the loss of hydraulic conductivity and  $\psi_{xyl}$  of shoots, the transition of cavitated into embolized xylem vessels, occurred at a  $\psi_{xyl}$ -value of -0.7 MPa. Embolized conduits stand for an irreversible impairment of the water transport system of drought-stressed shoots, whereas cavitations occur as well in well-watered plants. This critical  $\psi_{xyl}$ -level was determined in predawn at a  $\psi_m$  of -60 kPa. In comparison of this  $\psi_{xyl}$ -threshold with those of other species (JONES and SUTHERLAND, 1991; VOGT 1998) the shoots of asparagus demonstrated low xylem vulnerability. Stomatal conductance, transpiration and assimilation rates exposed an abrupt reduction beneath the uniform  $\psi_m$ -level of -50 kPa. Until this critical  $\psi_m$ -value was reached no impairment of the stomatal gas exchange has arisen. The maintenance of photosynthesis up to a  $\psi_m$ -threshold for constrictive soil water deficit and the feature of this threshold to emerge in a higher soil moisture level than that for xylem embolism validate the stomatal apparatus as a good sensor for drought stress in asparagus.

## Conclusion

The investigated hydraulic parameters are qualified to image the impairment of the hydro physiology of potted asparagus plants caused by increasing soil drought. With the derived thresholds for the onset of drought stress in plant hydraulics the susceptibility of asparagus can be assessed. In model experiments the water conducting system of asparagus exposes low drought susceptibility. The adapted stomatal aperture to severe water deficit indicates a drought tolerant character of this. Future trials will allow to determine drought stress thresholds for open field conditions and to optimize the irrigation practice of this species with little drought vulnerability at moderate but high vulnerability at severe soil drought.

# The ornamental tree *Picea glauca* ‘Conica’ as a model plant for uptake studies with the environmental pollutant trinitrotoluene

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## Introduction

Most of German former military sites (2.8% of the entire territory) are covered by woodlands dominated by conifers. On large areas of these sites soils are contaminated with explosive's residues, mainly with 2,4,6-trinitrotoluene (TNT). To explore the decontamination potential of conifers with radio-analytical methods, model plants are needed which show all features of adult trees.

The dwarf mutant of Canadian white spruce, *Picea glauca* ‘Conica’ combines low space requirements with easy handling. Therefore *Picea glauca* ‘Conica’ is suited for uptake studies with [<sup>14</sup>C]-radio-labelled TNT.

## Methods

Using glass fibre application systems the time course of input of water-solved, bioavailable pollutants (TNT) to the soil/tree system is precisely quantifiable (Schoenmuth & Pestemer, 2004).

For uptake studies, uniform ring-labelled [<sup>14</sup>C]-TNT was permanently applied via glass fibre wicks. After exposition to [<sup>14</sup>C]-TNT overall radioactivity of tree compartments was determined. Extractability of radio-labelled explosives from plant tissues was estimated by Liquid Scintillation Counting (LSC). Radio-labelled extracts were separated by radio thin layer chromatography (TLC).

## Results & Discussion

TNT is accumulated in Canadian white spruce. For TNT, highest concentrations of [<sup>14</sup>C]-TNT equivalents (eq) are found in roots (Fig. 2). Only a very small percentage is transported to above-ground tree compartments, i.e. wood (3%) and needles (2%).

The mass distribution of radio-labelled compounds shows that spruces are able to reduce the content of [<sup>14</sup>C]-TNT in soil. Substrates planted with conifers clearly contain lower contents of explosive equivalents than unplanted variants.

Extractability of TNT<sub>eq</sub> was very low in roots (10%) but higher in wood (25-30%) and highest in needles (30-40%). The bulk of TNT<sub>eq</sub> is non-extractable bound in root tissue and only very low amounts of metabolites are translocated to above-ground tree parts.

TLC analysis indicates that extractable TNT<sub>eq</sub> residual portions contain neither TNT nor known metabolites (e.g. ADNTs, DANTs), but TNT is metabolised to polar metabolites.

## Conclusions

<sup>14</sup>C-TNT-uptake experiments with *Picea glauca* show that conifers are excellent helper components to reduce the content of TNT in contaminated coniferous forest soils. Their “dendroremediation” potential opens a wide range of future sustainable sanitation possibilities for explosive contaminated areas.

## Reference

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# Verifiability and molecular analysis of *Cherry leaf roll virus* infecting Finnish *Betula* spp.

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## Introduction

*Cherry leaf roll virus*, CLRV, is a worldwide distributed plant pathogen that infects a variety of deciduous trees and shrubs. Recently, it was detected in Finland in several *Betula pubescens* (downy birch) trees exhibiting symptoms of a viral disease (Jalkanen et al. 2007); the virus could also be confirmed in *B. pendula* (silver birch), both are dominating deciduous tree species in the country. Furthermore, CLRV was found in *B. nana* (dwarf birch), *B. pubescens* spp. *czerepanovii* (mountain birch) which is considered to be a genetic hybrid (*B. pubescens* spp. *pubescens* x *B. nana*) as well as *B. pubescens* spp. *appressa* (Kiilopää birch) comprising important key components of the arctic ecosystem.

Traceability of CLRV by molecular means was investigated during the vegetation period and dormancy of *Betula* spp. growing near the Arctic Circle (Rovaniemi) enduring mean annual temperatures near the freezing point. Furthermore, CLRV isolates originating from different locations in Finland were compared to isolates obtained from *Betula* and other host plants from different geographic origins.

## Methods and Results

Fragments of the 3' coding region (3'NCR) were amplified by application of IC-RT-PCR using a specific polyclonal antibody raised against an elderberry isolate of CLRV (phylogenetic group E) and a conserved primer pair. During the year round testing 15 CLRV infected *B. pubescens* trees were sampled seven times during 2006 and 2007. Virus detection was possible during the vegetation period between May and September using leaf and twig tips, buds or catkins, while CLRV was not detectable in dormant tissues (buds or catkins) in winter.

CLRV specific fragments from 3 downy birches from Rovaniemi, 2 silver birch trees (East and West Finland) and one mountain birch (Northern Finland) were cloned and sequenced. Subsequently, genetic relationships were investigated by PCR-RFLP as well as sequence comparison with CLRV isolates characterised previously by Rebenstorf et al. (2006), who established 5 different phylogenetic groups (A-E) depending on the host plant.

## Conclusions

CLRV was not detectable by IC-RT-PCR outside the vegetation period in downy birches which may be correlated to a higher freezing tolerance during that stage. Remarkably, when trees were tested in May shortly after flushing, only in about one third an infection could be detected. Most reliable results were obtained in July, August and September.

Nine individual CLRV clones obtained from six different *Betula* trees revealed two different fragment sizes, 404 bp and 412 bp, which were in accordance with grouping of Finnish CLRV isolates by PCR-RFLP. Unlike clustering of CLRV strains from birches growing in the UK and Germany exclusively within group A, Finnish CLRV isolates exhibited highest sequence identities to isolates clustered in phylogenetic group B, D or E.

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# Impact of Substrates and Fertilization on Quality of Drainage Water of Extensive Roof Greening

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## Introduction

In addition to the retention of a huge part of the annual rainfall a substantial amount of the rainwater percolates the roof greening system and reappears as drainage water which may be discharged into canalization, drained away into the soil or used as water for domestic purpose and irrigation water, respectively. As the drainage water also returns to the hydrologic cycle a contamination of pure rainwater e.g. by substances released from roof greening substrates or applied fertilizers should be avoided.

## Material & Methods

Therefore the influence of common mineral roof greening substrates (lava/pumice, broken expanded shale, cinder oil shale) with and without mineral and organic additives (zeolite, peat, composted bark) as well as with and without moderate fertilization (coated slow-release fertilizer) on the quality of drainage water was tested.

A single layer of the substrates was installed in a thickness of  $100 \pm 3$  mm and planted with 20 herbaceous and gramineous perennials. The test started in May 1998 and was finished in October 1999. Electrical conductivity, pH,  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ ,  $\text{P}_2\text{O}_5$ ,  $\text{K}_2\text{O}$ , Na,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HCO}_3^-$ , As, Cd, Cr, Cu, Hg, Ni, Pb and Zn were analyzed in the drainage water.

## Results

With all substrates, additives and even with fertilization the average drainage water quality was in accordance with the very high requirements of the German Drinking Water Ordinance concerning the analyzed parameters. Merely the average coloration of the drainage water was higher than the limits of the ordinance.

Also the maximum values of the drainage water complied with the prescribed limits of the German Drinking Water Ordinance (2001) in most cases. An exceeding of the limit values could be detected for electrical conductivity,  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ ,  $\text{SO}_4^{2-}$ , As and coloration mainly in the first weeks of testing (see table).

In addition it was observed that fertilized plants showed a good growth by covering most parts of the substrates whereas not fertilized plants did not grow sufficiently.

To conclude, we can say that the drainage water of an extensive, single layer roof greening can feature a high quality on average. A moderate and purposeful fertilization which effects an appealing roof planting has just a marginal impact on the drainage water quality.

Table. *Some characteristics of drainage water (summarized data of all substrates and treatments)*

Parameter	Dimension	n	Maximum	Minimum	Mean	75%-Quartile	Limits of German Drinking Water Ordinance (2001)
Electrical conductivity	$\mu\text{S cm}^{-1}$	324	3220	25	437	310	2500
$\text{NO}_3\text{-N}$	$\text{mg l}^{-1}$	324	47	0,01	1,47	0,22	11,5
$\text{NH}_4\text{-N}$	$\text{mg l}^{-1}$	324	0,56	0,01	0,11	0,15	0,15
$\text{SO}_4^{2-}$	$\text{mg l}^{-1}$	324	2520	1,0	209	98	240
As	$\text{mg l}^{-1}$	89 <sup>1)</sup>	0,03	<0,001	0,009 <sup>2)</sup>	0,009 <sup>2)</sup>	0,01
Coloration	$\text{m}^{-1}$	324	27,6	0,05	2,51	2,72	0,5

<sup>1)</sup> Less number of analyzed samples, as analysis was not continued when the values constantly went below the detection limit (0,001  $\text{mg l}^{-1}$ ).

<sup>2)</sup> For statistical computation values below the detection limit were equated with 0,001  $\text{mg l}^{-1}$  to point out approximately the potential maximum size of arsenic concentration.

## Horticultural plants in unstable climate

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### **Introduction**

Temperature is one of the important environmental factors affected plant growth and development. To successfully transplant agricultural species in the spring, hardening prior to transplanting is of great significance. In particular, in situations with fluctuating temperatures common to early spring, low temperature treatment leading to increased cold tolerance prior to transplanting, might be critical for survival. It is well known that cold tolerance can be increased in many species by exposure to a period of low, non-freezing temperature. Temperature drop is used widely in horticulture. However, the influence of temperature drop on subsequent growth and development have not been well characterised for most bedding plant species. The aim of this work was to quantify the effects of temperature drop on growth, development and cold resistance of *Petunia* and *Tagetes* and to determine whether there were any residual effects of temperature drop on subsequent growth and development after transplant outdoors.

### **Material and methods**

Seedlings were subjected to 2 temperature regimes: constant temperature of 22°C (control treatment) and 3 h temperature drop from 24 to 12°C during the last 3 hours of night (drop treatment) for one week. To study the after-effect of temperature drop on subsequent growth and development plants were transplanted outdoors for the next 3 months. Plant dry weight, plant height, number of lateral shoots, flower and buds number and plant cold resistance (LT<sub>50</sub> method) were recorded.

### **Results**

Plant flowering was evaluated during their vegetative outdoor growth period following transplantation. Temperature drop at seedling stage accelerated subsequent flowering and improve plant quality in *Tagetes* but not in *Petunia* and increased cold resistance in both species.

### **Conclusions**

Greenhouse growers could use temperature drop at seedling stage to accelerate subsequent flowering and improve plant quality in *Tagetes* but not in *Petunia* and to increase cold resistance in both species. Enhanced and long lasting cold resistance of plants treated with temperature drop technique allows them to adapt better to outdoor conditions following transplantation from greenhouses in early summer as well as to survive at fluctuating temperature in Fall.

## **Influence of climate on flowering phenology of pome and stone fruit in Romagna (Italy)**

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### **Introduction**

In the last few years there has been an increasing interest in world climate variations, and also in our Country a rise in temperatures, especially those of winter months, was recorded. The 2006/2007 winter is an example of particularly mild winter in our country. The Fruit Tree Research Unit in Forlì (CRA-FRF), whose research activity is mainly devoted to fruit tree breeding and cultivar evaluation, from the early 1980' regularly records phenological data of the large number of varieties/advanced selections collected at its experimental farm in Magliano (Romagna, Italy), as well as climatic data. Since the flowering phenology of fruit

tree species is strongly influenced by climate, especially by temperature, it seemed interesting to find out if in the last 25 years, period in which we have punctual records of flowering time for the main pome and stone fruit species, some significant variations have occurred in this main phenological phase.

### ***Material and methods***

The phenology of flowering was recorded on hundreds of pear, apple, peach, sweet cherry, Japanese and European plum genotypes collected at the experimental farm of CRA-FRF (34 m a.s.l., 44,16 N 12,08 E). Starting from 1983 in peach, 1984 in apple, 1989 in plum and 1991 in pear and cherry, the dates of beginning (5% of flowers open), full (60-80%) and end of flowering (petals start to fall) were recorded yearly for each accession. Average daily temperatures were also recorded. The correlation between the average monthly temperature from November to March and the average flowering time of the accessions of each species grown at Magliano farm was also studied. We also verified the hypothesis of a tendency to time shift of this phenological phase in the different fruit species.

### ***Results***

In the 25-year period considered, we found a general, slight tendency to an increase in the average temperature of winter months in our area. For the same period, no univocal tendency to a change in the flowering date in the fruit species was found: although peach tended to anticipate the flowering date, the other species were un-influenced.

January and February average temperatures significantly affected the average flowering date of each species. In particular, the highest correlation values (from  $r=0,72$  in cherry to  $0,79$  in peach and Japanese plum) were found when correlating flowering date with the average temperature of the last week of February.

Regardless of the differences in winter climate, Japanese plum and peach cultivars (whose blossoming constantly overlapped) were always the first to flower followed, in sequence, by European plum, pear, cherry and apple. The average time lapse between the beginning of flowering of the earliest and latest species was on average about 20 days; the highest concentration took place in the years 1985, 1987, 1996 and 2006 (a two-week period); the lowest concentration (more than 4 weeks) took place in 2001 and 2007. The year with the earliest blossoming was 1990, then, in order, 2001, 2007, 1998 and 1997. The years in which flowering was most delayed were 1984, then, in order 1987, 1986 and 1996.

## **Influence of spatial variability of soil properties on the response of a citrus orchard under regulated deficit irrigation**

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Agriculture water consumption in Andalusia ascends to 4,761 hm<sup>3</sup> per year, of which almost a 10% are destined to the culture of citrus, with an approximated surface of 73,000 ha. The existing climatic conditions in SW Spain characterize by the shortage and irregularity of their precipitations, agreeing the period of greater evapotranspirative demand with the driest time of the year. In addition, structural cycles of drought exist that have caused, in the last 26 years, severe restriction in the availability of irrigation water with a high or very high negative impact on the crops.

These conditions, together with an increasing competitive demand (social, industrial and agronomics) for water resources, force to look for new strategies of irrigation that allow maintaining the sustainability of the agricultural systems of irrigated land. One of the strategies to increase irrigation efficiency consists in the application of regulated deficit irrigation (RDI) strategies based on a controlled diminution of the water supply without the yield and quality of the harvested product are seen significantly affected. Numerous contributions have recently documented the advantages of the use of these strategies to improve the efficiency of the use of the water in different fruit species. Nevertheless, these techniques have not been applied based



on the hydric state of the plant and/or causing a partial drying of roots zone, neither in relation with spatial variability of soil properties.

Have been studied in this work the response of a citrus orchard (*Citrus cinensis*, cv. Navelino) to different RDI based on the water state of the culture established through different xylem water potential levels. In the same way, we studied the effects of the spatial variability of soil properties characterized by an electromagnetic induction device (EM 38) that measures soil apparent electrical conductivity in both, horizontal and vertical ways.

RDI strategy allows obtaining yields similar to the treatment without water restrictions, and save water when it is applied properly. In our conditions, inherent factors to the spatial variability of soil properties have a great influence on tree responses to water deficits that can even prevail to water stress effects, mainly in situations of slight to moderate stresses.

# **Lectures and Posters of Theme 4**

**SUSTAINABILITY OF HORTICULTURE IN EUROPE  
(ENVIRONMENTAL, SOCIAL, ECONOMIC ...)**

# Sustainability of Horticulture in Europe (environmental, social, economic)

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## *Is sustainability a dead issue?*

Sustainability is a principle which eventually drives all technological development. It is only some 20-30 years ago (see e.g. Brundtland report in 1987), however, that sustainability has become an issue which is recognised as something that has to be met if the aim is to develop technology that shall generate a positive impact on society. Nowadays, sustainability has a very prominent place on the political agendas of many countries and also, e.g., in the European Union's research framework programme addressing agriculture. Food production standards such as GLOBALGAP are based on the conceptual framework of sustainability. Science has played a crucial role for giving sustainability the above mentioned recognition and acceptance as an overruling principle of development. Can science, therefore, focus now on other issues and delete sustainability from its agenda?

If looking at the actual hot topics of scientific meetings and new prominent projects in horticulture, one could have the impression, that sustainability has lost its important place on the agenda of horticultural science. Omics-technology, nutrition and health, food safety, or consumer acceptance are some of the actually hot topics on many research agendas. Luckily, there are at least a few international scientific meetings in Europe in 2008, that concentrate on sustainability in horticulture and continue to drive scientific thoughts along the lines of sustainability. Clearly, several facts demand, that sustainability has to stay a very important issue on any horticultural research agenda, such as "old" but still unsolved and therefore pressing problems (e.g. aspects of soil health) and the particularities of horticultural crops such as e.g. high market standards of fresh products that demand resource intensive production principles. Furthermore, new hot topics such as climate change, IT-mastered micro-technology and challenges of global markets will create new challenges but also opportunities for developing sustainable production systems in horticulture.

## *Exemplary cases*

The paper addresses selected examples of horticultural crops in Europe, that could be exemplary for the way how further, science-based technological development in horticulture should meet the principles of sustainability. Pesticide residues on horticultural products must continue to be an issue of importance for horticultural science for helping to develop technology that better addresses expectations of markets and consumers regarding drastically reduced pesticide residues. Innovative spraying technology in fruit-growing has the potential for contributing significantly to meet such expectation. The example of edelweiss (*Leontopodium alpinum*) in Switzerland demonstrates that the domestication of new horticultural plants and development of innovative products meeting the modern society's demands can help to make horticulture in marginal rural areas competitive and contribute to a sustainable rural development. A drastic reduction of energy demand in greenhouse horticulture may contribute to the sustainability of greenhouse production but also of the economy as a whole. In vegetable production, seed borne diseases are a critical factor with regard to the sustainability of crop growing. Innovative and easy to handle disinfection technology that is not based on pesticides can contribute to a more sustainable production. Disease and pest forecasting will continue to be an important tool for increasing the sustainability of a crop also in vegetable production, even if in a particular context, potential and limits of the technology must be explored individually.

Sustainability is an integrative concept and not a discipline-oriented branch of science. Therefore, discipline-oriented research sometimes may have difficulties to appreciate the role of sustainability in science and society. However, efforts should not be neglected to continue to address the integrative demands of sustainability in horticultural science, even if sometimes particular issues of ecology, economy or society might be overruled by the public and political argumentation of a certain time.

# Eco-social Certification in International Floriculture - A War of Labels?

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## *Introduction*

Floriculture has turned into a worldwide active business. Whole branches of production, and trade were transferred to tropical and subtropical countries in the northern as well as the southern hemisphere. Favourable climatic conditions, a second summer-season belong to the main reasons. On the other hand less restrictive environmental standards, lower labour costs, lower social standards, less stringent official control by states organs are motives too.

Reports on forced labour, children work, irresponsible use of agrochemicals and working conditions (especially of female workers) led in the past 10 to 20 years to activities driven mainly by non-governmental organizations to establish and guarantee environmental and social standards in flower-production and marketing. Several certification-systems and different labels were implemented in the industrialized countries, which in general are the main sinks of flower-consumption. Labelling has been promoted as the means of implementing and sustaining good practices of corporate eco-social responsibility, equity and ethics along the complex supply chains and alliances.

For the time being there are three certification & labelling systems active and present on the Austrian floricultural markets: The 'Flower Label Programme' (FLP; a NGO label), the Fairtrade label (Fairtrade organization, involved in state funded developmental cooperation) and the 'Fair Flowers and Fair Plants' label (FFP; introduced by the Union Fleurs and funded by the European Community). Negotiations between FFP and FLP were not successful to appear as a collaborative harmonized system. Fairtrade only markets cut flowers via supermarket-chains are already succeeding to find common standards to harmonize the different systems. The competition on the market of more sustainability, stronger ethics and economic effects has been started.

## *Methods*

The study combines economic, social and environmental perspectives as well as the presence among players in the chain. To collect data questionnaires were sent to decision makers and guided expert-interviews were conducted. Labelling in the International Floriculture: Innovation, ethics and with emphasis on effects and consequences on floriculture as well as the certification systems in the near future. A field-research was conducted (interviews) with main focus on gender issues of female workers.

## *Summary*

Attention will be drawn on the secondary and primary data from research and publications in Europe and Latin American to illuminate achievements, tensions and contradictions perceived by different actors in the industry from the labelling experiences to date.

A side-focus will be set on the possible function as parameter of quality, fitness to meet the requirements of the the players, especially the consumers – on reliability and traceability of the different systems concerning fair conditions and prices, social and environmental aspects, descriptive catalogues of crop management, pesticide use; to a reliable, evaluation of process and product quality and progress needs to be based on objective and measurable criteria, documentation and transparency.

Flowers and plants are natural products and they are bought because of sentimental, emotional and ornamental values. This symbolism must not be affected by negative publicity, which correlates to methods of production, transportation and marketing. Consumers now are becoming more and more aware and sensitive of environmental (“ecological”) and social issues.

We conclude with some reflections on the limitations of ‘ethical and fair’ behaviour in practice and the prospects of the near and midterm future of the labels – will one, two or three vanish, coexist or something else?

## **Economic efficiency of the use of plant protection products in Flemish horticulture**

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In this research the use of pesticides is studied in the different sub sectors of horticulture in Flanders. An inquiry was held on the holdings of the Flemish farm accountancy data network to collect the data on the use of pesticides in different crops in horticulture. The data on the quantities of plant protection products were transformed into the quantities of active substances they contain and then extrapolated to the population. Most of the active substances were fungicides. In the same study also a number of agricultural crops were studied. It was found that for agricultural crops the use of active substances per hectare is lower. On the other hand horticultural production is more intensive and has a higher production value per hectare. If we express the economical efficiency of pesticides as the production value that is obtained by the use of one kilogram of active substances then we obtain a totally different image. For every kilogram of active substances that is used for the production of horticultural crops the production value is much higher than for agricultural crops. These figures gave another insight in the use of pesticides in agriculture and horticulture. Many studies use the quantities of active substances that are used. But there is also a big difference of impact on the environment between the active substances. To have an idea of the impact of the use of pesticides the POCER indicator developed by professor Steurbaut and his team of the University of Ghent was used. The POCER indicator, or Pesticide OCCupational and Environmental Risk, is an indicator that measures the risk of the use of active substances on some components of the environment such as: the person that applies the product, the person that works on the field after the application, the bystander, the persistence, groundwater, water organisms, birds, earthworms, bees and useful arthropods. This kind of indicator takes into account the real impact on the environment and is more valuable to compare the use of pesticides. Using this indicator reveals that the economic efficiency per unit of indicator is much higher in horticulture than in agriculture. This means that although horticulture uses more pesticides per m<sup>2</sup> the impact on the environment per produced Euro is much smaller than in agriculture.

## **Re-discovering horticulture: An exploration from plant production to Social Capital**

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Horticulture is a term that evokes images of plants and gardening for most people working in the horticulture industry. For the general public and government policy makers at local, national and international levels the term is not completely understood nor is the impact that horticulture has on human activities been fully appreciated. In recent times the interaction between people and plants has received increasing attention within the horticulture research community. This concept has spawned further research and debate in the overall area of human issues in horticulture. This debate will assist in helping public and policy makers begin to understand the positive and pervasive influence of horticulture in human society.

The present study contributes to this effort by exploring and outlining the development of horticulture from the gathering of plants from the wild by primitive man, to the domestication of plants in the homestead, through the use of plants as a therapy to arrive at the most recently proposed paradigm; social horticulture. This has not been a simple linear development. Horticulture has and will always exist as a matrix of inter-relating areas with overlapping and complex relationships. However, modern mass communication requires a simple message presented in a language that is easily understood and immediately accepted by the public

and government policy makers. To this end a number of areas of interest within horticulture were examined to clarify and define their scope.

Defining the term Horticulture is a key factor in effective communication of the importance of plants, their cultivation and their use for sustainable human existence. It is evident that limiting the term horticulture to the popularist understanding of just a gardening activity fails to encompass the enormity of the impact that horticulture has on individuals, communities and society. And as such diminishes the ability of horticulturalists to convince policy makers to commit public funds to horticulture research and development. Describing the impact of horticulture on the physiological, psychological and social activities of people is key to expanding an understanding of what horticulture is. It is primarily an art but it is intimately connected with the sciences. Horticulture happens when people are in intimate contact with plants, it is the interface between people and plants and could be simply defined as “the art and science of plant cultivation for human use”.

The term “horticultural therapy” is regularly used as a catchall phrase applied to anytime anyone gardens and feels better or acts better. However, horticultural therapy is an activity that has pre-defined clinical goals similar to that found in occupational therapy. Therapeutic horticulture, on the other hand, is the process by which individuals may develop well-being through using plants and horticulture. This may be achieved by either active or passive involvement. Therapeutic horticulture has its roots in environmental psychology, the study of the influence of environment on human behaviour.

The use of horticulture in the development of human skills through the delivery of various educational curricula such as school science programmes or the use of horticulture to develop life skills in the education of persons with developmental disabilities contributes to the development of an individual’s “Human Capital”. This can assist a person to make more effective occupational and social contributions to society.

Modern political leaders have begun to recognise the vital contribution of “Social Capital” to economic development. Social capital is the “glue” that binds individuals, community groups and state agencies to form effective society. An opportunity exists to highlight the hidden role that horticulture has had and continues to play in developing communities and society. It is proposed that the term “Social Horticulture” be defined as being the application of horticulture for the development of social capital. Defining social horticulture in this context and expounding the contribution of horticulture to human well being will assist in advocating a wider understanding of the importance of horticulture in society, and thus ensure the sustainability of horticulture as a primary industry in Europe.

## **The family-firm life cycle and its impact on sustainable development in glasshouse horticulture**

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In Flanders glasshouse vegetables and ornamental plants are typically produced at family businesses. According to agricultural economics literature at this type of holdings the objectives and long-term firm developments are influenced by the so-called ‘family-firm life cycle’. The objective of the paper is to investigate the impact of the ‘family-firm life cycle’ on personal and business characteristics, objectives and sustainable development of the glasshouse holdings. As sustainable horticulture integrates the three P’s (People, Planet, Profit), special attention is paid to the environmental, social and economic aspects.

The empirical research was performed at 138 glasshouse holdings selected from the Flemish Farm Accountancy Data Network (FADN), permitting to couple accounting data to the results of a questionnaire measuring the importance of several objectives and management aspects by the firm manager. Principal component analysis (PCA) of the personal objectives resulted in the dimensions ‘instrumental objectives’, ‘expressive objectives’, ‘familial/social objectives’, ‘intrinsic objectives’ and ‘general objectives’. For the business ob-

jectives the dimensions 'financial independence of the firm', 'creativity and innovation', 'growth', 'stabilisation' and 'profitability' were distinguished.

The hypothesis that the life cycle of the firm manager parallels the life cycle of the family firm can be confirmed by the results. The glasshouse holdings in the different phases of the 'family-firm life cycle' show significant differences in age and education level of the firm manager, firm size, modernity of durable goods, solvency and investment pattern.

The results indicate that the emphasis on various objectives is changing during the firm manager's life time. At the start of the business the firm managers place a high priority on 'high level of income' and 'growth'. During the exit phase these objectives are replaced by the less ambitious objective 'survival'. Most of the expressive objectives such as 'job satisfaction', 'self-fulfilment' and 'personal independence' are more important at businesses with a long term perspective. In general, intrinsic objectives, covering the aspects of craftsmanship, receive a high average score, although at the older businesses they seem to be more important. Firms in the start and growth phase are attaching a higher importance to 'productivity' than in the other phases. No significant differences among the groups could be detected for the business objectives 'creativity and innovation' and 'profitability'. At the holdings in the later stages of the family-firm life cycle the availability of a successor has an important influence.

With regard to the environmental aspects the results show that the phase of the 'family-firm life cycle' has a significant influence on the quality of environmental management aspects, such as external comparison of the use of energy, fertilizers and pesticides. Human resource management (HRM) is an important aspect of the social dimension of sustainable development. Firm managers in the start phase declare to attach a higher importance to HRM, have more performance interviews with the personnel, a better rewarding policy and are more concerned about improving the labour conditions than those in the other phases of the 'family-firm life cycle'. However involvement of personnel in decision making is still low, independent of the phase in the 'family-firm life cycle'. With regard to the economic aspects no significant influence of the phase in the 'family-firm life cycle' on the income obtained per familial labour unit could be observed.

The insights derived from this research have important implications both for research and practice. They can enable glasshouse growers and advisers to take and/or support correct decisions and may help policy makers to differentiate on the base of the family-firm life cycle.

## **Conversion to organic horticulture: a multidimensional issue and an example for transitions towards sustainability**

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Organic farming (OF) can be considered as a prototype of innovative agriculture. Its time-proof principles and specifications, and the challenges organic food and farming has to face in terms of technical constraints and potential development, renew its interest for research and technical workers. The conversion process is a cornerstone for its development, and can also enlight the issue of transition towards sustainability. Conversion entails new relations with technology and environment, as well as consumers. It is seen as a process including a series of events, adaptive management and a new rationale. In this paper, we account for three types of results of an interdisciplinary project aiming at the identification of trajectories for conversion to organic horticulture ("Tracks").

First, comprehensive case studies exhibit how farmers' backgrounds and duration of conversion differs. Both a description and a classification of trajectories are proposed. Five types of trajectories were identified, described as more direct – for example following a health incident or economical difficulties- or more progressive, *e.g.* when a former rupture with conventional agriculture was identified long before conversion to OF was considered. This was applied both to conversion *per se*, *i.e.* with previous farming experience, and to direct entry to farming.

Secondly, from a technical viewpoint, experimental data in vegetable production show that soil conversion encompasses a longer time frame than 2 years, *i.e.* the formal conversion period. After 5 years of organic inputs supply, the effects on soil organic matter become tangible when significant amounts are supplied (14t/ha of commercial compost on a 2 years crop sequence salad/tomato/salads); conversely no clear effect was noticed on crops. This can be interpreted as gradual changes in soil balances. Moreover, cropping systems under greenhouses reveal high inertia due to past regimes, with strong mineralisation dynamics and persisting influence of past management. For example, the usual management of soil borne diseases with chemical disinfection in conventional cropping systems entails long-term biological imbalances, contributing to pathogen proliferation. In addition, most of soil borne pests and diseases exhibit conservation forms that cannot be suppressed in a short two-year period.

Thirdly, in fruit production inheritances are also prevalent, since before conversion orchards are usually managed with a high level of pesticides. Twenty interviews completed with fruit growers show that the 3 years period of formal conversion entails difficulties for 80% of the growers, due a decrease in commercial yield (sometimes half of the previous performances) and problems with pests and diseases. An anticipated adoption of eligible methods in organic farming facilitate this transition period. Trajectories for conversion encompass changes in both soil and trees, as well as for fruit growers. The orchard pattern is usually redesigned, especially in apple production, with the abandonment of some varieties (Golden, Early Red One, Starkimson) and the introduction of adapted ones (Juliet, Goldrush, Chouquette). Some fruit growers tend to modify their training systems, introducing vigorous rootstocks, larger planting densities, and to enhance an agroecosystem approach with the integration of hedges and livestock. Conversely, other growers tend to optimize the use of eligible inputs, following organic specifications. These two groups also differ according to the protection methods used. As a result, the production potential is usually reconsidered, although a lower commercial yield is partly compensated by processing (fruit juice) or direct selling, which enables a direct relation with consumers. Two extremes situations were thus encountered: diversified orchards and marketing systems, emphasizing agroecological orchard design and local resources; as opposed to mass production where inputs costs and efficiency are driving forces for a more intensive production.

Results are discussed in three directions: specifications for the redesign of horticultural organic systems, consequences for the development of organic food and farming, relevance for the sustainability of horticulture.

## **Husbandry - the ultimate means of control for soil borne pathogens**

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Sustainable disease control is achieved by minimising host vulnerability to pathogen invasion and symptom expression by integrated crop management (ICM). A primary component in ICM is husbandry. The efficiency and effectiveness of husbandry lies firmly in the hands of the grower and relies wholly on his / her knowledge, experience and skill. Soil borne pathogens cause substantial damage and loss to horticultural crops and are intractably difficult to combat. Reasons for this include:- large differences in vertical and horizontal distribution in the soil profile, widely varying responses to physical, chemical and biological soil properties, highly evolved capacities for robust and long lasting perennation, extended host ranges, limited availability of useable host resistance, abilities for the rapid generation and spread of physiological races tolerant to host resistance and agrochemicals.

The soil borne microbe *Plasmodiophora brassicae*, the cause of clubroot disease in the *Brassicaceae* presents an excellent example illustrating many of the difficulties inherent in controlling soil borne pathogens. Considerable progress has been made in combating this organism using ICM in which husbandry is now a major tool. This pathogen is favoured by excessive soil moisture permitting the movement of primary zoospores from the well - protected resting spores into the root hairs of host plants. Following a primary reproductive phase the pathogen colonises more deeply into root tissues and undergoes secondary reproduction resulting in the formation of resting spores that are discharged back into the soil. Soil management resulting



in deeply fertile well - drained soils inhibits zoospore movement. Growing crops on ridges and elevated beds improves soil drainage. Acid soils are associated with disease outbreaks. Consequently, the addition of lime in various forms has been associated with clubroot control for at least 200 years. Elevated levels of calcium in the rhizosphere have been correlated with diminished reproductive abilities of *P. brassicae* in root hairs. Calcium also promotes soil suppressiveness probably by changing the soil microbial composition towards species that are antagonistic to *P. brassicae* and reducing the germination capacity of resting spores. Boron has similar properties to calcium but is also repressive to symptom expression. Readily available calcium as an oxide or hydroxide is more rapidly effective than carbonate forms and reactive small particle sizes reduce clubroot severity more quickly compared with coarsely ground limestone. Fertilisers such as calcium cyanamide and calcium nitrate induce environments suppressive to clubroot and stimulate the yields of *Brassica* crops. Rotations with non - hosts such as cereals or *Allium* spp. help erode the soil inoculum potential. The half life of spore populations is estimated to be 4 - 5 years. Eradication of crop and weed brassicas by cultivation, stale seed and transplanting beds and use of herbicides, where available, is essential. Trap crops that encourage spore germination and penetration with subsequent mechanical and chemical destruction has been used in specialised situations.

Combinations of soil, fertiliser and crop care can be pyramided thereby reducing the impact of Clubroot Disease relative to concentrations of soil inoculum. As yet only bio - assay techniques are available in practice for measuring inoculum concentration but enzyme - linked and DNA assays are emerging. A few effective agrochemicals are available but are not permitted for use in Europe. The limited genetic resistance has been deployed into some selected specialist crops. There is a world wide epidemic of Clubroot Disease causing major losses to all *Brassica* crops and in the last few years it has entered the Canadian oil crop (canola) raising the prospect of destabilising the world vegetable oil market.

## **Does knowledge on fruit tree architecture and its implications for orchard management improve horticultural sustainability? an overview of recent advances in the apple**

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Orchard planting systems are often associated with tree shape management which has direct and practical implications on training and pruning strategies. The objective of horticultural practices is to reach the needed height and shape as fast as possible and to maintain this structural framework in time and space. Scientific studies consider the individual crown shape and the spatial organization of trees within the orchard, which encompass tree height, planting distances, alley width and/or the leaf area index (LAI). Therefore, ecophysiological studies are mostly dedicated to the optimisation of orchard agronomical performances, highlighting the importance of light interception and distribution. At orchard scale, the individual tree is often considered as a black box, and the turbid medium analogy hypothesized, i.e., tree crowns are filled with a uniform leaf area density. Isolines of canopy light distribution are computed.

An incremental improvement in our understanding of tree growth and fruiting strategies has been developed in the last three decades. Minimal pruning concepts have been proposed to fruit growers, minimising undesired vegetative reactions and taking advantage of the genetic variability of architecture. They improve early and more regular yield, and favour homogeneity of fruit quality. Recent studies also show that interactions exist between components of tree architecture, namely branching density and shoot growth dynamics, and development of orchard pests and diseases. Paralleling our better understanding of tree architecture, dramatic improvements of ecophysiological studies now include an explicit description of plant topology and geometry. This gives rise to computation of 3D tree mock-ups allowing more accurate modeling of light

interception and distribution at tree and orchard scales. These approaches are therefore capable of taking the within-canopy complexity into account and this can in turn open new hypotheses related to pest and disease occurrence.

Emerging concepts in fruit tree manipulation, and their impacts on architecture and ecophysiology, undoubtedly open new avenues in orchard management with the objectives to meet horticultural sustainability, i.e., optimising labor efficiency and fertilizer and water use, and demanding less pesticides.

## **How to manage nitrogen fertilization of the spinach-bean succession at the crop rotation scale - Case Study in the loam soils in Belgium**

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### ***Introduction***

Belgium is the first European exporter of frozen industrial vegetables (1 00 000 t) which represents 48 % of the European Union exportations. Main vegetable crops in Belgium are bean (12 000 ha and 99 000 t), pea (18 000 ha and 98 000 t) and spinach (4000 ha and 78 000 t) (Source: Unilet informations, 2006).

Since 1985, the establishment of a frozen industry in the Hesbaye area (Wallonia region) have led to the integration of some vegetable crops in most traditional rotation of arable crops as sugar beet, cereals and potato. This have required technical and cultural adaptations because of vegetables characteristics. For instance, all the fields in the area are now equipped for irrigation system to produce vegetables in spring and summer. Due to shallow roots development and a low nitrogen use efficiency, unbalanced nitrogen rates are often applied to vegetable crops. The most frequent vegetable successions are broad bean/autumnal spinach or spring spinach/bean. Two succeeding crops within a same year makes difficult to manage nitrogen fertilization. Problems appear at harvest as regards yield and quality (nitrate rate, bean size, ...) but also environmental hazards (nitrogen leaching) and subsequent traditional nitrogen crops management.

### ***The study***

The Case Study presented relates to spring spinach/bean succession followed by an autumn-winter period with or without catch crops before the sowing of a sugar beet.

In collaboration with the C.M.H. (Centre Maraîcher de Hesbaye), we adapted parameters of AZOBIL software (INRA, Laon, France) according to local characteristics to manage nitrogen fertilization of spring spinach in Hesbaye area. Nitrogen fertilization of spring spinach can be well controlled in terms of yield, quality of the production (respect of European nitrate concentration norms) and soil nitrogen residues directly at harvest. Difficulties are nevertheless encountered to estimate crop residues mineralization and its contribution for the subsequent crop which is variable according to the cutting height of the spinach plant (nitrate content is higher in petiole than in leaves). For bean, results confirm that no nitrogen is necessary after spinach. Excessive nitrogen fertilization can lead to a depreciation of their quality (increase of the bean size, for instance).

The management of the inter-cropping period after the spinach/bean succession gave interesting results. The sowing of catch crops appears beneficial to avoid the leaching of high rates of nitrogen not only during autumn and winter, but also during the growing period of sugar beet and after its harvest. Indeed, nitrogen kept in upper soil layers is rapidly taken up by sugar beet and contributes to the yield. With a delayed uptake of this nitrogen located in lower soil layers (inefficient catch crops or bare soil in preceding winter period) it is more accumulated in sugar roots and in leaves. In roots it can interfere with sugar extraction and in leaves (crop residues) it contributes to enhance nitrogen potentially at risk of leaching during the following drainage period. In both cases no direct effect was observed on sugar beet yield.

This researches show that in such cropping systems a global management of nitrogen inputs/outputs is hardly required at rotation scale to match yield/quality and environmental objectives in the context of sustainable agriculture.

## Newly Developed Plant Factories: a Low-Cost Approach Using Hybrid Electrode Fluorescent Lamps

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### Introduction

A plant factory is not affected by the weather, and it is possible to obtain a stable production of plants. At present, a serious problem of a plant factory is its energy cost. Electricity carries most of the expense; therefore it is necessary to reduce this cost.

Hybrid Electrode Fluorescent Lamps (HEFL), which were modified back lights for LCD television, are improved and adapted for plant cultivation purposes. Compared to fluorescent lamps (FL) and LEDs, HEFLs are superior concerning energy saving, heating, longevity, size and weight. HEFL's life is more than 10 times longer than fluorescent lamps. The price of HEFLs is lower than 1/4 of LEDs and the light efficiency of HEFLs is 2.5 times higher than that of LEDs (Table 1).

The growth of a plant is promoted or restrained by the color of the light source. Since HEFLs are possible to choose the wavelength, we conducted the cultivation of several kinds of plant under various light sources and investigated their efficiency in comparison with fluorescent lamps or LEDs.

### Methods

We compared 3 light sources: the first was composed by Cool White HEFLs (CW-HEFL) which emits white light and is more blue than FLs; the second was composed by Red HEFLs (R-HEFL) which is mostly red; the control was FL. Specific energy was shown by Fig.1. In order to obtain the same conditions for the photon flux, we set the PPF (photosynthetic photon flux) to  $85 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Under these conditions, the distance from the light source was 200 mm for FL, 280 mm for CW-HEFL and 210 mm for R-HEFL.

Spinach mustard (*Brassica rapa* L. Perviridis Group) 'Gokurakuten' was cultivated by Enshi-shohou (i.e. most popular nutrient solution in Japan).

### Results and Discussion

Under R-HEFLs spinach mustard grew the most, and it was produced low nitrate, but lower SPAD values and longer leaf stalks. On the other hands, under CW-HEFLs had the most leaves, higher SPAD values, shorter leaf stalks and voluminous look which have a commercial value. But under CW-HEFLs there was a delay of days to harvest compared to R-HEFLs.

From these results, it is possible to cultivate spinach mustard under HEFLs with a much lower cost and better or equal quality than FLs. In our future work, HEFLs we want to investigate that improve light quality and light efficiency. We examine other environmental factors which can allow earlier harvest and we also research the culture of various other plants.

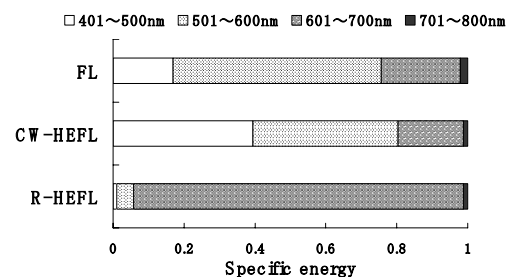


Fig.1. Specific energy of FLs, CW-HEFLs and R-HEFLs:

CW-HEFLs is more 401-500 nm than FLs;  
R-HEFLs is mostly 601-700 nm.

Table 1. Comparison between HEFL, FL and LED:

HEFLs are the longest life, highest light efficiency and lowest surface temperature. And HEFLs are cheaper than LEDs and 0.1 times maintenance cost of FLs. In this case, maintenance term is five years.

	Fluorescent Lamp	LED	Hybrid Electrode Fluorescent Lamp
life	below 6.000 hr	below 30.000 hr	above 60.000 hr
light efficiency	*70 lm/w	30 lm/w	80 lm/w
surface temperature	50 °C	cooling needed	30~40 °C
price ratio	1	5	1.5
maintenance cost ratio	1	0.1	0.1

\*40 W white fluorescent lamps ... Fixed luminous flux is 2.800 lm

## Improving vegetable yield and quality standards in organic farming by defining functional cultivation protocols

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### Introduction and methodology

Organic farming is increasingly expanding within the European Union, where it is practiced over 6.3 million ha. Typically, organic farming is associated to small/medium size farms with relatively low-technology, in which specific cultivation protocols that may guarantee high quality standards have rarely been defined. With the overall objective of providing a guideline that may assist organic farmers in their effort, we began a thorough experimentation aimed at defining cultivation parameters, under organic regime, that may affect yield and quality of three vegetable crops, namely endive (*Cichorium endivia* L. var. *crispa*), zucchini (*Cucurbita pepo* L.) and cauliflower (*Brassica oleracea* var. *botrytis*). In endive and zucchini, conventional and organic farming were compared respect to nitrogen fertilization (0, 100, 200 kg ha<sup>-1</sup>), soil type (sandy and clay) and presence/absence of plastic mulching. In cauliflower, the effect of conventional and organic farming was assessed on three cultivars, with white, red or green inflorescence, grown on different soil types (sandy and clay).

### Results and Discussion

**ENDIVE:** Organic farming caused a 40% yield reduction, yet it did not affect the leaf nitrate content, which was generally enhanced by high N levels (N200). The absence of mulching both reduced the leaf nitrate content and modified the antioxidant capacity of the commercial product. **ZUCCHINI:** The commercial yield was 14% lower in organic vs. conventional farming, with generally higher yield in clay soils, in mulched crop, and at high N rates. A moderate increase in K was observed in organic zucchini. **CAULIFLOWER:** The average inflorescence weight was higher in clay soils vs. sandy soils (+49%), under conventional vs. organic cultivation (+25%) and in both white and red cultivar compared to the green one. Hydrophilic and lipophilic antioxidant capacity was not affected by the cultivation regime, whereas it was mainly associated to the inflorescence colour (lowest values in the white cultivar compared to the others). A moderate increase in K was also found in organically grown cauliflowers. Based on these results we concluded that it is possible to modify and improve quality standards in organic farming by using functional combinations of agronomic factors.

### Acknowledgements

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## **Application of saccharose during blossom to attract honey bees and their influence on yield and quality of strawberries (*Fragaria x ananassa*)**

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The strawberry hardly can be called a desirable bee flora for the honey bee, since it entices them rather sparingly with colour, fragrance and not abundant offers of nectar and pollen. Hence, several trials had already been performed in order to raise the attractiveness of strawberries by spraying them with saccharose during blossom. The results of these trials showed that a better pollination by bees and a positive influence on the plant's health could be ascertained, and with a slightly raised yield additional profits were achieved.

On the basis of this knowledge, further fruit parameters were to be examined in a field trial. One of the aims also was the attraction of the honey bee with a lower concentration of saccharose and its application only after rain or artificial irrigation in order to lower the additional costs and to render the application more practice-oriented.

In 2002 three treatment variations, each plot measured 9 m<sup>2</sup>, with 4 repetitions, were performed in an organic managed strawberry field (planted in August 2000) in Vienna with the cultivar Malling Pandora. For variation 1, the surface of the leaves were wetted after every rain and artificial irrigation with a 20 % saccharose water, for variation 2, only water in the same quantity was given, in the thirds group the strawberries remained untreated (=control group).

The following results were accumulated during the season of 2002:

- The observations of the bees' flights at 17 dates showed a highly significant enhanced visit of honeybees (15 times more bees) on the sugar treated strawberries than in the water treated plants.
- The P-value of fruits on the sugar treated plants was lower, which indicates a higher vital quality, but there were no statistically significant differences. The strawberries of variation saccharose showed the lowest redox potential and, therefore, were exposed to less oxidative stress. There was a significant difference between variation water and variation saccharose. The measurement results of electrical resistance and pH-values showed no significant differences.
- Dry matter and sugar content were slightly, but not significantly increased in the fruits of the sugar treated plants, the content of vitamin C was similar.
- The lowest fruit infestation with the fungus *Botrytis cinerea* and the highest infestation with the fungus *Colletotrichum acutatum* was found in the saccharose treated variation. The differences for both criteria were statistically not distinguishable.
- The quantity of marketable fruits was significantly less on the saccharose treated plants. This was in contrast to former studies and most probably, the unexpected decline in marketable yield was due to the hypersensitiveness of the leaves of the used strawberry cultivar Malling Pandora to the saccharose treatment. In preceding trials, with other cultivars of strawberries, this effect had not been found.

In order to verify this assumption, further studies are necessary.

# Nano-structured silica based insecticides

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## Introduction

Many synthetically produced pesticides belong to a class of toxic chemicals known as persistent organic pollutants (POPs). They are among the most dangerous compounds ever produced, because they are long-lasting and travel long distances in global air and water currents. Despite their hazards, POPs continue to be produced, used, and stored in many countries. Therefore, there is a need for alternative pesticide products such as the physically active diatomaceous earth (DE). Until a few years ago DE's worked best under low humidity conditions. Newly developed formulations are effective even under higher relative humidity (r.h.). The purpose of the present study was to analyze the toxic effects of different modified silica products against two different insect pests: the granary weevil (*Sitophilus granarius* (L.)) and the mustard beetle (*Phaedon cochleariae* F.). The best performing substances are going to be selected for greenhouse trials in horticultural production systems.

## Material & Methods

In pilot tests with *S. granarius* as test insect a preliminary selection of several dusts was done. Only the most effective substances against this beetle were used for further studies: the commercial DE products Fossil-Shield® FS100, FS90.0s, FS80p, three synthetic amorphous silica's (SAK), and three different silica formulations developed in our own laboratory called "AL-06". The last mentioned substance, AL-06, is a natural derived product composed of small hollow spherules with a surface area of approximately 800 m<sup>2</sup>/g. We have tested AL-06 formulations with different particle sizes. The products tested contain more than 96% SiO<sub>2</sub> with different degrees of hydrophobicity and compaction. All experiments were conducted in petri dishes without food in a climate chamber at 25°C and 65% r.h. In different trials with six replications each, the substances were tested against 10 unsexed adult beetles of both species at five concentrations (0.1, 0.25, 0.5, 1.0 and 1.5 mg cm<sup>-2</sup>). Mortality and weight of the beetles were recorded over time. The survived beetles from the treatments were dried to estimate water content.

## Results

The pilot test showed that the different AL-06 formulations were all quite effective against *S. granarius*. But some of the formulations were not easy to handle considering future greenhouse applications. The highest mortality in the *S. granarius* trials was achieved with a commercially available SAK (92%) after 48 hours. In the *Phaedon* trials, already a mortality of 100% was achieved after 6 hours with one of the AL-06 formulations at 0.25 mg cm<sup>-2</sup>. The results of this study indicate that silica dusts can effectively control *S. granarius* and *P. cochleariae*. Apparently, the unmodified DE FS100 and one of the SAK's failed to control the beetles sufficiently in the same period of time with mortality rates ranging between 20% to 47%. The efficacy of some AL-06 formulations, especially in the trials with *P. cochleariae*, was astonishing good in comparison to the commercial dust FS90.0s. At higher dosages all materials resulted in a higher beetle mortality rate. In *P. cochleariae* trials weight loss correlated negatively with the rate of survival ( $r = 0.99$ ).

## Discussion

The poor performance of some substances supports the fact, that hydrophile substances saturate with water from the surrounding air and lose their insecticidal efficacy. In previous studies (unpublished data) we could prove that particles with a larger surface are more effective than particles with smaller surfaces. Further studies are planned to prove the relevance of water saturation of hydrophobic and hydrophilic substances in order to examine their effectiveness under typical greenhouse conditions. Additional greenhouse experiments are considered to study possible phytotoxic effects of silica dusts. It might be that silica dusts plaster stomata and reduce plant's photosynthesis.

# How to get potato plants resistant to low temperatures and nematode invasion

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## **Introduction**

In the field plants are routinely subjected to a combination of different abiotic and biotic stresses. The majority of abiotic stress studies performed under controlled conditions. Considerable gap seems to exist between the knowledge gained by these studies and the knowledge required to get plants with enhanced tolerance in field conditions. *Globodera rostochiensis* Woll. is one of the widespread potato cyst-forming nematode in the North-West Russia. Cold temperature combined with nematode invasion affect potato plants in the North. The aim of the study was to investigate the effect of short-term temperature drop on cold resistance formation of potato plants and development of potato cyst-forming nematode *Globodera rostochiensis* Woll. (PCN) in laboratory and field conditions.

## **Material and methods**

We studied the effect of short-term temperature drop on cold resistance formation of potato plants and development of potato cyst-forming nematode *Globodera rostochiensis* Woll. in laboratory and field conditions. Plant cold resistance was estimated by measuring the temperature that caused chloroplast destruction and cytoplasm coagulation in 50 % of palisade parenchyma cells after 5-min freezing (at temperature range from  $-15$  to  $-5^{\circ}\text{C}$ ) of discs from leaves in a micro refrigerator ( $LT_{50}$ ). The difference between temperatures that caused death in treated and control plant cells was accepted as plant cold resistance increment. The effect of different temperature treatments on plant cold resistance, yield and development of potato cyst-forming nematode were studied.

## **Results and discussion**

The level of plant cold resistance was twice as high under the temperature drop treatment than under long-term diurnal low temperature. It was established that nematode population in drop treated plants was much lower (3 folds) than in control and long-term treatments. Short temperature drop led to increase in plant productivity: tuber weight of drop treated plants exceeded control considerably.

## **Conclusions**

Cross-adaptation takes place in response to temperature drop: high level of cold resistance is combined with high tolerance to cyst-forming nematode. Little is known about the molecular mechanisms underlying the acclimation of plants to a combination of two different stresses. Tolerance to a combination of different stress conditions, particularly those that mimic the field environment, should be the focus of future research programs.

# Strategies for alternative energy supply in greenhouse production in Saxony, Germany - comparative cost methods for different combustibles and perspectives for a CO<sub>2</sub> reduction

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## **Introduction**

Due to a considerable price increase in the energy sector the existence of greenhouse enterprises are highly threatened in Northern Europe. At the same time there is also a strong need to reduce CO<sub>2</sub> emissions in re-

gard to current ecological and political requirements. An increasing intensified discussion on the use of renewable energy sources in the area of greenhouse production has taken place in recent years in Europe.

In general the use of alternative combustibles depends on several diverse factors, such as prices of oil/gas/alternative combustibles, fuel value, technical characteristics, size of greenhouses, heat requirement etc. Depending on the particular region these factors can differ to a certain extent. Potential strategies for alternative energy supply respectively energy saving in the Saxon greenhouse production were investigated in a survey carried out on behalf of the federal state Saxony, Germany.

### ***Material and Methods***

Current situation of Saxon greenhouse enterprises was investigated at the beginning by evaluating both secondary and primary empirical data. In the following step potential alternatives and energy savings were identified, based on an extensive literature research, which was amended by some expert interviews. Moreover comparative cost methods for the use of different combustibles were carried out by using software "Horteb" as well as an individual calculation model based on DIN / ISO 2067. In addition, total CO<sub>2</sub> issue volume was determined, referring to different heat requirements, when using fuel oil, gas or hard coal. Finally varying economic, technical and organisational factors were evaluated for several alternative combustibles.

### ***Results and Discussion***

An average-sized (2,500 m<sup>2</sup>) typical Saxon greenhouse enterprise emits about 114 t CO<sub>2</sub> per year assuming low heat demand supplied by oil-fired heating system. On contrary, CO<sub>2</sub> emissions amount annually to 390 t assuming a high heat demand. Generally, CO<sub>2</sub> emissions can be significantly reduced by using alternative combustibles like wood chips, straw or waste heat, e.g. generated by biogas plants. Total emissions might decrease by 80-90 per cent when alternative combustibles are used as a base load system.

However, calculations comparing alternative combustibles with fossil fuels indicate that use of alternative combustibles effects no or low reduction of costs, when assuming low or mid heat demand due to large investment costs. Assuming a high heat demand, cost savings, amounting to 15-20 per cent can be realised, depending on total amount of investment costs and price level for fuel oil (or alternative combustibles respectively). On the contrary, these cost savings cannot be realised while using wood pellets as they are characterised by high and increasing prices in Germany / Saxony.

In addition, it should be considered that modified requirements, referring to company organisation, space required and maintenance arise while using alternative combustibles. In particular wood chips constitute a well-engineered alternative among various alternative combustibles. Using waste heat, generated by biogas plants or industrial facilities constitute another opportunity to save energy, though it is not very popular yet in Saxony.

In addition, energy savings caused by improved insulation, climate control, adapted production methods and the like have to be proved regarding the high age of many Saxon greenhouses (about 40 % are over 25 years old). By doing so energy savings amounting to 10-70 % might be realised.

Though regarding the partly keen economic situation and considerable investment costs prospective use of alternative combustibles in Saxon greenhouse production also depends largely upon national support programmes aside from technical and organisational factors.



## **Living mulches in field tomato production**

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### ***Introduction***

Living mulches are being recognized as an important part of organic and integrated crop production system aiming predominantly the reduction of pesticide and mineral fertilizers use as well as protection the agricultural environment. On the other hand, they compete with vegetable crops for light, moisture and nutrients and for this reason often cause the reduction of crop yield. The efficiency of such production system highly depends on the proper choice of plant species for use as living mulches, time of undersowing and possible suppression of plant growth by mowing or herbicide treatment.

### ***Material and methods***

In a field experiment conducted in 2004 – 2006 there were evaluated the effects of living mulches on yield and nutritional value of tomato fruits. As the living mulches there were used white clover (*Trifolium repens* L.), seradella (*Ornithopus sativus* L.), perennial ryegrass (*Lolium perenne* L) and pot marigold (*Calendula officinalis* L). All these species were sown before tomato transplanting in early or late April, at planting time (20 May) or 3 weeks thereafter. In some treatments growth rate of living mulches was restricted by mowing or Roundup 360 SL application.

### ***Results***

Data of the study showed that irrespective of the kind species and term of undersowing, the living mulches reduced the yield of tomato fruits by 21.6% in comparison to the control. Among tested plant species seradella and perennial ryegrass were less competitive for tomato than white clover and marigold, due to lower production of biomass at the end of growing period. The delay of term of undersowing from 5<sup>th</sup> April to 10<sup>th</sup> June caused a significant increment of tomato yield by 51.8% while suppression the growth rate of cover crops by mowing or herbicide use – at the range of 6.2 – 14.1%. Chemical analysis did not show any significant effects of living mulches species, term of their undersowing or method of growth suppressing on biological value of tomato fruits expressed by the content of dry matter, vitamin C, sugars and minerals.

## **Evaluation of coated slow-release fertilizer for low temperature cultivation of ornamental plants**

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### ***Introduction***

Spring flowering plants with low salt tolerance, like *Primula vulgaris*, often are cultivated using soluble salt fertilizers. The application of slow-release fertilizer could simplify the fertilization management though giving a continuous nutrient supply to the plants. On the other hand, there is a general uncertainty among growers concerning the temperature dependence of the nutrient release from coated slow-release fertilizers.

### ***Materials and Methods***

Experiments in the greenhouse as well as in growth chambers were carried out to compare the influence of nutrition with coated slow-release fertilizer against nutrition with fertilizer in salt form on growth and quality of *Primula vulgaris* 'Tiara Rose with Red Eye'. The given amount of slow-release fertilizer with high potassium content (Osmocote High K 5-6 M, N+P<sub>2</sub>O<sub>5</sub>+K<sub>2</sub>O = 10+11+18) varied from 2 to 4 g/l. Besides treatments with merely salt or coated fertilizer also treatments with combined fertilization were tested. The

growing medium used was a mixture of white peat and clay with a low basic nutrient supply. Heating temperatures in the two greenhouse experiments were constantly (day and night) adjusted to 3 or 8 °C, respectively. The plants in the growth chambers were given constant temperatures of 3, 7 or 11 °C, respectively.

In a further experiment in growth chambers the nutrient release from two types of coated slow-release fertilizer (Osmocote High K and Basacote Plus K, N+P<sub>2</sub>O<sub>5</sub>+K<sub>2</sub>O = 10+11+18 or 11+9+19, respectively) was investigated at different temperatures (3, 7, 11, 21 and 25 °C). Both fertilizers were used as six months versions. Polypropylene cups were filled with a definite amount of weakly decomposed peat (German white peat), which was adjusted to 60 % of the water capacity over the whole duration of the experiment. The fertilizer granulate was placed in the middle of the substrate in packets made from curtain cloth. Contents of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O in the substrate were extracted with CAT and measured after several intervals according to EN 13266 (determination of nutrient release from coated slow-release fertilizers).

## **Results**

In both greenhouse experiments increasing fertilization according to the experimental design resulted in larger plants. The highest amount of fresh weight and at the same time the best quality were obtained with fertilization corresponding to a nitrogen supply of 210 mg N per plant. There was no significant difference between the fertilization techniques at a given fertilization level.

The plants from the growth chamber experiment showed the important influence of the cultivation temperature. Plants from 11 °C were the largest ones that came into bloom first of all, whereas those from 3 °C remained very small and developed almost no flowers. Nevertheless, the varying fertilization regimes did not result in significant differences of plant growth and quality at all tested temperatures.

The investigation of the nutrient release in peat substrate revealed significant differences in nitrogen and potassium supply from the two types of coated fertilizer. Nutrient release from Osmocote constantly occurred over the duration of the experiment and after three months had reached 30 to 40 % for nitrogen and 15 to 50 % for potassium, depending on the temperature, respectively. For Basacote, nitrogen and potassium release did not exceed 20 % within three months at all tested temperatures, except at 25 °C. With both types of coated fertilizer the influence of the temperature on the release of nutrients is smaller than commonly expected. The good results with slow-release fertilizer in the plant experiments can be explained with the considerable release of nutrients from Osmocote even at 3°C.

The experiments show, that cultivation of low temperature requiring plants with coated fertilizer brings about equal results as with fertilizer in salt form. If an appropriate amount of nutrients in coated form is supplied in the growing medium, cultivation is possible without liquid feeding later during the cultivation period.

## **Life cycle assessment (LCA) and food miles - an energy balance for fruit imports versus home-grown apples**

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The primary energy is calculated to provide apple (cv. 'Braeburn' and 'Golden Delicious') fruit for consumers in the densely populated (8 million consumers) Rhein-Ruhr area, Germany in April. Three sources of apple fruit are compared: a) home grown-apples harvested in mid-October and CA-stored for 5 months on-site at ca. 1°C until mid March and b) fresh apples imported from New Zealand and c) fresh apples imported from South Africa in March. This was compared with apples of the same cultivar grown in a Southern hemisphere in Hawke's Bay, New Zealand or in Grabouw-Elgin, Western Cape, South Africa. These apples were picked in March with subsequent 28 day, or 14 day transport, respectively, on reefers to Antwerp for sale in April in Germany. The primary energy required for the cultivation of cv. 'Braeburn' apples in New Zealand of ca. 0.7 MJoule/kg apple fruit represented 11% of their overall primary energy requirement, compared with 1.0 MJ/kg fruit in Germany or South Africa with smaller harvests of 40 t/ha cf.

90 t/ha in New Zealand. Apples (cv. ‘Braeburn’ and ‘Golden Delicious’), grown and stored locally in Germany, consumed nearly 4.1 MJ/kg fruit, which included ca. 0.8 MJoule/kg for five months CA storage during the winter. This compared favourably with 5.4-6.1 MJoule/kg for overseas shipment from New Zealand or South Africa, i.e. a 24-33% greater energy requirement for imported fruits. The CA storage of home-grown apples in Germany partially compensated for the energy required to import fresh fruit from overseas. To fully compensate for fruit imports from South Africa or New Zealand, home-grown apples had to be stored locally for ca. 9 or 18 months, respectively, i.e. in the latter case beyond the next harvest. The smaller primary energy required for domestic apple fruit is discussed with respect to providing local employment, fruit orchards preserving the countryside, fruit quality, food safety and quality assurance schemes such as QS and EUREP-GAP and food security of local fruit and networking favouring regional produce.

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## Comparison of big gun sprinkler and mini sprinkler irrigation systems for the Radicchio cultivation: evaluation of environmental impact and crop production

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## Introduction

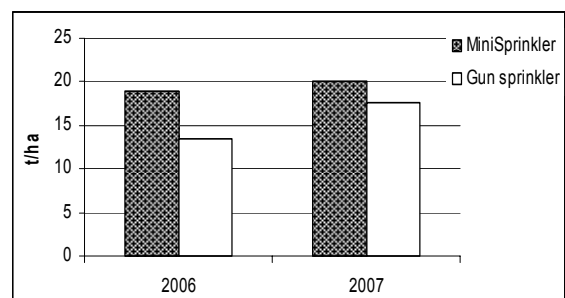
Big gun sprinklers are commonly used in Veneto Region (Italy) to irrigate horticultural crops, especially “Radicchio” or red chicory (*Cichorium intybus*, var. Bishoff, L. ). Even though this high-pressure irrigation system allows to irrigate large areas in a short time, it has some major disadvantages: the impact of the drops on the soil and on the crop can be very strong, producing soil crust and low crop sprouting; the high volume of water used tends to increase water and nitrogen losses (especially due to surface runoff). Further, water distribution uniformity is generally low: some areas do not receive enough water, whereas others can be over-irrigated, with negative effects on yield.

## Material and methods

In 2006 and 2007 an experimental field of about 4000 m<sup>2</sup> was set in a farm located in Conche di Codevigo (Padua, Italy) in order to evaluate the possibility of application of a different, more modern portable solid set system using no impact low-pressure (mini) sprinklers. The field was divided in two areas, one irrigated with mini sprinklers and one with the traditional high pressure system. The evaluated parameters were: water volumes (mm per season), water distribution uniformity (DU<sub>q</sub> coefficient calculated using the catch-can method) crop production (nr. of heads and kg/ha) and WUE (kg/ha · mm). The site was chosen both for its environmental importance, being situated inside the Venice Lagoon Watershed, and for the large diffusion in the area of this horticultural crop.

## Results and comments

The following preliminary results have been achieved: with the innovative mini sprinkler irrigation system the water distribution uniformity is higher (DU<sub>q</sub> 0.76 versus 0.43 with the traditional big gun sprinkler system); the average crop production is higher (see Graph below) mainly due to the higher number of harvested heads; the seasonal water volume applied is lower with a higher water use efficiency (WUE = 180 versus 109 in 2006, and 125 versus 74 in 2007).



## Conclusions

In the two monitored seasons the irrigation system with mini sprinklers allowed a higher yield and a better water distribution uniformity compared to big gun sprinkler, which in turn led to a better water use efficiency. During irrigation surface runoff along with nitrogen losses have been observed only in the area irrigated with big gun sprinkler.

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## Indicators of nitrogen status for ornamental woody plants

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## Introduction

Over-fertilization is frequent in ornamental plant nurseries. Indicators of plant nitrogen (N) status adapted to woody ornamental plants are essential to adjust fertilization to plant N demand. N supply influences the synthesis of both proteins and polyphenols, because their biosynthetic pathways share a common precursor, the amino acid L-phenylalanine (Jones and Hartley, 1999). The objective was to verify if optical measurements of leaf epidermal polyphenol (EPhen) and leaf chlorophyll (Chl) contents could be used as N status indicators for woody deciduous and evergreen ornamental plants.

## Material and Methods

One-year-old plants of *Lagerstroemia indica*, *Callicarpa bodinieri* and *Viburnum tinus* were grown outdoors in containers. They received high (TH, 105 mg. L<sup>-1</sup>) or low (TL, 4 mg. L<sup>-1</sup>) levels of nitrogen in June and July. EPhen content was recorded by fluorescence and Chl content by transmittance, with Dualex<sup>TM</sup> and SPAD-502 leaf-clip meters, respectively, on young expanded leaves exposed to direct sunlight. Chemical analyses of shoot and leaf N contents were performed regularly.

## Results

Shoot growth was reduced in TL treatment starting from day 28 and day 37 after treatment initiation in *Lagerstroemia* and *Callicarpa*, respectively, and only after 50 days in *Viburnum*. The mass-based leaf N content (Nm) of a sample of young expanded leaves exposed to direct sunlight correlated well with shoot N content in each species ( $r = 0.88$  to  $0.94$ , depending on species) and differentiated the treatments several weeks before shoot growth was reduced. Thus, Nm was used as an index of plant N status. In the three species, Dualex values were highly and negatively correlated with Nm ( $r = -0.92$  to  $-0.70$ , depending on species). Dualex values differentiated the treatments at least 15 days before any difference in shoot growth occurred, and these differences then remained significant throughout the experiment. SPAD values were positively correlated with Nm in *Lagerstroemia* ( $r = 0.85$ ) and *Callicarpa* ( $r = 0.44$ ), but not in *Viburnum*. SPAD values differentiated the treatments early in the three species; these differences lasted over time in *Lagerstroemia* and *Callicarpa* but were inconsistent in *Viburnum*. These inconsistencies and the absence of positive correlation between SPAD values and Nm were due to large variations in leaf mass per area (LMA) in *Viburnum*, which compensated for variations of mass-based chlorophyll content. The SPAD/Dualex ratio was used to assess changes in the proportion of leaf dry mass allocated to proteins and polyphenols in response to fertilization. It correlated to Nm ( $r = 0.92$  to  $0.65$ , according to the species) and differentiated the treatments early in the three species.

## ***Discussion - conclusion***

This study showed that Dualex measurements and SPAD/Dualex ratio could be used as N indicators in the three species (early diagnosis, consistency of the response over time), whereas SPAD measurements were only adapted to *Lagerstroemia* and *Callicarpa*. SPAD and Dualex are area-based values that incorporate (i) the proportion of the leaf dry mass invested in Chl or EPhen and (ii) LMA. Mass-based Chl and LMA are positively and negatively correlated to Nm, respectively, explaining why SPAD values could not accurately predict Nm in a species presenting high LMA variations, like *Viburnum*. On the contrary, mass-based EPhen and LMA both vary negatively with Nm. Dualex values varied more strongly with LMA than with mass-based EPhen in the three species, but the very general relationship between LMA and Nm (Wright et al., 2004) suggests that the relationship between Dualex and Nm is likely to be reliable in other ornamental species. The SPAD/Dualex ratio probes changes in leaf chemical composition and its response to fertilization was of large amplitude.

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# **Utilisation of cooling tower water of power plants in order to reduce expenses for greenhouse heating, to increase the primary energy efficiency, and to reduce CO<sub>2</sub>-emissions**

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## ***Introduction***

In the Rhine area near Cologne, a new brown coal fuelled power station is under construction. On behalf of a nearby municipality a case study about utilization of the rejected heat from the cooling towers is carried out in order to heat a 30-ha-greenhouse of a Horticultural Park that might to be erected. On basis of the experience of the 25 years old HORTITHERM greenhouse project, which uses cooling tower water (26 °C) for the heating of 66 ha of greenhouses, the heating installation for the direct use of low temperature water in enlarged air heating units was redesigned in order to reduce the electricity consumption for fans and pumps. The study shows that the factor of efficiency (the gain of heat in respect to electricity input) raises up to higher than 60 for the rejected heat utilisation. To find out the most profitable solution for greenhouse heating, this approach was compared to conventional heating resources as fossil fuel, and different configurations of gas and electric heat pumps using the rejected water as low temperature heat input.

## ***Results***

On condition of the 30-ha-greenhouse the consumed energy is calculated for the different concepts. Under German climate conditions the cucumber cultivation needs 54 MW heat capacity consuming yearly 91 GWh of effective heat for a single glazed house. To proof the excellence of the concepts, conventional heating with light fuel oil, natural gas, hard coal, and different configurations of heat pumps were assessed by their economic efficiency using annuity method for the investment over 20 years, maintenance, service, and fuel costs. Thereby, comparable heat generation cost prices are realised for all options.

The yearly full costs of heating are highest for conventional oil fuelled heating systems with 71 €/MWh, respective 22 €/m<sup>2</sup> greenhouse ground area, followed by natural gas driven heat pumps (for 100 % load capacity) with 20 €/m<sup>2</sup>, natural gas boilers 19 €/m<sup>2</sup>, gas heat pumps with gas boilers (each 50 % load) 18 €/m<sup>2</sup>, electrical heat pumps 17 €/m<sup>2</sup>, and electrical heat pumps in combination with oil boiler (each 50 % load) 17 €/m<sup>2</sup> too. Heating with coal and oil (each 50 % load) causes 13 €/m<sup>2</sup>. The use of rejected heat with adjusted heaters causes only 6 €/m<sup>2</sup> annual heating costs (price base: autumn 2006).

Basis of energy efficiency is the cumulated energy demand of the inputs for the different heating systems, incorporating the bias of former processing and transportation of the fuels, and the efficiency of heat generation and auxiliary power for the display in the greenhouse. The investigation of primary energy demand for the 30-ha-greenhouse shows that the coal and oil boiler supply consumes 138 GWh per annum whereas exclusively light oil gets by with 125 GWh. Setting the light oil as reference to 100 %, the coal boiler additionally consumes 12 %, and natural gas plus 3 %. Gas and electric heat pump, each with 50 % capacity, need 87 % and 69 % of primary energy respectively. Rejected heat has the lowest primary energy consumption with only 10 % referenced to light oil. The primary energy efficiency is then calculated by normalization of all energy inputs referred to the actual energy used. The emission of greenhouse gases expressed by the CO<sub>2</sub>-equivalents is calculated for the different inputs. The direct use of rejected heat reduces the CO<sub>2</sub>-emission by more than 90 %.

### **Conclusion**

Comparing rejected heat utilization for greenhouses with conventional heating by light fuel oil, natural gas, hard coal, or heat pumps, the low temperature heating with rejected heat is not only practicable but also most profitable, most efficient and causes lowest CO<sub>2</sub>-emissions.

## **Soil-plant N dynamics in processing tomato grown with different fertigation-irrigation frequencies**

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### **Introduction**

Fertigation can increase N fertilization efficiency and reduce environmental risk as it allows the localization of the fertilizer near the roots and the adjustment of the rate according to the crop nutritional status (Phene, 1999; Tei *et al.*, 2002). Achieving the maximum fertigation efficiency requires knowledge on crop specific water and nutrient needs in any site throughout the growth cycle and attention to the timing of water and N delivery to meet crop needs. At a given water and nutrient supply, the fertigation frequency affects the water volume and N rate per each application and thus soil moisture and nutrient concentration in the rhizosphere between irrigations with consequent changes in crop growth, N uptake and potential N leaching (Silber *et al.*, 2003). The aim of the experiment was to investigate the effect of the fertigation-irrigation frequency on soil-plant N dynamic.

### **Materials and methods**

An experiment was carried out in 2006 in a clay loam soil in Central Italy on processing tomato (*Lycopersicon esculentum* Mill., cv. PS1296). The crop was grown with 3 N-fertilizer rates (0, 100 and 300 kg N ha<sup>-1</sup>: N0, N100 and N300) applied with 3 different fertigation-irrigation weekly scheduling (F1: 1 fertigation; F2: 1 fertigation + 2 irrigations; F3: 3 fertigations) in the same water volume, according to potential ETc. The following determinations were performed: 1) aerial biomass and N accumulation and partitioning in stems, leaves and fruits; 2) soil N content at 0-0.6 and 0.6-0.9 m depth; 3) weekly soil moisture in each plot at 0.3 and 0.6 m depth by TDR probes; 4) continuous soil moisture in F1N300 and F3N300 at 0.1, 0.2, 0.3 and 0.5 m depth by capacitance probes (EnviroSCAN, Sentek); 5) N-NO<sub>3</sub> concentrations in the soil solution extracted from suction lysimeters at 0.6 m depth located close to the soil moisture probes. Soil moisture and N-NO<sub>3</sub> content in the soil solution determinations were used to estimate the daily drainage volumes and N leaching.

### **Results and conclusions**

The N rate and fertigation-irrigation frequency affected biomass and N accumulation. On average, increasing N availability increased crop dry weight (DW): at the final harvest DW was 760 g m<sup>-2</sup> in N0 and about +32% in N100 and +53% in N300. For any given N rate, DW in F3 and F2 were similar and higher

than in F1 (+11%). Crop N accumulation increased with increasing N availability (N300 was +62 higher than N0 and +37% than N100) and with the fertigation-irrigation frequency, but only within N300. At N300 the highest N uptake ( $384 \text{ kg ha}^{-1}$ ) and apparent N recovery (0.83) were observed in F3 (F3 N uptake was +24% higher than F1 and 13% than F2). Biomass and N partitioning were not affected by treatments. As compared to the critical N curve for processing tomato (Tei *et al.*, 2002) N100 showed an optimal N crop status while N300 a luxury N consumption. F1 increased the depth of the wetted zone and the variation of humidity in the upper 0.3 m soil layer in the time interval between two irrigations or fertigations suggesting an higher root development and efficiency than F3. The N-NO<sub>3</sub> concentration in the soil solution was affected by both the N rate and fertigation-irrigation frequency with the highest values recorded in N300 and F1. The amount of N-NO<sub>3</sub> potentially leached below 0.6 m was 35 and 53 kg ha<sup>-1</sup> for F3N300 and F1N300 respectively. The soil N content at the final harvest at each depth was not affected by treatments. Results indicate that the fertigation-irrigation frequency is not crucial for N uptake efficiency in case of optimal N availability while it can play an important role for improving N uptake efficiency and reducing N leaching risks in case of luxury N consumption.

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## Comparison of a standard climatic regime and a 24 h temperature integration regime in tomato soilless culture

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### Introduction

To save energy in glasshouse culture, one possibility for climate management is the application of temperature integration over a 24-hour period. The temperature integration is based on the plant's capacity to tolerate variations around an optimum temperature. It is thus a question of using solar energy during sunny days and of decreasing consequently the night setpoint temperature.

### Experimentation

In 2006, the trial was carried out in two identical greenhouses, each one with a surface of 90 m<sup>2</sup>. Two varieties of tomato (Altess and Albis both grafted on Maxifort) were grown either under a climatic regime with standard setpoints ( $T_{\text{day}}$ : 17°C,  $T_{\text{night}}$ : 19°C,  $20^{\circ}\text{C} < T_{\text{ventilation}} < 22^{\circ}\text{C}$  depending on radiation), or under a temperature integration regime ( $13^{\circ}\text{C} < T_{\text{night}} < 15^{\circ}\text{C}$ ,  $T_{\text{day}}$ : 17°C,  $20^{\circ}\text{C} < T_{\text{ventilation}} < 26^{\circ}\text{C}$  depending on radiation). The objective was to obtain average similar temperatures over 24-hour period in the two regimes. The plantation was done on February 16. And the differentiation between temperature management started on February 27. The influence of climatic control on the yield and the quality of tomatoes was measured.

### Results

Temperature integration did not have influence on the thickness of the stem or on the height of appearance of the bunch on the stem (development). The yield till June was reduced but the total yields at the end of the culture were comparable between the two temperature treatments. External quality (appearance of bunches and fruits) slightly decreased in spring but without economic consequences. No significant incidence on the analytical and sensory quality of tomatoes was noted. The energy saving estimated through the Horticorn software (simulation) reached approximately 40% over the periods of differentiation and 20% on the whole culture.

## **Conclusions**

This trial show that it is possible to make 24-hour temperature integration in tomato without negative influence on the yield and on the quality. The vigorous varieties seem to be better adapted to the large variations in day-night temperatures.

## **Mechanical weed control of the intra-row area with robotic system**

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As a component of successful non-chemical weed control intra-row weeding shall be considered as a final weed elimination procedure and not as a primary method. Conventional methods for inter-row weed control can handle with approximately 80% of the field area in row planted vegetables. However, the weeds occur in the remaining area between (intra-row) and around the vegetable plants (close-to-crop) have a much bigger impact on the development and yield of the plants.

Online detection of the single plant position and the plant/weed distinction are the bottlenecks of intra-row weeding but concerning the expeditious R&D in this field it is to expect that appropriate systems would be available on the market in near future. In the meantime, construction and adjustment possibilities of implements considering the role of soil properties and mechanics need to be optimised toward universal intra-row weeding tools, which can be used in different plant spacing systems, different plant intra-row distances and growth stages.

A virtual prototype of a system for intra-row weeding imitating the manual hoeing motions under the soil surface was designed. The hoeing tool consists of an arm holder and three or more integrated arms rotating around the horizontal axis above the vegetable row. Tests and simulations of the hoeing trajectories carried out with the virtual prototype have increasingly facilitated the design process and significantly shortened the path from the idea to the prototype. The physical prototype was realised using a servo motor with direct software control providing rotational speed adjustment according to the forward speed of the carrier, intra-row distance between plants and the observed position of the arms.

A simplified methodology and system for plant position detection based on the spectral characteristics of crop plants combined with the context information of the planting pattern was developed and tested. The experimental results showed that the combination of the RGB sensor and laser sensor can be used for accurate detection of the plant centre position independently from illumination conditions. In conducted experiments the maximum deviation of the estimated centre positions from the plant measured centre positions, detected by RGB sensor, was 31 mm, whereby 50% of the samples were inside the interval 0 to 5 mm and 90% of the samples were inside the interval 0 to 16,9 mm. For the laser sensor, the maximum deviation of the estimated centre positions from the plant measured centre positions was 25 mm, whereby 50% of the samples were inside the interval 0 to 3 mm and 90% of the samples were inside the interval 0 to 6.9 mm.

The servo system built in the physical prototype was operated in a mode with direct software control providing rotational speed adjustment according to the forward speed of the carrier, intra-row distance between successive crop plants and the observed angular position of the arms. The controlling algorithm and software solution were developed in the Labview® environment. The main task of the controlling software was permanent calculation, checking and change of the recent rotational speed of the hoeing tool in real time. The software solution used an extended version of the software previously developed for detection of the plants' centre position.

Tests have proved that depending on the angular adjustment of the duckfoot knives an uncultivated area big enough to avoid damaging of the plants can be left around the plants during the intra-row weeding with the developed system.



The system is able to autonomously adapt the rotational speed of the hoeing tool in case of non-intensive forward speed change. After rapid forward speed change, several plants can be damaged while the system stabilises its work, but stable state can be reached immediately after the stabilisation period.

The presented concept of the intra-row hoeing system can fulfil the requirements; it has sufficient degrees of freedom to allow full adaptation to different vegetable species, different plant intra-row distances and plant growth stages. In combination with an inter-row hoe or installed on an autonomous vehicle, the developed robotic system could be a solution for accurate and rapid mechanical weed control.

## Five years of the fungus *Monilinia* on blueberries in Europe: Status quo vadis

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### Introduction

Approximately 15.000 tons of blueberries are produced in Europe on about 4.000 ha with an increasing tendency (Gosch, 2006). Beside anthracnose fruit rot (*Colletotrichum* sp.) the mummy-berry disease (*Monilinia vaccinii-corymbosi*) is the most important fungal disease on blueberries in America and can cause major damages on vegetative and reproductive tissue (Eck, 1988).

### Status quo

*Monilinia vaccinii-corymbosi* was detected in Europe (Austria) in the recent past (Gosch, 2003) and is spreading continuously. Infections are also reported from Slovenia (A. Munda, 2005, pers. comm.). Disease management first concentrated on biological methods like covering the mummy berries with a layer of bark, which is effective but expensive. Removing of the mummy berries or mechanical destroying of the developing apothecia seem to be too inefficient for commercial plantations. Most efficient was the use of synthetic fungicides against primary (via ascospores) and secondary (via conidiospores) infections. Removal of infected vegetative tissue to prevent secondary infection or removing infected fruits to reduce the inoculum for the next year were also inefficient. Therefore, biological methods seem to be only useful in combination with the use of synthetic fungicides.

### Quo vadis

Prospective efforts concerning the disease management in Europe focus on deliberate site and cultivar selection for new plantations. Many highbush blueberry cultivars show some resistance to *M. vaccinii-corymbosi* (Stretch and Ehlenfeldt, 2000). For existing plantations the optimisation of plant protection methods is necessary. Alteration of different fungicides with different active ingredients and registration of new fungicides are important for resistance management. Additionally, fungicide application against the primary infection should only be started when susceptible tissue (after bud break) and ascospores are present together, to reduce total number of applications per year. Presence of ascospores can be evaluated by apothecia development. Discharge of ascospores from apothecia starts with cup openings of 1,2 – 2 mm in diameter, increases exponentially, and is levelling off at about 6 mm (Wharton and Schilder, 2005). Infection prognosis models including weather conditions, host phenology and apothecia development should be established.

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## **Yield and quality of field-grown vegetables at different sites in Europe as affected by fertilizer application together with the nitrification inhibitor DMPP and its environmental implications**

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Nitrification inhibitors (NI) delay the oxidation of ammonium to nitrate in soils for a certain period of time. DMPP (3,4-Dimethylpyrazol phosphate) (ENTE<sup>®</sup>) is a newly approved nitrification inhibitor in many European countries. The aim of this trial series was to detect its effects on yield and quality of field grown vegetables.

We carried out more than 100 exact field trials with small plots with four replications in Germany and Austria. Compared were two fertilizers which contained both 61% NH<sub>4</sub>-N and 39% NO<sub>3</sub>-N without or with DMPP. They were applied at each site at both the optimum N rate according to N<sub>min</sub> target values for the different crops and at a rate reduced by 25%.

Over all we found a yield increase due to DMPP of 10% in marketable weight. Among vegetables lambs lettuce and small radish responded particularly favorable to DMPP-containing fertilizers with increased yields of up to 20% and more. We also observed in many cases an increased inner and outer quality of the crops fertilized with DMPP. The edible parts of the plants had a tendency of lower nitrate content and the leaves showed mostly a darker green color compared to conventional fertilizer use, which may increase commercial value. In other crops, e.g. lettuce, a more compact habit was observed, which may extend the time frame of harvest time to optimize labor allocation and/or market prices. For vegetables with longer duration (e.g. head cabbage, Chinese cabbage, cauliflower) it could also be shown, that split fertilizer applications could be reduced to one single dress without loss in yield or N efficiency. Reduced N losses due to nitrate leaching after unexpected rainfalls or an over-irrigation were found both in model experiments and by the statistical evaluation of field data. It could be shown that a fertilization system with DMPP-containing fertilizers is less sensitive to weather conditions, owing to a higher flexibility in timing and less fertilizer applications.

The overall higher N efficiency in using ENTE fertilizers may be explained by reduced N losses, due to leaching of nitrate and volatilization losses of N<sub>2</sub>O, and may also account for both direct and indirect physiological effects, caused by a higher proportion of ammonium in the nutrition of vegetables.

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# Estimation of nitrogen release in garden soils

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## *Introduction*

Garden soils often show high organic matter contents of about 10 % and total nitrogen contents of 0.4 % on average. From this nitrogen pool mineral nitrogen ( $N_{\min}$ ) is mineralized continuously, which can be used by plants for their N-demand. N-release can achieve a considerable magnitude in intensively managed garden soils. Therefore, it should be considered for an appropriate fertilization strategy in recreational gardening. However, in German recreational gardening the users apply N-fertilizer according to approximate values, without considering the nitrogen release so far. Thus, the N-offer from N-release and additional fertilization can substantially exceed the demand of plants. This causes high  $N_{\min}$ -contents in the soil in autumn and thus a burden of the groundwater with nitrate by discharge with leakage water.

The current research project serves to acquire the magnitude of N-release from organic nitrogen. Parameters for the estimation of N-mineralization are looked for, in order to predict the amount of N released during the vegetation period in recreational gardens and to optimize previous fertilization strategies.

## *Material & Methods*

In-situ measurement of N-release (mineralization) was accomplished on 12 roofed fallow grounds in two allotments with different soil types.  $N_{\min}$  was measured monthly from May to October in 30 cm depth. If necessary, soils were watered and weeds were eliminated.  $N_{\min}$  was extracted with  $\text{CaCl}_2$  and detected photometrically by Continuous Flow Analyzer.

Furthermore, 31 garden soils have been incubated for 12 weeks under optimal conditions (25°C, soil water content at 60 % of maximum water capacity) in laboratory. Changes in  $N_{\min}$  contents were detected after 0, 2, 4, 6, 9 and 12 weeks of incubation.

## *Results*

120 kg N per ha on average were mineralized from May to October 2006 on 12 fallow fields with contents from 5.2 to 13 % organic matter. Maximum  $N_{\min}$  values of 294 kg ha<sup>-1</sup> were measured. The mostly very high N-mineralization in garden soils was only weakly correlated with organic matter and total nitrogen contents as well as with the C/N-ratio of the soils and thus could not be predicted from these parameters.

After 12 weeks incubation a high N-release of approximately 200 kg ha<sup>-1</sup> on average had been measured. The estimation of that N-release from organic matter or total nitrogen contents was only tendentious.

Due to the high N-mineralization on fallow grounds of 6 kg ha<sup>-1</sup> per week on average, further N-fertilization was dispensable in many cases on these soils. Thus, the common fertilization strategy using approximate values without consideration of N-release has to be refused.

The results originate from a current research project, which is supported financially by the Bavarian State Ministry of Agriculture and Forests.

# Assessing critical nitrate concentration in the root zone of a fertigated, soil-grown, tomato crop

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## Introduction

Despite the widespread use of the fertigation technique, the nitrogen fertiliser-use efficiency in fertigated, row crops is still low. In soil-grown crops under plastic tunnels or greenhouses, high water requirements associated with elevated planting density lead to the development of large wetted soil volumes that may not be entirely colonised by the plant root systems. Nitrate brought to the soil by fertigation moves to the periphery of the wetted soil volume, where, if no roots are present, it can be lost to the plants. To assess the optimal nitrate concentration and distribution in the soil, we monitored the root development, nitrate movement, and plant N status of a tomato crop.

## Material and Methods

Tomato (*Lycopersicon esculentum*) was grown in two plastic tunnels, one non-limiting (HN treatment, 445 kg N ha<sup>-1</sup>), the other limiting in nitrogen (LN treatment, 256 kg N ha<sup>-1</sup>), in March – August 2003. Rooting densities and soil nitrate concentrations were measured monthly in two-dimensional soil profiles, 90 cm wide x 130 cm deep. Plant DW and Nitrogen Nutrition Index (NNI) were also measured monthly.

## Results

Total plant DW was higher in the HN treatment. The root densities in the deep soil and under the alleys between rows were higher in the LN treatment. Root decay was observed during the last two months in the HN treatment, while continuous root extension was measured, until the end of the season, in LN. In the HN treatment, nitrate concentrations > 800 mg l<sup>-1</sup> were measured under the alleys, whereas concentrations remained uniformly low in the LN tunnels. A good correlation ( $p < 0.0001$ ,  $r^2 = 0.78$ ) was found between mean soil nitrate concentration and plant NNI, provided nitrate concentration was measured in a soil volume that included the zone of N accumulation. In this volume, a mean nitrate concentration of around 100 mg l<sup>-1</sup> was sufficient to ensure maximal plant growth.

## Conclusions

The monitoring of soil nitrate in wetted soil regions where no roots are present can help to evaluate the efficiency of N fertilisers. Avoiding N accumulation in non rooted soil areas can also reduce the risk of N leaching during subsequent crops.

# Influence of different techniques of fertilization on lettuce cultivated in southern Italy

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## Introduction

The use in agriculture of organic fertilizers and amendments (manure, anaerobic digestates, municipal solid waste compost, etc.) could develop a sustainable agroecosystem due to their importance in the conservation of organic matter in soil. Many studies showed that organic fertilizers can supply macronutrients and could

also improve the physical, chemical and biological soil characteristics because they contain high levels of organic matter. The aim of this research was to evaluate on lettuce production the effects of different organic amendments compared to mineral fertilizers.

### Materials and methods

The research was carried out during winter 2006/07 at Metaponto (MT) in southern Italy (lat. 40° 24' N; long. 16° 48' E and 8 meters above the sea level) located at the experimental farm of the Agriculture Research Council on clay soil (Typic Eptiaquerts according to Soil Taxonomy).

On lettuce (*Lactuca sativa* L., var. Bacio) crop, the effects of application of mineral fertilizer (FM) and of three treatments with organic fertilizers (commercial amendment based on stable manure -F1; anaerobic digestate based on stabilized wine distillery wastewater -F2; municipal solid organic waste compost coming from the separate collection -F3) were studied. The N dose of 140 kg ha<sup>-1</sup> was applied in only one solution (20 days before transplanting) for all treatments. The experimental design was a randomized block with six replications. Lettuce was transplanted on 6<sup>th</sup> November with a plant density of 6.7 plants m<sup>-2</sup> and the cropping cycle was of 142 days. At harvest, total and marketable yields, average weight tuft and leaf area index (LAI) were determined.

### Results and discussion

In table 1, total and marketable yields, average weight tuft and LAI are reported. The best productive responses were obtained with conventional mineral treatment, although the performance obtained in organic treatments was consistent with those found in other researches (Lombardi *et al.*, 2002), indicating that the use of organic fertilizers, as a N source for lettuce cropped in Mediterranean conditions, appears to be a viable practice. Furthermore, the organic fertilization could often be the amendment function more important than the nutritional one in a Mediterranean environmental (semiarid conditions), where the high mineralization rate quickly decreases the organic matter in the soil.

Table 1. Yields, average weight tuft and LAI of lettuce crop.

Treatments	Total yield (t/ha)	Marketable yield (t/ha)	Average weight tuft (g)	LAI
FM	57.3 a	56.2 a	980.1 a	6.3 a
F1	50.8 ab	49.5 ab	803.7 b	5.8 ab
F2	45.3 bc	44.2 b	729.5 bc	5.4 ab
F3	40.1 c	39.8 c	644.7 c	4.7 b

No significant difference was found between commercial organic manure (F1) and the experimental treatment based on anaerobic digestate (F2) in total and marketable yields. Conversely, the F3 treatment showed the lowest values of all measured parameters. These findings could be due to slow mineralization rate in soil of composts compared to the anaerobic digestate, which is characterized by low molecular weight organic acids and a high amount of mineralized N.

Finally, some studies show that the application of these residues did not seem to point out a potential risk for the agro-system, while their application in the soil improved both chemical fertility (Montemurro *et al.*, 2005) and plant growth by supplying the sufficient nutrients to plants (Togun and Akanbi, 2003).

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## **Controlling products in retailing market gardens**

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Retailing market gardens are one of the most complicated types of horticultural enterprises. They usually combine own production with retail, sometimes wholesale activities and several services like caring of graves. It is important for retailing market gardens to keep track of the flow of goods because the value of in-house products and trading goods plays an important role in relation to total costs. Therefore it is absolutely necessary to take action in respect of the economical assignment of goods. Nevertheless, there is usually no or little information about the flow of goods between various divisions of a retailing market garden. Due to this fact a detailed analysis of several aspects, e.g. cost centres, is impossible. Therefore, the author's intention is to submit a proposal especially for internal controlling of goods and their flows in complex structured retailing market gardens. The following proposal is part of a controlling-system which has been developed in co-operation with Bavarian market gardens. The application of a MIS (merchandise information system) is an essential part of the controlling system. MIS can give useful information for several economical analyses in order to optimize the operating profit of retailing market gardens.

The main focus in MIS application is the collection of data, namely data concerning the internal flow of goods. It is necessary to document the incoming products and outgoing products of the various divisions of a retailing market garden. By means of these data it is possible to receive information about the value of the own production and its internal distribution. In conjunction with additional data, contribution margin as well as profitability of the own production can be calculated. Furthermore it is possible to calculate specific KPIs (key performance indices) both in retail and wholesale and in product lines. KPIs provide the basis for composition of a customer-oriented and economical range of products. Therefore MIS are able to hold up long-term competitiveness in spite of increasing competition pressure in retailing market gardens.

### **Building sustainable cropping systems by combining scientific and empirical knowledge. The example of soil-born disease control on vegetable sheltered crops.**

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#### ***Introduction***

Farming systems must evolve very quickly to follow the economic and social changes. This is mostly true for protected vegetable crops that face new stakes such as limiting use of chemical products to avoid pesticide residues in the food products and in ground water, while avoiding any visual symptom of pests and diseases to agree with marketing-firm requirements.

The aim of this project is to build sustainable cropping systems for protected vegetable production by using alternative techniques such as diversified crop sequences, thermal disinfection or organic manure and optimizing their combination to enhance the natural regulatory processes in the soil. This goal cannot be reached using the sole scientific knowledge since systemic knowledge on that subject is very poor. However, a wealth of information and knowledge is available from local practitioners (farmers and technical advisors), especially on the interactions between techniques, environment and the expression of pathogens' activity on the plants.

## **Methodology**

Our approach relies on the hypothesis that it is possible to build new agronomical knowledge based on a synergy between empirical knowledge of producers and scientific knowledge. It develops along four steps.

The first step has been to design a uniform representation frame for scientific and empirical knowledge that also allows easy extraction of targeted pieces. This frame, designed as a database is filled with information from literature articles and from on-farm surveys. In both cases, the aim is to organise information in order to analyse the effects of each cropping technique on the evolution of soil-born pathogens, taking into account the pedoclimatic conditions in which the technique has been applied and the whole cropping system at hand.

The second step is to use this framework to extract all available information related to a given practice, analyse this knowledge by clarifying the *experimental conditions* and determine the main effects explaining variations in the expression of this knowledge. Experimental conditions both refer to its common scientific meaning and to the account by the grower of the conditions in which his observations are made. We checked the accurateness of the growers' observations by measurements of pathogen development in soil and damages on plants, on some selected greenhouses. For example, the analysis of the relation between the use of grafted plants and the damages due to nematodes (*meloidogyne sp.*) show that grafting is only efficient for low to moderate soil infestation levels but does not allow sufficient protection for high infestation levels. Similar analyses have been conducted on the use of nematicide *oil-cakes (neem or castor bean)*. Methods of AI, like evidence theory or argumentation framework shall be tested on these data. During this second step, the growers are regularly informed of the results of the analysis and the reformulation of their knowledge. This confrontation is to be constructive, both to improve the growers' knowledge and to prevent the creation of a gap between them and the scientists.

The third step will be triggered when no conclusion can be drawn on the effect of one practice on the development of pathogens, because there are too many contradictions between growers and/or with scientific current knowledge. Then, group discussion between growers and advisers will be conducted to get a more experienced insight and to attempt to identify the roots of the contradiction. If necessary, experimental plots will be designed with the growers to conduct a focussed study of the problem.

The last step is to build a dynamic model of the interactions between cropping techniques, biological processes and environmental conditions to simulate the evolution of soil born pathogens in given cropping systems. This model will be used, with growers and advisers, in an iterative process of design, evaluation by the model and improvement of new cropping systems (Navarrete et al., 2007).

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## **Effects of strategies for N fertilization and fixed ploughing date on field vegetable cultivation and nitrate leaching**

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## **Introduction**

Nitrogen (N) is the most often applied nutrient to vegetable crops as mineral fertilizer solely or combined with other nutrients to ensure yield quantity and quality. Nitrogen – as nitrate – also is the most likely nutri-

ent to contaminate ground and surface waters. In order to minimize N leaching due to mineralization after turnover in winter, ploughing is not allowed before 1<sup>st</sup> of February in water protection areas in Baden-Württemberg, Germany. For more efficient use N fertilizers are often recommended which release nitrate slowly e.g. ENTEC<sup>26</sup>. To figure out if these two strategies really have benefit for groundwater quality without yield and quality decreases on field vegetable production a perennial experiment was started to collect data on plant and soil parameters.

### ***Material and Methods***

Since November 2003 effects of three fixed ploughing dates (1.12., 1.1., 1.2.) and two strategies using two different N fertilizers (KAS, ENTEC<sup>26</sup>) were investigated on a field plot at the experimental station for horticulture at Stuttgart-Hohenheim, Germany. In that experimental array (35 m x 35 m) two vegetable crops per year were grown from early spring until autumn followed by a cereal catch crop. To investigate effects on growth and development most comprehensive different plants were cultivated by sowing (spinach, radish) and by using transplants (lettuce, broccoli, leek, amaranth, fennel). For using KAS the total amount of nitrogen was split in two rates: applied in the first rate at setting and in the second rate after four weeks of culturing. For ENTEC<sup>26</sup> there was one application at the setting of transplants. N fertilizer were broadcast and incorporated at each treatment. Plant parameters for yield quantity and quality were collected and N in plant material was quantified. Nitrate N was determined from soil samples taken at distinct stages during growing season. Using this data N balances were computed. Nitrate leaching was measured using SIA (Self Integrating Accumulators) placed in depth of 60 cm. With this method it was possible to monitor Nitrate N leaching during season (March to October) as well as during off season (October to March) accumulatively.

### ***Results and Conclusions***

Results of the ongoing field experiment reveal effects of both fixed ploughing date and N fertilization strategy. Fixed ploughing date had influence on the amount of dislocated nitrate N. In two years of four-year-experiment there was less amount of nitrate N dislocated into the subsoil in the plots which were ploughed on 1.2. In this experiment array this effect emerged after two years of intensive vegetable production. As expected and also mentioned in literature late ploughing date (1.2.) was reason for lower yield quantity of vegetable crops in several years and especially by culturing vegetables in early spring period. The N fertilizer strategy had influence on yield quantity when culturing vegetable crops like broccoli or spinach. For these plants higher yields were reached by using the N fertilizer ENTEC<sup>26</sup> with single fertilizer application when setting the transplants.

For effects on the amount of nitrate N dislocated into the subsoil there was no tendency up to now favouring one of the both tested fertilisation strategies in this experiment.

## **Improving mechanical weed management as an alternative for chemical weed control in selected medicinal herbs**

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### ***Introduction***

Using herbicides in medicinal herbs is getting more and more difficult because processing industry for medicinal products demands high raw material quality with low residues of plant protectants. Mechanical and thermal weed regulation provides an alternative to conventional cultivation where just a few herbicides are licensed.



Intention of this project is to compare and improve established strategies as well as new machines and instruments for interrow and intrarow weeding under economic aspects especially by reducing expensive manual work.

This project is supported by the FNR – Fachagentur Nachwachsend Rohstoffe e.V.

### **Materials and methods**

Field trials are carried out since 2007 at Bonn University on three different experimental stations. Besides differences in climate, soil and crop rotation and thereby weedpopulation at the stations, water, nutrition and treatment are under same conditions.

Three important medicinal herbs in Germany *Matricaria recutita* `Bodegold` (chamomile), *Valeriana officinalis* `Artener Züchtung` (valerian) and *Melissa officinalis* `Citra` (lemon balm) are cultivated. They stay representative for blossom, root and leave drug plants where the essential oil is located. The characteristic growth and the different sections of the plant which are used request for adapted weeding technics. Therefore each of the medicinal herbs are treated in six different ways, including one herbicide control and one manual treated control. In addition to collecting data manually also computer imageguided systems are used to show the success of the different weed treatments and their influence on the three crops.

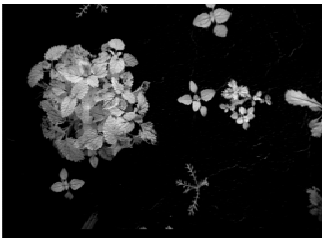


Figure 1: Dominance of culture crop and identification of weeds at a photograph by moving bispectral camera system.

### **Results**

It turned out that the fragmentation of the stations with their typical crop rotation affected weed populations as well as unequal quantity of weeds. Vegetable crop rotation offers the most species and large numbers of different weeds compared to agriculture crop rotation. The different treatments reduced weeds with varying percental results. Some mechanical treatments seem to decrease weeds with a positive lasting effect. The influence by machine use often showed no effects at the crop yields (FM/DM).

The analyses of the imageguided pictures are still in progress. At one hand the pictures demonstrate the tolerance of the crop by dominance after the treatments. On the other hand it is possible to identify different kinds of weed to compare manual control samples. In addition to that outstanding quality analyses of dried raw material may get more important to show the differences between weed treatments especially by contamination of impurities (basically soil and weedparts).

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## Control of *Trialeurodes vaporariorum* with parasitoid wasps and the predatory mite *Amblyseius swirskii* in cut flower gerbera

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Chemical and biological control of whiteflies (*Trialeurodes vaporariorum*, Homoptera: Aleyrodidae) in cut flower gerbera (*Gerbera jamesonii*) often fails in practice. In the frame of a r&d project (Nützlinge II), funded by the German Federal Ministry of Food, Agriculture and Consumer Protection, a strategy should be developed to implement biological control with beneficial organisms, parasitoid wasps as well as predatory mites. Investigations took place from 2003 to 2006 in three nurseries. The most important beneficial used was the parasitoid wasp *Encarsia formosa* (Hymenoptera: Aphelinidae). Because this wasp has not been sufficiently effective in practice, additional studies were conducted in greenhouses at the Federal Biological Research Centre (BBA) in Braunschweig. In these studies other beneficials were included such as the wasp *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) and the predatory mite *Amblyseius swirskii* (Acari: Phytoseiidae).

For the first greenhouse trial in 2006 twelve tents made of curtain cloth serving as separate cabins with 10 gerbera plants each were provided. These plants were infested with 2-3 adult whiteflies each and after two weeks beneficials were introduced as i) 5 *Encarsia* per plant, ii) 75 mites per plant and iii) a combination of both. After 2 weeks the first parasitised larvae of *T. vaporariorum* were found and the whitefly population remained on a low level. *A. swirskii* could keep infestation level very low as well. This study lasted 12 weeks.

A parallel study started in May 2006 and is to be continued. In this study, four greenhouses were provided with 80 young gerbera plants each and these plants were also infested with 2-3 whiteflies. Four different treatments were tested in 2006: i) *Encarsia formosa* and ii) *Eretmocerus eremicus*, both wasps were released with 5 individuals per plant fournightly, iii) *A. swirskii* released two times with 75 mites per plant in bags and iv) a chemically treated greenhouse with six insecticidal sprayings. Each treatment was sufficiently effective. In October 2006 it became obvious that *A. swirskii* had the best, long lasting effect on the whitefly population. In those greenhouses where wasps had been released only two additional treatments with selective insecticides were carried out. In March 2007 all greenhouses were chemically treated before new beneficials were introduced. During 2007 infestation levels of *T. vaporariorum* increased in all greenhouses compared to 2006.

Until October 2007 twenty-three treatments with selective insecticides, often in a combination of two insecticides, were necessary in the chemically treated greenhouse to keep infestation at a sufficiently low level. The insecticides used were pyrethrum, potassium soap, buprofezine, teflubenzuron and azadirachtin A. In the *A. swirskii* greenhouse the situation was different in comparison to 2006. Mites were released in bags every six weeks from the end of March. Starting in July infestation level increased heavily so that six additional treatments with insecticides were necessary until October. We used predominantly pymetrozine. *E. formosa* exerted the best control. Wasps were released ten times and apart from those three treatment in March no additional chemical treatment was necessary. Infestation levels were low until September, when infestation increased as it did in 2006. Hence, even if infestation levels are low during summer wasps should be released during the whole growing period. It may be possible to reduce their number or frequency of release. Definitely, from August onwards wasps have to be introduced into the greenhouses again. A complementary tool to lower whitefly infestations and to support beneficials is the partial defoliation of the plants which we carried out three times in this year.

The wasp *E. eremicus* was not able to keep whitefly infestation on a practical low level. Beginning in May we had to add insecticide treatments. We aborted this part of the investigation, when infestation levels became too high at the end of August. The quality of the wasps (e. g. hatching rate) was sufficient, but given that they are more expensive and less sufficient than *E. formosa*, there was no sense in continuing this

alternative under North German conditions. These results were comparable to those carried out with *E. mundus* to control *Bemisia tabaci* in potted poinsettia plants.

## Competitiveness of Fresh Produce Production in Europe

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Horticultural enterprises in Europe are exposed to higher price as well as non-price competition due to processes such as Globalization and European Integration. There are two major trends in European agribusiness to be recognized: the increasing importance of standards and an increasing concentration at multiple points in agribusiness value chains. Of high importance thereby is the concentration in retailing with demanding global buyers setting requirements. Horticultural enterprises are increasingly tight into sophisticated agribusiness value chains, so that questions like cooperation and coordination along the chain become more important. Determining factors of competitiveness are also increasingly seen to be situated at the regional level, making the spatial organisation of production an important parameter for knowledge transfer, development and diffusion of innovations and trustful cooperation.

The study focuses on the analysis of the coordination of agribusiness value chains and the spatial organisation of horticultural production as the decisive parameters for competitiveness.

The theoretical background of the analysis consists of the Global Value Chain Approach (Gereffi, Humphrey, Schmitz) combined with the ideas of the Cluster-Approaches (Porter, Becattini, Lundvall). The application of Global Value Chain analysis to agribusiness allows the causes and consequences of vertical coordination to be explored further whereas the Cluster Approach permits to analyse effects of regional proximity.

In addition to the exhaustive consultation of the corresponding technical literature the study will be based on the analysis of secondary statistical data and on qualitative empirical research methods, with the objective of isolating variables which are decisive for the explanation of differences in the competitive capability of the consulted horticultural regions.

The study will be conducted in the form of three case studies in different European regions with important, intensive horticultural production. The Palatinate Region (Germany) has been successfully analysed so far based on 20 open interviews with experts of the horticultural sector. The field studies in Italy started in autumn 2007 with expert interviews in the Veneto and Emilia-Romagna region and will be followed by the survey in Murcia, Spain planned to be conducted in spring 2008.

The research design allows to present the first results regarding the role of lead firms in the value chain, the governance of inter-firm relationships, the distribution of incomes and functions between enterprises at different points in the chain and the role of regional cooperation and trust.

# Pathogenicity of entomopathogenic fungi to endophytic leaf miner moth

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## Introduction

Entomopathogenic fungi are microbial antagonists of different insects. The infection process takes place prevailing over the spores on the integument of hosts. Endophytic species are prevented from spores contact in their habitat through the plant tissue. Still in the mines infected individuals are founded regularly. In bio-tests should be investigated, if a leaf application of entomopathogenic fungi can increase the mortality of population.

## Material and Method

Investigations take place with three entomopathogenic fungi *Lecanicillium muscarium* (V 24), *Paecilomyces fumosoroseus* (P 6) and *Beauveria bassiana* (B 412) ( $1 \times 10^7$  up to  $1 \times 10^9$  conidia/ml) and larvae/pupae of chestnut leaf miner moth *Cameraria ohridella* and larvae of black locust leaf miner moth *Phyllonorycter robiniella*.

Biotests were carried out either as petri dish method or as chestnut seedling method in growth chamber. In case of petri dish method application take place outdoors on *Robinia pseudoacacia* trees in summer or on leaf litters of chestnut in the autumn. After different times (14 d respectively 164 d) leaf lets were incubated in a wet chamber at 25°C. Seedlings with eggs were sprayed and putted on in a growth chamber. Dead individuals and such with mouldiness were counted up to 21 days after application seedlings respectively incubation of leaf lets in petri dishes.

## Results

On seedlings the mortality of larvae of *C. ohridella* in mines was nearby 100% in every treatment and appeared on the end of larval development. Only in few cases pupation took place. Each larva respectively pupa was moulded and mycelium of all applied fungi grew out of mines (Kalmus et al. 2007).

Application on leaf litters with pupae of chestnut showed, that selected entomopathogenic fungi are able to infect the pupae in her pupal chamber at low temperature (Richter et al., 2007 a, b). Under this conditions *L. muscarium* reached the most favourable infection results in mines. In semi field trial this fungus reached 40% mouldy pupae in wet chambers after hibernating as well. In the further development it was to seen, that the fungus has an effect on moth also. The moth died earlier than in control and mould appeared quickly. The difference to control was significant. Together pupae and moth reached 60 - 70% mortality.

The larvae and pupae of *Ph. robiniella* were infected from entomopathogenic fungi in her mines also. In all development stages we found mouldy individuals. Most susceptible were the smaller larval stages L1 - L3. At all *L. muscarium* reached in this trial nearby 80% mortality (Wendt et al., 2007).

## Conclusion

In different trials it was proved that entomopathogenic fungi, especially *L. muscarium*, are able to infect larval stages and pupae of leaf miner moth. Infection doesn't come from spores (Richter et al., 2007 b). It is probably that the fungi overcome the epidermis on damaged cells and grow inside of mines. This infection process depends on wetness of leaf. So, it is to explain that on green leaves in early summer more infected individuals were found than in hibernating on high damaged and dry leaves. The results encourage carrying out further experiments in the field.

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## **The use of brassicas to reduce inoculum potential of soil-borne pests in Austrian horticulture**

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Soil-borne fungi form persistent structures such as sclerotia, chlamyospores or oospores which allow them to survive in the soil even though conditions are unfavourable (that is when appropriate plant hosts are missing or when climatic conditions are unfavourable...). Chemical soil sterilisation to control these pathogens is not permitted in Austria; therefore soil-borne diseases are often a limiting factor in horticultural crops. Glucosinolate containing Brassica species are known for the capability of releasing volatile Isothiocyanates. When grown as green manure crops and incorporated into the soil they are an option for biologically-based pest suppression. A research project scheduled 2006-2009 and funded by the Austrian Ministry for Agriculture aims at developing strategies to resolve problems caused by soil-borne pests. The project is in collaboration with local extensionists and growers. One Work package is dedicated to test the implementation of biofumigation in Austrian horticultural crops.

Factors assumed to influence the efficacy of biofumigation, such as the use at different temperatures, the combination of biofumigation with treatments of urea and/or sugar beet molasses and the covering of the pots with a plastic film after initiation biofumigation by water addition, were tested in in-vitro trials. These trials were conducted by using Brassica pellets: BioFence<sup>®</sup> for biofumigation. The target organisms used in the tests were *Verticillium dahliae*, *Fusarium graminearum*, *F. culmorum* and *Stellaria media*. The results showed that combining the biofumigation with a urea and/or molasses treatment had no additional effect on the soil pathogens. The efficacy against *V. dahliae*, *F. culmorum* and *Stellaria media* was high independent from the adjusted temperatures, which was ranging between 08°C and 25 °C. Inoculum of *F. graminearum* in the test soil was not significantly reduced. Again, temperature did not influence efficacy. The covering of the pots with a plastic film significantly improved the efficacy against all target organisms. In the remaining years the project will be focussed on transforming the experiences gained during the in-vitro trials into Austrian horticulture by conducting on-farm trials.

# Influence of the soil, fertilization and plant protection management systems on the apple ecosystem resilience

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A sustainable agriculture in the hilly areas of Romania, disfavored zones due to the natural “hindrances”, implies somehow a rational exploitation of the soil and plant natural resources within the limits of resilience ability of ecosystems providing these resources so that a normal recover of the resources, ecological perenity, ecosystem dynamic balance, consumer welfare and efficiency should be ensured. The general objective of the paper is to establish framework technological systems for the apple culture which can be supported by the ecosystem resilience ability. To achieve the objectives expected, a trifactorial experiment was organized (Idared cv., grafted on M106, 1000 trees/ha), where the specific indicators of the fruit ecosystem resilience ability were observed: the average annual increase of the trunk cross area, average annual biomass of wood resulted from pruning, average annual number of flower buds per tree at the beginning of growing season, number of fruit, mean percentage of fruit picked in autumn (of the flowers per tree in spring), annual average weight per fruit, mean yield of fresh fruit, biennial bearing index, average biomass removed yearly of the tree by pruning and fruit picking, annual average ratio between fruit yield biomass and wood biomass removed by pruning, fruit dry matter percentage, frequency and intensity of disease and pests. The experiment factors were as follows: A) Disease and pests control management of this species, with the graduations: a1 – standard chemical control methods; a2 – chemical control of “low risk”; a3 – biotechnical control methods; a4 – biological control; B) Mineral fertilization schemes, soil applications in all orchard with the graduations: b1 – mineral unfertilized; b2 – NPK fertilization at optimum rates from the experimental standpoint; b3 – NP fertilization, same rates as in b2; b4 – NK fertilization; b5 – PK fertilization; C) Soil management system along the tree row with the following graduation: c1 – tilled cultivation; c2 – herbicides application; c3 – leguminous species crop used as “green” manure; c4 – soil mulch application. The mineral fertilization of soil, chemical control of “low risk”, and leguminous species crop along the tree row had positive effects on the resilience indicators versus the other treatments, although the number of flower buds was the same in all of them, the fruit set percentage was better, the physiological fruit drop was lower and the fruit showed a higher mean weight. Diseases management strategies using biotechnological and biological tool lead to even better response of the apple trees. In biotechnological and biological treatments the attach frequency of *Venturia inaequalis* and *Podosphaera leucotricha* on leaves and shoots ranged between 0.55% and 1.88% face to 4.00 – 5.44% in untreated plots. Also in low risk and biotechnological treatments the frequency of *Erwinia amylovora* attack on shoots was lower (F = 7.66% and 3.44%) compared to untreated plots (F = 65.55%).

# Attempts to save energy in cultivation of bedding plants by a simple alternative temperature strategy

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## Introduction

In order to save energy in horticultural plant production dynamic climate strategies like Intelligrow® have been developed. In these dynamic strategies energy consumption is mainly reduced by lowering the culture temperature in times of low light intensity. Therefore, we tested the effects of a simple alternative temperature strategy - realized by raising the ventilation set point and lowering the heating set point - on growth and quality of different bedding plants. In addition, the impact of carbon dioxide fertilization for the two temperature strategies was analyzed.

## Materials and Methods

The plant species under investigation were *Pelargonium zonale* cultivars, *Argyranthemum frutescens*, *Ageratum houstonianum*, *Sutera* Cultivars, *Bidens ferulifolia*, and *Impatiens* Cultivars New Guinea group. Young plants were potted in week 3 2007 into potting substrate (TKS1) in three replications of 15 plants each. After two weeks they were submitted to the different temperature strategies, and CO<sub>2</sub> fertilization was started in variants 3 and 4 by adjusting the CO<sub>2</sub> concentration to 700 vpm during the light period at closed ventilation. Variants 1 and 3 comprised conventional temperature control of 16 °C heating (HT) and 21 °C ventilation (VT) set points, while in variants 2 and 4 set points of 12 °C heating and 27 °C ventilation were chosen (Table 1).

Table 1: Climatization variants tested

	16 °C heating/21 °C ventilation	12 °C heating/27 °C ventilation
without CO <sub>2</sub>	variant 1	variant 2
with 700 vpm CO <sub>2</sub> during light period	variant 3	variant 4

## Results

The climate in winter and spring 2007 was characterized by high irradiation and mild temperatures. This resulted in average day temperatures (week 5 to 17) of 18.8 and 17.5 °C for variants 1 and 3 and variants 2 and 4, respectively. Relative humidity was higher in variants 2 and 4 (66 %) than under conventional temperature control in variants 1 and 3 (53 %). In variants 1 and 2, CO<sub>2</sub> concentrations of about 400 vpm (average of weeks 5-17) were measured in contrast to 500 and 550 vpm recorded in variants 3 and 4. By the alternative temperature strategy about 30 % of energy were saved as compared to the conventional strategy.

In all variants the plants had reached marketable quality at the end of April. Different effects of the temperature regimes and CO<sub>2</sub> on plant growth and development were observed for the species under investigation. In *Pelargonium zonale* cultivars higher fresh mass and higher numbers of flowers were found in variants with 12/27 °C (HT/VT), whereas there was hardly any effect on plant diameter or plant height. In contrast, *Bidens ferulifolia*, *Ageratum houstonianum*, *Argyranthemum frutescens*, and *Impatiens* cultivars New Guinea group had significantly smaller plant diameters and less fresh mass when cultivated under the alternative temperature strategy. Also in these species temperature strategies did not influence plant height.

Taking the results of our investigations into consideration, CO<sub>2</sub> application can not be recommended.

In conclusion, the alternative temperature strategy resulted in plants in marketable quality in all species tested in the season of 2007 with 30 % less energy consumption. However, the experiments have to be repeated to show the effects of other environmental conditions.

## Microclimate patterns under different shading nets

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### *Introduction*

Covering crops with screen materials is a common practice used to attain a number of agricultural objectives. In recent years, the use of light shading screens above orchards is rapidly expanding because of the possibility of saving water and the increasing demand for high quality produce. Obviously the existence of a screen modifies the exchange of radiation, momentum and mass between crop and atmosphere. Main drawback of these structures was the significant rise in internal temperature caused by low ventilation rates. Screen mesh size and screen colour, had the largest influence on temperature, ventilation and heat transfer properties of a screen. Only few research reports are available on microclimate patterns under screenhouses, due to the difficulty to measure the spatial distribution of the climate parameters. Computational fluid dynamics (CFD) can be an alternative method to substitute the difficulty.

### *Materials and Methods*

In the present study a commercially available computational fluid dynamics (CFD) code was used in order to numerically investigate the influence of different types of screens on microclimate patterns in a screenhouse with a tomato crop. The finite volume method was used in a three dimensional model. Average values from measurements and from the Hellenic National Meteorological Service were used in order to adopt realistic boundary conditions. Special items like the mechanical behaviour of screen or the climatic behavior of the rows of tomato crop are determined using a customization routine included in a used defined file (UDF) and built using the C language. The SIMPLE (Semi-Implicit Method for Pressure-Linked Equations) algorithm was used to couple flow and pressure field and a 2nd order scheme for the discretization of the solution domain.

Based on the meteorological data of July for the region of Volos (Greece), two parametric studies were carried out:

- (a) changing the mesh of the screen material (40, 50 and 60) and
- (b) changing the shading intensity of the screen material (40,50 and 60% shading)).

### *Results and Discussion*

The results show the influence of the properties of screen materials on the distribution of solar radiation, air velocity, air temperature and humidity inside the screenhouse. Increasing shading screen porosity resulted in an increase of air temperature and air humidity and in an decrease of air velocity under the screenhouse. The Such knowledge would be important for the growers in tuning their cultivations techniques (i.e irrigation scheduling) and for screenhouse designers as a basic tool for future improvements of this and similar intensive agricultural systems

## Organic mulch in tree rows as an alternative to herbicide in apple

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The objective of the present work was to examine possible alternatives for herbicides in fruit orchards. The overall aim is to improve both fruit quality and the properties of the top soil, where the root system of the fruit tree is located, in terms of sustainable horticulture.



Four-year-old trees of the popular Russian apple cvs 'Gigulevskoye' and 'Bogatir' on B.62-396 rootstock and 'Krasivoye' on B.57-545 rootstock were grown at the experimental orchard of Michurin Research Institute of Horticulture in Russia. In March 2002, the 1-1.2 m wide apple tree rows were mulched with sawdust, pine bark, conifer wood chips at ca. 25-30 t DM\*ha<sup>-1</sup>, i.e. a rate within the recommended limit of 30 t DM\*ha<sup>-1</sup> \*3 years<sup>-1</sup>, or mowed grass as environmentally-friendly alternatives to herbicide to improve fruit quality and soil properties. Manual weeding or herbicide (4 L glyphosate \* ha<sup>-1</sup> in June) served as controls, while the grass alleyways were regularly mowed.

The herbicide progressively decreased the bank of weeds seeds within the top soil over the 4 years of the experiment to a final reduction of 51% in the last year of the experiment (2005) relative to the year when the trial was established (2002); similarly, the four organic mulches reduced these seeds, but to a much lesser extent, to 72 %.

In any of the four years of the experiment, the herbicide treatment consequently reduced the weed cover in the tree rows to 2-25 % and their dry mass to 3-27 %. The four organic mulches decreased the number of emerged weeds to 5-30 % relative to the control (manual weeding = 100%) and comprised of 25-30 main weed species making organic mulches an environmentally-friendly alternative to herbicide.

The four organic mulches in the tree rows enhanced vegetative growth, measured as length of current shoots, leaf area on the current shoots and tree trunk diameter, and yield more than herbicide did. The organic mulches increased the leaf area on one-year-old shoots by 27 % and the herbicide by 12 %. The organic mulches enhanced growth of current year shoots by 27 % compared with 23 % by the herbicide. The mulches increased trunk diameter by 23 % relative to 13 % with the herbicide. Fruit yields were improved by 35 % the organic mulches and by 17 % by the herbicide and mean fruit mass by 17 % by the mulches and herbicide by 8 % relatively to the manual weeding.

The four organic mulches enhanced the microbiological activity in the top soil compared with both the herbicide treatment and control (manual weeding); apple trees with mulch developed a shallower root system. The four organic mulches improved the physical properties of the soil and its aeration, conserved soil moisture, increased N, P, and K content in the soil compared with the herbicide fallow.

The four organic mulches also influenced the quality of the apple fruit. Fruits from cv. 'Krasivoye' apple trees with mulch accumulated 2.5-fold more phenolic substances in their peel, while the other two cvs 'Bogatir' and 'Gigulevskoye' contained ca. 15 % more phenolic substances than fruit from the herbicide treatment. Ascorbic acid (vitamin C) content of the fruit was also increased by 63 % in apples from 'Gigulevskoye' and by 22 % 'Krasivoye' both from sawdust mulch compared with fruits from trees with herbicide strips of each variety in the 2003 harvest, but without any differences in case of 'Bogatir'. Thus, sawdust mulching of tree rows enhanced the anti-oxidant potential making these apple fruit from trees with organic mulch healthier for the consumer.

Apple fruit from mulched trees assimilated more calcium and phosphorus relative to the herbicide trial, which improved the storability of these fruit. This effect was most pronounced in cv. 'Gigulevskoye', but without significant difference in cvs. 'Bogatir' and 'Krasivoye'. The appearance of the apple fruit from trees with sawdust mulch was more attractive for the consumer due to increased red colouration of the fruit skin in bi-coloured apple cultivars ('Gigulevskoye', 'Bogatir' and 'Krasivoye') due to 2,5-fold increased light reflection at 1 m height above the sawdust.

## **Regulation of fruit set by mechanical flower thinning in fruit crops**

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A new device was developed, which comprises three rotors with adjustable angles and vertically rotating ropes to remove excess flowers in a variety of fruit crops. The objective of the present study was to thin flowers without use of chemicals, in order to improve fruit quality, reduce labour for hand thinning and overcome alternate bearing for sustainable fruit production in horticulture.

Three year experiments were carried out with the new device in Klein-Altendorf near Bonn, Germany. Apple cv. 'Braeburn', 'Elstar' and 'Gala' trees were thinned in 2005-2007 at flower opening (BBCH growth stage 61). Adjacent untreated, hand-thinned or chemically (benzyladenine-) thinned apple trees of the same rows served as controls. Tree branches remained un-damaged by the vertically rotating ropes. The photosynthetically active leaf area was reduced by less than 8% at the fastest rotor speed of 320 rpm, which also gave the best thinning efficacy. Ethylene efflux was monitored as an indicator of stress imposed on the fruit tree and as a potential cause for further fruitlet drop.

The portion of class one fruits >70 mm viz the fruit quality was improved by 10 % without yield loss and by up to 20 % with yield losses of ca. 5-10%, depending on the settings, relative to the untreated control. This was equivalent to fruit mass gains of 10 g without yield loss and of 20 g with 10 % - 20 % yield loss with economic gain in both cases. The environmentally-friendly mechanical thinning required ca. 1 h ha<sup>-1</sup> at a tractor speed of 5 km h<sup>-1</sup> and reduced the subsequent hand thinning by 45% (by 15 h/ha or its cost by 135 €/ha). The new device removed up to one third of both peripheral and central flowers at a cost of less than 100 €/ha and with a negligible risk of over-thinning and without adverse effect on return bloom. The work showed that the newly developed, environmentally-friendly device can overcome alternate bearing, improve fruit quality and substitute chemical thinning using apple as model crop for sustainable horticulture.

## **Assessing the possibility of biomass use on greenhouse heating using GIS**

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### ***Introduction***

GIS's (Geographic Information System's) and precision agriculture applications are almost absent in the protected agriculture sector. In this paper we try to describe necessary steps and a methodological framework for GIS application development for protected cultivations, in Greece and abroad, as well as to underline the necessity of relevant systems. For doing so, we present some results based on an ongoing research in the Greek Prefecture of Magnesia, aiming on using local biomass resources for the substitution of conventional fuels for greenhouse heating.

GIS's are specially designed systems for simultaneous processing of spatial and related attribute data, helpful for monitoring, analyzing and optimally allocating of protected cultivation's related resources. Our research consists of three main steps: (a) the digital acquisition of a base map, mainly based on aerial orthorectified maps provided by the department of aerial photography of the Greek Ministry of Agriculture. On the above mentioned digital images greenhouses were identified and recorded, (b) the field observation, conducted in order to test base map's integrity and accuracy, as well as acquire descriptive data about greenhouses cultivations concerning greenhouse structure, cultivations, equipment, farm management etc. In parallel, newly constructed greenhouses were recorded by exploiting the advantages of GPS (Global Positioning System) technology and (c) Current biomass source data entry in the geographical data base

### ***Material and Methods***

The ARC VIEW-GIS and TATUK-GIS software have been used to elaborate simultaneously both spatial data and related attribute data.

GIS applications for protected cultivations development, will establish a sufficient mechanism for digital maps production, based on queries about the spatial and numerical distribution of the acquired information. Our aim is to underline that modern geographic systems, can prove to be integrated elements for creating updated cartographies.

## ***Results and Discussion***

Accounting for an energy consumption of about 8% for drying (mean moisture content 40%) and 10.5% for harvesting, collection and transportation in a distance of 20 km, the energy produced, theoretically, from the combustion of pruning is about ten folds greater than the energy actually consumed for greenhouse heating. Annual variation of pruning availability is not so important as for olive stone and thus fuel availability problems occurring from one year to the other may be avoided. With geographic information system (GIS)-based analyses, multifarious renewable energy sources can be evaluated according to actual local land uses in order to provide more-integrated and accurate decision-making information for policymakers greenhouse growers. The established model may help localities explore their exploitable resources, and this can possibly be expanded to conduct comprehensive surveys at the national level in order to estimate the entire potential of a country.

## **Criteria-based and value-oriented horticultural and agricultural practice in crop-growing**

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The voluntary introduction of environment management systems – as well as ISO EN 14001 (1996), the ECO-Audit of the EU (Regulation EC 761/2001, EMAS), the social management system SA 8000, and the development of practical criteria for the assessment of companies in view to their ecological and social competence and performance – were the results of the ‘Conference for environment and development’ in Rio de Janeiro in 1992 and the connected and guiding principles of the Rio-declaration within the framework of Agenda 21.

In comparison with already existing systems, and also in regard to adaptability in agricultural enterprises, the Guideline Frankfurt-Hohenheim (FHL) has proved particularly helpful. This is, at present, the most extensive ‘criteria catalogue’ for management according to ethical-ecological standards.

The voluntary integration of environment management systems, and the use of ethical-ecological criteria in the production, trade and service industries is gaining increasing significance in manufacturing. On a voluntary basis, internationally rating agencies and certification organisations assess ethical-ecological performance and supervise the adherence to principles and criteria. In the meantime, the ethical-ecological assessment model became established in economic practice, and is increasingly used by internationally operating companies which employ ranking methods. Thus, on the basis of epistemological considerations (together with practical possible applications) they developed ‘ethical-ecological criteria’. FHL focuses on entire society-related company results, by taking the cultural compatibility of actions into consideration, as well as considering environmental and social compatibility. Apart from ecological and social dimensions, the cultural dimension was also introduced to the discussion on ethical conduct. Ecologically and socially compatible actions are always related to economic compatibility of commercial entrepreneurial activities. Our examples (Co-operative Pahren and Flor Verde Programme) fit ideally into this scheme. International discussions are expanding towards including voluntary assessment and evaluation of social and, lately, cultural standards. The aim is an evaluation of the extensive social performance of a company, according to ethical-ecological criteria.

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## Use of industrially made Growing Media in Horticulture - a Survey

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### *Introduction*

Most of the horticultural enterprises in Germany use industrially made growing media for the cultivation of plants. Knowledge about these materials and their applications by plant growers with indirect sale was investigated with a written anonymous survey. Besides the survey gave an interesting insight into the annual turnover, staff structure, training and age of the owners as well as the dimension of the greenhouse production area.

### *Materials & Methods*

A questionnaire was sent to around 1,000 companies of German floriculture plant growers in the autumn of 2006. It was divided into two parts: standardized questions about operating data of the companies and special questions concerning clay, peat-substitute materials and important characteristics of different growing media.

The response rate was 25.3 % resulting in 253 filled-in questionnaires.

### *Results*

93.2 % of the respondents with indirect sales of plants use industrially made growing media. Only 6.8 % of the owners mix growing media on their own. The mentioned average production area is about 18,217 m<sup>2</sup> per company. The total costs for industrially made growing media accumulate in average to 25,332 € per annum.

The following table shows the annual turnover structure of plant producers with indirect sales:

annual turnover in €	% plant growers
< 100,000	6.8
100,000 bis 250,000	15.1
250,000 bis 500,000	24.7
500,000 bis 1,000,000	26.0
> 1,000,000	27.4

Differences could also be seen in the training of the owners: 68.9 % are master craftsmen, 17.6 % have a primary degree, 8.1 % are gardeners and 5.4 % are technicians.

It could be shown, that 71.8 % of the growers have changed their growing media supplier during recent years. The main reasons (more responses possible) were high prices (50.0 %), a wrong structure of the industrially made growing media (25.0 %), bad cultivation results of plants (23.3 %) and problems with the substrate delivery service (24.7 %). 13.3 % of the plant growers mentioned weeds in the growing media as a problem in spite of raw material control by the suppliers. Packing (1.7 %), fungal infections of the media

(3.3 %) or wrong raw material (5.0 %) seem not to be relevant for changing the distributor of growing media.

Knowledge of growers concerning the use of clay as a constituent of industrially made growing media was studied. For example, the used measuring unit for clay content in growing media depends on the training of the owner. So master craftsmen (75.6 %) and graduate engineers (75.0 %) mostly use vol.-%, while by gardeners kg/m<sup>3</sup> (66.7 %) is most commonly used.

A comparison of different characteristics of peat/clay mixtures with pure peat growing media showed that special attributes such as buffering of nutrients (97.3 %), water capacity (89.5 %), pH-buffering (79.5 %), water uptake after drying out (73.2 %) or compact growth of plants (67.2 %) are expected to be positively influenced with the addition of clay. On the other hand some plant growers expect peat/clay mixes to be high priced (29.6 %) or to have problems with shrinkage (22.7 %) In this context it is interesting to know, that 38.1 % of the growers vary the clay content depending on the cultivar.

## **Treatments with effective microorganisms on apple flowers and influence of fungicides on microflora of effective microorganisms**

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EM (effective microorganisms) is a mixture of different bacteria and fungi, which used as a nutrient agent has a positive effect on growth and yield of plants. In organic apple growing it is also used for treatments on leaves. But so far it has not been cleared, if fungicides (copper, sulphur) that are used in organic apple growing could have a negative influence on growing of EM typical microflora. Another question was to find out, if EM treatments during blossom could have a fruit thinning effect on apple flowers. To solve these questions two trials were established in 2005.

The tested culture of microorganisms was EM A („effective microorganisms activated“). The following fungicides used in organic apple growing were tested against several bacterial cultures and spread on adequate culture media (MRS agar for lactobacilli, anaerobic, 37°C, 48 h; Yeast extract agar for *Rhodopseudomonas*, day light lamp, aerobic, 28 °C, 72 h; YGC agar for yeasts, aerobic, 25 °C, 72 h): Cuprofor (50 % copper) 0,1%, Cuprofor 0,02%, soluble sulphur 0,3%, lime sulphur 1%, coco soap 0,8%. The antagonistic activity was examined by agar spot test (two variations) and well diffusion test (with and without pre-incubation). Zones of inhibition were evaluated visually. Lime sulphur (1%) exhibited weak inhibitory activity on YGC agar against the yeasts in EM A when performing the agar spot test (variation a) and the well diffusion test without pre-incubation. No inhibition was observed with all other fungicides irrespective of culture media or antagonism test.

The flower spraying trials were made in a 15 year old apple orchard, cultivar ‚Golden Delicious‘ (spindle, M9) in the research orchard of the institute in Vienna. For each variant four branches on 8 trees with a high amount of flowers were marked at the beginning of blossom and flower buds were counted (minimum 100 per tree). The trees were treated the 2.5. 2005 at full blossom with a knapsack sprayer dripping wet. On 2nd and 27th June (before and after June fall) the remaining fruits on the branches were counted. No hand thinning was done. At harvest (on 21st October 2005) the number and the weight of all harvested fruits on the marked branches was registered. Statistical analysis of data was made with spss 11.0 (Anova with SNK – test,  $\alpha = 0,05$ ). The trees treated with formic acid showed lesions on leaves and flowers maybe caused by low pH-value of the spraying; in all variants treated with EM no leaf damages could be found. Concerning the thinning effect on flowers and fruits, on all variants treated with EM no difference to control could be found, whereas treatments with formic acid especially when higher concentrated, led to a statistically lower fruit set compared to untreated control during summer and at harvest.

## Fertilizers with nitrification inhibitors reduce deleterious effects of salt stress and over-fertilization in horticultural crops

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Nitrification inhibitors slow down the microbial process of nitrification. Hence ammonium-based fertilizers containing nitrification inhibitors are also called ammonium-stabilized fertilizers, which are able to provide a higher ammonium to nitrate ratio to plants for a longer period of time than equivalent fertilizers without nitrification inhibitor. Known positive effects of prolonged ammonium abundance in soil systems due to a nitrification inhibitor may comprise a better nitrogen supply owing to less  $\text{NO}_3$  leaching and less volatile N losses ( $\text{N}_2\text{O}$ ,  $\text{N}_2$ ) due to both reduced nitrification and denitrification under given climatic conditions. Physiological effects of a prolonged ammonium abundance and hence a higher proportion of ammonium uptake by plants include among others a relative acidification of the rhizosphere with consequences in a better availability of calcium phosphates, Fe, Mn, Zn, Cu or Si to plants. The preferential uptake of ammonium over nitrate by plants, if spatial availability is not a limiting factor, is also associated with a lower energy demand of ammonium versus nitrate uptake.

Deleterious salt effects to plants may be due to osmotic effects, direct toxic concentration of ions as *e.g.*  $\text{Na}^+$  or  $\text{Cl}^-$  and/or due to induced nutrient deficiencies.

In the present studies it could be demonstrated that nitrification inhibitor-containing fertilizers were much safer in usage than the equivalent fertilizer without inhibitor. An over-fertilization up to five times the recommended rates resulted in pot experiments with onions, iceberg lettuce or strawberries in yields that were from 60% up to 8 times higher than without nitrification inhibitors under otherwise identical growth conditions. Under these conditions the new nitrification inhibitor DMPP (ENTE<sup>®</sup>) was also more plant compatible than the old nitrification inhibitor DCD which increased yields somewhat less than ENTEC fertilizers. Vegetables over-fertilized with DCD-containing fertilizers showed necrotic lesions on margins of older leaves whereas no such symptoms were detectable with DMPP. If vegetables were grown in NaCl affected soil, ammonium based fertilizers with DMPP also proved to be superior to fertilizers without DMPP in model trials. From physiological effects as *e.g.* energy charge up to effects on ion uptake this may also be due to the spatial distribution of ions in the soil profile. The deeper rooting zone of vegetables fertilized with ammonium-stabilized fertilizers showed a somewhat lower electrical conductivity than nitrate-based fertilizers or ammonium-based fertilizers without nitrification inhibitor after a certain period of time. For less obvious reasons in many but not in all cases also the germination rate of crops in salt affected substrates was higher if the nitrification inhibitor DMPP was included in the substrate.

On farmers fields salt affected soils are often alkaline. The acidifying effect in the rhizosphere of an ammonium nutrition of plants using an ammonium-based fertilizer with a nitrification inhibitor may above all further contribute to a better plant performance.

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# Laser light beams for open field weed control in horticulture

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The objective of this research is the investigation of the effect of laser treatment on young plants. Lasers are to be used as a tool for weed control. Such a technique offers a means of environmentally friendly production by reducing the application of herbicides and it decreases manpower and energy used for manual or thermal weed control, especially in cultivation of vegetables in ecological farming.

In this investigation stationary laser systems (diode: waveband 940 nm; CO<sub>2</sub>: waveband 10600 nm) are tested. Comparing to existing publications new factors were examined, such as the long wavelength of the carbon dioxide laser, a large spot diameter - which should simplify the positioning of the laser beam in relation to the plant - and the distribution of the uncoupled energy, as well as the use of dicotyledonous (*Nicotiana tabacum*) and monocotyledonous plants (*Echinochloa crus-galli*), each within three growth stages (see figure 1). Within the test procedure, the fresh biomass of every plant is measured for four replications after the laser treatment within a given spot of 6 mm to the centre of the plants and compared to non-treated plants. The effects of the treatments follow logistic dose-response-curves by rising energy doses [J/mm<sup>2</sup>]. The growing stage of the plants seems to be an important factor: the larger the plant, the more energy is needed. The absorption of the diode laser irradiation in the plant material is relatively weak (7 %, respectively 40 %), while the carbon dioxide laser irradiation is absorbed at a high level up to 95 %. Between the species, the observed differences are given due to different absorption spectra and growing principles. To handle *Echinochloa crus-galli*, more energy is needed than for *Nicotiana tabacum*. The necessary laser power for carbon dioxide laser application is given in figure 1. The results for diode laser application for *Echinochloa* is similar, but for *Nicotiana* the necessary beam output power and the lethal dose is up to 4 times higher.

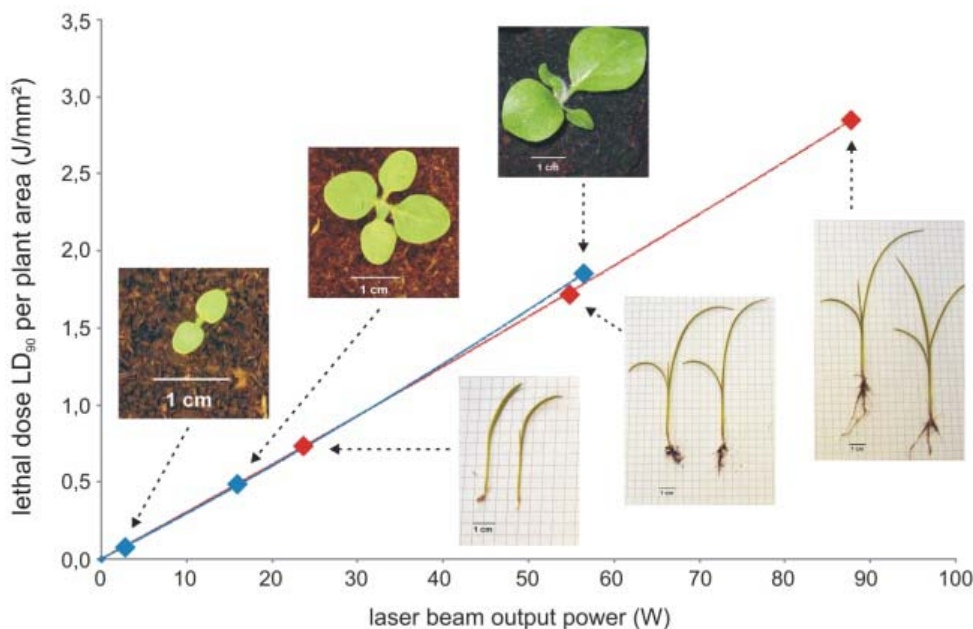


Figure 1: Growth stages and resulted relationship between lethal doses and output power for carbon dioxide laser application.

It can be concluded that both systems can work with economical energy doses (maximum connected load including cooling: 500 W) to delay and to stop the plant growth. This is only possible until the true-two-leaves stage is reached, which is also the point when an alternative herbicidal application is more or less at the edge of good results. The investigations of the underlying processes within the plant material, as well as many differences between different plant species need further research and development.

## **Characterization of physiological responses in strawberry to drought stress**

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Drought stress is one of the most important environmental factors that limit plant production. Plants can respond and adapt to drought stress by altering their cellular metabolism and invoking various defence mechanisms. Identification of these responses along with the genetic and molecular controls, help breeders to improve new varieties with a higher resistance level. Overall aim of our research is a better understanding of molecular and physiological mechanisms conferring drought tolerance in strawberry this to support breeding strategies.

In a first experiment, cold stored strawberry plants cv. Elsanta were grown in the greenhouse at 18-20/12-14°C day-night temperature. After an adaptation phase of nine weeks, drought stress was imposed by withholding irrigation to the plants while control plants remained well watered.

Under increasing drought stress leaf water potential changed from -1.00 MPa at the start to -1.63 MPa at the end of the experiment (near wilting point of the leaves). At 6 intervals during the experiment (7 weeks), leaf samples were taken for analysis of physiological parameters in drought stress induced and control plants. Results indicated that with the progress of drought stress leaf protein, proline content and total antioxidant capacity (TAC) increased and the activity of some stress related enzymes such as catalase (CAT) and ascorbate peroxidase (APX) decreased. Activity of superoxide dismutase (SOD) increased for a moderate drought stress, but decreased with drought progressing.

Morphological traits such as leaf area, dry and fresh biomass were lower in the stress treatment compared to the control. The results indicate that the main plant defence responses to drought stress in strawberry 'Elsanta' are the increasing of osmolytes such as proline and changes in antioxidant defence mechanisms such as the increasing of total antioxidant capacity and changes in the activity of stress enzymes.

## **Comparison of customer satisfaction in different types of distribution channels of horticultural products in Austria and Bavaria**

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### ***Introduction***

An enterprise can only be commercially successful if it succeeds in satisfying customers' needs thus resulting in repeated sales for its products or continuous demand for its services. An effective and efficient customer satisfaction management programme can be regarded as an important measure for maintaining existing customers and for winning new customers.

### ***Material and Methods***

Two diploma thesis carried out in 2006 at the University of Applied Sciences of Weihenstephan identified the needs, behaviour and motives of customers at different types of selling places for horticultural products. In addition differences between the Austrian and Bavarian customer structures in retail trade with horticultural products were extracted. A total of more than 1,500 customers of 25 different purchase outlets for horticultural products were asked about their purchase behaviours and their satisfaction with the performance of individual retail businesses in the two surveys. In order to get an adaptable analysis of the satisfaction rate, several categories of relevant buying criteria have to be evaluated by the customers. The criteria are representing the assortment and price policy of the retailers as well as the interaction with the customer and the



design of the location. Since the scales of the two surveys were adjusted a direct comparison of the data sets can be accomplished. Furthermore, by including the assessment of the individual importance of the upraised buying criteria it is possible to calculate and compare the 'global satisfaction' in the different retailing shops.

### **Results**

Managing customer satisfaction in horticultural firms can only be effective if meaningful retail performance indicators can be determined. Customer satisfaction significantly differs between garden centres, horticultural retail stores and DIY-stores and also between the Austrian and Bavarian customers of horticultural retail shops.

In general the level of the satisfaction values of the different categories in the Austrian traditional gardening stores is higher than in the Bavarian comparable enterprises except in the categories 'Plant quality' and 'Special offers'. The evaluation of the traditional gardening retail stores is evidently better compared to DIY-stores and garden centres in both countries, especially in the categories 'Plant quality', 'Assortment', 'Quality of consulting' and the location design criteria. Only for the attribute 'Special offering and activities' there are advantages for the DIY-stores and garden centres. Concerning the attributes 'Advertising and information' and the 'Price-performance-ration' the satisfaction levels are quite equal - two categories, which are regarded in general as advantages for the non-traditional retail sector.

The offsetting of all requested satisfaction values linked with their stated importance levels to a 'global satisfaction indicator' shows a high standard for all analysed retail types but also the discrepancy between the different distribution channels in the two countries. The value of 1.685 for the Austrian horticultural retail shops (for 1.000 is 'very satisfied' and 4.000 is 'unsatisfied') shows a marginal difference to the Bavarian traditional retailers (1.695). For the garden centres and DIY-stores the values for the 'global satisfaction' decrease considerably (1.816 and 1.838).

### **Conclusion**

The customers of traditional horticulture retail stores were much more satisfied both in Austria and in Bavaria than the customers of DIY-stores and garden centres. Only 'Special offers and events' got a higher satisfaction rate in DIY-store and garden centres compared to traditional gardening retail stores. The lead of the gardening retail stores particularly results from excellent consumer assessments of the plant quality and a better consultation of the employees. With the help of a calculated 'global satisfaction index' the value at the customers could be pointed out for all enterprises which have taken part in the survey.

## **Clustering of groups of regular customers in horticultural retail stores in Austria**

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### **Introduction**

In recent years there were significant changes in the distribution structures in horticulture retail trade. Thereby the decrease of traditional specialized retail gardening traders in favour of the chain stores/garden centers and the DIY sector forces the gardners to orient their service and their marketing strategies more closely to their customers. A vague knowledge about the needs, wishes and motives of the own customers and their shopping behavior permits only a "grey-in-grey"- marketing, which does not take into account the specific characteristics of differing customer groups in horticultural retail stores.

### **Material and Methods**

The basis of this study forms a diploma thesis, in which a written survey of regular customers of 18 horticultural retail stores in Austria was performed in autumn 2006. The respondents were mainly identified out of the companies' customer databases and can so be considered as regular customers who have knowledge and

reference to the dedicated companies. After transferring the data in applicable variables and scales a hierarchical cluster analysis could be implemented. With approximately 800 data records, customer groups were clustered taking into account their shopping behaviour, certain attitudes to the purchasing of plants and the shopping place and socio-demographic characteristics. This classification of the customers allows a quick and concise overview of differences in their relevant purchasing criteria like the purchase frequency, the expenditure in the stores or the average age of the customer groups.

### **Results**

According to the fixed procedure the hierarchical cluster analysis method finally differentiated seven characteristic customer groups. The consumer clusters range from "Loyal regular customers" (7.6%), who visits its gardening retail store weekly and is interested in the entire assortment, up to the "Pure gift buyer" (6.4%), who buy only occasionally a bunch flowers as a gift. With 12.4% the "Good deal hunters" are another important customer group, asking strongly on special offers, whereby the consulting service is not in the centre of interest of this group. In contrary, the "Purchase enjoyer" (20.8%) likes the purchasing activity as its own, while the range of the assortment can be strongly limited for this group. A special cluster constitute the "Spring-time vegetater" with 9.9% of the customers who have their focus on plants for bed and balcony and have therefore only a low frequency in purchasing plants. The "Must-acquirer" (13.8%) apply little time and interest in plants but have to buy them because of the high dues of possessing presentable houses and gardens. Nearly one-third of all respondents are "Loyal everything-purchaser" who are interested in the whole assortment of the horticultural retail shops and are willing to bridge longer distances to buy the plants in their favourite store.

By putting all information in portfolio schemes, the two clusters "True regular customer" and "Loyal everything-purchaser" can be considered as important and well-funded customer groups for the traditional horticultural retail stores. They predominantly identify themselves as regular customers of the store, have a superior buying frequency and represent with an average age of around 52 years customers which are not too old to offer further on a major purchase potential in the future.

### **Conclusion**

By the identified differing characteristics between the groups (like e.g. monetary level of the annual expenditures for gardening products, the service requirement at the point of sale or the wishes concerning the assortment), the enterprises are able to set up a catalogue of measures to bind individual groups of clients more strongly to the gardening retail store. By knowing the purchase preferences and their individual demand on the assortment and the point-of-sale, direct marketing measures can be applied exclusively on each group and can ensure an effective and successful customer relationship.

## **Growth stage and conditions affect sorbitol: sucrose ratio in peach source and sink organs**

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### **Introduction**

Polyols are hypothesized to have a wide range of possible functions, such as enhancement of photosynthesis, storage and translocation of carbon, osmotic adjustment, cold hardiness, stress tolerance, and protection of enzymatic systems and membranes (Loescher, 1987). Along with sucrose, sorbitol represents the major photosynthetic product and the main form of translocated carbon in peach. Once synthesized in the mature leaf, sorbitol is either translocated or stored since the main catabolic pathway (oxidation to fructose by NAD-dependent sorbitol dehydrogenase) is not expressed in the mature leaf (Merlo and Passera, 1991). On the contrary, the ability to synthesize and accumulate sorbitol and sucrose increases with leaf age or distance from the apex until full maturity. In peach leaves, sorbitol content can be from 1.3 to 4.2 times that of su-

crose (Escobar-Gutiérrez and Gaudillère, 1994). Moing et al. (1997) detected a maximum sorbitol:sucrose molar ratio (SOR:SUC) of about 7:1 in the leaf main vein and 4:1 in the phloem sap. Results of a number of experiments are reported here to elucidate whether peach trees partition different amounts of fixed carbon into sorbitol or sucrose in response to varying resource levels, and ultimately whether the ability to alter SOR:SUC may represent an advantage for adapting to different growth/stress conditions.

### **Materials and Methods**

Sorbitol and soluble carbohydrate levels were determined by gas chromatography in a variety of tissues (mature leaf blade, main vein, and petiole, shoot apex, shoot internodes, bark, fruit peduncle, flesh, and non-lignified pit, seed, and roots at various developmental stages) of different peach genotypes. Sorbitol and carbohydrate levels were also measured after imposing water deficit, tree crop load manipulations, source-sink manipulations at the shoot level, phloem stream disruption by girdling, and at different stages of fruit development.

### **Results and Conclusions**

Data from 'Encore' peach show that SOR:SUC is highest (3:1) in the leaf blade, remains fairly constant from the main vein all the way to the fruit peduncle (2:1), and drops sharply in the fruit flesh and the seed. In the internodes of a growing shoot, SOR:SUC increases with distance from the apex, while SOR:SUC of root apices (3:1) is not associated with tissue development. In fruit flesh, SOR:SUC is highest (1:1) at cell division, decreases sharply (0.2:1) at pit hardening, and remains low until maturation stage, due to greater accumulation of sucrose than sorbitol. In the seed, SOR:SUC is generally low and decreases from cell division (0.3:1) to pit hardening (0.03:1) and cell expansion (0.01:1); this trend is mainly attributable to very active sorbitol degradation (Lo Bianco and Rieger, 2002). Leaf SOR:SUC increases in response to fruit removal and water deficit, due to sorbitol rather than sucrose accumulation. Low crop loads (fruit thinning) lower SOR:SUC in the flesh but not in the seed, due mainly to changes in sucrose content. Flesh SOR:SUC is also inversely related to changes in shoot leaf:fruit ratio imposed by defoliation, and girdling reduces significantly SOR:SUC of the fruit located downstream. Both those two trends are associated with reductions in sorbitol, but not in sucrose content. Overall, our data show that partitioning of carbon into sucrose is generally favored under optimal non-limiting resource and assimilate availability, whereas partitioning into sorbitol is preferred under unfavorable conditions. The resulting changes in SOR:SUC may provide some adaptive response to varying growth and stress conditions generally associated to photoassimilate availability in peach.

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# Vision based plant recognition under overlapping situations in horticulture

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Much research has been undertaken in the field of machine vision in order to identify objects based on their color, shape and texture. Overlapping objects make the task of recognition even more difficult. Therefore, the automation of several tasks, like sorting, robotic harvesting or automated weed control is very challenging.

The goal of this research is to identify young plants under overlapping situations such as those shown in Figure 1(a). This goal should be reached by combining a Hough-Transformation to recognize possible single elliptic leaves with a model based algorithm to detect possible plants, which are created by combining the detected leaves.

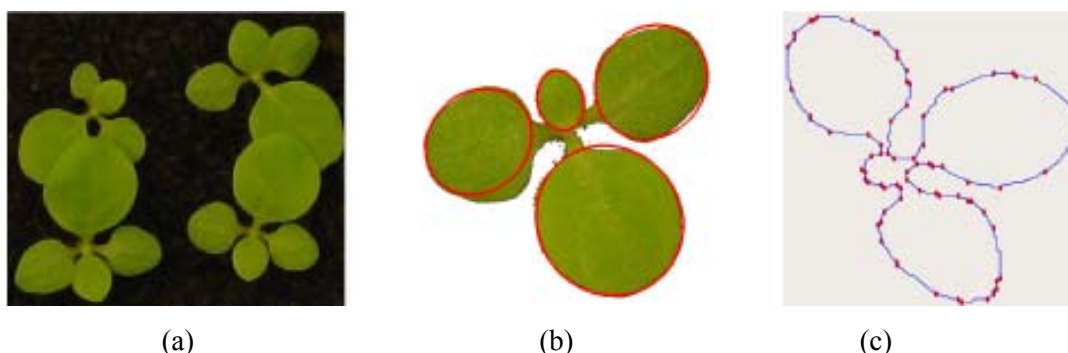


Figure 1: (a) Example of plants with elliptic leaves overlapping each other. (b) Ellipse detection with the Hough-Transformation. (c) Placing landmarks over a plant shape.

In order to evaluate the shapes of the exemplar plant *Nicotiana tabacum*, a detection of the possible leaves (ellipses) was carried out. Considering that the tobacco leaves have, to a certain degree, an elliptical shape, the Randomized Hough Transform (RHT) (McLaughlin 1998) is extended, adapted and used to separate the leaves that overlap and the ones that are occluded, see Figure 1(b). Due to the several real and false detections of the RHT, a cluster analysis (CA) was necessary in order to identify the ellipses that best superimpose the shapes.

Using Active shape modeling (ASM) (Cootes and Taylor 2001) with landmark detection, the closest leaves are grouped together in order to form the possible plants, see Figure 1(c). The necessary land marking was carried out by a special recursive search, as described by Apiwat et al. (2003).

The combination of these 3 methods (RHT  $\rightarrow$  CA  $\rightarrow$  ASM) is able to overcome the general overlapping problem in plant image processing for sorting, automation and biotechnology.

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## Stabilisation of the colour of red fruit juices by admixture with apple phyto-components

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The colour of red juices linked to presence of anthocyanins is highly susceptible to evolution, mainly browning. Literature reported that this colour may be better retained in presence of phenolic acids by formation of more stable addition products, with two mechanisms involved, namely co-pigmentation and formation of pyranoanthocyanin. Within the European Project "ISAFRUIT", we intended to try this hypothesis for black currant nectars, and started by evaluation the impact of addition of phenolic acids on anthocyanin and anthocyanidin solutions, then of apple extracts rich in phenolic acids on a black currant extract.

Delphinidine and its glucoside myrtilline at 0.1 mM were put in contact with caffeoylquinic acid (CQA, chlorogenic acid) at 10 mM or ferulic acid (FeA) at 6mM. In a second experiment CQA, ferulic acid and a CQA-rich extract from Val-de-Vire were added in variable concentrations to a black currant pigment preparation, which was a gift from CHR Hansen. They were incubated at 37°C in 0.02 M citrate buffers at pH 1.5, 3 and 4.5. The colour was followed by the absorbance at 540 nm and the composition of the solutions by HPLC-DAD.

Deglycosylation of anthocyanins leads to loss of colour (and molecule) stability. It is therefore of major importance that enzyme preparations used in juice extraction be devoid of glycosidases. Addition of phenolic acids in an anthocyanin solution increases and stabilises the colour; this is actually accompanied by an acceleration of the disappearance of the native molecules, and formation of coloured adducts with the pyranoanthocyanin structure .

The glycosyl residue of anthocyanins is necessary for colour stabilisation, which appears due to formation of new pigments. Caffeoylquinic acid appears more efficient than ferulic acid, and a CQA-rich extract from apple also gave good stabilisation of the colour of black currant extract.

## Characteristics of early Ripening Strawberry Cultivars as tolerant alternatives to 'Elsanta' in *Verticillium* infested soils

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Soil-borne pathogens, above all *Verticillium sp.*, cause plant losses and yield decreases in many Austrian strawberry regions. As part of a research project 13 cultivars were planted in 2005 at 11 sites on 9 farms in 5 different Austrian regions. The aim was to test new cultivars for tolerance to soil-borne pathogens and leaf/fruit diseases, high yield, winter hardness and good fruit quality, to serve as alternative to the highly susceptible cultivar 'Elsanta', regarding soil-borne diseases.

Following parameters were assessed in 2006 and 2007 on 2 sites: marketable yield, percentage of different categories of unmarketable fruits, vigorousness of plants, incidence of leaf diseases and of the blossom weevil. Infestation with *Verticillium dahliae* was assessed in 2005 and 2006 on 7 sites. In 2006 fruit characteristics and consumer acceptance were studied.

'Elsanta' and 'Sonata' showed the highest infestation with *Verticillium dahliae*. All other cultivars were less susceptible. 'Salsa' and 'Daroyal' showed the lowest infestation and were significantly less infected than 'Elsanta'. 'Dora', 'Eva', 'Queen Elisa' and 'Daroyal' recorded significantly higher losses by the blossom weevil than 'Alice'. 'Alba' and 'Divine' were the earliest cultivars in ripening time, 'Clery', 'Daroyal', 'Queen Elisa', 'Darselect', 'Dora' and 'Eva' started with the first picking nearly the same time as 'Elsanta', all other cultivars started later. Highest marketable yield per plant had the late ripening cultivars, particularly 'Salsa' and 'Sonata'. Of all early ripening cultivars tested, 'Elsanta' showed the highest productivity, followed by 'Alba', 'Darselect', 'Daroyal' and 'Eva'. 'Salsa', 'Elsanta' and 'Divine' were rather tolerant to *Botrytis cinerea* whereas 'Record' and 'Queen Elisa' were heavily infested. Many gummy fruits (fruits that couldn't ripen properly because of water stress and therefore had a gummy texture and bad taste) were harvested from 'Salsa', 'Alice', 'Elsanta', 'Sonata', 'Eva' and 'Divine'. In comparison, 'Alba' and 'Daroyal' produced very few gummy fruits.

A significantly higher average fruit weight than 'Elsanta' showed 'Record', 'Queen Elisa', 'Alba' and 'Dora'. 'Divine', 'Darselect' and 'Clery' had rather small fruits. Some new cultivars (e.g. 'Eva', 'Queen Elisa' and 'Alba') showed a much higher fruit firmness compared to 'Elsanta'. 'Eva' and 'Clery' had a very high content of ascorbic acid. While storing 10 days at 2°C, fruits of 'Alice' and 'Queen Elisa' showed high shelf life qualities in contrast to fruits of 'Daroyal', which are not suitable for storage.

## Seasonal changes in photosynthetic activity and carbohydrate content in leaves of three fig cultivars (*Ficus carica* L.)

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### Introduction

The fig is an important fruit tree for Europe since its production averages 166 million tons per year and its fruit have a high nutritional value. Its relative drought and salinity resistance are also attractive characteristics. The purpose of this study was to investigate the photosynthetic capacity of the Kalamon cv. (one of the most important in Greece and Europe) in comparison with two European cultivars, Fracasana and Mission. Mission and Kalamon produce one crop per year while Fracasana produces two.

### Materials and methods

The study was performed at the orchard of the Agricultural University of Athens. Four trees per cultivar, 15- to 20-years-old, were randomly selected with uniformity in tree size, shoot length and diameter. Net photosynthesis (Pn) and stomatal conductance (gs) were measured using a portable closed infra-red gas analysis (IRGA) system (LI-COR, model LI-6200). Sugars and starch in leaves were also measured to evaluate the photosynthetic capacity of the three cultivars during leaf and fruit development. Soluble sugars were analysed using HPLC and starch by an enzymatic method. Additionally, total chlorophyll (a+b) and nitrogen concentrations, as well as specific leaf weight (SLW), were measured. Measurements were performed from leaf emergence through the period of leaf expansion and fruit development, at 10- to 15-day intervals.

### Results and discussion

Pn rates increased rapidly from leaf emergence reaching its maximum value for all cultivars at full leaf expansion, then decreasing with small fluctuations, probably due to temperature changes. The Kalamon cv. generally had significantly higher Pn than the other two cultivars, although the Fracasana cv. carried fruit (first crop) from mid April. Maximum Pn was 18.2  $\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  for the Kalamon c.v. and 12.9  $\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  for the other two cultivars. Similar results were found for gs. Nitrogen and total chlorophyll (a+b) concentrations, as well as SLW, were also higher in the Kalamon cv. than in the other two cultivars. Maximum values of nitrogen were 0.37, 0.29 and 0.27  $\text{mg cm}^{-2}$  for the Kalamon, Mission and Fracasana cultivars, respectively, with chlorophyll (a+b) values of 940, 640 and 730  $\text{mg m}^{-2}$ . The Kalamon cv. had significantly higher sucrose, glucose and starch concentrations than the other cultivars, while fructose concentrations were similar in all three. Maximum values of sucrose were 1.02, 0.72 and 0.70  $\text{mg cm}^{-2}$  for Kalamon,

Mission and Fracasana cultivars, respectively, with corresponding values for total sugars of 1.34, 1.0 and 1.16 mg cm<sup>-2</sup>. In all cultivars, sugar and starch concentrations increased until leaves reached their maximum area then decreased from this time. This period (late May) coincided with the emergence of inflorescences and fruits. The photosynthetic activity of the fig tree is influenced by both the cultivar and leaf age. The Kalamon cv. had a much higher Pn than the other two cultivars, indicating possible higher productivity. The higher Pn may be due to the higher gs and SLW, as well as nitrogen and chlorophyll concentrations.

## **Annual balance of CO<sub>2</sub> exchange in an olive orchard: assessment by eddy covariance and biometric methods**

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The carbon balance of an olive orchard (14 ha) in the western part of Sicily (Castelvetrano – TR), has been measured for 12 months in order to understand the potential role of a common agricultural orchard towards the carbon reduction goals as expressly required on Kyoto Protocol.

The orchard under study is composed by different plots, characterized almost all of them by an age variable from 12 to 16 years old.

Net ecosystem exchange at orchard level was monitored with the eddy covariance technique. Moreover the orchard capacity to CO<sub>2</sub> storage has been directly analyzed by estimating the biomass production on above and below ground components: amount of vegetation removed by winter pruning, oil and olive production, increase of trunk cross sectional area, winter production of grass between trees during the same period were measured directly on the field. Other components (yearly leaves senescence and biomass accumulated on root) were estimated to complete the analysis of all components.

On the same site, a micrometeorological station was positioned in a central part of the orchard in order to measure the photosynthetic active radiation, wind speed, air temperature and humidity, soil temperature and moisture: these information were used to analyze the effect of microclimate factors on yearly dynamic of CO<sub>2</sub> accumulation.

Results shows the capacity of olive orchard to act as a sink of CO<sub>2</sub> during all year and also the comparable values of yearly CO<sub>2</sub> accumulation with ecosystem characterized by forest species.

## **Characterisation of sainfoin tannins by degradation studies and MALDI-Tof MS**

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Tannins are polyphenolic compounds with high molecular weights, which accumulate in plants as natural products of secondary plant metabolism. They have many interesting biological activities, e.g. antimicrobial, antiviral, antitumour, anticancer, nematicidal or antioxidant. Some tannins can also produce important benefits for ruminant nutrition and the environment<sup>1</sup>. When tannins bind to dietary proteins, they generate ‘ru-

men-escape' protein, which leads to better protein utilisation and less environmental pollution. This process also leads to environmentally safer forms of excreted nitrogen, i.e. lower urinary N, but slightly higher faecal N, which contributes to build up of organic matter. However, some tannins are anti-nutritional and impair protein utilisation. Our research attempts to investigate why sainfoin (*Onobrychis viciifolia*) tannins tend to be particularly beneficial in ruminant nutrition and health. However, there are some contradictory reports in the literature concerning the structures and molecular weights of sainfoin tannins<sup>2,3</sup>. Therefore, detailed chemical analysis is required to identify the most active, beneficial tannins. This information will be of use in future plant breeding/selection studies.

Tannins were isolated from sainfoin (Cotswold Common variety) by Sephadex LH20 chromatography and separated into low, medium and high molecular weight fractions according to Meagher et al<sup>4</sup>. This variety contained 11.6% tannins (on dry matter basis). 11.8% of the tannins eluted in the low molecular weight fraction, 9.8% in the medium molecular weight fraction and 78.4% in the high molecular weight fraction. These fractions were analysed further by degradation with HCl/butanol, phloroglucinol, thiobenzene and by MALDI-TOF mass spectrometry in order to validate each of these techniques.

Further work on this topic is in progress.

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## **Formation of tannins in sainfoin (*Onobrychis viciifolia*)**

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Sainfoin (*Onobrychis viciifolia*) is a forage legume with high content of tannins, which are polyphenols occurring also in several foods and drinks. Many of these condensed tannins have a documented beneficial effect on human and animal health (Mueller-Harvey, 2000; Xie *et al.*, 2003).

The high content of tannins in sainfoin have not only been ascribed to an increase in animal performance, due to enhancement of dietary nitrogen utilisation by ruminants, but also to have a determinant role in the prevention of bloat (Mueller-Harvey, 1999; Xie *et al.*, 2003). Moreover, the use of sainfoin has been reported to increase soil fertility, by its ability to increase nitrogen fixation, as well as to facilitate the build up of soil organic matter, through a shift from urinary to faecal N. Consequently, this leads to a reduction in nitrogen emissions (Mueller-Harvey, 1999). Finally, tannins may also play an important role as antihelmintics, in veterinary medicine, revealing its potential to prevail over nematode drug resistance (Mueller-Harvey, 1999). Due to its unique nutritional and environmental properties, sainfoin was chosen as the model plant species for these investigations.



Condensed tannins are widespread plant natural products composed of linear or branched C<sub>4</sub>-C<sub>8</sub> or C<sub>4</sub>-C<sub>6</sub> linked chain of flavan 3-ols (Singh *et al.*, 1997; Xie *et al.*, 2003). Primarily, two types of flavan 3-ols are used as building blocks, (2R,3S)-(+)-flavanols (catechin-type, figure left) and (2R,3R)-(-)-flavanols (epicatechin-type, figure right). Catechin-type flavanols are derived from dihydroflavonols by the sequential action of two NADPH-dependent oxidoreductases: dihydroflavonol reductase (DFR), and leucoanthocyanidin reductase (LAR) (Joseph *et al.*, 1998). Epicatechin-type flavanols are formed from anthocyanidins by anthocyanidin reductase (ANR) (Xie *et al.*, 2003). Recently, it has been shown that the anthocyanidin synthase (ANS), involved in the conversion of leucoanthocyanidins to anthocyanidins, could additionally accept catechin as substrate (Wellmann *et al.*, 2006). It remains to be further confirmed, if the reaction observed *in vitro* has any physiological relevance and whether or not ANS is directly involved in the synthesis of condensed tannins in plants.

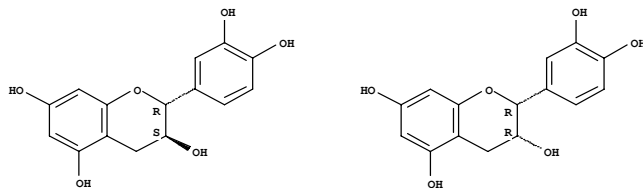


Figure: Chemical structures (absolute stereochemistry) of catechin (left) and epicatechin (right)

Our work is aimed at the screening for and characterization of the enzymes involved in the flavonoid and tannin biosynthesis. To this end, we purpose to set up a suitable method for HPLC analysis of several flavonoids, flavan 3-ols and tannins present in sainfoin. Currently, we are validating the optimal HPLC conditions for an efficient separation of flavan 3-ols. In addition, the LAR reaction of sainfoin could be shown successfully. Further work on this topic is in progress.

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## Polyphenols and Other Natural Products in the Forage Legume Sainfoin (*Onobrychis viciifolia*)

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Sainfoin is a forage legume with excellent nutritional and veterinary properties, namely prevention of bloat-ing and controlling the nematode parasitism in ruminants. These beneficial effects are thought to be due to the particular tannins structure, characteristic of sainfoin. However, since the formation of other polyphenol classes may promote or interfere with tannin biosynthesis, they also need to be investigated.

These investigations report the isolation and identification of a number of polyphenols and other natural compounds from sainfoin, variety Cotswold Common.

The acetone/water extract of leaves and stems from sainfoin plants was concentrated by evaporation under vacuum and defatted using chloroform. The remaining aqueous extract was further partitioned using water/ethyl acetate to remove the polar impurities such as sugars. Fractionation of the ethyl acetate phase by column chromatography on Sephadex LH-20 using a methanol-water step gradient yielded 50 fractions. All fractions were examined by TLC, HPLC and HPLC-ESI-MS/MS for their phenolic constituents. Phenolic acids including cinnamic and benzoic acids, glycosides of the flavonols quercetin, kaempferol, myricetin and isorhamnetin, dihydroflavonols and flavon-C-glucosides were characterised. In addition to these phenolic compounds the aminoacid L-tryptophan and the hydroquinone arbutin were identified. Flavan-3-ols monomers as well as dimeric and trimeric condensed tannins were also selectively detected using HPLC with post column derivatisation [Treuter, 1989; Treuter et al., 1994].

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### **Acknowledgements**

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## **Agronomic characterisation of European germplasm of sainfoin**

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Agronomic characteristics of twelve varieties coming from all over Europe and eleven Spanish accession of sainfoin (*Onobrychis viciifolia* Scop.) were evaluated in a small plot trial in Zaragoza (41° 3'N, 0° 47'W), Spain. In spring 2007, the plant material was sown in an alkaline silty-loamy soil and regularly irrigated by flooding. In order to explore the diversity on morphology, physiology and disease resistance within and between varieties, the time and intensity in flowering, growth habit, size, health state and re-growth ability after cutting were regularly assessed.

For most traits considered, a large intra-variety variability was observed that showed in some cases a variation coefficient up to 40%. This may suggest a lack of stability of these varieties, which could probably be due to a contamination by other foreign varieties. The variability amongst the variety was also particularly evident, especially at flowering time. While most plants were able to flower this first year, some did not. Moreover, within the plants that were able to flower in the first year, there was a delay of up to one month concerning the full flowering. The size of plants ranged from 25 to 65cm average and it was significantly correlated ( $P=0.0004$ ) with the flowering intensity. From this first year results, it was possible to discriminate between two sainfoin populations. A group of plants showed a precocious and intense flowering that appeared to be associated with a larger size, quicker and thicker re-growth whereas the other group either did not show flowers or a decreased and delayed flowering. However, due to the variability observed, only six varieties could be clearly allocated to one of the two types. The other varieties were behaving between the two extremes.

The investigated sainfoin material also showed a full range of growth habit from prostrate to erect. The erect plants are more likely to show the full flowering character.

Although the irrigation system used did not seem to be the most adequate for sainfoin, the plant showed a good resistance to pests and diseases. Oïdium was the only disease to occur with a level of incidence, which was far more correlated to the phenological stage ( $P$ -value = 0.0004) than to the variety or flowering type (not significant). The plants seem to be more sensitive to oïdium as they progressed through flowering and seed ripening.

Further work on this topic is in progress.

### **Acknowledgements**

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## **Methane production of different varieties of sainfoin (*Onobrychis viciifolia*) in dairy cows measured by in vitro technique**

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The Leguminosae sainfoin (*Onobrychis viciifolia* Scop.) spreads as wild plant throughout the continent of Europe to the Middle East and up to the last century it was commonly used as a fodder crop. Because of its low persistency and low regrowth rate after the first cut sainfoin gradually disappeared and was replaced by crops like lucerne (*Medicago sativa* L) that have a better performance (i.e. yield and persistency). Nowadays, there is a renewed interest to include sainfoin in animal diets, amongst others because of its high nutritive value (Parker and Moss, 1981), its apparent high palatability with as a result a high level of voluntary feed intake (Griggs and Matches, 1991), and its positive effect on protein use efficiency (Thomson et al., 1971), its anthelmintic and bloat reducing properties (Hoste et al., 2005; Min et al., 2006), and its potential to reduce methane emission (Puchala et al., 2005). The positive effects on methane reduction can be attributed to the phenolic secondary compounds (condensed tannins) present in sainfoin. From an environmental point of view methane formation by ruminants forms a major concern as it is one of the important greenhouse gasses with a high global warming potential (72 times more powerful than CO<sub>2</sub>; IPCC, 2007).

Many studies have been conducted to investigate the effect of tannins on methanogenesis and by which mode of actions tannins may alter methanogenesis by the ruminants. Tannins may have a direct inhibiting effect on the growth of proteolytic and cellulolytic rumen bacteria (Jones et al., 1994), and hence, alter the type of fermentation occurring in the rumen. Methanogenic bacteria live to large extent in symbiosis with protozoa and therefore, the reduction of the protozoa in the rumen forms another strategy to reduce methane production.

Our work focuses on assessing the effect of different sainfoin cultivars and the specific tannins they contain, on total gas and methane production using *in vitro* batch culture systems by which the cumulative gas production can be measured. The procedures used by our lab are a manually operated so-called "pressure transducer technique" (Theodorou et al., 1994) and a semi automated system (Davies et al., 2000; Cone et al., 1996). In both the techniques ground sainfoin (1mm particle size) is incubated in air tight bottles with rumen liquid and a pre-warmed semi-defined buffered medium. During the incubation period the cumulative gas production is measured. In addition at regular intervals the bottles headspace is sampled using a gas-tight syringe (10µm) and methane concentration is determined by gas chromatography. Methane production is calculated from the pressure corrected headspace volume and methane concentrations of the headspace. Fermentation kinetics are determined by fitting a modified nonlinear Michaelis-Menten equation (Groot et al., 1996) to the gas profiles. As this work is in progress at the moment further data on the fermentation kinetics of sainfoin, and the effects of tannins on total gas and methane production and production kinetics will be presented.

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## **Pre-Breeding and Development of Sainfoin (*Onobrychis viciifolia*)**

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We are working as part of an EU funded project entitled; The re-invention of sainfoin: an example of a novel resource for sustainable agriculture (Acronym: HealthyHay). This Marie Curie Research training Network is comprised of 10 research institutions and 2 SMEs in 10 countries. HealthyHay will lay the foundations for a new sainfoin breeding programme in the EU, whilst maintaining its unique nutritional, environmental and veterinary benefits. This will contribute towards, developing a new source of home-grown protein, reducing environmental pollution from ruminants (NH<sub>4</sub> and CH<sub>4</sub>) and developing a fodder legume suitable for sustainable agriculture.

The new sainfoin breeding programme has been initiated by establishing an extensive collection of sainfoin germplasm, which has been amassed from many nationally and internationally held collections. Currently, we are evaluating the agronomic and physiological characters of this germplasm collection and the genetic biodiversity represented in the collection. Plant microbial interactions are also being considered and their relative importance in establishment and longevity of a sainfoin crop. This will link into development of novel screening tools to underpin future sainfoin breeding programmes. Other partners in the consortium will evaluate nutritional and veterinary properties of the germplasm, which will be important drivers in the future breeding programme.

### ***Acknowledgements***

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## **Flavonoid biosynthesis in sainfoin (*Onobrychis viciifolia*)**

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Sainfoin (*Onobrychis viciifolia*) is a fodder legume which was grown in Europe before the widespread use of commercial fertilizers. It is a valuable and preferred forage for cattle, sheep and horses and has proved to be anti-bloating and anti-parasitic. It also increases the absorption of amino acids and the efficiency of protein utilisation. Sainfoin contains high levels of flavonoids which are secondary metabolites with physiological importance for plants. They act as phytoalexins by protecting the plant against parasites, fungi and diseases. The beneficial effects on ruminant's health can be ascribed to sainfoin's flavonoids, especially to its tannins. For this reason particular attention is paid to the biosynthesis of flavonoids in sainfoin.

For an evaluation of the flavonoid metabolism, we investigated four different sainfoin tissues: young leaves, old leaves, young stalks and old stalks. Due to the high content of polyphenols in sainfoin, enzyme preparations made with standard procedures (Halbwirth et al., 1997) showed only moderate activities for most of the enzymes which are involved in flavonoid biosynthesis, with exception of flavonol synthase (FLS), which was highly active in both leaves and stalks. Therefore, an optimization of the extraction method was necessary. Extraction methods (Dellus et al., 1997; Claudot und Drouet, 1992), which are adapted to the high content of polyphenols showed high activities for most of the enzymes in all the tissues. A considerable activity could be detected in leaves for the phenylalanine ammonia lyase, which is located at the interface between primary and secondary metabolism. Chalcone synthase/chalcone isomerase, the key enzymes for flavonoid biosynthesis were highly active in leaves and stalks especially in young tissues. Flavanon-3-hydroxylase (FHT) was more active in leaves than in stalks, whereas the dihydroflavonol reductase (DFR) showed high activities in both tissues. Due to the high values of polyphenols, the preparation of membrane bound enzymes turned out to be rather difficult. From the polyphenols present in sainfoin (Marais et al., 2000) the presence of at least two membrane bound enzymes was expected: flavonoid 3'-hydroxylase (F3'H) and isoflavone synthase (IFS). However, up to now we were not able to detect IFS activity in any tissue and the F3'H activities observed were extremely low.

In order to study the flavonoid metabolism also at the level of gene expression, procedures for RNA and cDNA preparation were required. Methods based on magnetic beads ( $\mu$ MACS (TM) mRNA isolation Kit, Milteny Biotech, Germany) did not result in RNA of suitable quality for further investigations. In contrast, a protocol optimized for pine trees (Chang et al., 1993) showed RNA of good further quality for further molecular biological investigations. Currently, cDNA fragments of FHT, FLS and DFR are available. The final aim of the studies is to identify target genes or enzymes of the flavonoid pathway, which could be correlated to optimal agricultural or veterinary medicinal attributes. This would allow to rapidly screen different sainfoin varieties during the breeding process or to select varieties for specific purposes.

Further work on this topic is in progress.

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## **Direct nozzle injection system for a real-time site-specific pesticide application**

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A real-time site-specific pesticide application requires the spraying system applying demanded volumes of different pesticide concentrations on the target area. Direct injection systems (DIS) maintain the chemicals in a reservoir separate from the water carrier and meter them into the carrier immediately before entering the nozzle orifice. The ability to change chemical type and concentration makes the DIS suitable for site-specific pesticide application. In a real-time controlled application the DIS response time is a crucial factor determining spatial accuracy of spray application.

The focus of this work was on developing of the DIS with acceptable response time by achieving desired mixture homogeneity of CoV 5 % on the nozzle orifice inlet. To enable a real-time controlled application,

the time to prepare and apply the demanded mixture by DIS should not exceed the time span until the spraying nozzle reaches the previous position of sensor when sampling (0.33 s). To reduce the response time of the system, the injection process was optimized by minimising the distance between the injection point and the nozzle orifice. Furthermore new injection valve has been developed for full range metering of active ingredients.

The homogenising process in the mixing space was considered between the injection point and nozzle orifice. The mixing process as limiting factor for reducing the response time of a DIS has been studied using Computational Fluid Dynamics (CFD) software and the results were verified by experimental tests.

Combination of injection system and two different mixing devices has been designed and tested for desired mixture homogeneity. The response time was by carrier flow  $20 \text{ ml.s}^{-1}$  lower than 250 ms for 1% pesticide concentration.

## **“Bio”system grapevine breeding in the Murfatlar vineyard – Romania**

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The recent integration of the Romanian state in the European Union generate market problems related with the competitiveness of the Romanian wines face of the European or international ones. The ones of the solutions done by the specialists in the area are the promotion of the autochthon, original varieties and the wines “BIO” obtained in ecological, certified breeding system.

Taking into consideration the subsistence agriculture – with lack of appropriate funds – practiced in the last two decades – it is obviously that in Romanian vineyard it isn’t a considerable problem of the environmental protection because it wasn’t applied chemical fertilizers and the number of treatments with pesticides was decreased until 4 to 5 in side of 9 to 10.

All these fact created very good premises to determine some of producers to inscribe their vineyards, or a part from them, in a controlled conversion program, with the aim to obtain certified ecological grapes for wines or for fresh consumption.

At the Research Station for Viticulture and Enology Murfatlar, located in the one of the most famous and big vineyard of Romania – Murfatlar, beginning with the 2006 year was registered as the surfaces in conversion 34 hectares of vine planted with Columna variety – an original creation of our research team – designed for to obtain white quality wines and Feteasca neagra variety, an autochthon, ancient vine variety designed for to obtain red high quality wines. The surface in conversion is tuckered in observation by I.C.E.A. – an international organism for the certification the quality “BIO” in accordance with Reg. CEE 2092/91 of the agricultural products.

The climatic conditions of the year 2007 was different face of those from the passed years, the known dryness present in each summer in this vineyard being in the 2007 summer excessive – in three mounts, May, June and July there was registered only 25% of precipitation in comparison with normal and the air temperatures was higher with  $5$  to  $8 \text{ C}^0$  than the monthly mean in these months. . This climate stopped the evolution of the cryptogamic diseases (manna, botrytis) but determining the development of the main pest of vineyard – the grape moth – this developing 4-5 generations instead of 3 in one vegetative period.

The management of the weeds was simplified, using the manual works for removing.

The phytosanitary treatments were applied only with admitted substances based on copper and/or sulfur. It was made only 5 treatments instead of 10 (used in a normal year).

In the August were registered high quantities of the precipitations being necessary the fifth treatment for to protect the grapes against the botrytis attack.

For grape moth, the population control was made with sexual synthetic pheromone traps type atraBOT made in Romania.

In comparison with the year 2006 the harvest obtained on the ecological vineyard in this year was decreased with 10 to 15 %, but the dryness from the berry development favorished the concentration of the aromas and of the sugars in must, the obtained wines having a very high potential to become top quality wines, and also “BIO” wines.

## **Lectures and Posters of Theme 5**

**QUALITY MANAGEMENT FROM MONITORING IN  
PRODUCTION TO MANAGEMENT IN SHOPS**



# High-throughput techniques for measuring taste and aroma of horticultural produce

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## *Introduction*

Flavour is becoming one of the most important quality attributes of horticultural products to be optimised in breeding, cultivation and postharvest processes of fruit and vegetables. For example, in a typical product development cycle, consumer panels are used to measure consumer preferences which are then mapped to flavour attributes scored by a sensory panel. Breeding programs then aim at providing products which match these flavour attributes. Often, the capacity and cost of such sensory panels restrict large scale screening programs, and instrumental techniques have been developed to measure flavour attributes. The aim is usually not to replace the human sensory panel completely, but to provide tools for large-scale prescreening of new cultivars. Laboratory techniques such as GC-MS and HPLC are available for analysing aroma volatiles and taste attributes, respectively. However, while such techniques might provide useful qualitative data, they are often too slow to analyse the large number of replicate samples which are typically required to provide statistically meaningful data – a typical analysis time is of the order of magnitude of 30 minutes. Also, the required laboratory skill to operate such equipment and the amount of sample preparation is considerable and often beyond reach of horticultural research stations. There is, hence, a need for high-throughput techniques to analyse flavour of fruit and vegetables which require minimal sample preparation, are easy to operate and as cheap as possible.

## *High-throughput techniques for flavour*

Optical methods are of particular interest for high-throughput measurements of taste components of horticultural produce (mainly sugars and acids in fruit). Near infrared (NIR) reflectance spectroscopy seems to be particularly useful as it allows estimating at the same time the SSC and the firmness of the fruit or vegetable. It is based on the nondestructive measurement of NIR light absorption and scattering in intact fruit and relies on multivariate statistical analysis to relate the reflectance or absorbance spectrum to, e.g., the soluble solids content of the fruit or the sweetness appreciation of a sensory panel. NIR devices are now commercially available for most commercial grading lines and are claimed to allow online sorting of fruit based on their sugar content or firmness. Alternatively, FT-IR spectroscopy in attenuated total reflectance or transmission mode has been advocated for measurement of SSC and even individual sugars in juices. This method lends itself well for high-throughput applications. Other optical techniques include space or time resolved reflectance methods which allow to measure light absorption and scattering properties simultaneously. While the first provide information about the chemical composition of the fruit, the second are related to the histology and, hence, the firmness of the tissue.

Other methods to measure taste components in a high-throughput way are based on enzymatic biosensor arrays which are typically implemented in 96 or 384 well microtiter plates. In contrast to the optical techniques which are essentially fingerprinting techniques, biosensor arrays provide information on individual sugars and acids. They can be further miniaturised in so-called multi-analyte lab-on-chip systems.

High-throughput techniques for aroma profiling are based on modifications of traditional GC-MS such as headspace fingerprint mass spectrometry, in which the headspace with volatiles is directly injected in the source of the mass spectrometry. The aroma profile is then related to a sensory aroma appreciation using statistical techniques. Other emerging techniques are based on fast GC-MS and soft ionisation methods such as proton transfer reaction ionisation (PTR).

We will conclude this presentation with an overview of recent developments in electronic noses for fast aroma profiling of horticultural produce.

# Predicting the weekly yield fluctuations of greenhouse bell pepper

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## *Introduction*

The production of bell pepper is characterised by large fluctuations in the number of fruits to pick over time. This phenomenon is called ‘flushing’: weeks with high yields are alternated by weeks with low yield and this is a reflection of the fluctuating pattern of the fruit set. The flushing is more or less the same for every grower and results in periods with high market supply and subsequently low prices and periods with a low supply and high prices. The fluctuating production pattern also has an impact on the labour allocation at the grower’s site and at the co-operative auction sites in Flanders since a large amount of the bell peppers produced here are graded and packed centrally where the product is sold. There is a need to have a good insight in the expected production highs and lows for at least three weeks in advance: to establish better prices with retailers and to guarantee the demand for periods in which bell peppers are sold in advance for a fixed negotiated price, to optimise packing logistics and to ensure additional labour when needed.

## *Fruit set and growth period*

A method was developed to predict bell pepper production on a weekly basis on a greenhouse level. It is based on the registration of the weekly fruit set and the prediction of the number of days from fruit set until harvest. The maturation of bell pepper fruits of several cultivars of red, yellow and green bell pepper was followed in 2005 and 2006. During the entire growing season the date of fruit set and the harvest date of each fruit were monitored to determine the length of the growth period of a fruit. The number of days until harvest followed a parabolic curve over time, fruits needed the most time to mature at the beginning and towards the end of the growing season and matured the quickest in summer time. The growing period of a fruit was mainly influenced by temperature and fruit load. On average the number of days until harvest decreased with 3 days for red bell pepper, 4 days for yellow bell pepper and 2 days for green bell pepper with an increase of the average growing temperature by 1° C. Due to competition for assimilates between a larger number of fruits, the fruits that grew when the average fruit load was high needed more time to mature than when the average fruit load was low. By using this information a model was built to predict the number of days until harvest as well as the harvest date of fruits and the total yield. Since fruit growth takes on average 6 to 8 weeks it is possible to predict the weekly harvest within this time horizon.

## *Fruit colouring*

Another method was evaluated to predict the production based on the colouring of the fruits. The final maturing of a bell pepper fruit starts with the colouring, from green to red, yellow or orange, of a small part of the surface of the fruit which gradually expands until the fruit is fully coloured. Pictures were taken of different stages of fruit colouring, from 10 % colouration of the fruit surface to 90%, the stage in which the fruits are usually harvested in practice. This was done for red and yellow bell pepper and all the pictures were then put together to make a colouring chart. In 2005 and 2006 the period of time to reach full colouring starting from each of the colouring stages was measured for several red and yellow cultivars during an entire growing season. An average bell pepper fruit needed 3 to 5 days from the beginning of fruit colouring to reach a harvestable stage, depending on fruit colour and cultivar. Red peppers needed approximately a day more to colour fully. There was a tendency towards slower colouration in spring and faster colouration in summer but this was not consistent for every year and cultivar. Once the fruit colouring started, this process didn’t seem to be affected by average greenhouse temperature or amount of daylight. Since most of the fruits coloured within a week, a count of the colouring fruits in the crop at the end of one week can give a good insight of the expected production of the following week.

# Monitoring and modelling the postharvest behaviour of horticultural produce

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As a global trend, requirements on quality, safety, and traceability of fresh horticultural produce have been increased. Studies pointed out, that one third of the cultivated produce deteriorates before reaching the consumer. However, it can be assumed that all batches suffer a loss of quality related compounds as a result of inappropriate handling after harvest. To assess these losses and the produce quality stage extensive and costly measurement procedures are necessary. Information on the expected postharvest behaviour cannot be given. The aim of the present study was the development of a monitoring system concerning the complete logistic chain from the producer up to retail. The modelling part of this system will allow drawing conclusions about the current produce stage and the prediction of the expected shelf life.

In two successive years storage-experiments on unripe and ripe tomatoes of two different cultivars ( $n_1=324$ ,  $n_2=486$ ), applying 3 fertigation levels in the production and three levels of shading, respectively, were conducted. After harvest, three temperature regimes were executed during a storage period up to four weeks. Twice a week the changes in the produce properties were quantified by means of non-destructive methods regarding mass loss, respiration [ $\text{mgCO}_2/\text{cm}^2\text{h}$ ] and transpiration rate [ $\text{mgH}_2\text{O}/\text{cm}^2\text{h}$ ]. By means of these data the tissue water conductivity respectively resistance were estimated. Optical properties were analyzed by measuring the tomato diffuse reflectance spectra in the visible and near infrared wavelength range (400-1100 nm). Additional destructive readings were used for the water content [%] and soluble solids content [%] as well as the elastic modulus [ $\text{N}/\text{mm}$ ] analyses.

In this study, it was confirmed that the crucial parameter for marketability of tomatoes is a decrease in pericarp elasticity by means of an expert panel, while the  $\text{CO}_2$ -efflux provides a sensitive parameter for the maturity stage of climacteric tomato fruit. Generic and statistic approaches were tested and a dynamic algorithm was developed. The dynamically adjusted coefficients are expressions of easily measurable parameters such as time after harvest, temperature, mass loss, pigment information, and the soluble solids content. All data are included in a web-based internet application that offers a tool for continuous modification of these coefficients. The interaction of the recorded environmental data with the information about the produce quality allows the predicting of the shelf life of the produce depending on the thresholds of marketability given by the sensory panel.

The analysis of the collected data showed that different but constant temperature treatments ( $12^\circ\text{C}$  and  $20^\circ\text{C}$ ) result in changes of certain product properties that can be described by equal models as a function of time and temperature. Similar results were found for both cultivars marginally influenced by the preharvest conditions or the maturity stage at harvest. In contrast, a fluctuation of temperature (8 h at  $20^\circ\text{C}$ , 16 h at  $12^\circ\text{C}$ ) caused a different postharvest behaviour, as shown in the figure 1 below for the accumulated carbon dioxide efflux of the fruits:

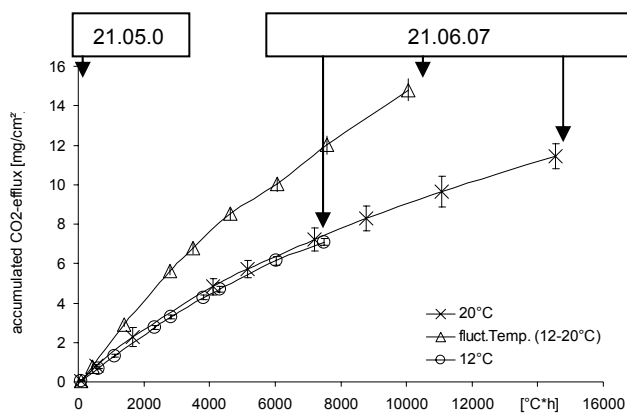


Fig. 1: accumulated  $\text{CO}_2$ -efflux of 'Liberto' tomatoes in different postharvest temperature regime as a function of temperature impact.

## **Towards the use of modelling in genetic improvement: example of peach fruit quality**

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Improving fruit quality raises major difficulties. To overcome these difficulties, an interdisciplinary approach has been developed which consists in forwards and backwards between modelling, ecophysiological analysis and quantitative genetics. We applied such an approach to peach fruit quality. A population of 140 genotypes derived from a clone of wild peach (*Prunus davidiana*) by three generations of crosses with commercial varieties of nectarine (*Prunus persica*) was studied and genotyped with 80 markers. The ecophysiological model predicts fruit and stone dry and fresh masses and total sugar concentration in relation to environmental conditions. We detected QTLs for all the model parameters and in many cases they were common to both years of experimentation and co-locations between QTLs for quality traits and QTLs for parameters were observed. QTL results were used to predict, for any genotype of the studied population, the values of each parameter which were integrated into the ecophysiological model. This approach could provide a framework for the understanding of physiological and biological phenomena, via the dissection of the quality traits into elementary processes. The integration of genetic information into the ecophysiological model may be used for practical purposes, such as predicting the genotypic variations of a plant response to environmental conditions. It may help to solve G x E interactions and to predict the behaviour of plants from the population with any combination of alleles under any climatic scenario.

## **Polyphenol analysis in horticultural products based on laser-induced fluorescence spectroscopy**

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During recent years several research groups focussed on the development of non-destructive product monitoring methods to improve the process management for horticultural products in the entire supply chain. Optical methods have been applied for fruit monitoring in production and postharvest processes using mobile measuring equipment or NIR sorting lines. Besides the quality aspect of horticultural products also the demand of foodstuffs promoting a better healthiness such as functional foods has increased, due to actual discussions and new findings of the relation between nutrition and healthiness based on clinical studies. In this context fruit and vegetable became more and more popular, since they possess valuable compounds built up in the secondary metabolism. Polyphenols constitute one of the most important groups of these health promoting compounds in horticultural products. They have specific activity in the human metabolism regarding bioavailability, antioxidative capacity, interactance with enzymes and influence on gene expression. The aim of the present study was to quantitatively determine health promoting native fruit polyphenols by means of laser-induced fluorescence spectroscopy.

On different horticultural products (carrot, apple, strawberry) the fluorescence and reflectance spectra were detected, while the polyphenols contents were analyzed chromatographically for developing calibration models. The influence of side-effects (temperature, concentration) on the fluorescence signal was studied in phenol standards, fruit extracts and sliced fruit tissue. In the complex fruit matrix the quantitative determination of fruit compounds by means of fluorescence signals is influenced by the compounds' fluorescence quantum yield as well as reabsorption and quenching effects of the fruit tissue. To take these re-absorption and fluorescence quenching effects into account, partial least squares (PLS) regression has been used to build calibration models for the polyphenol analyses on the fruit fluorescence spectra (apples,

strawberries) using the chromatographical analyses of phenols as a dependent variable. Different pre-processing methods such as normalization, derivation, genetic algorithms, and direct orthogonal signal correction (DOSC) have been applied to enhance the robustness of the calibration models. The uncertainty of the models was evaluated by their root mean squares errors of calibration and cross-validation. Autoscaling the spectral data along the samples and subtracting 2 DOSC factors from the matrix of, e.g., strawberry fluorescence spectra led to  $r^2 = 0.98$  for the content of p-cumaroyl-glucose in the fruits.

The feasibility of the non-destructive analysis in practice is influenced by the high variability of horticultural products. To evaluate the robustness of the calibration, the models were validated on an independent test set, which leads to  $r^2 = 0.94$  and  $\text{rmsep} = 15.03\%$  for the strawberries and their p-cumaroyl-glucose content. Thus, the mathematical data pre-processing method of DOSC removed the non relevant information in the spectral data and resulted in the lowest errors. In comparison, the often applied empirical approach in fluorescence spectroscopy to correct the fluorescence spectral data with the simultaneously recorded reflectance spectra to reduce re-absorption effects did not improve the calibration models. This data processing step leads to less robust models in the before mentioned example of strawberries and their p-cumaroyl-glucose content ( $\text{rmsep} > 55\%$ ).

## **Approach for Nondestructive Analysis of Carotenoids Contents in Horticultural Products using Scattering and Absorbance Information**

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Non-destructive quality analyses are presently introduced in practise for an adapted process management in the areas of production and postharvest handling of perishable products. Particularly spectroscopic methods have been often reported for non-destructive analyses of soluble solids as well as pigment contents. Beside major benefits of the method, an unsolved disadvantage is the need for re-calibration on new plant material, which is caused by the measuring principle. Photons detected in the turbid sample are not exclusively influenced by absorption, but also scattering properties of the fruit tissue.

In the present work, time-of-flight information was used to address the influence of photon scattering and to calculate the effective pathlength between light injection and detector. With the pathlength information additionally to the information of wavelength-dependent absorbance intensities obtained with the common spectroscopic analysis, it might be possible to apply Lambert-Beer law for directly analyzing absolute pigment contents without a time-expensive re-calibration step.

This hypothesis was evaluated on liquid phantoms mimicking the optical properties of tomato fruits and carrots. The phantoms represented (i) typical absorbance intensities similar to the carotenoids absorption as well as (ii) varying scattering properties in the range found in tomato and carrot tissues.

A calibration was built on phantoms with different absorption, but equal scattering properties. In comparison, spectroscopic results and combined application of spectroscopic and time-of-flight measurements were  $r^2=1.0$  and  $0.99$ , respectively. When applying the calibration on phantoms mimicking additional differences in the scattering properties such as they appear in fresh products at different maturity stages resulted in the following validation results:  $r^2=0.87$  and  $r^2=0.96$ , respectively. The improved results by the additional use of the effective pathlength obtained from time-of-flight readings were confirmed on real-world samples such as tomatoes and carrots (table). Non-destructively obtained data were compared to chromatographical carotenoids analysis leading to  $r^2$  values of  $0.31$  and  $0.43$  when applying direct Lambert-Beer law using a single wavelength in comparison with combined application with time-of-flight measurements, respectively. In batch-wise estimation  $r^2=0.66$  and  $r^2=0.74$  were obtained, respectively.

Table: Calibration and validation results regarding the coefficient of determination ( $r^2$ ) and percentage of bias ( $\%e_b$ ) and root mean square error ( $\%rmse$ ) of carrot carotenoids content analyses by means of direct Lambert-Beer law using a static pathlength of 15 mm or the effective pathlength calculated from time-of-flight readings at 758 nm calculated at the half width at half maximum ( $L^*s1$ ) or peak maximum ( $L^*s2$ ).

	calibration results for individual carrots			validation on individual carrots			batch-wise validation on means of carotenoids content classes		
	$\%rmse$	$\%e_b$	$r^2$	$\%rmse$	$\%e_b$	$r^2$	$\%rmse$	$\%e_b$	$r^2$
L=15mm $y=0.3674x+15068$	19.22	0.01	0.393	35.87	13.42	0.314	14.21	3.89	0.660
$L^*_s1$ $y=0.5334x+17678$	20.67	0.00	0.451	22.61	5.11	0.429	8.41	1.21	0.742
$L^*_s2$ $y=0.6782x+21079$	20.20	0.00	0.468	9.61	15.07	0.259	3.84	8.48	0.683

## Monitoring of post-harvest Orange Fruit disease by Fluorescence and Reflectance Hyperspectral Imaging

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### Introduction

The composition and content of pigments like chlorophylls, carotenoids and phenolic compounds determine colour and appearance of fruit. These pigments are strictly related to the physiological conditions and their variation can give information about health of fruit. The assessment of the quality and safety of food are very important for both production and marketing. The express and non-destructive methods to monitoring in real time physiological changes are helpful in food safety inspection. When the pigments are excited with UV radiation the pigments emit in the VISible range. The fluorescence and the reflectance spectra emission by pigments can be changed for different injuries, as well as pathogens attack. Spectroscopic assessments with imaging techniques can provide spatial information about defects and safety of food products. In order to detect early and non-destructively the disorder due to pathogens attack in orange fruit surface the Laser Induced Fluorescence (LIF) and Reflectance Hyperspectral Imaging were utilized. The development of Imaging Analysis provides a promising powerful tool to assess the post harvesting fruit quality is also discussed.

### Material and methods

The hyperspectral measurements were performed on orange fruit (cv Navelina) harvested at the same stage of ripe from a commercial orchard in Sicily. The fruit were inoculated with two important post-harvest pathogens: *Phytophthora citrophthora* ( $2 \times 10^4$  sporangia/ml) and *Penicillium italicum* ( $2 \times 10^6$  spores/ml). The inoculated fruit were compared to healthy orange fruit and with fruit inoculated with distillate water. The analysis were performed on the same fruit during time: before inoculation and 24h and 48h after inoculation. The Nd:YAG laser fluorometer with excitation wavelength 355 nm capable of imaging operation was utilized to measure LIF spectra of whole fruit. Reflectance 2D spectral imaging was carried out on the same whole orange fruit using the spectral scanner ImSpector within the visible spectral range.

### Results and discussion

Already 24h after inoculation both LIF and Reflectance images of the orange fruit show the disease evolution in terms of the surface pigment spectra variation due to the pathogens attack. In particular the spectral changes are dominated by the emission on the blue region around at 540 nm and on the red region at 680 nm. In the ripe orange peel fruit the phenolic compounds are the main components and their absorption and

fluorescence is around 540-550 nm. In fruit inoculated with *P. citrophthora* was observed a decrease of reflectance value at 540 nm while in fruit inoculated with *P. italicum* the reflectance values increased by more than 10%. The visible symptoms were evident 72h after inoculation. The increase of absorption obtained at 540nm highlight the accumulation of phenolic compounds in early response of stress

The chlorophyll content decreases in each fruit inoculated compared with not inoculated fruit, as see in reflectance and in fluorescence emission (680nm). Changes in spectral features of the inoculated area are consistent and can be put in evidence by making use of differential imaging technique by considering the time differences. The results obtained indicate the feasibility of non-destructive assessment of chlorophyll (680nm) and phenolic compound (540nm) changes in early response to pathogens interaction. The potential of quantitative in vivo measurements of plant pigments and phenolic compounds on orange fruit surface is also discussed.

## Non-destructive evaluation of high-pressure processing effects on fresh produce

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The application of high hydrostatic pressure has the potential to selectively affect food related enzymes and microorganisms while retaining the characteristic structure and functionality of fruits and vegetables. To reach this goal, the impact of the selected process parameters (p, T, t) on the relevant systems must be known. Studies on the effect of high hydrostatic pressure on quality attributes of fruit and vegetables focus mainly on changes in colour, texture, flavour, and nutritive value. Changes in colour, flavour and nutritive value due to high pressure processing of foods (tomato, broccoli, avocado, etc.) are negligible at room temperature while texture properties of the treated fruits and vegetables changes in dependence of both pressure level and duration of pressurization. It was reported that pressure up to 350 MPa can be applied to plant systems without any major effect on texture and structure [1]. An extensive study on the effect of pressure on the texture of fruits and vegetables has been carried out [2]. In general, the softening curves revealed that texture changes caused by pressure occurred in two phases: (1) a sudden loss as a result of the pulse action of pressure, followed by (2) further loss or gradual recovery during pressure-holding phase depending on the product treated [3]. However, high pressure effects are product specific. To characterize property changes of the native biological material fast and non-invasive methods can be helpful tools.

Lamb's lettuce was cultivated at the ATB and harvested immediately before the experiments. Two leaves were selected, their physiological activity evaluated using chlorophyll fluorescence image analysis (CFIA), packed into plastic pouches and transferred into a pressure vessel. High pressure experiments were performed in a vessel (Uhde GmbH, Germany) with an inner volume of 0.6 l and a maximum working pressure of 360 MPa. Pressure transmitting medium was silicon oil. Inner vessel temperature and pressure were measured at a rate of 1 Hz during experiments. The vessel temperature was set to selected values using an external cooling and heating system. Pressure up to 250 MPa was investigated and holding times between 10 s and 15 min. Subsequent to the treatment CFIA of the leaves was performed using a FluorCam 680MF (PSI, Brno, Hungary). Measurements were taken during 24 h post treatment time. Results obtained in these experiments were compared to thermal treatment in a water bath set to temperatures of 40, 45, 50, 60 °C.

CFIA allows to determine the maximum photochemical efficiency  $F_v/F_m$  of the fresh produce and consequently to indicate treatment related changes of the physiological activity. The obtained kinetics of  $F_v/F_m$  show minor effects of thermal treatment at pressure below 125 MPa and temperatures lower than 45 °C but significant property changes after pressure treatment of 150 MPa and temperatures of 45 °C and above. Regeneration effects of the leaves can be seen for different temperature holding times (45 °C) up to 120 s, but after 24 h the photochemical efficiency decreases to 65 % (120 s) or 79 % (90 and 60 s) when compared to the initial  $F_v/F_m$  value of 0.7. In conclusion, CFIA is a very sensitive non-invasive measurement method to

detect the treatment related property changes of fresh plant leaves due to thermal and pressure effects. CFIA allows the immediate evaluation of photosynthetic activity and integrity as an indicator for cell and tissue vitality. Proper function of the entire photosynthetic apparatus strongly requires intact membrane systems. Hence, any perturbation of cell integrity and functionality due to high hydrostatic pressure treatment can be inevitably detected by analyzing photosynthetic activity.

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## **Young families as buyers of horticultural products**

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### **Introduction**

Often, the well-funded buyers of horticultural products are more than 60 years old and thus it is quite obvious that this customer group will only buy and demand the respective products for a limited time period. Therefore, it is essential for retail trade nurseries – which are continuously losing market shares in favour of Do-It-Yourself-stores, garden centers and food retailers – to search in time for younger customers in order to sell them plants and other horticultural products for a longer time period. At least part of the target group “young family” earns high household net incomes, is often underrepresented among the buyers of horticultural products and offers high potentials for future sales not least since the families often influence their children’s prospective consumption behaviour.

### **Material and Methods**

Data for the diploma thesis was generated via a standardised questionnaire consisting of 17 questions which has been distributed to 1,000 households within the urban area of Munich and in the rural districts of Rosenheim and Lindau/Bodensee in summer 2006. Those chosen households had to give indications for children, or otherwise data from the Federal Statistical Office of Bavaria had been used in order to find appropriate households. Statistical distribution of 223 answered questionnaires could be calculated by the use of the statistical program SPSS. Among the returned questionnaires 85% belong to the selected target group “young family”.

### **Results**

Young families have a keen interest in using their garden for recovery, as a playground for their children or for celebrating with friends. Obviously, there is a desired high demand in horticultural products. Those are mainly bought in garden centers, retail trade nurseries or DIY-stores, which are favoured especially for special offers. Mainly 31 to 40 year-old women with an average household consisting of four persons frequently buy horticultural products for about 100€ to 300€ per year. Young families demand high quality products, friendly and competent consulting, a wide selection of plants and other horticultural products, as well as a reasonable price-performance ratio and enough parking sites. Very good plant quality can be primarily found in retail trade nurseries and in garden centers, both also turned out to provide friendly personnel and expert consulting. Retail trade nurseries already fulfil most of the customer needs, but prices are too high and opening hours are not appropriate. In DIY-stores however, customers are less satisfied with the



product quality, but more content with the price-performance ratio. Still, half of the respondents have to queue too long there, cannot find qualified personnel and if so, they are unfriendly.

Families that are shopping together with their children wish to find a playground or at least a kid's corner. Child care can at present only be found in retail trade nurseries. Family-specific criteria such as an ideal routing within the store, long and family-friendly opening hours especially at weekends can already be found in garden centers and DIY-stores. While one third perceives the shopping atmosphere in garden centers and retail trade nurseries as very satisfying, friendliness and high availability of personnel can mainly be found in retail trade nurseries.

### **Conclusion**

Polled families possess an over-average purchasing power, are highly interested in gardening and plants and will by reason of young age be potential buyers for a long time - as opposed to many elderly persons interested in horticultural products.

Therefore, purchasing together with one's children should become as barrier-free as possible which might implicate changes in the range of products, marketing, paths (length, broadness), family-friendly opening hours, queue times and articles for sale at the paying desk, the existence of car parks and the availability of child care.

Aware of the shopping habits, behaviour, preferences and needs of young families, adjusted marketing measures can provide forward-looking support and ensure a successful continuity of the business companies.

## **Effect of cultivar on dry matter and sugar content in tomato fruits**

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Six tomato cultivars, three tall-growing (Mi-10, Arletta and Saint Pierre) and three small-growing (Narvik, SP-109 and Arizona) were investigated. Tomato was grown under field conditions (Belgrade region) and analyzed with regard to dry matter and sugar content. Tomatoes harvested on August 15 were used in the trial. Fresh tomato fruits were analyzed. A two-year trial was conducted in 2005 and 2006.

Dry matter content ranged from 4.96% (Arletta) to 6.51% (Arizona), i.e. 5.62% on average. Small-growing cultivars were richer in dry matter content (6.09%) compared with tall-growing cultivars (5.16%). The difference amounted to 0.93% which is a substantial difference in dry matter content for tomatoes.

Sugar content ranged from 3.06% (Arletta) to 4.07% (Arizona), i.e. 3.57% on average. Cultivars richer in dry matter content were richer in sugar content as well. This may be attributed to the dry matter content in tomato fruits known to be primarily composed of sugars (approx. 60%). Small-growing cultivars synthesized about 3.86% of sugar on the average, whereas tall-growing cultivars 3.29%. The difference amounted to 0.57%.

Therefore, tomato cultivars were found to contain different amounts of dry matter and sugar. However, small-growing cultivars were far richer with regard to dry matter and sugar content.

# Relationship between cropping system and compositional parameters of early sweet corn

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## *Introduction*

The sweet corn is the vegetable crop grown on the largest acreage in Hungary. It is grown in the open field, primarily for the purposes of the processing industry. When produced for the fresh market there are good reasons for having cropping system components (seedling raising, temporary plastic film cover) that enhance earliness.

Considering the compositional parameters, significant differences can be observed from one variety type to another relative to the carbohydrates, lipids and proteins.

The vitamin C content of sweet corn is not very high, as compared to tomato, nor its carotene content as compared to carrot, however the respective amounts are significant which provides diversity in our diet. Among the mineral elements, there are significant sources of K, Ca, Mg, P, Cu, Zn and Fe.

## *Material and method*

The objective of our trial was to study the effect of some cropping system components that enhance earliness (seedling raising, temporary plastic film cover) on different morphological parameters (height, ear weight, ear length and kernel length), as well as on the major compositional properties of sweet corn (*Zea mays* L. convar. *saccharata* KOERN.).

The following cropping technologies were compared by using the variety Spirit (normal sweet with very early growing season): 1. plants grown from transplants under floating row cover (2 planting dates), 2. plants grown from transplants with no row cover and 3. covered and 4. uncovered direct seeding (2 sowing dates in both cases).

Besides determining the macro nutrient content (NPK) of the leaves and cobs we also determined the mineral composition by using an ICP-OES Thermo Jarrell Ash, as well as the total phenolic content, the antioxidant capacity and the reductive sugar content by spectrophotometric methods.

## *Conclusions*

Based on the results it can be concluded that the row cover, besides improving earliness and reductive sugar content, had a favourable influence on the total antioxidant capacity and phenolic content which are very important for a healthy diet. The aforementioned conclusion was reached also in the case of the earlier propagation date.

In view of morphological parameters as height, ear weight and ear length the direct seeded treatments produced significantly better results as transplants.

The measurement's results in case of kernel's length the plants from earlier propagation time produced higher results compared to the traditional technology.

In case of macro nutrient (NPK) content of leaves and cobs we didn't find any significant differences, excepted the leaves of earlier sowed, uncovered treatment had significantly lower N and K content as other treatments.

# Effect of some agronomical factors on nitrates (V) content in early potato tubers

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## Introduction

On the nutritional values of potato to consist the content of nutrition components, especially vitamin C, protein and mineral compounds, and also relatively low content in tubers of unhealthy for human compounds, such as nitrates and glycoalkaloids. The nitrates (V) toxicity for human is not large, but big threat to make the products of their change – nitrates (III), at of which share can be arise cancerogenic nitrosamines, to belong the strong of cancerigenic substance (Leszczyński 2000). The content of these compounds in tubers depends on agronomical factors and climatic conditions in the vegetation period. In case of this same cultivar, the physiological earlier tubers contain more nitrates (Cieślik 1995, Frydecka-Mazurczyk & Zgórska 2000, Hlušek et al. 2000, Lachman et al. 2003).

## Material and methods

The effect of the potato cultivation way for early crop (no covering and under polypropylene fibre Pegas Agro 17UV) and nitrogen fertilization (0, 30, 60 and 90 kg N ha<sup>-1</sup>) on the N-NO<sub>3</sub> content in early potato tubers was investigated. The field experiment was established in the splitblock-splitplot method. The 6-week seed potatoes presprouted of Aster and Drop cultivars were planted on 9-12 April and harvested 60 and 75 days after planting, respectively. For laboratory studies, 50 tubers of different size, according to the proportional participation in the yield of each treatment were taken. The nitrates (V) content estimated the colorimetric method. The results gave in mg N-NO<sub>3</sub> in kilogramme of fresh matter in tubers. The results of the experiment were analysed statistically by means of analysis of variance. The significance of differences was verified using the Tukey's test at P=0.05.

## Results and conclusion

The forcing of plants vegetation by polypropylene fibre covering contributed to improvement the tubers quality by decreased N-NO<sub>3</sub> content, especially at a very early date of potato harvest. At this way of potato cultivation for early crop, N-NO<sub>3</sub> content in the tubers harvested 60 days after planting was lower on average by 6.40 mg N-NO<sub>3</sub> kg<sup>-1</sup> FM (5.24%) in comparison with the cultivation with no plant covering. After 2-week delay of potato harvest the difference was smaller and not statistically confirmed. N-NO<sub>3</sub> content in tubers to a higher degree depended on the level of nitrogen fertilization. The increase of nitrogen dose caused an increase in the N-NO<sub>3</sub> content. After 60 days from planting, independently from cultivar, only just the dose 90 kg N ha<sup>-1</sup> caused significant increase the N-NO<sub>3</sub> content in tubers, on average by 10.75 mg N-NO<sub>3</sub> kg<sup>-1</sup> FM (9.41%), in comparison with the cultivation without nitrogen fertilization. After 2-week delay of potato harvest already the dose of 30 kg N ha<sup>-1</sup> increased the N-NO<sub>3</sub> content in tubers of Aster cultivar, on average by 9.11 mg N-NO<sub>3</sub> kg<sup>-1</sup> FM (8.19%), however, significant increased this compounds content in tubers of Drop cultivar, on average by 7.22 mg N-NO<sub>3</sub> kg<sup>-1</sup> FM (7.21%), occurred at the dose of 60 kg N ha<sup>-1</sup>. The more nitrates accumulation the tubers of Aster cultivar, than the tubers of Drop cultivar. After 60 days from planting the nitrates content in tubers of Aster cultivar were higher, in the three-year period, on average by 18.51 mg N-NO<sub>3</sub> kg<sup>-1</sup> FM, and after 75 days from planting by 18.57 mg N-NO<sub>3</sub> kg<sup>-1</sup> FM. The higher temperature and the shortage of rainfalls in the vegetation period of potato increased the N-NO<sub>3</sub> content in the tubers. In both dates of potato harvest the nitrates (V) content in tubers not exceed the admissible content 200 mg N-NO<sub>3</sub> kg<sup>-1</sup> FM.

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## **Ferrous oxide saves the world**

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Iron is indispensable in photosynthesis, respirations, nitrogen assimilation, etc.; and the deficiency symptom of plants, chlorosis, often occurs in case of alkaline soil and/or low-temperature term. About one third of all the land in the world is the alkaline soil, so "how Fe is supplied to crops" is a big problem in inadequate soil. According to the WHO, iron is most insufficient nutrient in the world, and approximately 70% of the global population is in the iron deficient condition. To make iron abundant crops is considered to become the solution. Although the improvement of growth and quality of crops are recognized by iron application also in Japan, the mechanism and optimum application method are not clear yet.

Then, the new ferrous oxide material TetsuRiki that dissolves the iron bivalent ion in the long term stably was examined. In tomato, the yield and the Brix increased 5% only by addition more than 3.5g L<sup>-1</sup> of TetsuRiki FE to transplant substrate. In soilless culture of many leafy vegetables, significant increase of growth and iron content was achieved by addition of more than 0.5 ml L<sup>-1</sup> of TetsuRiki AQ instead of usual FeEDTA. In addition, it was very effective under high pH nutrient solutions, because the growth in the TetsuRiki AQ treatment was slightly retarded although that in FeEDTA was retarded remarkably.

## **The influence of the various conditioning treatments on the compositional parameters of rose wines**

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Among the large varieties of wines obtained in our country - as a natural results of Romanian exceptional climate and a remarkable vini-viticultural tradition – the rose wines are missing, also this wines are intensively desired by fine-tasted consumers.

In the great European wine-marking countries (and especially in France) numerous researches had been developed regarding rose wines obtaining.

Rose wines had already acquired their reputation by fresh taste drink, fresh grape flavor, qualities typical for white wines, but not for red wines.

To preserve these qualities it is necessary to condition the wines early after alcoholic fermentation and before the malolactic fermentation, in order to obtain the perfect clarity and biological and physico-chemical stability, and then directly bottling.

This proceeding provides the keeping of malic acid in wines, with a proved physiological value, and also a good part of monomer polyphenols forms, with a positive role for the heart dizziness.

The necessity to conservation integral of kind characteristic, the freshness and expressivity of the rose wines, high by a delicate coloratura, very attractive suppose a early bottling, but only in a perfect limpidity and physico-chemical and biological stability. In this way must be implicate the treatments, for obtain the limpidity and stability but with minor effects on composition and taste of the rose wines.

About the compositional and sensorial characteristics was effectuated many researches overseas. In Romania this researches was effectuated in Dealu Mare area and, also in some vineyard by Dolj and Olt County.

The researches were effectuated on some rose wines who are obtaining from different red grapes kind cultivated in Sâmburești-Olt vineyard.

The experimental variants are:

Experiment I: testifier; treatment with bentonite (50g/hl); treatment with gelatin (6g/hl); filtration (EK); combinative treatment: gelatin (6g/hl) + bentonite (50g/hl); combinative treatment: bentonite (50g/hl) + filtration; combinative treatment: gelatin (6g/hl) + filtration; combinative treatment: gelatin (6g/hl) + bentonite (50g/hl) + filtration.

Experiment II: filtration through membranes with different porosity (8; 0,6; 0,5; 0,45; 0,2 $\mu$ ).

Experiment III: the influence of microfiltration on evolution in time of poliphenolyc complex in rose wines at 3, 6 and 9 month.

The influence of treatments was established by chemical and spectrophotometrical determination, pre-scripted by O.I.V.

The most used enological materials in winemaking industry, like gelatin and bentonite, applied singular and in moderation doses reducing the color intensity with approximate 20% and in combinative treatment with 24%.

The operation of early filtration of the rose wines determines diminution of antociani contents and also at the colorant intensity values. In situation of wines without filtration, the antociani sedimentation, in a bigger proportion, under oxygen action, and also because the combination with acetaldehida. The acetaldehida formed in bigger contents under microorganism actions, especially acetic bacterium, which in filtration cases it no more exit.

Through filtration are retain the polymer forms, who contain a part of mauve, violet and blue pigments, realized in this way a better chromatic structure.

This aspect is attest by bigger values of the tonality of the filtration wines, in all situations (3, 6 and 9 month).

## **The influence of different type of maceration on flavor complex in Romanian flavors wines**

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The flavored wines which are obtained from the varieties of grapes that have the capacity to biosynthesize and to accumulate the flavor substances (linalool, geraniol,  $\alpha$  terpenol) in the superficial or profound stratum of the peel reclaim an elaborate technology, specific to increasing their flavored potential

Further, the flavored wines technologies refer at two fundamental objectives: the extraction of primary flavors from the grapes and the favorisation of the formation of the secondary flavors in fermentation. Therefore, the prefermentative phase is crucial in obtaining flavored wines.

The maceration, fermentation-maceration is a complex process with a biochemical and biophysical character and also a technological link with important applications for obtaining some typical flavor wine. For possibility to extraction of flavor substances from peel grapes supposes the keeping touch, for a time, the two phases of mixture resulting after pressing.

In the course of time, it realized many researches regarding this aspect and the conclusion was that using the enzymatic preparation has positive consequences in increasing the flavored potential of wines through the liberation of terpenes from the bounded compounds

Terpenic flavors are those which dominate in grapes and the terpenics monohydroxilic alcohols are the most important, from an olfactory point of view. Yet, any of those volatile compounds, considered as individual, can not provide a typical flavor of "muscat" kind, while their general mixture lead to a flavored, typical note of wines.

In the last period, the research was about the specifically precursors, most the terpenic precursors. By reason of chemical comportment of the crush berries in different condition of pH, it was observed that, beside vola-

tiles monoterpene exist in “muscat” grapes, bound forms of these compounds, unvolatile, sensible at acids. So, through enzymatic studies it was established that exist bound forms of sugar with aglicons, with importance linalool, geraniol, nerol, constituted the adequate monoterpenic glycosides.

Foreknowledge the specifically precursors, their distribution in the berry, their evolution during the ripening process and also their evolution during making wine process present an scientific and practical interest for an rational conduct of the harvest and making wine process.

In this study, we propose different vinification variants of the flavor grapes Tămâioasă românească and Muscat Ottonel for obtaining a high quality flavors wines. This grapes rise from Drăgășani vineyard. The experimental variants for Muscat Ottonel grapes are: V<sub>1</sub> – white vinification; V<sub>2</sub> – classic maceration for 36 hours; V<sub>3</sub> – maceration in metallic rotary cistern (ROTO) at 20<sup>0</sup> C, time to 12 hours and V<sub>4</sub> – the treatment of mixture resulting after pressing with thermic shock at 45<sup>0</sup> C, return at 25<sup>0</sup> C and maceration in metallic rotary cistern (ROTO). The experimental variants for Tămâioasă românească grapes are: V – white vinification; V<sub>1</sub> – classic maceration for 24 hours by frozen grapes; V<sub>2</sub> – cold maceration for 24 hours; V<sub>3</sub> – cold maceration for 24 hours and enzymatic preparations; V<sub>4</sub> – classic maceration for 24 hours; V<sub>5</sub> – classic maceration for 24 hours and enzymatic preparations; V<sub>6</sub> – classic maceration for 48 hours; V<sub>7</sub> – classic maceration for 24 hours and enzymatic preparations.

The maceration process determined the important growths in the flavor potential at the wines through increases at the free volatile terpenes and also bound terpenes contents. Through low temperature of maceration process, the extraction of flavor compounds is more slowly. The application thermic shock by maceration gives enrichments in free volatile terpene and bound terpene contents.

In the maceration in metallic rotary cistern (ROTO), the extraction of the flavor compounds by a solid fraction is highest comparative with the classic maceration process. The alcoholic fermentation produced a diminution of the free and bound contents because the adsorption or rallying CO<sub>2</sub> resulting at alcoholic fermentation by yeasts cells.

## **The changing of axin content in the hardwood cuttings during the storage and the rooting period by HPLC**

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The aim of the work was to study IAA content changes in four parts of the oval leaf privet cuttings during the storage of plant material and the rooting period of hard-wood-cuttings. We tried getting more information about plant hormones translocation and changing regulation is one way of getting conclusion. The analysis of plant growth factors as like the indol-acetic acid (IAA) is not too easy, because the low concentration in the plants, and easy and rapid oxidation and the photodecomposition. There are few possibilities to determine the biological active substances the used HPLC methods one of the most sensitive possibility to determine the quality and quantity of plant hormones.

Hard wood cutted oval-leaf privet (*Ligustrum ovalifolium* L.) was used as plant material in our experiment. The plant cuttings were collected during the dormancy period, every 10 days. First collection was made in the beginning of December. The cuttings were stored in the plastic-foil at 5 °C until the time of cutting. The propagation was carried out in the (first week) middle of April, cuttings were planted in the Research field of the Department in Soroksár in Hungary, to 5X5 cm, onto the black plastic-foil covered soil. The IAA content in the new collected and stored plant material too has been determined until the developed new shoot lengths were 10 cm long. The plants were collected, and cca. 1 cm long basal and internodal parts were cutted from it, and analysed monthly during the storing, and weekly during the rooting period.

The IAA was extracted in cool (4 °C), dark place to avoid the IAA degradation with 10 ml ethanol in one day, added BHT (butylated hydroxy toluene). The content of chlorophyll was removed from the solution with petroleum-ether. The solution of extraction was filtered on filter-paper, and the solvent was moved in rota-

dest on 20 °C. The residue was redissolved onto 1 ml methanol. The solutions were taken onto the Eppendorf-tube, and centrifuged for 10 minutes on 15.000 rpm. The well centrifuged extraction was filtered on 0,45 µm MILLEX-HN Syringe Driven Filter Unit and injected onto the HPLC. A WATERS High Performance Liquid Chromatograph equipped 2487 Dual Detector, and 1525 Binary HPLC Pump, controlled with EMPOWER™2 software. A SYMMETRY C18 5µm 4.6 x 150 mm column was installed. Mobile phase methanol:water 60:40 v/v% containing 0.05 % acetic acid. The flow rate 1 cm<sup>3</sup>·min<sup>-1</sup>, the pressure on the column was 1800±15 psi at ambient room temperature. The each injected volum was 20 µl. The IAA was monitored at a wavelength of 280 nm. The retention time of IAA in standard solution was 2.813 min.

During the storing until planting time IAA concentration did not change sensibly in the cuttings there were only small differences found between the four internodial parts in IAA concentration. The quantity of indole acetic acid was more or less 0,07 µg·g<sup>-1</sup> both of part the cuttings, only in the top part could be measured higher (0,076 µg·g<sup>-1</sup>) concentration. At 17 of April cuttings were carried out and planted into the field. Since this time, the analyses of plants were made every week. We found, the higher concentration of IAA can be detected in the topside. In our experiment we found lower and lower IAA concentrations analysing the internodial parts from the top part to the basal part. At 17 of May cuttings formed callus, the examined hormones concentration were again on the top internodium the highest. Toward to basis it had lower and lower levels. At the end of May the cuttings had a few adventitious roots as well. Being able to get fast water and minerals by the roots, inner transport mechanism has become much faster, either hormonal transports. The RSD value of the determination was about 5% (n=5).

As a conclusion, we can say, the native IAA concentration in the case of oval leaf privet is enough high to promote the rooting of cuttings.

## **Effect of Nitrogen and Potassium Fertilization on Productivity and Fruit Quality of 'Crimson Seedless' Grapes**

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The effect of the nutritional status of grapevine 'Crimson Seedless' on growth, fruit set and quality was studied in two growing periods. The vines were treated with three nitrogen rates (24, 36, 48 kg/ha.) combined with three potassium levels (240, 285, 330 kg/ha). Increasing N supply enhanced N-concentrations in the petioles. N-fertilization also increased K concentrations in the petioles. The K-fertilization variants themselves did not alter the K content of the petioles. High N-fertilization improved vegetative growth (leaf area, cane diameter) and reduced bud burst, bud fertility and fruitfulness. Excessive N-supply negatively affected yield per vine by reducing the number of fruit clusters. Cluster size, however, was improved with increasing N-nutrition whereas the number of berries per cluster and the cluster compactness remained unchanged. The single berries were bigger (size, weight) and their shape remained typically for the cv. The amount of juice per berry increased and fruit firmness decreased in the high N-variants. The only effect of high K fertilization was an increase of total soluble solids and a decrease of acid concentration. Anthocyanin contents generally show a high variability in the experiment. Therefore, the colour of berries might be more regulated by the ripening process than by the fertilizer treatments.

# Managing Quality in Finnish Ostrobothnian Horticultural Supply-chains

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## *Introduction*

Finland is located between the 60th and 70th degrees of Northern latitudes. Plant production is possible due to the warming effect of the Gulf Stream, the use of natural resources (E, g. peat) for thermal use in the highly intensified greenhouse production. Finland is one of the northernmost countries producing significant quantities of horticultural products. Many fields of horticultural plant-production can be found: Greenhouse cultivation of vegetables, flowers and small berry- and fruit-growing.

Winter period's low temperatures favours control of pests and diseases and the usage of pesticides is moderate. Long summer days (up to 24 hours) compensate the unfavourable winter-season in the growing year. Demanding conditions have led to a highly developed implementation of technology (artificial light) and training and education of the growers on a high professional level.

The predominantly Swedish speaking Närpes region in the South of Vaasa in the Finnish Österbotten district is one of the most important horticultural production areas in Finland. Production of vegetables (major supplier of tomatoes, cucumbers, salads), herbal spices and ornamental plants is conducted in protected greenhouse cultivation systems through the whole year. Its presence dates back to the 19<sup>th</sup> century and can today serve as an example of regional adjustment to agricultural decline and increased economic pressure on the population. There is a strong pressure on the domestic production in the context of the entrance of Finland to the European communities - this opened market access for strong European competitors and suppliers resulting in a heavy pressure on the prices.

## *Methods and Material*

We chose the qualitative method of free and guided interviews with growers, experts (growers association, education institutions) to elucidate the situation of the players in the supply chains, their motives, decisions and policy making.

The scientific problems were defined as: How to ensure / improve the quality of processes and end products (horticultural produce) in the Ostrobothnian horticultural supply chains. Significant cases/examples are described and analyzed by the means of supply chain and quality strategies.

## *Results*

Production in greenhouses totalled 432 hectares, of which two thirds was in production for more than 7 months of the year. Vegetables were grown on 338 and flowers on 94 hectares. Both light and heavy fuel oil was common sources of heating energy in greenhouses. The covering material in greenhouses was in most cases either plastic or glass. The Närpes growers provide 45 % of total cucumber-, 50 % of bell pepper- and 60% of tomatoe-production of Finnish total vegetables-production. The produce is mainly delivered to domestic markets. It is the center of the main area of greenhouse horticulture in Finland.

We try to look at the difference of the end-product-quality which is rather well taken care of but then the process quality and the profitability issues of the companies are not as well pre-planned. Stepping up of efforts in Intensification of production, improving and adapting crop-quality to exceed market requirements (labelled Finnish high quality products), implementing a quality label and improve awareness of domestic goods are introduced as activities to strengthen the supply-chains.

The empirical data can serve as a basic evidence for judging the actual situation as one of economic transition in depressed rural areas – with the loss of horticultural enterprises on one hand and the gain of size on the other hand – and the fight for survival through the improving of supply chains and implementation of strict quality policies. Accompanied by the increased pressure on issues of environmental protection.



# Occurrence of viruses in asparagus (*Asparagus officinalis* L.) in North Germany

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## Introduction

Asparagus growers have reported on a decline syndrome, which leads to reductions in the profitable life of asparagus plantings. The syndrome causes asparagus crops to lose their economic value within 5 to 10 years of cultivation. The decline is associated with a reduction in spear number and size, and even death of the crown. Several pathogens as well as abiotic factors are believed to act individually or in concert to cause the decline. So far viruses which have frequently been detected in asparagus fields are *asparagus virus 1* (AV-1), *asparagus virus 2* (AV-2), *cucumber mosaic virus* (CMV) and *tobacco streak virus* (TSV). This survey was conducted to determine the incidence and degree of single and mixed virus infections in asparagus crops in North Germany and to ascertain the effects of field location, plant age and variety.

## Material and Methods

Fern samples from selected commercial asparagus stands throughout Lower Saxony (Germany) were tested individually for presence of AV-1, AV-2 and CMV by enzyme-linked-immunosorbent assay (ELISA). Thereby the samples were randomly collected in selected asparagus stands differing in age and supposed yield level. Additionally fern samples of six year old asparagus plants from a stand located in Berlin were taken to evaluate the sensitivity of three different varieties: 'Eposs', 'Rambo' and 'Ramos'.

An indirect ELISA protocol was used to detect AV-2; antibodies as well as the test system were obtained from Agdia (Linaris GmbH, Wertheim, Germany, prod. no. SRA71000). A double-antibody sandwich (DAS) ELISA was applied to detect AV-1 and CMV. Specific antibodies against AV-1 as well as CMV-specific antibodies were provided by Dr. Frank Rabenstein (Federal Centre for Breeding Research on Cultivated Plants, Quedlinburg, Germany) and alternatively a CMV-specific antibodies by Dr. Joachim Hamacher (University of Bonn, Institute of Crop Sciences and Resource Conservation, Bonn, Germany). The optical densities of the samples were measured in a microplate reader set at 405 nm. The samples were scored positive for the presence of the specific virus if the optical density value was at least twice that of the negative control. ELISA identifications were confirmed by symptom development after rub inoculation of the indicator plants *Chenopodium quinoa* Willd., *Cucumis sativus* L and *Nicotiana tabacum* var. "Samsun" with sap extracted from randomly-selected fern samples. Indicator plants were inoculated at the 4 – 8 leaf stages.

## Results and Discussion

In general, asparagus ferns of infected plants did not show characteristic virus symptoms. Some of the ferns exhibited chlorosis or were stunted, but these symptoms did not correlate with infection.

Only a sixth part of the tested plants could be addressed as virus-free. An infection with a single virus was displayed by approximately half of the fern samples whereas infections with AV-1 dominate. Remarkable is the fact that only four months after planting, 12% of the plants were already virus-infected, and that these infections were exclusively by AV-1. This frequency of infection likely reflects the level of AV-1 in asparagus crowns used to establish the crops. Subsequent dispersal within a field – up to 98% within 7 years - may be caused by aphid transmission, and by mechanical transfer of virus on knives used to harvest spears and machinery used to cut down foliage. Mixed infection with two viruses were detectable in a third of the asparagus plants. An infection with CMV and AV-1 occurred most frequently. None of the investigated plants indicated a triple infection nor a single infection with AV-2. The proportion of virus-infected plants, especially mixed infections increased with the age of the asparagus crop.

No significant difference in the proportion of AV-1-infection could be determined in regard to the asparagus variety. In contrast there supposed to be a correlation between the susceptibility to a CMV-infection and the asparagus variety. Whereas about 80% of the fern samples of the varieties 'Ramos' and 'Eposs' were tested CMV-positive, only 42% of the variety 'Rambo' did so.

## **Influence from the leaf area on the vase life in cut roses**

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The vase life of cut roses is determined by a variety of factors. Most important is the water status management during postharvest, especially under drought stress conditions. The hypothesis that the amount of water lost by transpiration is directly related to leaf area appears to be sound. The larger the leaf area the more stomata are available. This may finally result in an increased stomatal transpiration. Even when stomata close found cuticular transpiration becomes the dominant factor, with a larger leaf area the cuticular transpiration is higher, too. The aim of this experiment was to determine the influence of leaf area on postharvest vase life of cut roses.

The vase life of the cut rose cultivars 'Akito', 'Aloha', 'Milva' and 'Red Giant', kept with and without fresh flower food, was determined in climate chambers at 20°C, 60 % rH and 14  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD. In the variants with leaves the roses were trimmed to three leaves.

The analysis of all variants indicated no correlation between total leaf area and vase life of cut roses ( $R^2 = 0.002, 01. \text{ and } 02.07.; R^2 = 0.098; 03. \text{ and } 04.07.$ ). Also the individual evaluation of each cultivar and each treatment revealed no significant correlation between these two parameters.

However, as expected, there were significant differences between the vase life of cut roses with and without leaves. Vase life increased significantly when the leaves were removed. After detachment of leaves it rose from 11.5 d to 15.1 d in 'Akito', and from 17.5 d to 24.3 d in 'Aloha', from 24.5 d up to 27.8 d in 'Red Giant' and from 22.4 d up to 23.3 d in. However, in 'Milva' this increase was not significant. The large difference in 'Aloha' expressed their drought stress susceptibility. In this cultivar and in 'Red Giant' end of vase life was mainly determined by discoloration when leaves were removed and flower wilting in the variants with leaves. The fall of turgid flower leaves was the dominated factor that determinates vase life in leafless 'Akito' in contrast to leaf wilting in 'Akito' roses with leaves. In leafless samples of all cultivars the flower turgidity at the end of vase life was higher than in roses with leaves still attached. Factors for further investigations are the morphology and function from the leaves and from the abscission zone at the base of the peduncle.

## **The effect of iron chelates on yielding and nutritional state of greenhouse tomato grown in peat substrate**

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### ***Introduction***

In horticultural practice it often happens a problem with correct iron feeding because of its easy transfer into less available form. Principle meaning in iron protection against retrogradation is using chelate forms, from which Fe cations gradually realised to soil solution or can be absorbed by roots as organic-mineral complex. Durability and availability of iron from chelats depend on ligand properties. The aim of the present investigation was the evaluation of suitability of three iron chelates and different Fe concentration in the medium for greenhouse tomato grown in peat substrate.

## Material and methods

The aim of the study was to estimate the effect of different kinds of iron chelates: Fe 8 Forte (EDTA+DTPA), Top 13 (EDTA), Librel Fe DP7 (DTPA) and iron concentration: 50, 75 and 100 mg Fe per 1 dm<sup>-3</sup> of peat substrate on yielding and nutritional state of greenhouse tomato cv. 'Merkury F<sub>1</sub>' grown on benches. Seedlings were planted in the beginning of April in spacing 0.6x0.5 m and grown for 5 clusters. The medium contained the following amounts of nutrient (in mg dm<sup>-3</sup>): N - 220, P - 180, K - 350, Ca -1500, Mg - 200, Mn - 20, Zn - 20, Cu - 5,0, B - 1,5, Mo - 1,5, pH 6.0 and EC 1.80 mS·cm<sup>-1</sup>). In 3-rd decade of June plants were additionally fertilized by 5g N and 10 g K for 1m<sup>2</sup> of the cultivated area. The samples of leaves were collected 3 times during the growing season at one month intervals starting from May. Total forms of microelements (Fe, Mn, Cu and Zn) were determined after digestion in the mixture of H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub> and HClO<sub>4</sub> by AAS method.

## Results

Data of the study proved that the highest total and marketable yields of tomato fruits were obtained in the case when Fe 8 Forte (EDTA+DTPA) was used as an iron source, followed by treatments with they use of Fe 13 Top and Librel Fe DP7 which provided lover and similar to each other results. Increasing Fe content in the growing medium from 50 to 75 and 100 mg Fe·dm<sup>-3</sup> resulted significant increment of total yield from 12.11 to 12.62 and 13.27 kg·m<sup>-2</sup>, respectively. Kind of iron source affected significant differentiation of iron content in tomato leaves. The highest mean concentration of this nutrient characterized plants fertilized by Fe 8 Forte-111.6 mg Fe·kg<sup>-1</sup> d.m. followed by Fe 13 Top – 107.0 mg Fe·kg<sup>-1</sup> d.m. and Librel Fe DP7 – 111.2 mg Fe kg<sup>-1</sup> d.m. Nutritional status of tomato plants depended on initial concentration of iron in the growing medium. The highest mean Fe level in leaves recognized as the index parts of tomato was recorded in May 132.2 mg Fe kg<sup>-1</sup> d.m. and July 129.6 mg Fe kg<sup>-1</sup> d.m. at the iron content in 75 mg Fe·dm<sup>-3</sup> of peat substrate. Increment of iron content in growing medium resulted in the decrease of manganese concentration in tomato leaves. The level of 75 mg Fe·dm<sup>-3</sup> in peat substrate was superior for Cu uptake by plants.

## Light transmissions (PAR-, UVA- and UVB-radiation) of different greenhouse glass-materials and their effects on plant-physiological and plant-morphological parameters

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The radiation intensity is strongly reduced in low-light seasons in our regions. This is more reduced in interiors of a greenhouse with glass-materials and low degrees of transmission. The reactions of plants to lower radiation intensity and radiation quality are species-specific. Glass impurities, caused by high iron, change the radiation quality. Among other things they decrease the light transmission in the PAR- as well as in the UVA- range and prevent the transmission of UVB-radiation.

Five different glass-materials on the south side of a greenhouse were built and examined for their transmission characteristics into the PAR-, UVA- and UVB- range. Among the selected glass-materials were: Insulation glass (VSG), thermal insulation glass (VSG), normal floating glass, Planilux Diamant (white glass) and micro-structured Centrosolar (white glass). Apart from that special boxes were developed, which were made to fit tightly on the glass-materials. In these were put *Impatiens-Neuguinea-Hybride* 'Rivera Orange Night', in order to examine the effects of the glass materials for plant-morphological and plant-physiological parameters during the low-light seasons.

The highest transmissions in the PAR-, UVA- and UVB-range were measured at Planilux Diamant and Centrosolar, followed by normal floating glass, insulation glass and thermal insulation glass, which does not let a UVB-radiation transmit. Glass materials with a higher PAR-, UVA- and UVB-transparency lead to more compact *Impatiens Neuguinea* hybrids 'Riviera orange Night' plants. Leaf area and the length of the middle

internodes were reduced. Further positive characteristics were the strongly shining bloom colours, the higher dry substance and shoot amount. A chlorophyll reduction in leaves and other quality-reducing damages due to the influencing UVB-radiation was not shown.

## **Effect of EMAP storage on the respiration, carbohydrate balance and quality of unrooted azalea cuttings.**

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Florist azalea (*Rhododendron simsii* cv.) is commonly propagated by means of leafy cuttings. Optimal cold storage of these unrooted cuttings is limited to 4-5 weeks although a longer storage period would be advantageous for the production planning. Therefore, the applicability of equilibrium modified atmosphere packaging (EMAP) was assayed for azalea in order to improve cutting storage ability.

An assay, in which 3 different cutting amounts [ $\pm 30$  g,  $\pm 15$  g and  $\pm 7$  g] were packed in bags (0.072 m<sup>2</sup>) with 4 different foil O<sub>2</sub> permeabilities [0, 970, 2290 and 3529 ml O<sub>2</sub>/m<sup>2</sup>.atm.day], showed that the O<sub>2</sub> foil permeability, required for optimal EMA storage conditions, lies between 970 and 2290 ml O<sub>2</sub>/m<sup>2</sup>.atm.day. When cuttings were stored in foils with a permeability lower than 970 ml O<sub>2</sub>/m<sup>2</sup>.atm.day, the enclosed gas composition swiftly moved to anaerobic conditions, causing a metabolic shift from the cutting's respiratory pathway to the fermentative way. As a result, external cutting quality and rooting ability decreased rapidly. In contrary, when foils with a permeability higher than 2290 ml O<sub>2</sub>/m<sup>2</sup>.atm.day were used, almost no alterations in head space gas concentrations were observed. Storage under these conditions was very similar to open air storage and, as a result, no decrease in product's respiration rate was observed.

Based on these results, a packaging experiment, in which  $\pm 30$  g azalea cutting material was packed in bags (0.072 m<sup>2</sup>) with an O<sub>2</sub> foil permeability of 1850 ml O<sub>2</sub>/m<sup>2</sup>.atm.day, was set up. After two weeks of storage, an equilibrium atmosphere of  $\pm 0.2\%$  CO<sub>2</sub> and  $\pm 18\%$  O<sub>2</sub> was reached. It can be concluded that under these packaging conditions, an O<sub>2</sub> foil permeability of 1850 ml O<sub>2</sub>/m<sup>2</sup>.atm.day is still too high to achieve the aimed optimal EMA storage conditions. However, under these conditions, a significant decrease in carbohydrate depletion was observed.

As small changes in head space gas composition could prolong the post-harvest life of stored cuttings, optimized storage of unrooted cuttings in packaging foils highly depends on the selected O<sub>2</sub> foil permeability. For azalea, storage of approximately 30 g of cutting material in semi-permeable bags (0.072 m<sup>2</sup>) requires an O<sub>2</sub> foil permeability between 970 and 1850 ml O<sub>2</sub>/m<sup>2</sup>.atm.day.

## **Biochemical and histological analysis of storage-mediated root formation in *Petunia* cuttings**

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Intermediate dark storage and transport of leafy stem cuttings are frequently used in vegetative propagation of many ornamental plant species. Depending on plant genotype, storage conditions (e.g. temperature, dura-

tion) and also other environmental factors, adventitious root formation (ARF) can be impaired after storage. Such responses of pelargonium were correlated with depleted carbohydrate levels in leaves, supporting the view that ARF in stems is dependent on leaf-derived influx of sugars and is limited by carbohydrate reserves when genotype and/or environmental conditions do not allow for sufficient current photosynthesis. However, if this is not the case, rooting of cuttings can be improved by storage as shown for chrysanthemum and carnation. Kinetic of carbohydrates during storage and also auxin transport may contribute to such responses.

Within the scope of a joint project on molecular physiology of ARF (see also Druège et al. 2008 in the same book), the objective of the present study was to analyse the influence of dark storage of cuttings of *Petunia hybrida* 'Mitchell' on the kinetic of carbohydrates in leaf and basal stem tissues as related to cytological events and final intensity of ARF. For this purpose, cuttings were either used immediately after harvest, or by way of comparison, first stored in darkness (10 °C, 7 d), before they were rooted in perlite in climate chambers (temperature: 22/20 °C day/night, light: 10 h day length, PPFD 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Concentrations of carbohydrates (glucose, fructose, sucrose, starch) in leaf and stem tissues were measured by means of enzymatic assays. Cytological stages were determined by light microscopy.

Dark storage of cuttings greatly promoted ARF in terms of number and length of roots. Storage reduced the time period required for sufficient rooting from 16 to 9 days. Furthermore, higher number and length of roots was recorded for the stored cuttings even after 9 days of rooting when compared to the respective values of the unstored cuttings after 16 days of rooting. Concentrations of sugars were significantly reduced after one week of storage but they conformed to the levels of the unstored cuttings within the first week of rooting, which reflected a high current photosynthesis of cuttings. Kinetic of carbohydrates during the early periods of storage and rooting will be presented together with corresponding histological data. Results will be discussed considering the possible involvement of carbohydrates and auxin in storage-promoted ARF in leafy cuttings.

The project is granted by the Pact for Research and Innovation of the Leibniz Science Association (WGL).

## **The joint project "Molecular physiological analysis of adventitious root development in *Petunia*"**

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The wound-induced generation and subsequent growth of adventitious roots in stem tissues of excised leafy cuttings is a crucial physiological process in propagation of many ornamental plant species. Despite intensive control of environmental factors in the modern propagation industry, high economic losses still occur due to insufficient rooting. It has been repeatedly shown that adventitious root formation can be improved by the control of mineral supply to the stock plants, of storage conditions and also by application of microorganisms such as arbuscular mycorrhizal fungi. However, the lack of knowledge of related molecular physiological events hampers most efficient utilization of these relationships and development of new sophisticated technologies.

*Petunia* is a plant genus of high economic importance in European horticulture and a substantial proportion of this bedding and balcony plant is propagated vegetatively by rooting of leafy cuttings. Considering the qualities of this genus to serve as a model system for studying plant development, an interdisciplinary joint project has been started on *Petunia* to analyse the molecular physiology of adventitious root formation. The

main objective is to elucidate the involvement of particular genes, of carbohydrate metabolism and of plant hormones in development of adventitious roots, in particular considering the pre-conditioning of cuttings by the control of nitrogen supply, storage, phosphorus nutrition, and application of microorganisms.

Technological, plant physiological and microbiological treatments are combined with methods of cytology (histological characterization), biochemistry (analysis of compounds/precursors/enzymes, application of interacting chemicals) and molecular genetics (analysis of gene expression by use of micro arrays and reporter genes, modification of key processes by gene transfer, forward and reverse genetics). The project is further linked to the worldwide network [www.petuniaplatform.net](http://www.petuniaplatform.net).

The project is granted by the Pact for Research and Innovation of the Leibniz Science Association (WGL).

## **Biochemical and histological analysis of nitrogen-mediated root formation in *Petunia* cuttings**

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It has been shown that adventitious root formation (ARF) in chrysanthemum, pelargonium and also poinsettia is increased with higher supply of nitrogen to the stock plants and positively correlated with nitrogen level in cuttings, even though, depending on plant genotype, storage and light conditions, this relationship can be uncoupled by carbohydrate shortage or secondary osmotic stress. Considering the observed increased partitioning of leaf carbohydrates towards sucrose in response to high nitrogen supply and the correlative dependence of ARF on leaf-derived carbohydrate, it is assumed that changed carbohydrate fluxes contribute to nitrogen-mediated ARF. Furthermore, regarding the dependence of ARF on auxins and the possible interactions between nitrogen, carbohydrates and plant hormones, both changes in carbohydrate and auxin transport may be involved in nitrogen-mediated ARF.

Within the scope of a joint project on molecular physiology of ARF (see also Druege et al. 2008 in this book), the objective of the present study was to analyse the influence of preharvest nitrogen supply to stock plants of *Petunia hybrida* 'Mitchell' on the kinetic of carbohydrate and auxin levels in cuttings as related to cytological events and final intensity of ARF. For this purpose, stock plants were cultivated in greenhouse in organic substrate and received either adequate (high, but without strong accumulation of nitrate and salt in the substrate), or by way of comparison, low (deficient) nitrogen supply via fertigation using graduated doses of ammonium nitrate. Cuttings were rooted in perlite in climate chambers (temperature: 22/20 °C day/night, light: 10 h day length, PPFD 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Concentrations of carbohydrates (glucose, fructose, sucrose, starch) and indole-3-acetic acid (IAA) in leaf and stem tissues were measured by means of enzymatic assays and GC-MS/MS, respectively. Cytological stages of ARF were determined by light microscopy. Number and length of roots were determined after a rooting period of 16 days.

Both, number and length of roots determined at the end of rooting period were significantly reduced in nitrogen deficient cuttings. Kinetic of carbohydrate and IAA levels during the rooting period will be presented together with corresponding histological data. Results will be discussed considering the possible involvement of carbohydrates and auxin in nitrogen-mediated ARF in leafy cuttings.

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# Fruit quality and aroma of tree ripe and detached, sun-exposed 'Annurca' apples

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## **Introduction**

Annurca is an old cultivar mainly grown in Southern Italy, specifically in Campania, highly appreciated for its flavor and firmness (Bignami et al., 2003). After intensive genetic work, two interesting clones have been selected for better peel coloration, namely 'Annurca Rossa del Sud' and 'Annurca Bella del Sud' (Limoncelli and Testoni, 1984). The Rossa del Sud clone gets the best peel coloration in the climatic settings of the Sicilian hinterland (Lo Bianco et al., 2004). Harvest and postharvest practices are quite traditional and specific, due to scarce fruit peel reddening, rather unattractive (Forte, 1975). In fact, after harvest fruits require a period of sun exposure to get good red peel color. The aroma profile of intact 'Annurca' apple fruits has been determined before and after reddening and only quantitative changes of high volatility compounds have been observed (Lo Scalzo et al., 2001). The aim of this work was to test the effect of postharvest sun exposure (reddening) on fruit quality and extractable volatile composition of three clones of 'Annurca' apple.

## **Materials and Methods**

The study was conducted near Caltavuturo (37° 49' N and 850 m a.s.l.), Sicily, using eight-year-old 'Annurca' apple trees of clones AICA, Bella del Sud (ABS), and Rossa del Sud (ARS). Trees were grown on M.9 rootstock, planted in single rows, spaced at 4 x 1.5 m, trained to a central leader, and received conventional cultural cares. Fruits were collected at commercial harvest, and number of fruits and yield per tree were recorded. One half of the fruit of each tree was analyzed immediately, whereas the other half was positioned under direct sun light for 15 days (rotating every 3 days) before analysis. Fresh weight, diameter, firmness, peel color, soluble solids, pH, titratable acidity, starch index, and volatile compounds were measured on fruits of each tree. Peel color and flesh staining for starch quantification were determined by digital image analysis using an algorithm that quantifies color characteristics as the weighed distance (CIE L\*a\*b\* space) of each pixel in the image from a reference sample. The output is a color index and a starch index ranging from 0 (no red color or staining) to 1 (max red color or staining). Volatile compounds were determined in fruit peel and flesh by Solid Phase Micro-Extraction technique in Head Space followed by Gas Chromatography/Mass Spectrometry.

## **Results and Conclusions**

The reddening treatment imposed in this experiment induced significant changes in fruit quality, regardless of clones. Specifically, both intensity of red color and percent of cover color increased after reddening, as expected; also, soluble solids increased whereas starch index, pH, and titratable acidity decreased. No change in flesh firmness was detected. Despite any reddening effect, ARS exhibited the largest fruits in terms of weight and diameter, while AICA exhibited greater soluble solid content than ABS, with ARS showing intermediate levels. The percentage of cover color and the intensity of red color were similar in all clones, as well as flesh firmness, starch index, titratable acidity, and pH. The volatile fraction was always more concentrated in the fruit peel than in the flesh, regardless of reddening or clones. Clones responded differently to reddening. Specifically, reddening tended to decrease both number of volatiles and their concentration in AICA and ABS fruits, whereas no substantial change was observed in ARS fruits, regardless of fruit tissue. Our results show that 15 days of reddening improve fruit peel coloration and internal quality without further decrease in flesh firmness in all clones. Yet, only ARS fruits maintain the initial rich flavor after the reddening treatment and may fully express their distinguishing characteristics that make this apple unique in the local and national markets.

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## **How to kill an asparagus plant?**

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### ***Introduction***

A common harvest duration of 60 days can be assumed for white asparagus under temperate climate conditions. The harvest period stops at the middle of June. This stop criterion has evolved from practical experience over many years and ensures proper fern growth to provide new assimilates for the next year harvest period. The fixed harvest duration explains the lack of knowledge about the theoretical yield potential of an asparagus plant with respect to cumulative spear weight and number.

### ***Materials and methods***

For estimation of yield potential we extended the harvest duration from 60 to 200 days. The asparagus plants were four years old. Two widely used cultivars – Gijnlim and Grolim – have been considered besides the grouping of plants with quite different fern growth durations in the previous years. Gijnlim and Grolim are two asparagus cultivars with different growth characteristics. 'Gijnlim' is an early sprouting, high-yielding cultivar, whereas 'Grolim' is a late sprouting cultivar with thicker spears and lower spear production than 'Gijnlim'. Spear number, weight and diameter and important quality traits as the number of rusted spears, and hollow/club-shaped spears were recorded daily. The concentration of carbohydrates within the rhizome was estimated at three weeks intervals.

### ***Results***

Gijnlim produced the highest number of spears per plant (102 spears) for the treatments with longest fern growth duration in the previous years, while Grolim produced 60 spears, respectively. There were no differences in yield (Gijnlim: 33 t/ha, Grolim: 34 t/ha). A stronger decrease in spear diameter can be observed for Gijnlim up from about 12 t/ha and for Grolim up from 8 t/ha cumulative spear yield.

The carbohydrate concentration was for all treatments 550 mg/g storage roots at the start of spear harvest and diminished to 250 mg/g after 200 days of spear removal.

### ***Discussion***

Comparison of yield thresholds for acceptable spear diameters to the overall obtained yield suggest, that under practical conditions yield limitations may arise more from spear bud size development in the previous year than from carbohydrate content of the rhizome. But both processes are probably strongly correlated.



# Improving Fruit Quality of Sweet Cherry (*Prunus avium* L.) through Flower thinning: A two-year field study.

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Sweet cherry (*Prunus avium* L.) is economically cultivated in temperate climatic regions producing non-climacteric fruits for local and national markets. For the production of high quality fruits with at least 20 mm of diameter and adequate colouring as defined in EC regulations intensive horticultural management is required. Fruit thinning is a well recognized strategy and has been successfully applied in a variety of fruits (e.g. apples, peaches, apricots). Fruit thinning effects through flower reduction cause increased fruit sizes, improved fruit quality, prevention of alternate bearing and balancing of the fruit-to-shoot ratio, leading to an increase in assimilates in fruit and shoots, whereas excessive fruit set may result in small fruit size and low quality, breakage of branches, exhaustion of reserves and reduced cold hardiness. Field study research on flower or fruit thinning with sweet cherries is in the beginning and published studies indicate the complexity of physiological and genetic interactions and difficulty of data interpretation. Published Studies give an account of reduced yield and increased fruit sizes (application of 5 % ammonium thiosulfate ATS) respectively did not find improved fruit sizes despite of a reduction of fruit set after the treatment with ATS (0.9 %). Though fruit thinning is an often used management strategy, the multiple interacting elements affecting fruit formation in the field need to be assessed. Timing, technique and the degree of thinning are key factors for developing a thinning strategy.

The first field study was performed in 2006 in a sweet cherry plantation employing a flower thinning strategy to address the questions (1) whether flower thinning can be achieved on sweet cherries employing the chemical flower thinning agent ammonium thiosulfate; and (2) if flower thinning affects fruit size of three respectively four sweet cherry cultivars. In the first study the effects of flower thinning were investigated by the application of water and ATS with 8.82 g l<sup>-1</sup> and 17.64 g l<sup>-1</sup> at the stage of 100 % opened flowers of the cultivar “Blaze Star”, “Samba” and “Techlovan” (rootstock “GiSelA 5”). Laboratory Analyses included crop load, fruit size, fruit weight, soluble solids (in ° Brix), pH-values and the content of anthocyanins (expressed as cyanidin-equivalents, analysed by spectrometer).

To confirm the results of 2006 the study was repeated in 2007. Derived from the results of the first study of 2006 the experimental setup was advanced: In 2007 the application of ATS with 8.82 g l<sup>-1</sup> and 17.64 g l<sup>-1</sup> at the stage of 100 % opened flowers and the application of ATS with 17.64 g l<sup>-1</sup> ATS at the stages 40 % and 80 % opened flowers, but no application of water were implemented. Additionally to the 2006 cultivars variety “Merchant” was included in the trial. In addition to the parameters of 2006 the stone: fruit – ratio and the content of titratable acidity of fruits were analysed.

In 2006 significant reduction in crop load was assessed by chemical flower thinning though fruit size and fruit weight were not significantly increased. The content of soluble solids and the pH-value were significantly directly or indirectly affected by the application of the thinning agent. In 2007 the average fruit weights of the four cultivars Blaze Star, Samba, Techlovan and Merchant were 4,0 g / 5,3 g / 9,0 g / 4,9 g (control), 3,9 g / 5,3 g / 10,2 g / 6,7 g (17.64 g l<sup>-1</sup> ATS, 40 % opened flowers), 4,5 g / 6,9 g / 8,8 g / 6,1 g (17.64 g l<sup>-1</sup> ATS, 80 % opened flowers), 6,4 g / 5,7 g / 8,5 g / 5,6 g (17.64 g l<sup>-1</sup> ATS, 100 % opened flowers) and 4,5 g / 5,4 g / 7,4 g / 6,1 g (8.82 g l<sup>-1</sup> ATS, 100 % opened flowers) with the cultivar Techlovan always achieving highest weights. Concerning fruit sizes fruits of Blaze Star had the smallest (20,3 mm – 21,2 mm) and fruits of Techlovan the greatest sizes (25,2 mm – 28,6 mm) in all treatments. Further data work will be done as the experiment proceeds.

Examining the data of 2006 and 2007 we conclude significant effects of the variety and the degree of flower thinning on various fruit quality parameters. As with any chemical thinning agent effects are hard to predict and more basic work has to be done to elucidate the morphological and molecular effects of thinning agents to the flower and fruit considering each variety in particular concerning timing, concentration and the general need of flower thinning agents.

# Quality Management for Vineyards through intra-vineyard and inter-plant monitoring of physiological plant indicators

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On site variability in vineyards is a well-known fact caused by natural geological processes, soil properties and environmental factors. Recognition of these fine-scale variations is essential for grapevine management, especially for defining stressed vs. non-stressed areas, since stress seems to play a key role in producing high quality fruits and wines. The aim of this study is to define (1) intra vineyard variation; (2) to assess and evaluate a set of plant indicators reflecting the vine's physiological status and (3) to define parameters that correlate the plants status with fruit quality.

Intra vineyard - and inter plant variation was monitored analysing the physiological condition of vines by water potential, gas exchange, chlorophyll fluorescence and nutritional status. The vineyards (Pinot noir/5BB) in the age of five to six years, located at Vienna and Sooß (Lower Austria) were randomly sampled (four times/year) and individual vines (133/ha) were randomly measured. Additionally a greenhouse trial with one-year old grapevines was conducted to differentiate inter plant variation employing two different Pinot noir clones (four replications/plant; 15 replications/clone) under controlled environmental conditions. Fruits were randomly sampled at maturity and analysed by FTIR-spectroscopy. Data analysis has been carried out by interpolation of sampled data to predict parameter values of non-sampled grapevines in the vineyard. Interplant variation was measured to evaluate plant physiological parameters: only Parameters with insignificant variance between plant materials of same age and physiological status was used for mapping intra-vineyard variation.

Execution of data analysis basing on correlations with stress impact factors (e.g. reduced photosynthesis efficiency) resulted in the definition of "stress" zones which facilitate efficient vineyard management for the growers. Analysed data shows a high correlation between water stress zones and grapevine ingredients. Special vineyard managements are possible to increase certain grape berry ingredients.

## Sanitation of root vegetables

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Plenty of literature exist dealing with sanitation of fresh fruit and vegetable during post-harvest processing. Human pathogens as well as plant pathogens should be inactivated to ensure consumption of safe produce and to prolong produce shelf. Different treatments are proposed like dry heat, hot water, chlorinated water, ozone and others (Artes and Allende 2005). But the effect of these treatments is often limited. Therefore, Zagory (1999) recommended to improve hygienic conditions and to assure a closed cold chain from harvest to the point of sale (POS) without such active treatments.

Carrots are harvested in big bins, stored, washed, polished, packed and cooled down for several hours. After reaching a core temperature of 7 °C the carrots are transported to the retail store and presented without cooling at the POS up to several days. Very often, such carrots will start rotting and get dark spots within few days.

In recent studies also limits in bactericidal effects of chlorinated water and aqueous ozone was presented regarding postharvest processing steps. But to prevent cross-contamination wash water sanitation is recommended. Usually wash and rinsing water in washing plants are loaded with organic substances (chemical oxygen demand (COD)) up to 10 000 mg of COD, which limit the sanitation effect of oxidizing agents. For example 1 g of carrot per litre water represents already about 100 mg COD. The task of this experimental study was therefore to investigate the remaining inactivation potential of chloride and ozone when applied to

*Pectobacterium carotovorum* and *Escherichia coli* suspended in tap water and loaded with different concentrations of COD.

Fine shredded carrots (0.1 to 10 g l<sup>-1</sup>) were mixed with *P. carotovorum* resp. *E. coli* suspension (10<sup>4</sup> to 10<sup>6</sup> cfu ml<sup>-1</sup>) and put into chlorinated (1000 ppm free Cl<sup>-</sup>) resp. ozonated (up to 4 ppm ozone) water. After thinning, resp. stirring the suspension plate count of the bacteria was performed after 48 h incubation on McConkey agar at 37 °C.

The threshold for inactivation of *P. carotovorum* was about 0.7 g l<sup>-1</sup> shredded carrot for the chlorinated water and 1 g l<sup>-1</sup> for ozonated water. The threshold for *E. coli* in ozonated water was about 0.5 g l<sup>-1</sup>. For higher concentrations of COD the effect was negligible.

The results show, already little concentrations of organic matter in washing or rinsing water in modern vegetable processing plants inhibit the effect of sanitation treatments. It is assumed that the oxidizing agents first react with the organic matter without influencing bacterial growth. High hygienic standards, i.e. closed cool chain, fixed cleaning cycles and frequent water change, can not be compensated by the use of chemical treatments resp. oxidative agents.

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## **Multi-element (H, C, N, O) stable isotope characteristics of soft fruits and strawberries**

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The stable isotope ratios of soft fruits were never extensively explored, whereas several recent papers show the usefulness of stable isotope analysis in determining the genuineness and geographical origin of orange juices. In this study we analysed two strawberry cultivars and several cultivars of soft fruits, in particular red raspberry (*Rubus idaeus* L.), blackberry (*Rubus fruticosus* L.), highbush blueberry (*Vaccinium corymbosum* L.), and currant (*Ribes rubrum* L.) produced in the North Italian region Trentino and in two sites in Romania and Poland. The measurement of the ratio D/H of the fermented alcohol and of the ratios 18O/16O of juice water, 13C/12C of sugar, acid and pulp fractions and 15N/14N of pulp was performed by Site specific Natural Isotopic Fractionation – Nuclear Magnetic Resonance (SNIF-NMR) and by Isotopic Ratio Mass Spectrometry (IRMS), respectively. About 170 samples related to the 2005 and 2006 seasons were considered, taking into account factors like genotype and/or species, altitude of the production area and cultivation systems: in soil and soilless conditions were assessed, together with controlled and uncontrolled microclimate by use of tunnels. According to the species, typical ranges of 18O/16O, (D/H)I and in some cases 13C/12C values were observed.

These differences are probably due to the adopted cultivation practice and/or to the plant physiology. The samples produced in soil show significantly higher 15N/14N values than those produced in soilless conditions probably due to the organic and/or mineral fertiliser applied. Finally, the possibility to distinguish the production in typical areas of Trentino from other sites was checked.

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## Water status gradients in cut roses

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### **Introduction**

The vase life of cut roses is largely determined by the postharvest water regime. Cut rose cultivars with a low mechanical strength need an effective protection against turgor loss to achieve a long vase life and low bent neck susceptibility. A well established parameter to describe the water status is the water potential ( $\Psi$ ). In this study we proofed the hypothesis that the lower the water potential difference between leaves and flowers buds the better is the water status management in postharvest.

### **Material and Methods**

For this purpose the relevant water relation parameters of the cut rose cultivars ‘Akito’ (low mechanical strength and low vase life), ‘Milva’ (low mechanical strength and long vase life) and ‘Red Giant’ (high mechanical strength and long vase life) were investigated with a scholander bomb.

### **Results**

In June average flower bud water potential of roses after one day cool storage and 3 h acclimatisation at 20 °C and 60% air humidity was approximately -0.11 MPa in all investigated cultivars. In contrast, in December plants of ‘Akito’ (-0.12 MPa), ‘Red Giant’ (-0.09 MPa) and ‘Milva’ (-0.07 MPa) showed a significant different flower bud water potential. Average leaf water potential was always significantly lower, ranging from -0.64 MPa in ‘Milva’ to -1.15 MPa in ‘Red Giant’. The difference between flower and leaf water potential was always significantly lower in ‘Milva’ than in the two other cultivars. Leaf water potential did never depend on the leaf position on a stem. This finding was valid for roses of all cultivars. The magnitude of the water potential difference between flower bud and leaves might be explained by a safety function of the abscission zone at the base of the peduncle, or simply by the extent of leaf transpiration.

### **Conclusion**

From these results it may be concluded that the long vase life of ‘Milva’ is due to the highly effective water status management, resulting in higher leaf water potential and a lower flower to leaf water potential difference. The long vase life of ‘Red Giant’ plants may be based on the high mechanical strength of their peduncle tissue. ‘Akito’ roses had a low mechanical strength and an ineffective water status management and, hence, showed a short vase life.

# ***Chalara* Black Root Rot: A serious Challenge to the Swiss carrot Production and Processing Industry**

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Carrot is one of the most important open field vegetable produced in Switzerland. As many of the soils of the Swiss plateau used for vegetable production are possibly infested with *Chalara elegans* and *Chalara thielavioides* the occurrence of *Chalara* black root rot on stored and processed carrots is a latent risk. In the last years this quality problem led to increasingly frequent complaints from consumers and retailers. Black root rot symptoms appeared often during distribution or - even more unacceptable - after purchase by consumers. The aim of our project was to identify factors affecting *Chalara* black root rot along the entire chain from the field to the table. Investigations included analysis of the distribution of the fungus in the main carrot production areas. The effect of field contamination by *Chalara* sp., cooling and storage techniques, washing processes on yield of marketable carrots and shelf life was investigated during two harvest seasons.

Our investigations in 6 carrot processing plants showed that of 671 tested samples of stored carrots from 116 producers 52 % were contaminated with *Chalara* sp., in 70 % of the cases *C. thielavioides* was detected, in 24 % *C. elegans* and in 5 % there were mixed infections of both species. In order to prevent black root rot on carrot, attention must therefore be focused on post harvest operations. The washing process with recycled water favoured cross contaminations. It could be shown that the use of clean water in the last steps of the washing process prevented infections by *Chalara* sp. and the subsequent development of black root rot on the processed carrots. In addition maintaining temperatures below 8 °C after washing and packaging was crucial especially if the water quality used for washing was poor.

## **Monitoring of cut roses for freshness during post harvest process by cavitation and transpiration studies**

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### ***Introduction***

In floriculture, the introduction of quality management concepts along with the implementation of freshness and vase life guarantee at different trade levels has stimulated demand for new methods to quantify the inner quality of cut roses. Easy techniques like the declaration of harvest date or monitoring the flower's weight loss from harvest to end-user are not enforceable at present. Water potential, generally accepted as a freshness indicator of perishables describes only the actual water status of plant segments, and this method is usually invasive (e.g. according Scholander). In contrast to that, monitoring of stomata function by transpiration or cavitation studies is non-destructive and describes mechanisms controlling plant water relations/freshness during post harvest life continuously. The simultaneous measurement of cavitation and transpiration processes, provided by UAE-techniques in a gas exchange chamber, and its potential for prediction of cut roses stress management is discussed in the paper.

### ***Results***

Experiments with different cultivars have shown, that fresh cut roses are characterized by 1) generally high transpiration during light phases, 2) large amplitudes of transpiration rate under fluctuating light/dark conditions, and 3) nearly no occurrence of cavitations. Additionally, after exposure of the cut surface of fresh roses to air, a high Iwanoff effect (temporary passive increase of transpiration rate) could be observed along with a short-time (usually about 1hour) strong increase of the cavitation rate. Subsequently, a drop-off of

both transpiration and cavitation rates to a very low-level reduced water loss due to the stomata closure to the minimum, and shows control of water stress.

In the following stages of post harvest life, though still before visible deterioration of cut flowers (according to cultivar, leaf area and post-harvest handling), more frequent occurrence of cavitations during light phases could be registered indicating a slightly imbalanced water status. Such continuous increases of cavitation rate are usually accompanied by continuously decreasing transpiration rates at the same time. These results support recently published findings about the role of cavitations as signals for stomata closure to keep the plant water relations in balance.

Decreases of transpiration rates to a relatively low level, marginal differences between light and dark cavitation rates along with no Iwanoff effect during exposure of the cut surface to the air were typical for the final stages of cut rose's vase life.

### **Conclusions**

Monitoring water relations in this way reveals a better understanding of the water stress management of cut roses during post harvest life and shows potential for determination of flower freshness.

## **Effects of saline irrigation water on growth, physiology and quality of *Mesembryanthemum crystallinum*, a rare vegetable crop**

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Commercial use of halophytes as fresh food is limited. On the other hand, world wide increased desertification due to recent global changes enhances the need of irrigation, which, in turn, provokes the risk of soil salinization. Furthermore, limited fresh water resources may increasingly constrain the use of low quality irrigation water. Hence, intensified use of halotolerant crop plants will be necessary, even in Europe.

Several facultatively halophytic members of Aizoaceae are nowadays used as special crop plants. Since about 200 years *Tetragonia tetragonioides* (Pall.) O. Kunze has been grown as a spinach-like vegetable or fresh salad. A rare lettuce is the common ice plant *Mesembryanthemum crystallinum*, a CAM species, which is mostly cultivated in India, California, Australia, and New Zealand. It is also known in Europe as a quickly cooked tender vegetable. With their succulent, mellow, slightly salty tasting leaves and young shoots, *Mesembryanthemum crystallinum* is getting interesting as delicious cool flavored salad greens during recent years. However, it is a perishable product and shelf life is short.

In a cooperative project we studied whether moderate salt treatment affects physiology, growth and yield. Furthermore, we investigated whether such treatment that enhances the irreversible C3 to CAM shift in young leaves of this CAM species, potentially prolongs shelf life. Results show that moderate salt treatment does not negatively influence growth. When in CAM, leaves show reduced transpirational water losses and CAM also reduces carbon losses during storage.

# Impact of UV and ozone as physical elicitor on cell wall composition and mechanical properties of white asparagus (*Asparagus officinalis* L.) in postharvest

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The fleshy spears of white *Asparagus officinalis* L.) are developmental immature and rapidly growing shoots. After harvest they retain their physiological activity and even continue growth at high rates. As asparagus spears are purchased not only as fresh commodity but increasingly as convenience product (i.e. sliced, fresh-cut), quality assurance has to focus on the retardation of metabolic processes in postharvest on one hand and on the other hand has to meet hygienic requirements. Losses in food industry due to microbiological spoilage (e.g. *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*) have been estimated as being as high as 30%. Therefore and due to new food safety regulations (HACCP concept, traceability), the optimization of postharvest treatments and storage requirements is an essential tool for the food supply chain management of asparagus.

The application of short-term UV radiation and ozonated wash water is known for disinfection of pathogenic organisms in numerous food products. Hence, the aim of our investigation was to evaluate the stress mediated plant responses of physical elicitors such as UV and ozone on textural properties and on the storability of asparagus spears.

Immediately after harvest, asparagus spears cv. Gijlim were subjected to a) a short-term UV-B application (UV dosage: 8.2 J m<sup>-2</sup>) for 60, 90 or 120 min using an UV-B fluorescence light source (FL 20SE, 305-310 nm) or b) additionally were submerged in ozonated water (4 ppm) for 2 min. After an adaptation time of 2 and 22 h cell wall composition (mono- and disaccharides, cell wall content of pectic substances, cellulose, hemicellulose, lignin and protein) were analysed and mechanical properties (dynamic stiffness coefficient, tissue strength) and as well as water status determined. Non treated spears were used as control. An aliquot of postharvest treated spears were stored at 2°C and 20°C for up to seven days.

It was found that UV and ozone treatment had a pronounced effect on the synthesis of cell wall composition and mechanical properties of asparagus spears. Results will also be discussed and outlined in terms of possibilities and constrains for the application and transfer in food chain systems.

## Studies on Phyllody in *Parthenium hysterophorus* and detection of phytoplasmas within important crops cultivated in Ethiopia

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### Introduction

*Parthenium hysterophorus* L. is an annual weed exhibiting high competitiveness and adaptability to different climatic and soil conditions. It is thought to originate from Middle America and is now widely spread in tropical regions including parts of East Africa. It was introduced to Ethiopia in the 1980ies and became the major invasive weed in both arable and grazing lands. In Ethiopia a disease caused by phytoplasmas was commonly observed in *Parthenium* (up to 75% field incidence). Diseased plants show excessive branching, reduced plant height and leaf size as well as modification of petals into leaf-like structures that lead to sterility (phyllody). More than 700 plant diseases are associated with phytoplasmas. Phyllody symptoms caused

by phytoplasmas were already found on different crops, This suggests that Parthenium phyllody also affects a wide range of crops in Ethiopia.

### **Methods and Results**

In order to test whether Parthenium plants harbour phytoplasma, which may also infect important agricultural crops in Ethiopia, diseased Parthenium as well as cultivated plants were collected from locations in Ethiopia along the Rift Valley. Sesame (*Sesamum indicum*), grass pea (*Lathyrus sativum*), lentil (*Linum esculentum*), chick pea (*Cicer arietinum*), and fenugreek (*Trigonella foenum-graecum*) showed extensive phyllody symptoms. Peanut (*Arachis hypogaea*), faba bean (*Vicia faba*), papaya (*Carica papaya*), orange (*Citrus sinensis*), and tangerine (*Citrus reticulata*) with phyllody, witches'-broom (small chlorotic leaves, proliferating shoots, shortened internodes) as well as die-back symptoms were included in the study.

Phytoplasma infection of plants was assessed by polymerase chain reaction (PCR). Amplified fragments were sequenced allowing species identification of the pathogens. Obtained rDNA sequences revealed that phytoplasmas detected in Parthenium plants were also present in sesame and peanut. Sequence identities of 1488 bp of the 16S rDNA sequence were above 99%, covering strains infecting sesame and peanut in other countries. Ethiopian Parthenium, sesame and peanut phytoplasmas exhibited sequence similarities of 98% to phytoplasma strains within the 16SrII species group (Peanut witches'-broom group) including a phytoplasma originating from Ethiopian papaya with die-back symptoms (Arocha et al. 2006), faba bean phyllody (FBP) and the reference species *Candidatus* Phytoplasma aurantifolia, causing witches'-broom disease of lime (WBDL, Zreik et al. 1995).

### **Conclusions**

Phytoplasmas detected in Ethiopian crops are closely related, which suggests that Parthenium represents a pathogen reservoir for the phytoplasmas affecting agricultural crops in the country. Since phytoplasma infections can lead to sterility of the florescences, severe losses in yield of crops can be expected. Thus, it seems unsuitable to utilise phytoplasmas as biological antagonists in integrated Parthenium management as suggested in Taye et al. (2004).

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## **The effect of ethanol treatment on removal of astringency in stored persimmon fruit (*Diospyros kaki* L.)**

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Persimmon fruit is a very important crop in Indonesia, but the post-harvest handling of persimmon is limited. The astringent taste of most cultivated persimmon, due to naturally occurring polyphenols in the fruits, causes a lack of acceptance by consumers in Indonesia and Europe. To remove astringency, a post-harvest treatment with lime solution is applied, but it impairs appearance and thus also limits acceptance of fruits.



The aim of this study was firstly, to evaluate, whether an ethanol treatment would remove the astringent taste of persimmon fruit without impairment of fruit appearance and, secondly, which ethanol concentration would be most appropriate during storage to meet consumers' needs. In addition, the influence of ethanol treatment on further quality parameters was investigated in order to achieve the best quality of persimmon fruits.

To review the working hypothesis, the fruits of Junggo persimmon were obtained directly by the farmer in Batu (Indonesia). These fruits are typical for East Java and are used for direct consumption as well as for cooking and processing purposes. The persimmon was cultivated under typical conditions for this fruit in Indonesia. The work was performed from June to September 2005 as a two-factorial complete randomized design with different ethanol concentration (0%, 20%, 40% and 60%) and durations of storage (harvest, 5, 10, 15 and 20 days) with 3 replications.

Duration of storage and ethanol treatment reduced significantly the content of tannins in persimmon fruit as well as astringent taste, with duration of storage being more effective. The reduction reached over 80 % in case of the application of 60 % ethanol and a storage of 20 days. However, a decrease of ascorbic acid of up to 28 % was also observed. Changes in taste occurred not only due to the degradation of tannins, but also due to the increase of content of total soluble solids (up to 97 %) and softening processes (changes of texture). However, too soft fruits as well as fruits with senescence symptoms were rejected by panellists, especially after 20 days of storage. With respect to the content of tannins and the results of the sensory panel, the best results were achieved using 40 % ethanol and 10 days of storage. The so-treated Junggo persimmon fruits were sweet, firm, with a good aroma and preferred by consumers.

## **Study of a tomato growth model for optimization of greenhouse microclimate in a tomato seedlings greenhouse**

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### ***Introduction***

Today producers have to utilize all available inputs on the optimum way in order to achieve the sustainability of their farm. That requires, when it comes to nurseries, advanced control and decision support systems. These systems rely much on models to estimate the impact of today decisions in future production, based on limited real world data. Therefore, among others, a seedlings crop model is needed. Despite a number of tomato growth models have been developed, none of them has been used to model the growth of tomato seedlings. The objective of this work was to evaluate the efficiency of a mature tomato crop growth model (TOMGRO) to model the growth of a tomato seedlings crop.

### ***Materials and Methods***

Since certain differences exist between a plant in the production stage and a seedling some adjustments on the TOMGRO model had to be done. Seedlings do not have reproductive organs, so functions or part of functions concerning fruit development and truss appearance were removed. Attention was paid not to affect the rest functions. The organs that exist at any time are not old and do not have different performance due to age. Also, seedlings crop growth period is much less than the growth period of a productive crop, thus the number of age classes of organs is smaller. Hence, age class number was decreased and functions concerning senescence were removed.

In order to evaluate the model, a number of experiments were conducted under various temperature and solar irradiance regimes. The experiment was held in Velestino (Thessaly, Greece), during spring (23 February – 19 March 2007) and the mean daily temperature was 25°C and the mean night temperature was 19.4°C. Plant density was about 312 plants/m<sup>2</sup>. The mean daily solar radiation was about 8.2 MJ/day.

## **Results**

Comparison between measurements and modelled results reveal that TOMGRO can not be used to model the growth of tomato seedlings, unless significant modifications are made. At the end of the experimental period the predicted dry matter of the crop was four times lower than the measured one. That could be attributed to the fact that the potential growth rate table, used by the model, uses values lower than the measured one. Despite that the leaf area index (LAI) of the crop finally underestimated, the simulation data agree well with the measured until 18<sup>th</sup> day since emergence. LAI and dry matter (DM) are strongly correlated and since DM is underestimated LAI is also underestimated. For the same values of measured and estimated DM, the estimated values are 30% lower than the measured. On the contrary the number of leaves, is overestimated up to twice the real value. That must be due to high potential leaf appearance rate that is used by the model for a mature crop. Dry matter partitioning is relative well estimated. The dry matter partitioning to the roots is underestimated, but this is due to a maximum rate of DM partitioning to the roots set to the model, which seems not to apply for seedlings. The above affects the dry matter partitioning to leaves and shoots. The ratio of leaves dry weight to shoots dry weight is well estimated for the half of the experimental period while later it seems that the model progressively favours leaves DM.

## **Conclusions**

In conclusion, the TOMGRO model fails to simulate the growth of tomato seedlings but a number of further adjustments could be made to incorporate in the model the characteristics of a seedlings crop, such as the light extinction coefficient. The values of the potential growth rate could be corrected to reflect better the exponential growth rate of a seedling. The potential leaf appearance rate could also be adjusted to reflect the leaf appearance rate of seedlings. The ratio of leaf dry matter to shoot dry matter has also to be adjusted. Age classes and number of organs per class also affects the behaviour of the model. After the above adjustments the model might be able to simulate the growth of tomato seedlings.

## **Environmental and productive zoning of apple-growing (Golden delicious cv) in western Piedmont district**

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## **Introduction**

The zoning concept was first introduced in the viticultural sector in the '90s. It has developed quickly and after few years it has been adopted by other agricultural sectors, particularly fruit-growing. In this sector, zoning aims to optimize the relationships between cultivar, territory and pedoclimatic conditions. This new approach allows to get over the unilateral and partial conceptions that characterize either cultural techniques and agronomic practices in orchard management. Apple culture has spread over Piedmont in different pedoclimatic conditions: in plain, hill and lowland mountainous areas, thus bearing fruits with a wide range of qualitative attributes. The aim of this research was to zoning the fruit-growing area of western Piedmont, focusing on Golden delicious apples cultivar.

## **Materials and methods**

The study was carried out in three successive years (2004-2006), considering 12 different typical zones of the fruit-growing area of western Piedmont, characterized to have a real turn for apple culture. Two plots per zone, with Golden delicious cv, have been selected in all areas, characterized by the same cultivation techniques. Geomorphological, pedological, agrometeorological, phenological, productive and fruits qualitative features aspects have been carried out on these places.

Different statistical analysis were performed using all the variables detected: Cluster Analysis, Discriminant Analysis and Multiple Linear Regression.

### **Results and discussion**

Starting from 12 zones located in the fruit-growing piedmontese district a Cluster Analysis was performed. The region was divided into three macro-areas characterized by specific bioclimatic indexes (STA, SET, Tx, Tn, Ta) and fruit quality indexes (flesh firmness, total soluble solids, titratable acid, chrome and hue).

Moreover correlations and linear regressions among the variables detected were carried out and the obtained equations allowed elaborating thematic maps about fruit qualitative features, exploiting the data spatial analysis techniques (tab.1).

Indices	$R^2$	Signif. F	Latitude	Longitude	Altitude	Exposure	Slope
STA	0.537	0.664					
SET	0.34	0.881					
NRD	0.964	0.023	***	***			
ERS	0.903	0.093	***				
PVR	0.855	0.164					
SFR	0.978	0.011	***				***
LVP	0.654	0.489					

Tab.1- Coefficient of determination ( $R^2$ ), significativity and variables employed in multiregressive linear models of bioclimatic indices. STA= sum of the active temperatures, SET= sum of daily thermal excursions, NRD= number of rainy days, ERS=effective rain summation, PVR=period of vegetation renewal, SFR=spring frost risk, LVP=length of vegetative period.

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## **Quality comparison in different storage times between Golden delicious apples coming from various pedo-climatic areas**

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### **Introduction**

Golden delicious is commonly considered the cultivar preservable for the longest time, either in normal or in controlled atmosphere. It is also known that the storage period depends on the fruit quality characteristics detected at harvest. Since in Piedmont apple-growing spreads over areas with different soils and climatic properties, it is possible to find out a wide range of different organoleptic characteristics.

The aim of this work is to analyze fruit quality traits at postharvest in order to compare storage properties of Golden delicious apples coming from 12 piedmontese farms placed in plain, hill and mountainous areas.

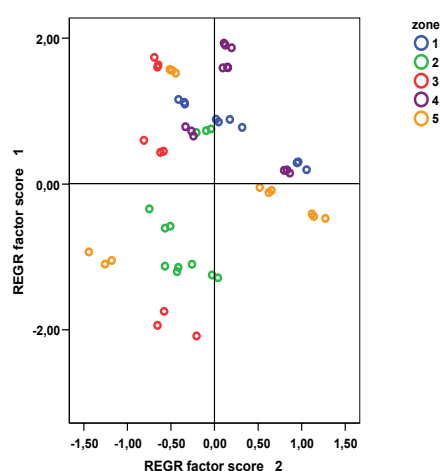
## Materials and methods

Experimental activity was carried out for two years (2004-2005) in western Piedmont apple-growing district (Cuneo province). Fruits were stored either in normal (3-3,5 °C; 95% R.H.) or in controlled atmosphere (1-1,5°C; 3% CO<sub>2</sub>; 2% O<sub>2</sub>), then, at the end of the storage period, fruit quality was assessed via destructive and non destructive analysis and correlated with bioclimatic indices and pedological parameters, such as the presence of soil macroelements. In order to evaluate the consumers point of view, a sensory evaluation was performed by a testers panel in contemporary with chemical quality analysis. Moreover, sensory analysis results were correlated with destructive analysis via Principal Component Analysis.

## Results and discussion

Data analysis confirms that growing environment affects fruit quality even after storage period.

In particular, first year results show that soil Ca/K ratio is positively correlated with total soluble sugar in stored apples and with fruits flesh firmness. At the end of the second year a strict correlation between bioclimatic indices and fruit quality was also detected.



Statistical analysis indicates a strong link between daily thermal range and total soluble sugar: apples coming from areas with a wider thermal range have higher Brix degree. Moreover, a positive correlation between the sum of active temperature and apples titratable acidity was obtained. A higher content of titratable acidity, responsible for a longer storage period, was also detected in fruits coming from areas with higher calcium content in soil and wider thermal range. Using the PCA analysis it appears the division into areas using the panel test and the destructive analysis (fig.1).

Fig.1- PCA of two years sensory analysis correlated with destructive analysis.

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## Effect of hypoxia on yield and quality of leafy vegetables grown in floating system

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Floating system is a soilless cultivation technique particularly suitable for growing small-size, short-cycle (40-60 days) species like leafy vegetables, especially if aimed to produce ready-to-use salads. As well as in other hydroponic systems, plants grown in floating system may have problems of oxygen deficiency (hypoxia) at root level, with obvious consequences on the development of their aerial part. The phenomenon is surely more urgent in the summer, as with higher temperatures the quantity of oxygen dissolved in the nutrient solution decreases and root respiration rate increases. In the specific case of floating system, the stillness of the solution, that may increase the problem, is compensated by the large volume of solution available for the single plants and the shortness of the growing cycles. Nevertheless, in order to avoid any risk, the nutrient solution is oxygenated, so resulting in additional costs. It is commonly thought that oxygen concentration must be kept around values of 5-7 mg l<sup>-1</sup>, yet physiological response (yield and quality) of the different vegetable crops to root hypoxia when grown in floating system has been little investigated. As far as quality

is concerned, it is well known that for leafy vegetables low nitrate content is demanded. Based on the recent evidence that, in hypoxic conditions, plants are able to activate an alternative respiratory pathway in which nitrate they contain is used as an electron acceptor, and so consumed, oxygen deficiency in the nutrient solution, if demonstrated not to be detrimental for yield, may be used to control nitrate accumulation in the product.

On the basis of these remarks, some experiments have been carried out in order to investigate the possibility of growing successfully leafy vegetables in floating system without devices to oxygenate the nutrient solution, thus attaining an economic benefit (reduction of investments and running costs) and to verify whether hypoxic conditions may cause a reduction of the vegetable nitrate content, thus achieving an improvement of the product quality.

Experiments were performed on spinach (*Spinacia oleracea* L.) and rocket (*Eruca sativa* Miller). Plants were cultivated in floating system, with or without an oxygen supplying device, in spring or summer period. Yield and product quality were evaluated on harvested plants by collecting the following data: leaf and root fresh and dry weight, leaf area and leaf nitrate, chlorophyll and carotenoid content.

Results indicated that the oxygenation of the nutrient solution is not indispensable to grow rocket and spinach in floating system for the production of ready-to-use salads. In fact, although oxygen concentration in the non-aerated solution decreased progressively to values, at harvest, of 0.5-1 mg l<sup>-1</sup> (compared to average levels in the aerated solution ranging from 4.72 to 8.11), with negative repercussion on root development more evident in the spring cycle, no yield reduction was observed in hypoxia grown plants.

As far as product quality is concerned, only in the spring cycle of rocket higher leaf dry weight percentage and chlorophyll content (attested also by higher SPAD values) were detected in the non-aerated thesis, while in no case oxygen deficiency cause the expected nitrate reduction.

Probably the short cycle (29 days in spring and 24 days in summer) and the high volume of the nutrient solution (250 l m<sup>-2</sup> of cultivated area) prevent the plants from feeling the effects of hypoxia. About nitrate content, it is also possible that the concentration of the nitric ions in the solution (although that normally recommended for fresh-cut vegetables) was excessive compared to plant needs – values observed in the leaves were in fact very high – so that differences could not be detected between the compared theses.

## **Freshbag® for Transport of Cut Flowers**

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### ***Introduction***

Consumers often transport cut flowers after purchase for some time without water. Thereby the length of time as well as transport temperature may vary in the individual case. This period of stress due to a lack of water and its adverse effect on cut flower quality should be reduced with Freshbag® – a type of 'transportable vase' with a cellulose pad and a leak proof plastic foil. The aim of this study was therefore to investigate the effect of Freshbag® on cut flower vase life in comparison to dry transport and transport in water.

### ***Materials & Methods***

Several trials were carried out to show the impact of Freshbag® in comparison to a dry, waterless transport. Therefore, popular cut flowers (roses, chrysanthemums, alstroemerias, carnation, gerbera and leather fern) were bought at a wholesale market, arranged into bouquets with 10 stems each and recut to a uniform length. The flowers were stored either dry, in a water-filled glass vase or in a water-drenched Freshbag® to simulate different transport and temperature conditions.

A constant temperature of 20 °C and a relative humidity of 60 % were kept during the transport simulation in a growth chamber and in the following vase life test. The bouquets were exposed to 12 hours daylength

(between 7 am and 7 pm) with 1,000 lux. Tap water from the city of Freising (pH 7.5; EC 665  $\mu\text{S cm}^{-1}$ ; 2.86  $\text{mmol l}^{-1}$  of total hardness; 5.71  $\text{mmol l}^{-1}$  acid capacity at pH 4.3) was used for the investigations.

## Results

First experiments with different transport times (3, 6, 12, 24 and 48 hours) showed that in the case of dry transport without Freshbag<sup>®</sup> first wilting symptoms could be seen with the alstroemerias already after 3 hours. Some roses and the leaves of the chrysanthemums started to wilt after 6 hours. Only the carnations and the fern were without damages after 12 or 24 hours of dry transport. Flowers of all species in the bouquets wilted and showed partial damages over 24 hours. If the flower stems were placed in a Freshbag<sup>®</sup> during the different transport phases first wilting symptoms appeared after 24 hours at the earliest. Hardly any differences were observed between the cut flowers in the control vase (permanently stored in water) and the ones in Freshbag<sup>®</sup> up to this time. Moreover, the flowers transported in the dry state recovered in most of the cases after transfer back to water within 3 to 6 hours.

It was shown, that Freshbag<sup>®</sup> prevented flowers from wilting under varying conditions in further experiments of higher transport temperatures (30 and 40 °C) and different transport periods (3 or 6 hours). In general, the wilting symptoms increased with transport length and temperature (table 1).

Table 1. Percentage of wilting flowers per bouquet depending on treatment (transport method/period/temperature)

Treatment	3 h / 30 °C	6 h / 30 °C	3 h / 40 °C	6 h / 40 °C
Dry	40 %	70 %	60 %	90 %
Freshbag <sup>®</sup>	5 %	10 %	15 %	25 %
Water (Control)	5 %	5 %	15 %	15 %

The vase life of the mixed bouquets after a dry transport phase at 30 °C decreased somewhat and at 40 °C significantly in comparison to transport in Freshbag<sup>®</sup> or with water. Average vase life has been diminished with the high transport temperature (40 °C) from 8.3 to 6.7 days. A dry transport shortened the shelf live significantly from 8.6 (with water) and 8.4 (with Freshbag<sup>®</sup>) respectively, to 5.5 days.

## Sooty blotch fungi on German apples: new insights in phylogenetic diversity of species

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In some parts of Germany organically grown apples may show symptoms of sooty blotch fungi affecting heavily fruit quality and consumer acceptance (German: “Rußfleckenpilze”, as part of the “Schwärzepilze”). Sooty blotch fungi also may occur on scab resistant apple varieties in integrated production systems, if fungicide application can be omitted.

In former times researchers thought that these symptoms were caused by one single fungus called *Gloeodes pomigena*, later redescribed as *Peltaster fructicola*. This species indeed can be found on some of sooty blotch affected German apples, but thorough studies meanwhile have shown that diversity of German pathogenic species is far higher than originally assumed. Morphological determination of these fungi was hampered by a lack of good microscopical characters, but parsimony analysis of nuclear rDNA (LSU and ITS) of these fungi proved that many phylogenetically different species of several genera can cause these symptoms. Meanwhile in Germany we could detect anamorphic species of two different orders of Ascomycota, the *Chaetothyriales* and *Dothideales*:

*Phylogenetic affiliation of anamorphic German sooty blotch fungi on apple fruit*

- Chaetothiriales*: (1) *Phialophora sessilis* de Hoog (1999) (growth form: hyphomycetes)  
 (2) *Phialophora* sp. (hyphomycetes)
- Dothideales*: (3) *Peltaster fructicola* Eric M. Johnson, T.B. Sutton & Hodges (1996) (fruiting bodytype: coeleomycetes)  
 (4)(5) *Peltaster* sp. 1 & sp. 2 (coeleomycetes)  
 (6)(7) *Pseudocercospora* sp. 1 & sp. 2 (*Mycosphaerellaceae*) (hyphomycetes)  
 (8) *Pseudocercospora* sp. (*Mycosphaerellaceae*) (hyphomycetes)

Some further sooty blotch or sooty mold fungi could be detected like (9) *Tripospermum camelopardus* In-gold, Dann & P.J. McDougall (1968), (10) *Tripospermum myrti* (Lind) S. Hughes (1951) (growth form: hyphomycetes) and some species which could not yet be characterized sufficiently and whose ITS rDNA regions were different from all the fungi that are deposited in GenBank. Besides the sooty blotch fungi also fly-speck fungi often can be found on apple surfaces (*Schizothyrium* sp./ hyphomycete anamorphs: *Zygo-phiala* sp.; German: “Fliegenschmutz-Pilze”). Most interesting are our findings of three members of *Mycosphaerellaceae* (6)-(8) with totally new rDNA sequences, a very big family of mostly plant pathogenic fungi which are known only in part till now.

A deeper insight in biology of this, what we now call, “eco-group” of phylogenetically quite unrelated sooty blotch fungi should help the organic apple grower to control these fungi by interrupting their hitherto unknown yearly life cycles.

## **A new method to separate the VIS/NIR-absorption of chlorophylls from fruit spectra of tomato, apple and grapes**

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Content and composition of nutritional valuable compounds of fruit and vegetable depend essentially on cultivation measures, cultivar, maturity stage, and post-harvest conditions. A sensitive indicator for fruit maturity provides the content of chlorophylls *a* and *b* and their ratio. Chlorophylls *a* and *b* are light absorbing pigments that can be measured by different methods. Using colour cards for measuring the skin colour of many fruits (*Solanum lycopersicon* L., *Malus x domestica* L., *Vitis vinifera* L.) are inexpensive and non-destructive, but erratic and subjective. In contrast, spectrophotometric methods are more precise, objective and well correlated with the pigment contents.

However, the determination of individual pigments by spectrophotometry is complicated due to the influence of scattering. Furthermore, the pigments are always presented in mixtures that result in overlapped absorption bands of chlorophyll *a* and *b*, as well as other pigments to a considerable extent. In recent studies methods for analyzing of in-situ measured spectrophotometric data statistical methods based on variation of spectral intensities were proposed. In this work, an approach was done, for calculating chlorophyll *a* and *b* separately by means of iterative multiple linear regressions with respect to the specific absorption coefficients of pigment standards.

Spectroscopic readings were carried out with a dual-beam UV/VIS scanning spectrophotometer (Lambda 950, Perkin-Elmer, U.S.A.). Spectral data were recorded in the visible wavelength range between 400 and 850 nm with a resolution of 1 nm. All spectra were base line corrected.

For analyzing the spectral data a software tool based on MATLAB Release 14 (The Mathworks, U.S.A.) has been developed. Standards were dissolved in diethyl ether (0.1-1.0 mg/L) to check linearity. The absorption coefficients of standards (Roth, Germany) from the pigments under question were recorded. The intensities were used as b-vector corresponding to the specific pigment signature. The sum of spectral intensities (400-

850 nm) of chlorophyll *a* and *b* built the data basis for multiple linear regression to fit the measured fruit spectrum by means of least square errors. Iteratively, in the first step chlorophylls were fitted, while other pigments were fitted after calculating the chlorophyll absorption influence in the blue wavelength range.

Results of the new approach were compared with standard methods (Porra et al., 1989; Lichtenthaler and Wellburn, 1983). For comparison, standards of pigments were used for calculating the specific molar extinction coefficients (400–850 nm) by means of Lambert-Beer law. The specific molar extinction coefficients were applied directly on the measured fruit spectra. In preliminary tests using the method developed, chlorophyll *a* and *b* contents were separately analysed in tomatoes and grapes of different maturity stages. The fit between calculated and added spectra of pigment standards correlated well with spectral remittance data from tomato. The coefficient of determination was  $R^2 = 0.82$  and  $R^2 = 0.80$  for tomato and grape, respectively. In all samples of grapes chlorophyll *b* was not detectable. In tomatoes, by using spectral signatures of carotenoids a separate determination of  $\beta$ -carotene and lycopene content was achieved with low measuring uncertainty. The comparison of the application of extinction coefficients according to commonly applied method (Porra et al., 1989; Lichtenthaler and Wellburn, 1983) resulted in higher coefficients of determination and lower errors.

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## **Effects of temperature and light level on primrose flowering**

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### **Introduction**

Primrose flowering depends on temperature and light level, but varieties differ in their response to temperature. In primrose production scheduling is difficult to manage, little knowledge is available about the effect of temperature on different cultivars.

### **Overview of experiment 1, glasshouses LVG Heidelberg**

Six (yellow and blue) varieties: 'Fruelo Gelb', 'Salome Golden Yellow' 'Unistar Gelb', 'Luxor Yellow', 'Eblo Blau', 'Euro Gelb'. Sown in week 25 (early varieties) and week 29 (late varieties). Two temperature regimes: 'cold' (heat 4 °C and vent at 6 °C / average temperature 17 °C), 'warm' (heat 18 °C and vent at 22 °C / average temperature 21 °C). Two light levels (natural daylength) in the cold and warm compartments: no shade, moderate shade (ca. 50%)

### **Overview of experiment 2, climate chambers LVG Ahlem**

Six (yellow and blue) varieties: 'Fruelo Gelb', 'Salome Golden Yellow' 'Unistar Gelb', 'Luxor Yellow', 'Eblo Blau', 'Euro Blau'. Potting in week 36, Salome and Luxor in week 38. Three temperature regimes: 'cold' (heat 8 °C), 'warm' (heat 16 °C), 'changing temperature' (8 °C and 16 °C). Two light levels (natural daylength) in the compartments: 3 klx and 9 klx



### **Results of experiment 1, glasshouses LVG Heidelberg**

'Fruelo Gelb', 'Salome Golden Yellow', 'Unistar Gelb' and 'Luxor Yellow' flowered weeks earlier in the 'cold' compartment. 'Eblo Blau', 'Euro Gelb' flowered slightly earlier in the 'cold' compartment. Shading delayed flowering by a couple of weeks.

### **Results of experiment 2, climate chambers LVG Ahlem**

'Fruelo Gelb', 'Salome Golden Yellow', 'Unistar Gelb' and 'Luxor Yellow' flowered weeks earlier in the 'warm' compartment than in 'cold' compartment. Flowering in the 'changing' compartment lay mostly between. 'Eblo Blau', 'Euro Blau' flowered 2 -5 weeks earlier in the 'cold' compartment. Shading delayed flowering up to 4 weeks

### **What causes differing results?**

'Fruelo Gelb', 'Salome Golden Yellow', 'Unistar Gelb' and 'Luxor Yellow' don't need temperature below 16 °C for early flowering. Average temperature of approx. 16 °C in the 'cold' glasshouse compartment in Heidelberg corresponded with the 'warm' temperature regime in the climate chambers of Ahlem. 'Eblo Blau' and 'Euro Blau' need temperature below 16 °C for "early" flowering, 'cold' treated in Heidelberg caused only slightly earlier flowering, but 'cold' treated in Hannover led to flowering weeks before.

## **Evaluation of Combined Use of Fog systems and CO<sub>2</sub> enrichment in Greenhouses by using Phytomonitoring Data**

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With global warming, higher external temperatures can be expected, which will demand greater attention to climate control in greenhouses, especially in summer. Air exchange by forced or free ventilation is mainly used for greenhouse cooling. However, many side effects are incurred with air exchange via open ventilation.

With fog systems an efficient method is available to increase vapour pressure difference between surrounding air and leaf. In times with high global radiation a perceptible cooling effect occurs by evaporating of floating small water drips. If fog systems and CO<sub>2</sub> enrichment is operating at the same time a significant change of microclimate occurs. On the one hand for protecting plants against fungi diseases relative humidity should not increase above 90 % and for a continuous greenhouse cooling effect the vapour should remove from the greenhouse. On the other hand with ventilation opening the vapour removes very quickly together with CO<sub>2</sub>. For managing these coupled systems a precise control of the microclimate is necessary. An essential prerequisite is to have more information's from the plant. Using a phytomonitoring system the microclimate between a greenhouse with CO<sub>2</sub> enrichment and fog system was compared to a greenhouse with CO<sub>2</sub> enrichment without fog. To evaluate the mass transfer conditions a new quantifying parameter - the gas exchange efficiency - was defined. For the calculation of this parameter the ratio between measured and potential CO<sub>2</sub> uptake at given light intensity was calculated. For visualisation of the difference the mollier plot method was used. In summer 2006 the influence of different air humidity while CO<sub>2</sub> enrichment was conducted with tomato (*Lycopersicon esculentum*). The experiments were undertaken in two 70 m<sup>2</sup> greenhouse cabins with special control algorithm and phytomonitoring systems. Beside short term measurements also fruit yield and quality were recorded. In the result higher gas exchange efficiency (GEE) was measured in the cabin with fog system. This was attended with a 16 % higher fruit yield and increase in fruit quality. Analysing the stomatal movement and boundary layer condition it can be assumed that higher relative humidity is lowering leaf transpiration and feeds CO<sub>2</sub> transport through the boundary layer.

With the Mollier plot method, ranges with a maximum GEE can be located for several temperatures and relative humidities. Plant yields and quality were also recorded. A preliminary test identified an increase in GEE with higher relative humidity, with a dependence on leaf transpiration.

A special control algorithm was developed to remove only the vapour from the greenhouse. Combining fog system and short time opening ventilation CO<sub>2</sub> can keep longer inside the greenhouse in times with higher global radiation. Using the calculated plant temperature the fog system was also controlled to keep a defined dew point distance to protect the plants against diseases.

### **References**

Schmidt; (2004). Microclimate Control in Greenhouses based on Phytomonitoring Data and Mollier Phase Diagram. Acta horticulturae 691:125-132

## **Effects of exogenous stress hormone abscisic acid on cut roses**

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### **Introduction**

The market quality of cut roses is determined exclusively by optical parameters like stem length or flower condition. Vase life hardly plays a role on the cut rose market. So are too many cut roses in marketing, which are optical correct, but have deficits in their vase life.

The vase life of cut roses is mainly affected by the water management. Water uptake and transpiration are steered by different physiological control circuits. On drought stress conditions the cut rose must protect itself as effectively as possible against water loss. A main factor is thereby the reduction of the stomatal conductance for water. ABA plays a substantial role with drought-stress-conditioned stomatal closure and is therefore important for our cut rose quality research project..

### **Materials and Methods**

The transpiration and CO<sub>2</sub>-exchange was recorded with a gas exchange measuring system (Walz, Efeltrich), which works with a bypass humidity control procedure. In this way all water in the gas exchange chamber (15 l), delivered by the plant, is removed and water vapour pressure remains constantly. For the measurement cut roses from own production were used, whose history was well known. The vase life tests were accomplished in fully air conditioned climatic chambers.

### **Results**

Concentration of ABA pre-treatment in the vase and the time of application are regulating the intensity of stomatal closure and the following opening. A calculated ABA uptake of 65 µg leads to almost complete and fast stomatal closure and there is a long delay until transpiration rate in pure vase water increases to the initial value. At an ABA uptake of 3 µg the reaction of stomata is slow and the following transpiration without ABA increases immediately and to a high level. The ABA induced stomatal closure is thus dose-dependently reversible.

A short term ABA pre-treatment of cut roses before a drought stress phase (22 °C, 55% rF, 60 µmolm<sup>-2</sup>s<sup>-1</sup>) reduces the water loss during the drying period up to 20%. That can mean that the cut roses may be better protected against stress effects.

The vase life of the cut roses after drought stress is often improved by a short term ABA pre-treatment. A continuous exposition of cut roses in a ABA solution during the test leads to a clear reduction of vase life.

### **Discussion**

Externally supplied ABA can weaken the effect of a following drought stress phase and can reduce negative effects on vase life. The application must be still optimized regarding time and dose. Cultivar differences are to be expected. An ABA pre-treatment can work only with high-quality cut roses.

# Controlled atmosphere affects the fruit quality during storage of ‘Kensington Pride’ mangoes

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The Europe is one of the prime export destinations of Australian mangoes. The sea freight involves a transit period of three- to five- weeks. The controlled atmosphere (CA) storage technology for long distance transportation of mango has been adopted on commercial scale. Mango fruit undergoes rapid softening and colour changes during storage at its optimum storage temperature (13°C). The present study was aimed to study the changes in textural properties and skin colour of mango fruit during CA storage. Mature green fruit of Australia’s commercial cultivar, ‘Kensington Pride’, were stored in CA containing 3% O<sub>2</sub> concentration combined with three levels of CO<sub>2</sub> (4%, 5%, or 6%) at 13°C (RH 80–85%) for four weeks. Evaluation of texture, skin colour, changes in sugars and organic acids profile of fruit was carried out at weekly intervals for four weeks. Mangoes stored under CA were firmer than those stored under normal atmosphere. After four weeks of storage, the firmness retention in fruit stored in CA containing 3% O<sub>2</sub> plus 4, 5, and 6% CO<sub>2</sub> was 30, 40, and 30% in contrast to normal air-stored fruit. The skin colour changes were largely affected by the storage atmosphere conditions. CA-stored fruit remained green in colour compared to the complete yellowing of fruit stored in normal air. There was a negligible change in the hue angle value of CA-stored fruit. However, it declined from 105.49° at the initial stage to 87.57° at the end of normal air-storage. CA-stored fruit had high sucrose, fructose and glucose content at the end of 4 week storage, while air-stored fruit showed a significant decline at the week 4 compared to week 3. Succinic acid, being the major organic acid, was retained at higher level in CA-stored fruit compared to normal air-stored ones. Citric and malic acids, the minor acids, also showed higher levels in CA-stored fruit at the end of 4 week storage. In conclusion, CA storage containing 3% O<sub>2</sub> and 5% CO<sub>2</sub> at 13°C seems to delay the softening, maintain the green colour, and affected the sugars and acids composition of ‘Kensington Pride’ mango during storage and transportation.

## Apple fruit quality monitoring by means of light remission and laser-induced chlorophyll fluorescence under shelf life conditions

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### *Introduction*

The objective of the present study was to assess the potential of Laser-Induced Fluorescence (LIF) and light remission techniques for detection of senescence-induced changes in apple peel chlorophyll content and internal fruit quality characteristics under shelf life conditions.

### *Materials and methods*

Apple fruits of cvs. ‘Golden Delicious’ and ‘Jonagold’ were harvested in the Experimental Station Klein-Altendorf, University of Bonn, Germany, on October 4, 2006 and after 2 weeks of cold storage at 4 °C, were transferred to a laboratory room (20±2 °C) for simulating shelf life conditions. From October 19 to November 13, ten apples of each cultivar were taken at a 3-4 day interval for the measurements. For all non-destructive and destructive analyses, a circle of approximately 12 cm<sup>2</sup> was marked on the sunlit and shaded

fruit sides. LIF measurements were done with a portable fluorometer (MiniVegN, Fritzmeier Systems GmbH & Co KG - Umwelttechnik, Germany). Chlorophyll fluorescence was induced with a red (662 nm) light emitting diode laser and detected with two photomultipliers at 692+2 nm (F690) and 730+2 nm (F730). For remission measurement in apple fruit, a hand-held photodiode array spectrophotometer (Pigment Analyzer 1101, CP, Germany) was employed. Remission spectra were recorded with calculation of Normalized Difference Vegetation Index ( $NDVI = (R780-R660)/(R780+R660)$ ) and Normalized Anthocyanin Index ( $NAI = (R780-R570)/(R780+R570)$ ) for the in vivo evaluation of apple peel chlorophyll and anthocyanin content, respectively. After non-invasive spectral measurements, destructive analyses of apple peel chlorophyll content, fruit firmness, total soluble solids, titratable acids and starch breakdown were conducted.

### **Results and Discussion**

Results obtained with 'Jonagold' and 'Golden Delicious' indicate that fruit ground colour alterations due to chlorophyll breakdown can be successfully monitored by LIF and light remission technique. NDVI and F730 showed strongest correlations with chlorophyll content in the apple peel with  $r$  in the range of 0.87-0.93. The intensity of red pigmentation of apples could be estimated by light remission Normalised Anthocyanin Index (NAI). Since the occurrence of anthocyanin pigmentation was accompanied by increased concentration of underlying chlorophyll, red patches of 'Jonagold' displayed higher NDVI, F690 or F730 and lower F690/F730 values than those apparently green. The multipoint scanning mode of LIF provides information on fruit colour heterogeneity. Among internal fruit quality parameters, the strongest correlation with the apple peel chlorophyll content was found for fruit firmness. For the green 'Golden Delicious', there were no differences in the Pearson's coefficients calculated for the data from sunlit ( $r = 0.78$ ), shaded ( $r = 0.77$ ) or both sides ( $r = 0.77$ ). For 'Jonagold', in contrast,  $r$  calculated for the data from both fruit sides was lower ( $r = 0.65$ ) as compared to those from sunlit ( $r = 0.74$ ) or shaded ( $r = 0.76$ ) sides due to the different chlorophyll content and same firmness values on the sunlit and shaded sides of this cultivar. The correlation coefficients between the non-destructively evaluated indices of apple peel chlorophyll content and Streif fruit maturity index could be significantly improved in both tested cultivars by considering differences in pigment content and flesh characteristics on the sunlit and shaded apple sides.

### **Conclusion**

The reliable detection and prediction of fruit quality or storability based only on the non-destructive pigment detection seems to be problematic in that, a robust relationship between internal fruit quality and coloration cannot be expected. However, the investigated methods are ideal for sensitive and rapid monitoring of senescence-induced changes in peel chlorophyll and may enhance both the potential and accuracy of non-invasive external and internal fruit quality evaluation.

## **Quality evaluation of ready to use fennel slices during storage**

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Ready to use products (RTUP) are fresh, raw fruits and vegetables refrigerated and minimally processed: trimmed, peeled or cut, washed and sometimes disinfected. RTUP are usually packaged in plastic trays sealed with polymeric films or in plastic sealed bags; a shelf life of several days at low temperature storage (2-4 °C) is required to transport and retail RTUP to the final consumer. These products are more perishable than the original raw materials because the tissue integrity is altered during processing; the microbial spoilage is one of the main causes of quality loss by formation of off-flavour and tissue decay. Fennel (*Foeniculum vulgare* Miller var. *azòricum*) is a vegetable widespread grown in Italy whose consumption is increasing in many European countries.

The aim of this work is to evaluate the quality of ready to use fresh fennel slices during storage at 4 °C under self controlled atmosphere (SCA).

Fennel slices 2 cm thick were washed in running water, drained and then dipped in citric acid 1% solution for 1 min to avoid browning on cut surface; after dipping the slices were drained, packaged in sealed PE bags and stored at 4 °C for 3 weeks. For the control, no dipped slices were used. Changes in respiratory activity, color, browning decay, microbial growth and sensorial attributes were monitored.

Fennel slices respiration rate was very high during the first seven days of storage and then, due to CO<sub>2</sub> increase, a reduction of aerobic metabolism was noted. At the end of the trial CO<sub>2</sub> concentration reached the value of 25-28 %, while the O<sub>2</sub> concentration was depleted to about 2 %.

The predominant microbial load was characterized by bacteria, following by enterobacteriaceae and yeast; moulds were not found during the trial. In any case, the viable cell count was lower than legal limit for safe consumption. Pre-treatment with citric acid had an important effect on total aerobic bacteria, dipping was able to reduce the microbial load of 2 log cfu g<sup>-1</sup> about. So, it was possible to extend to 11 days the shelf-life of fennel slices ready to use.

Generally, at the end of shelf-life changes in texture, aroma and flavour were similar for dipped and undipped samples; however an increase in browning was observed in untreated samples.

It was shown that pre-treatment with citric acid has an important effect on microbial count and browning inhibition. Anyway other experimental trials are in progress in order to detect the optimal storage and process condition to improve fennel slices ready to eat shelf-life.

## **The role of arbuscular mycorrhizal fungi on nutrient uptake of Cape gooseberry (*Physalis peruviana* L.) under salt stress**

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The Cape gooseberry, *Physalis peruviana* L., is native to South America but has received increased attention in the European Union recently. *P. peruviana* fruits are a rich source of provitamin A and vitamin C. Therefore, the influence of different environmental stressors on plants is of special interest in order to improve fruit quality. Salinity has been recognized as a major agricultural problem in arid and semi-arid regions. Differences in salt tolerance among plant species have also been long recognized. However, the role that salt tolerance plays in causing differences in nutrient uptake is still a major concern among investigators, and has not been fully understood.

A group of soil micro-organisms that live in very intimate contact with the root are the arbuscular mycorrhizal fungi (AMF). These fungi are known to assist the plant in nutrient uptake to improve plant growth. Aim of the present study was to examine the impact of AMF on nutrients uptake (N and P) of *P. peruviana* L. (Ecotype Colombia) under salt stress conditions. Field experiments were conducted from August 2006 until January 2007. Sixty-day-old seedlings were transferred to the field with a spacing of 2.5 m between plants and rows in one experiment and 3 m in a 2<sup>nd</sup> experiment. Some plants have been previously treated with AMF (Mycoral®). The experiments were set up as complete randomized block design and replicated three times. Physiological growth parameters associated with AMF inoculation were determined. Furthermore, N and P content in roots, stem, leaves, and reproductive organs (flowers and goblets) were analyzed.

In AMF treated plants the N content was significantly higher for roots, leaves, and reproductive organs than in the controls. For the stems we could not find any differences in N content between treatments. The opposite was true for phosphorous. Here we determined only for stems a significant increase in P content but not in leaves, roots, and reproductive organs.

The AMF treatment showed a positive effect on N uptake of *P. peruviana* in saline soils. In contrast, under phosphorous rich soil conditions AMF treatment did not have a significant impact on plant growth parameters.

## Effect of variety and cultural practices on pit fragment formation of clingstone peaches during processing

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A major problem in the peach fruit canning industry is the formation of pit fragments in peach flesh during the process of cutting the fruit in two halves by machine. Pit fragments are dangerous for the consumer and therefore they must be eliminated by hand even though the production cost is dramatically increased. The effects of cultivar, fruit thinning (fruit density, as well as time of application), fruit size, stage of maturity at harvest on pit fragment formation were studied during the years 2003 – 2006. Samples of one hundred fruits of each of 6 clingstone peach varieties grown mainly in Greece were taken at each harvest. Fruits were separated in three groups according to their diameter ( 4,95 – 6,35 cm., 6,36 – 7,6 cm., 7,61 – 8,2 cm., small, medium and big fruits, respectively) and also in three groups according to their stage of maturity based on the external color of fruits (green , yellow-green and full yellow ). For studying the effect of fruit thinning on pit fragment formation three levels of fruit density (one fruit / 5, 10 or 20 cm.of shoot length) and three stages of fruit growth(two weeks before pit hardening , during pit hardening and two weeks after pit hardening) were applied. Cultivar Andross, the most widely grown in Greece, was the most susceptible (maximum 32,27%, under growers conditions ), whereas the less susceptible proved to be Catherina (maximum 3,1%). The percentage of fruits with pit fragments in «Andross» varied from year to year (14,89% ± 9,6, average of four years). The larger the fruit the more susceptible proved to be in pit fragment formation. The percentage of fruits with pit fragments in large size fruits was 24,17%, while in small size fruits was 14,47%. Pit fragment formation was not affected by the stage of fruit maturity. Delayed fruit thinning reduced pit fragment formation significantly in all years applied. When fruit thinning was applied early, two weeks approximately before pit hardening, the percentage of fruits with pit fragments was 28,08%, during pit hardening was 11,72% and when fruit thinning was delayed by two weeks approximately after pit hardening the percentage of fruits with pit fragment was only 8,77%. Fruit density also affected significantly pit fragment formation. Heavy fruit thinning (one fruit per 20 cm. shoot length) resulted in 21,25%, while light fruit thinning(one fruit /5cm shoot length) resulted in 8,65%. Conclusively every factor which could increase fruit size proved to increase the percentage of fruits with pit fragments. Pit fragments were deposited mainly close to the fruit pedicel.

## Nitrogen nutrition determines quality of pelargonium stock plants and rooting of cuttings

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### Introduction

The global production of vigorous *Pelargonium x hortorum* L.H.Bailey young plants requires reliable rooting capacity of axillary shoot tip cuttings. This intrinsic quality trait is most unrelated to the external appearance of cuttings and is determined before they are harvested from stock plants. Particularly, it is known to be sensitive to nitrogen nutrition although conflicting results were found.

### Materials and methods

Therefore, the influence of nitrogen form and dosage on growth of stock plants and rooting capacity of cuttings was investigated in several experiments which examined (i) three graduated nitrogen dosages or (ii) six graduated ammonium to nitrate ratios at an equal N dosage either in (i,ii) aerated nutrient solution culture or

(i) thin layer peat substrate. All targeted N-form ratios and dosages were maintained at weekly intervals by (i) fertigation to the peat substrate and (i,ii) total replacements of nutrient solutions. To control stable N-form ratios in nutrient solutions a nitrification inhibitor was supplied exemplarily. Natural irradiation and different shading regimes during spring and summer cultivation of the stock plants (i,ii) supported sufficient light while (ii) limiting irradiation during autumn was allowed to interfere with the N-form treatments.

## **Results**

The moderate substitution of nitrate by ammonium and a nutrition at mixed N-form ratios containing 20 and 40%  $\text{NH}_4$  provided optimum growth and quality of cuttings regardless of natural irradiation changes. It promoted (a) branching of stock plant roots, (b) nitrate accumulation in shoot tissues, (c) overall cutting yield and (d) adventitious root formation in cuttings, when compared to 100%  $\text{NO}_3$  in nutrient solution culture. Though a further substitution of  $\text{NO}_3$  by  $\text{NH}_4$  with ratios above 60%  $\text{NH}_4$  impaired fresh mass and number of tip cuttings per plant, the rooting capacity of those cuttings was promoted further as long as sufficient light was available. Hence, we hypothesize that the N-form ratio shifts hormone levels and carbohydrate fluxes which influence both root and shoot morphology of stock plants as well as rooting in shoot tip cuttings.

The cultivation of stock plants in peat substrate resulted highest yield and best rooting of cuttings at adequate medium N dosages, which were well adjusted to the actual N demand. In contrast, the high N dosage did not enhance yield and reduced the number and length of adventitious roots under non-limiting irradiation. In addition, it also caused increase in  $\text{NO}_3\text{-N}$  and electric conductivity in peat substrates. Moreover, while a reduced level of rooting at the low N dosage indicated N-limitation, the observed impaired rooting at the high N dosage may be induced by osmotic stress to the stock plants. Thus, N dosage closely should meet N demand.

## **Investigation on ‘Clementine’ mandarin rind-spotting in maturity**

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Severe damage of ‘Clementine’ mandarins in the Assini area of Argolis in Greece, was investigated for two successive years. Irregular-shaped necrotic spots on the rinds of mandarins, mainly at the fruit styler-ends, are observed from the color break till harvest time. In the first year study, in order to investigate the correlation of the injury with the fruit inorganic nutrition, the concentrations of N, P, K, Ca, Mg, Fe, Mn, Zn, Cu and B in mandarin rinds with and without symptoms were determined in several orchards in the area. Significant differences were found in the concentration of some nutrients between damaged and healthy rinds, but they were different in every orchard. Significant differences were also found among trees in a given orchard, regardless of the presence of symptoms. From the second year relevant results, after modifying the sampling of mandarins, no significant differences were found in the concentration of the majority of nutrient element determined. The results related to fruit inorganic nutrition from both year studies did not show its constant effect on symptom performance. Using Electron Scanning and Fluorescence Microscopy, cracks were observed at the cuticle and the external strata of the rind from injured fruits, macroscopically invisible; however, oil glands remained intact. On the spots of the diseased fruits there were also observed secondary infections by fungi, mainly of the genera *Alternaria*, *Cladosporium* and *Penicillium* that caused their rot followed by the whole fruit decay. Consequently, the results of chemical analyses, microscopic observations, as well as, information given by growers showed that this disorder might be attributed to sudden changes of climatic conditions such as temperature and relative humidity and not to nutritional imbalance.

## The malolactic fermentation and the sensitive features of Red Wines obtained in the vineyards of Oltenia County Hills from Romania

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The researches made by a team of the Oenological Department of the Horticulture Faculty of Craiova University, in cooperation with the Romanian Authorized Testers Association, revealed a strong relation between the conditions the MLF is made in and the essential organoleptic features of red wines obtained in the mains vineyards of Oltenia hills – Sâmburesti, Dragasani, Craiova hills and Vânu Mare- from Romania.

The researches were made between 2004-2007 years, very different as climatic aspects. From the last 50 years for the Romanian viticulture, the 2005 year was the worst, due to excessive rain and coldness. The 2004 and 2006 years were good viticulture years. The 2007 was an excellent viticol year referring the climatic conditions in the growing up of the vine and in all ripeness and over ripeness periods of the grapes. As a result it was obtained a very good wines quality production in the area studied.

It was studied the influence of the spontaneous MLF and the different ways of starting and stimulating of the controlling MLF on the organoleptic features of the obtained wines. When were used the selected lactic bacteria, the MLF have had a fast starting, the malic acid was more fast metabolized and finally the MLF period was shortened. This permitted an early biological stability of the obtained wines. Organoleptical, the obtained wines, were appreciated as balanced, with a very good smell and taste, with a lot of aromas, especially from the second categories, of fermentation, depending of the kind of lactic bacteria used and the moment and mode of inoculation.

Spontaneous MLF had different results, depending on several factors, the most important being the climatic conditions of the year, which influence the maturity and the healthy conditions of the grapes in the harvest moment. During the good harvest years, with sunny and long autumns - a fast MLF ends in about 45-50 days after the grapes harvest. The obtained wines are agreeable in taste, have roundness, suppleness and fructuosity, complex flavor, the dominant feature being the flowering one. In the years with rainy and cold autumns favorable for *Botrytis cinerea* mould development, spontaneous MLF is slowly and last about 120 days, sometimes more or can remain unfinished. The obtained wines are lightly, raw, with a vegetal feature, even grassy, unpleasantly, sometimes with a large volatile acidity.

As a researches conclusion, in the good viticol years, when the grapes are ripened and healthy, the using of the selected lactic bacteria is optional, due to the fact that the spontaneous lactic bacteria realize the MLF in good conditions, and the obtained wines have an aromatic highest complexity, comparatively with the wines obtained with selected lactic bacteria, which have intense aromas but less complex. But in the rainy and cold years, the using of the selected lactic bacteria is compulsory due to the fact that the obtained wines are much better from the organoleptic features point of view.



## **N, P and K content in radish leaf and root**

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Radish was grown in glass-houses located in the city of Belgrade (city nursery stock garden). Plant samples were taken in the full growing stage with the aim of determining N, P and K content. Leaf and root samples were taken (dry samples). Trials were conducted in 2004 and 2005 and the results were given in percentage.

Nitrogen content in radish amounted to 4.38% which is the average for the whole plant. Radish leaves were richer in nitrogen (5.39%) compared with roots (3.37%). The difference amounted to 2.02%.

The content of phosphorus was substantially even (0.67 and 0.64%) which is known to be a common feature in the case of root vegetables. Thus phosphorus content may be said to be evenly distributed in radish. After all, this element is known to be stable in both soil and plant.

Radish was very rich in potassium content. Namely, its potassium content amounted to 4.21%. In comparison to nitrogen, potassium was far more available in radish roots than in leaves. This difference amounted to 2.14%. Potassium is known to be the major element available in great quantities to plant tissues. It is not a constituting element but is known to be unreplaceable in living organisms.

Thus, radish may be said to be rich in nitrogen, phosphorus and potassium. It is usually grown because of its root used in the preparation of various salads. Radish is a useful vegetable (food) and medicinal product.

## **Improving fruit quality and microclimate under hailnets in an apple orchard**

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With the increase of hailstorms as a possible result of global warming, fruit crops are increasingly grown under hailnets. This may result in lesser fruit quality in terms of colouration, fruit mass, firmness, starch and taste, i.e. sugar and acid as well as vitamin content under hailnet due to altered microclimate and particularly light deprivation. Hence, reflective mulches (Extenday and Daybright) were spread to improve fruit quality and light utilisation under hailnet at Klein-Altendorf Research Station near Bonn, Germany. A monophosphate (Seniphos) was applied twice for the same purpose; untreated apple cv. 'Elstar' trees served as control.

Under the translucent 'white' hailnet, humidity was increased by ca. 6%, soil temperature increased by ca. 0.5°C and light reduced by 11-15% resulting in lesser fruit quality of 2.5% less sugar and less taste. The two reflective mulches increased light reflection at 45° and 90° angles by 2.5-6.3-fold. No significant differences in fruit ripening and firmness were observed, but fruit from trees under hailnet with reflective mulch contained up to 2.4 % (from 13.3 to 15.7 %) more sugar than those of the control (uncovered grass alleys). A less negative NDVI (normalised differential vegetation index) of -0.3 on the red compared with -0.5 on the green fruit side indicated more chlorophyll in the outer, sun-exposed red side of the apples relative to the shaded side. Monophosphate-treated fruits maintained the peel chlorophyll with a greener ground colour of 92-97°hue and a NDVI of -0.3 as in the grassed control. Fruit in the lower canopy with reflective mulch were darker red (a value 30; 22° hue) relative to the grassed control with a=25 and 43° hue (light red), expressed in a 4-fold increase in NAI (normalised anthocyanin index), but showed enhanced chlorophyll breakdown (NDVI was declined from -0.2 to -0.5); similarly, the monophosphate increased the NAI by up to 2.5-fold.

Overall, the effect of both reflective mulches was most pronounced on apple fruit in the lower canopy under hailnet, which synthesized large vitamin C contents and developed a dark red top colour relative to the grassed control with enhanced chlorophyll breakdown. Both reflective mulch cloths improved the fruit quality by increasing the percentage of class I fruit with >25 % colouration by 12% (from 82 to 94%) without

and under hailnet by 23 % (from 69 to 89 %) relative to the grassed control resulting in financial net gains of up to 1,300 €/ha.

*We thank Deutscher Akademischer Austauschdienst DAAD for a grant to the first author.*

### **Literature**

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## **Time-resolved Reflectance Spectroscopy as a management tool in the fruit supply chain: an export trial with nectarines**

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### **Introduction**

Kinetic models are widely used to describe quality determining behaviour of fruit. Biological variation is always found in fruit, and it is mainly due to differences in growth conditions, which cause differences in maturity stage at harvest. With the novel technique of Time-resolved Reflectance Spectroscopy (TRS), the maturity at harvest of nectarines can be assessed by measuring the absorption coefficient at 670 nm ( $\mu_a670$ ), near the chlorophyll peak, in the fruit flesh. A kinetic model has been developed linking  $\mu_a670$ , expressed as the biological shift factor, to firmness decrease during ripening. The degreening of fruit flesh (i.e. the decrease of  $\mu_a670$ ), in nectarines, is synchronized with softening, thereby allowing the prediction of shelf-life of individual fruit. Here, an validation experiment is presented that tests the applicability of the TRS technique in the supply chain., Individual fruit softening at the end of the supply chain is predicted from the TRS measurement of  $\mu_a670$  at harvest.

### **Experimental plan**

At harvest, the absorption coefficient at 670 nm was measured by TRS on about 1000 nectarines (cv. Spring Bright, sizes A, B and C), which were graded into six classes of usability, based on the kinetic model calibrated on data sets of previous years. A sample of 40 fruit, representing the whole range of  $\mu_a670$  (0.65-0.046  $\text{cm}^{-1}$ ), was measured to obtain seasonal parameters of the kinetic model. The graded nectarines were transported from Milan (Italy) to Wageningen (The Netherlands) using a regular, temperature controlled, truck. At arrival, fruit were put at 20°C and tested for firmness by sensory evaluation after 5 (after transport) and 13 days (8 day shelf-life period).

### **Results and Discussion**

Using the  $\mu_a670$  and firmness values of the 40 fruit sample representing the whole range of  $\mu_a670$ , it was possible to estimate the seasonal parameters of the kinetic model for fruit softening according to Eccher Zerbini et al. (2006) and Tijssens et al. (2007). Afterwards, the time of the individual softening was predicted for each fruit and all fruits were graded into six classes, varying from 'will never ripe', 'dangerously hard', 'transportable', 'ready to eat-firm', 'ready to eat-ripe' to 'overripe'. The sensory analysis of fruit firmness carried out after transport and after a shelf-life period showed that the fruit softening was predicted correctly, as the classes 'will never ripe' and 'dangerously hard' did not soften and the class 'overripe' was too soft and subject to rot. The intermediate classes showed sufficient firmness to transport the fruit and sufficient ripening potential to satisfy consumers..

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# **Lectures and Posters of Theme 6**

**BIODIVERSITY AND LOCAL GENETIC RESOURCES:  
FROM KNOWLEDGE TO EXPLOITATION**

## Biodiversity and local genetic resources

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Since the years '90 of the last century, worldwide efforts concerning the safeguard and the sustainable utilization of agricultural biodiversity have been strongly enforced by numerous initiatives, in particular the Agenda 21, the Convention on Biological Diversity (CBD) in 1992, the Global Plan of Action (GPA) in 1996 for the conservation and the sustainable use of plant genetic resources for food and agriculture and the FAO International Treaty on PGRFA (2001).

A common aspect of these documents and treaties are the joint efforts of Nations to promote the safeguard and the sustainable use of genetic resources in general, while the FAO International Treaty legally binds its Contracting Parties to facilitate the access to PGR considered essential for global human nutrition and which are listed in Annex I of the Treaty, as well as to equitably share the benefits arising from their utilization, in harmony with the CBD.

To implement these above-mentioned agreements, a series of initiatives, at the international, regional and national level, have been undertaken.

The European meeting on plant genetic resources held in Nitra (Slovakia) in 1995 recommended that the European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR) be used as a platform to facilitate the implementation of the GPA. ECP/GR, a collaborative programme on PGR among most European countries, was founded in 1980 and is running within the Consultative Group on International Agricultural Research (CGIAR), coordinated by Bioversity International (formerly IPGRI) in Rome.

Also the European Union is supporting initiatives aiming at the recovery, conservation, characterization, documentation and exploitation of plant genetic resources by funding projects within its AGRI GEN RES programmes, even though the funds available have been, up to now, rather limited and insufficient to meet applications from European countries.

In addition, the European member states have developed national projects to exploit their local PGR, an important source of genetic traits for breeding activities, but which are also of direct economic (traditional products, agritourism) and environmental (landscape, soil preservation) importance.

Examples of utilization of genetic resources for breeding purposes are numerous: one of the most interesting, relevant to fruit trees, are the old apple cultivars of the local germplasm, found in several European countries, carrying the polygenic resistance to scab, the fungus disease caused by *Venturia inaequalis*. This resistance is actually used for obtaining apple cultivars carrying both vertical resistance (from *Malus floribunda* for example) and horizontal resistance (from the old cultivars).

Another very interesting source of resistance to dangerous pest of the pomefruits, the bacterium *Erwinia amylovora*, has been found in several old pears grown locally in Italy and France. These varieties are also very tolerant to *Psylla pyri*, an insect not easy to control chemically, and are used in breeding programmes to improve commercial varieties.

An example of valorisation of a peculiar quality trait of some old peach varieties is the "bloody" flesh of vineyard peaches particularly rich in antocyanins, powerful antioxidants; in France the bloody peaches have been crossed with a nectarine to obtain a series of bloody nectarines which are grown commercially as Nectavigne.

## Exploring the genetic determinisms of architectural and functional traits in an apple progeny

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The domestic apple (*Malus x domestica* Borkh.) is the most cultivated temperate fruit tree. To maintain its position in Europe, apple breeding programs aim at introducing regularly cropping cultivars with high fruit quality and pest and disease resistance. The consideration of other traits such as tree architecture and tolerance to abiotic constraints represents a complementary way to select innovative cultivars in the contexts of Integrated Fruit Production (IFP) and global climatic changes. Genetic control of tree form and leaf functioning is thus a desirable breeding objective, but efforts have been hampered by a poor knowledge of its determinism in apple. In the last four years, we investigated the genetic determinism of architectural and ecophysiological traits in a F1 apple progeny ('Starkrimson' x 'Granny Smith' cross) of 123 genotypes. On the one hand, a fine phenotyping was performed over the first 3 years of growth in the open field, collecting both topological and geometrical traits along the axes and at the whole tree level. On the other hand, the leaf gas exchange responses submitted to different air droughts was studied in a growth cabinet, through five increasing leaf-to-air vapor pressure deficits (VPD, from 0.5 to 3 kPa). Stomatal conductance (gsw), net CO<sub>2</sub> assimilation (An) and transpiration (E) rates were measured, and instantaneous Water Use Efficiency (WUE, An / E) computed. All traits were analyzed through mixed linear models in order to estimate broad sense heritability values (h<sup>2</sup>). Moreover, to in-depth investigate the genetic determinisms, a QTL analysis was carried out using a consensus 'Starkrimson' x 'Granny Smith' genetic map. For each trait, QTL detection was performed on the best linear unbiased predictor (BLUP) of genotypic values, computed from the linear mixed model. For architectural traits, high h<sup>2</sup> values were estimated and QTLs were detected for many topological and geometric traits: (i) at tree scale, surface and volume; (ii) on the trunk, mean internode length and number of sylleptic and proleptic axillary shoots; (iii) on lateral shoots, orientation, mean internode length, and number of proleptic axillary shoots. Regarding ecophysiological traits h<sup>2</sup> values were very high, ranging from 0.77 to 0.90, indicating a good repeatability of ecophysiological measurements in controlled conditions. QTLs were detected for An and E suggesting a strong genetic control of these two traits, whereas genotype x environment interactions were expected for gsw and WUE. The putative agronomic interest and potential value for selection of the architectural and ecophysiological traits will be discussed.

## Plant biodiversity recovery, conservation and valorisation: a case study from the Val di Vara (La Spezia - Liguria)

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### *Introduction*

Plant biodiversity recovery, conservation and valorisation are crucial processes for the preservation of ancient crop varieties and for production practices in small and marginal areas like those located in mountainous regions. Moreover, in modern agriculture the re-discovery of ethnobotanical utilisation of natural and cultivated plant species can be very useful for developing new products for the European and international markets.

### *Materials and Methods*

This research activity was performed in the Val di Vara, a mountainous area in Liguria (Italy). The study was performed for three years (2004-2006) at different knowledge scales: a) documental - bibliographic and cartographic material; b) interviews - on site analysis carried out by contacting local agencies, agricultural

companies, and single individuals; c) phenotypic characterization - in situ relief of the main morphological and biometrical traits of crops. Moreover, several farms/sites studied has been geo-referenced and described by analytical records containing their main characteristics, the cultivated species/varieties, the agronomical techniques applied, and the product destinations. All the information has been also elaborated and organised in an on-line database, which enables an effective and continuous data registration and consultation.

## **Results**

The Val di Vara has proven to be a very fertile area of popular tradition. During the study, a total number of 323 persons (65 males and 258 females; 71% age between 61-80 years old) were interviewed. The data collected has been used for a census of the wild and cultivated flora of local interest and we found more than 150 plant species still utilised for agricultural and ethnobotanical uses.

The ethnobotanical utilisations more frequently registered are for medical (30%), food (17%) and ornamental (10%) purposes. All the information collected has been integrated and cross validated with other bibliographic data, then organised in specific synoptic cards containing pictures and figures for plant identification and specific details on plant uses. Seeds and other genetic materials (germplasm) were collected during the spring-summer-autumn 2006 and conserved at +4 °C for long term storage.

Concerning the plants used in agriculture, we have identified several cultivars of herbaceous and woody species. In particular apple, pear, chestnut, and grape are much diffused in the hilly and mountainous areas of the Val di Vara. More than thirty apple and twenty pear cultivars were identified, geo-referenced and biometrically described. Many of them were also propagated by grafting and planted in the Val di Vara Botanical Garden in 2006 where local varieties and wild species are conserved for further molecular and biochemical analysis.

The main cultivated tree in Val di Vara is chestnut. During the project 29 sites were described in detail, recording the most relevant farm characteristics, the varieties in cultivation, the crop management techniques and the product destinations. Chestnut cultivation in many sites is suffering from pathologies related to poor crop management techniques. Grafting and pruning intensity was very low and in several occasions almost absent, whereas planting density was often suboptimal. The main destination of the product was for farm uses or for farmer direct marketing.

In order to valorise the Val di Vara germplasm potential a strong program aimed to teach and sustain the ethnobotanical utilisations, the most suitable crop management techniques, and the more profitable product distribution strategies were undertaken. This integrated approach should enable to preserve plant biodiversity, recover ancient crop varieties and improve production income in this marginal agricultural area.

## **Strategies for breeding for Plum pox virus resistance in European Plum (*Prunus domestica* L.)**

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Since the early report on the Sharka disease in 1935, breeding for Sharka (Plum pox) virus resistance is considered to be the most promising way of minimising the outstanding economic damage caused by the disease in stone fruit production. Several resistance breeding strategies are embarked by breeders throughout the world. Those used in European plum (*Prunus domestica*) breeding were compared concerning their potential use in both minimizing the economic losses and avoiding the dissemination of PPV in orchards and over long distances.

Varieties tolerant to PPV are able to prevent the decline of plum production in areas where PPV is prevalent. However, they contribute to the dissemination of PPV into regions up to know free from PPV via latently infected plant material.

For a long time, breeding for PPV resistance has been limited due to the lack of sources of resistance. Up to know, two different types of natural resistance have been found in *P. domestica*: The so called quantitative resistance and the hypersensitivity resistance. The latter inhibits the viral replication and dissemination within the plant. Hypersensitive genotypes are, under natural inoculation conditions, no source of PPV. Therefore, they do not contribute to the dissemination of PPV both via insect vectors within an orchard or via infected plant material. On the contrary, quantitatively resistant genotypes, even if they have low virus titer after inoculation with PPV, are sources of infection and hold the same disadvantages than tolerant varieties concerning the spread of PPV.

Genetically modified plum genotypes were created aiming at generating PPV resistant plants. Due to the lack of known resistance genes, the strategy of pathogen derived resistance is used in transformation efforts introducing parts of the PPV genome into the plum genome. Up to know, no field resistance could be achieved using these plants. Therefore, they do not exceed the practical use of well known quantitatively resistance varieties.

## Agro-horticultural biodiversity in mountain oases of northern Oman

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### Introduction

Several botanical studies have been conducted in different parts of Oman, but knowledge about agro-horticultural biodiversity in the rapidly changing ancient mountain oases remains scarce. This is perhaps because cultivated plants are usually neglected by botanists and collectors of crop genetic resources, who often follow crop-specific programs and are therefore less interested in the wealth of species found.

### Materials and Methods

To fill this gap we inventoried the crop species of three mountain oases in the Oman Mountains using a GIS-based field survey and farmer interviews. While arid conditions prevail throughout the mountain range, the different elevations of Balad Seet (950-1020 m a.s.l.), Maqta (930-1180 m a.s.l.) and Al Jabal al Akhdar (1750-1930 m a.s.l.) provide markedly differing agro-climatic conditions.

### Results and Discussion

Overall, 107 different crop species were identified belonging to 39 families. Species number was highest among fruits (33 spp.), followed by vegetables (24 spp.). Intensive irrigation allows cultivation of a broad range of species at all oases. However, the number of species varied significantly between sites. Fruit species diversity and homogeneity of distribution of individual fruit species was highest at Balad Seet and lowest at Maqta as indicated by respective Shannon indices of 1.00 and 0.39 and evenness values of 32% and 16%. At Balad Seet and Maqta, date palm (*Phoenix dactylifera* L.) was the most common fruit crop with 2690 and 2128 specimens, respectively, while in Al Jabal al Akhdar pomegranate (*Punica granatum* L.) was most frequent with 5894 specimens. For date palm, 16 and 13 varieties were cultivated at Balad Seet and Maqta, with an average yield of 32 and 8 kg (palm yr)<sup>-1</sup>, respectively. Mean shoot circumference of date palm was significantly higher at Balad Seet compared to Maqta with 177 and 118 cm, respectively, indicating a lower palm vitality in Maqta. In Balad Seet 18 cultivated black mulberry (*Morus nigra* L.) trees were identified with a considerable tree-to-tree variation regarding fruit size. Average fruit length and diameter varied from 11 to 79 mm and from 8 to 10 mm, respectively. However, farmers did not distinguish between different varieties. Temperate fruit trees such as walnut (*Juglans regia* L.), apple (*Malus domestica* Borkh.), apricot (*Prunus armeniaca* L.), plum (*Prunus domestica* L.) and pear (*Pyrus communis* L.) were only cropped at the high altitude of Al Jabal al Akhdar. However, long-term temperature records indicate that the number of chilling hours decreased dramatically over the past 24 years, with a mean decrease of 17.4 hours per year leading to recent crop failures in the traditional horticultural systems at Al Jabal al Akhdar. In Balad Seet and Maqta all fruits are consumed by members of the households, whereas fruits produced in Al



Jabal al Akhdar are sold outside the oasis. Century plant (*Agave americana* L.), faba bean (*Vicia faba* L. var. minor Peterm. em. Harz) and lentil (*Lens culinaris* Medik.) were identified as relict crops, supporting oral reports of past cultivation and providing evidence of genetic erosion. Some species, such as the temperate fruits of Al Jabal al Akhdar, were exclusively found at the coolest site, while others only occurred at the hotter locations. Overall greatest species similarity was found between Balad Seet and Al Jabal al Akhdar as indicated by a Sørensen coefficient of similarity of 67%. Most cultivated terraces had different vegetation layers making them typical agroforestry systems. The top layer (canopy layer, > 5 m) was composed mostly of date palms and some mangoes (*Mangifera indica* L.). In an intermediate layer (understory layer, 1-5 m), woody perennials such as lemon (*Citrus limon* [L.] Burm. f.) and peach (*Prunus persica* L.) were found mixed with bananas (*Musa x paradisiaca* L.) and papayas (*Carica papaya* L.). In the ground layer (shrub and herb layer, 0-1 m) different vegetables, spices and medicinal plants such as garlic (*Allium sativum* L.), onion (*Allium cepa* L.), red pepper (*Capsicum annuum* L.), castor bean (*Ricinus communis* L.) and fringed rue (*Ruta chalepensis* L.) were cultivated. Greatest species richness was recorded in the lowest stratum.

### **Conclusion**

Overall the study shows a location-specific but surprisingly diverse mosaic of crops in Omani mountain oases which merits further studies and conservation efforts.

## **Integrating ex situ and in situ and on-farm conservation approaches in the management of local vegetable diversity in Austria**

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Traditional on farm management of plant genetic resources (PGR) has almost disappeared in developed countries. PGR of local origin is limited to gene banks. In rare cases relicts of landraces or heirloom varieties have persisted on farm.

In contrast to this development, there has been an increased interest in “old varieties” over the past 15 years. Increased demand for vegetables in various market niches has paved the way for a certain amount of revival of land races and “heirloom varieties” -- particularly in direct marketing schemes. However, exclusively targeting the commercial sector as a carrier for on farm management will not suffice. Many of the local PGR were developed for the purpose of subsistence farming, under corresponding conditions, and will perform best in comparable small-scale settings (i.e. in garden).

The Austrian NGO “Arche Noah” pursues both ex situ and on farm conservation strategies. The organisation runs a gene bank with a strong focus on vegetable varieties. 360 accessions from this collection have been identified as PGR of particular relevance for Austria. Ex situ conservation strives to maintain a maximum of original genotypic diversity in these accessions. Long term storage and technical minimum standards form a well established framework for regeneration in the gene bank.

A current project aims to establish gene bank accessions in gardens and on farms. Objectives are to ensure the “fitness” of existing PGR and to allow for further adaptation and diversification. The project is based on a network of approximately 400 people, mainly farmers and home gardeners. 151 gene bank accessions were selected for potential on farm management based on a set of criteria (regionality, usability, status of conservation). The selection is flexible and can be expanded by participants of the network on the basis of the selection criteria.

By the end of 2007, 52 people were cooperating on a regular basis within the project, which currently encompasses 89 different vegetable varieties. The appropriate varieties were identified in a step-by-step process. Additional measures include training, counseling and regular meetings to exchange experiences. Various aspects of the project, such as varietal identity, seed quality and regularity of regeneration, continue to be monitored. A reliable and productive working atmosphere is created by personal contact and incentive measures.

## Striking a new path for conservation of crop genetic resources: First results of a pilot project to re-introduce old *Lactuca* varieties into the market

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Within the past decades, numerous plant species and varieties disappeared from agricultural and horticultural use because the demands of intensive production rely on a relative small number of modern elite varieties. The decrease in agrobiodiversity endangers heirloom cultivars and other genetic resources on the one hand and on the other hand the cultural heritage of traditional ways of use.

In a pilot project, we examine the chance of maintaining plant genetic resources by commercial utilization of old varieties and the re-animation of traditional ways of use. We choose *Lactuca sativa* as a model plant because relative to other crops it is easy to grow, has a short period of cultivation and there is a huge number of various accessions available from genebanks. Further, the traditional use as cooked salad has fallen behind the use as fresh vegetable and should be invigorated. In addition, we intend the use of Stem lettuce (*Lactuca sativa* var. *angustana*) to stimulate diversification.

In the majority of cases, information about genebank accessions is restricted to passport data. Therefore we conducted a field trial to evaluate the characteristics of an assortment of genebank accessions. Our aim was to establish useful descriptions of the varieties or to verify those descriptions that were available.

We identified a number of attractive cultivars and examined their suitability for market gardening in cooperation with local gardeners in the region of Berlin and Brandenburg, Germany. The market gardens tested the cultivars for field performance, yield and quality attributes in practice and brought the harvest to the market in their manner customary to test for marketing success.

The objective of the pilot project is to establish a collection of *Lactuca* cultivars suitable for commercial utilization in local market gardens as a tool to maintain old varieties on-farm. Complementary, the pilot project focuses on public relations concerning crop genetic resources and aims at stimulating consumer's interest for rare crop varieties.

The pilot project is funded by the Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (BMELV/BLE) FKZ: 05BM007/2).

## Molecular markers as an assisting tool for characterisation of local apple varieties

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While in the past several hundreds of different apple varieties were grown in traditional systems, the modern apple production of South Tyrol (northern Italy) is dominated by a handful of commercial cultivars. The complex technical demands on cultivation have led not only to a decrease of genetic diversity, but also to the loss of genetic resources available for future breeding programs. In order to save the gene pool of the still remaining local apple trees a collection and conservation program was initiated. Beside South Tyrol, the study area also included the Austrian County of Tyrol, where apple are mainly grown in extensively managed orchards. In addition to determination of pomological and morphological traits, each variety was characterised by a set of 14 microsatellite markers. In South Tyrol genetic variability, expressed as the number of different genotypes, was twice as lower in contrast to Tyrol, clearly reflecting the adverse impact of intensive production on the local apple gene pool. A comparison of the genetic profiles of the sampled apple

varieties to those of well determined cultivars from several European germplasm collections, allowed identification of many trees, for which names could not be assigned by traditional means. In addition, several cases of misdetermination were resolved and valuable guidance for the establishment of local germplasm collections could be provided. Our study gives an example on how management programs for genetic resources can benefit from the combined use of both, traditional methods and modern molecular genetic tools.

## **Characterization of a gene pool of old broad leaf *Rhododendron* hybrids by means of STMS markers**

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*Rhododendron* L. represents the largest genus within the family *Ericaceae* and it is well known for the beauty and diversity of floral and vegetative form of its more than 1000 species. This large genus has created systematic problems about intrageneric ranks. The genus was recently divided into 8 subgenera, being *Rhododendron* (lepidote rhododendrons) and *Hymemanthus* (elepidote rhododendrons) among the most important ones.

During the 19<sup>th</sup> century many broad-leaf woody evergreen *Rhododendron* cultivars (subgenus *Rhododendron*) were introduced in Italy as ornamental garden plants. The most comprehensive collection is located in the Special Natural Reserve of Burcina Park "F. Piacenza" located in Biella (Piedmont), that represents a rich reservoir of germplasm for its favourable climatic and pedologic conditions. Burcina Park is a landscape historical garden realized around 1850 in Pollone, near Biella (Italy). It extends over an area of 57 hectares covering almost completely the slopes of a mountain called Brik Burcina. The most important attraction of Burcina Park is the rhododendron valley, extending over a surface of about 2 hectares and blooming around the half of May.

Mr. Felice Piacenza started to collect rhododendron hybrids during the last decade of XIX century and bought very precious and unique cultivars selected in Europe. It took him 40 years to improve the collection and to gather over 120 different cultivars mainly derived from Belgian and French nursery of Van Houtte and Croux & Fils and Barbier.

During the 20<sup>th</sup> century nomenclature of these hybrids was lost and the present difficult to identify them is associated to a poor knowledge of these plants in Italy. In 2003 a book on morpho-botanical characterization of these rhododendron hybrids was published. The study consisted in filling in specific identification cards in which the following features were considered: blooming, truss, flower, stamen, pistil and leaf. Also historical and bibliographical information referred to place and year of origin, name of the breeder, previous descriptions and quotations were included.

The aim of the present work is to deepen the knowledge about these cultivars that represent an important genetic patrimony, since a number of them are deceased world wide. In order to characterize this germplasm, the genetic diversity of 24 rhododendron hybrids was investigated using a polymerase chain reaction (PCR)-based DNA fingerprinting method. Four Sequence Tagged Microsatellite Site (STMS) markers developed by Dunemann *et al.*, (1999) in *Rhododendron* 'Cunningham's White' were used. With the aim to elucidate the phylogenetic relationship among all these hybrids, 8 species (*R. arboreum*, *R. catawbiensis*, *R. fortunei*, *R. ponticum*, *R. edgeworthii*, *R. griffithianum*, *R. caucasicum* and *R. maximum*) were also included in the STMS analysis.

The genetic and morpho-botanical characterization of the rhododendron hybrids located in Burcina Park, besides helping their safeguard, allows the rediscovery of an interesting genetic heritage, the recovery of which could be of advantage to historical garden restoration and in modern horticulture, as these cultivars are surely adapted to local ecopedological conditions. Moreover, the obtained information could be useful for further genetic improvement of these plants.

# Cultivar susceptibility assessment and breeding for resistance: possible ways to fight the sharka in peach

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## *Introduction*

Sharka, caused by Plum pox virus (PPV), is the most dangerous stone fruit viral disease in Europe. In Italy, sharka was first found in Trentino Alto-Adige region on apricot (1973) and plum (1975). On peach, the virus was first isolated in 1992 in southern Italy. Since then, PPV has been found in several peach growing areas along Italy, seriously threatening two of the most important ones for the national peach industry: Verona (Veneto region) and Cesena (Emilia-Romagna). In the susceptible cultivars, indeed, this virus can cause heavy financial losses because symptomatic fruits, showing ring patterns on flesh and skin and mild or severe fruit deformation, are unmarketable. The strain most frequently isolated in the infected orchards is the PPV-M, the most aggressive for peach. The quarantine measures taken to prevent the PPV diffusion, such as eradication of infected trees or of entire orchards, the use of healthy propagation material and tree protection from virus transmission by aphids haven't been as successful as hoped so far. For this reason, in 2004 the region Emilia-Romagna founded a study aimed to investigate the level of susceptibility to this virus on the most important peach cultivars grown in Romagna area and in 2007 the Italian Ministry of Agriculture sponsored a new project aimed to prevent the spreading of sharka, through breeding actions and virology studies.

## *Materials and Methods*

Sixty-three cultivars (33 peaches, 28 nectarines and 2 canning peaches, white and yellow-fleshed) grafted onto peach x almond GF677 were grown under screen-house to isolate the trees from aphids. In 2004, 3 one year-old trees of each accession were inoculated with PPV M strain; the fourth tree was left un-inoculated (control). From blossoming onwards, the trees were periodically monitored to detect symptoms on flowers or on leaves and appropriate analysis, based on serological (ELISA) and molecular (real-time PCR) tests, were carried out to confirm the virus infection in symptomatic and non symptomatic samples. In addition, the vegetative growth and the fruit quality in inoculated and non inoculated trees were evaluated.

In 2007 the new interdisciplinary project (PPV-CON) started, involving 9 scientific institutions and aiming to increase resistance or tolerance to PPV in peach. The susceptibility to sharka of different Italian varieties and selections tolerant to other diseases (powdery mildew, leaf curl and brown rot) are assessed using the same methodology. As parents for resistance, selections obtained at the INRA of Avignon (Summer Grand x *Prunus davidiana*) and at the Department of Pomology of Davis University in California (hybrids 'Hesse' peach x 'Padre' almond) have been used.

## *Results*

A large number of inoculated trees showed discoloration and mottling on the young leaves in spring; furthermore, many peach varieties with rosaceous flowers appeared infected with strong symptoms on petals and fruit deformations with typical rings and mottling. From observations and analyses carried out over 3 years including two dormancy cycles, over 95% of inoculated trees showed symptoms of the disease. There were significant differences among genotypes in the length of latency period, site of appearance and/or severity of symptoms. Cultivars like Maria Anna, Neve, Rose Diamond, Spring Bright, Diamond Bright, Maeba Top, Max, Nectaross, Maria Marta, Tendresse, Royal Glory, Rich Lady, Rubirich, showing uncertain symptoms on leaves or flowers, were positive to the analysis. Sweet Red and Alix had the longest latent period, showing the first symptoms only in 2006 and 2007, respectively. Maria Dolce, Summer Lady and Morsiani 90, never showing symptoms nor resulting positive to serological or molecular test, have been scored as highly tolerant and will be further observed. Most of the fruit qualitative traits analyzed (weight,

titratable acidity, harvest data) were unaffected by the virus, but soluble sugar content was lower in symptomatic fruits.

As far as the PPV-CON Project is concerned, more than 800 seedlings have already been obtained and are being assessed for resistance in the different UO involved in the project.

## Screening of tomato root endophytic fungi for potential application in Biocontrol

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Endophytes are microorganisms that are able to colonize internally plant tissues and roots without causing visible disease symptoms for at least part of their life cycle establishing neutral or mutualistic associations. Positive effects on the plant are increased tolerance to abiotic-stress, disease resistance or plant-growth promotion. According to these effects on the host plant, it was aimed to isolate new fungal endophytes from tomato roots, which might improve plant development and health and are therefore useful for application in plant production systems.

Fungi were isolated from surface disinfected tomato roots of four different greenhouse sites in Colombia. 51 potential root-endophytic fungi were roughly characterized and potential pathogens like *Fusarium* spp. were omitted. 14 potential endophytes were further analyzed concerning their phylogenetic position, their root colonization patterns and their impact on plant growth. Additionally, barley seedlings were inoculated by the 14 endophytes. Interestingly in comparison to tomato, different isolates promoted barley growth.

Based on the results three new isolates (two mitosporic fungi and one identified as *Leptodontidium orchidicola*) with neutral or positive effects were selected and are currently tested for their ability to induce local and systemic resistance against tomato pathogenic fungi and for their colonization in other plant hosts such as barley and Arabidopsis. On barley was confirmed that these isolates do not show any effect when are inoculated to seedlings but when are inoculated on seeds, the two mitosporic endophytes significantly promoted the production of shoots and one of them also incremented the mass of shoots and roots. On the other hand, *L. orchidicola* showed a neutral effect on number of shoots and a negative effect on shoot and root biomass. Preliminary results on tomato resistance against *Verticillium dahliae* indicated that one mitosporic isolate (E49), *L. orchidicola* and the root endophyte *Piriformospora indica*, which was used as positive control, not only promoted plant growth after 6 weeks of inoculation but significantly avoided the loss of shoot biomass after infection with the pathogen.

## Characterization of local fig genetic resources in Slovenia

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The fig tree (*Ficus carica* L.) is a typical Mediterranean fruit species and probably the first intentionally cultivated plant during the Neolithic revolution of which domestication preceded cereals by about thousand of years as indicated by recent fossil records. The genus *Ficus* contains several hundreds of different spe-

cies, which are widespread in different climate zones. Cultivation of common (edible) figs is mainly limited to the countries of the Mediterranean Basin, where it represents a significant source of agricultural income. Mediterranean food products are increasingly recognized as being beneficial to health, and the exotic character of the fig fruit and its functional properties motivate producers to enhance and to promote fig growing in marginal areas. The common fig could be considered as underutilized fruit species, especially in the region of the north Adriatic Sea, where the economic potentials of its cultivation have been poorly addressed. Re-emergence of minor crops could lead to an agricultural diversification, to a greater use of marginal lands, to a better maintenance of agrobiodiversity heritage, and to a better preservation of cultural identities and traditions. Besides that, growing of minor crops also provides additional sources of income to farmers and opportunity of employment in agriculture and related sectors.

An attempt is being made to revitalize and promote fig cultivation in Slovenia. Such expertise requires an inventory list and characterisation of available fig germplasm in the region, in order to select fig varieties well suited for the production of fresh and dried figs of commercial value. Sampling in the field was performed during the growing season and collected plant material have been evaluated for some morphological descriptors of fruit (shape, skin colour, flesh colour, volume, weight, stalk length) and leaf (number of lobes, degree of leaf lobation) developed by The International Plant Genetic Resources Institute (IPGRI). As expected, traditional cultivation and exchange of planting material in the region contributed to the confusion of variety denomination and several synonyms and homonyms have been noted. To confirm the identity of local varieties, we also developed new microsatellite markers from fig genomic DNA libraries, enriched for GA/TC and GT/AC motifs. Fifteen newly developed primer pairs were used for amplification of microsatellite loci and all sampled fig varieties have been successfully identified by the allelic polymorphisms. Preliminary results of molecular and morphological evaluation of local fig genetic resources will serve for propagation of certified planting material of figs in nurseries and for establishment of national collection for further fig genetic resources investigation.

## **Clonal variability for agronomic traits in local table olive variety ‘Meski’**

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The local variety "Meski" dominates the sector of table olive in Tunisia with 60% of planted areas. It's endowed with good technological characteristics (high average weight and pulp/pit, taste not very bitter, white flesh not adhering to the pit) and prepares itself perfectly to the conservation. It enjoys a good assessment from the Tunisian and foreigner consumers. Nevertheless, this variety had problems on the agronomic plan, with a weak and irregular production, a weak vigour and sensitiveness to the cycloconium. These defects explain largely the weakness of the national production of table olives. On the other hand, we reported that this variety is very heterogeneous and represents rather a variety population. Thus, we launched a clonal selection program to attenuate these defects.

A total of nearly 1000 clones from four different orchards conducted under irrigated conditions are followed since 2006 at the level of production and fruit characteristics.

For each clone, we recorded the olive production and several fruit parameters: weight and volume of the fruit, weight of the pit and the flesh, ratios of flesh to pit and weight to volume. The follow up concerned all sites in 2006 and only 2 sites in 2007. The olive production was noted for all clones and fruit characteristics only for productive ones (367 in 2006 and 185 in 2007).

The descriptive and correlation analyses revealed:

- A wide phenotypic variability between clones and sites for the most parameters.
- Significant correlations between traits with the dominance of pulp weight in the detected variability.
- The possibility to define reliable selection criteria

# **Vegetative and reproductive traits evolution of olive varieties under irrigated conditions**

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The in situ prospection and characterization of olive varieties in Tunisia allowed detecting a wide genetic variability on the agronomic and technological plans. The performances of these varieties in collections began in 2001. One of the installed collections in Ettaous Station (Sfax, Tunisia) contains forty varieties differed by their origin and end-use. The agronomic follow up of these varieties concerns the olive production, the vegetative growth and the reproductive development.

In this paper, we have focused on the vegetative growth and reproductive development of olive varieties. Thus, we have chosen three varieties representing the three regions of Tunisia (Chemlali Sfax in the Center, Chetoui in the North and Zalmati in the South) and three trees were chosen for each variety. Ten shoots of one year old were randomly selected within the canopy of each tree and the traits related to vegetative growth and reproductive development were recorded in the period going from the end of February to the end of May. The traits are the number of vegetative and generative buds on the one year shoot, the length of the apical shoot and the number of its vegetative buds, the length of resulting vegetative extension on the two shoots and the fruit setting per inflorescence.

The collected data of two years showed significant differences between the varieties and the years for all the traits. In 2006, Zalmati and Chemlali Sfax were similar for most of the parameters. On the other hand, Chetoui had a higher vegetative lengthening of the buds on the one year shoot and on the new apical shoot and a very weak production of clusters. In 2007, the differences between Zalmati and Chemlali Sfax were significant for the followed parameters and Chetoui showed a notable production of clusters.

In the favourable conditions, it seems that Chetoui preserves his alternating character for the production. On the other hand, the competition between the vegetative growth and the reproductive development is always aspects that influence the behaviour of the olive tree, while varying with the variety.

## **Agronomic behaviour of olive varieties under irrigated conditions in Sfax region (Tunisia)**

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Tunisia is rich in olive varieties and local ecotypes. Most of this germplasm is stationed in its origin centres and their behaviour is practically unknown outside these regions. Since 2001, we have installed olive varietal orchards in different oleic regions of Tunisia in order to evaluate the genetic variation of olive varieties and to select the best of them.

Among these orchards, we have the Ettaous collection in the Sfax region which contains 40 varieties and conducted under intensive and irrigated conditions. The follow-up concerns the agronomic and technological aspects. On the agronomic plan, we were interested to the vegetative growth, the reproductive development and the olive production.

For 11 varieties, we have chosen three trees by variety and we followed during two years:

- The lengthening of the growth parameters of every tree in the spring period (trunk diameter, canopy height and diameter).
- The development of the buds into clusters or shoots at the end of the spring phase for 10 shoots of one year old of every tree, as well as the number of produced clusters and shoots per cm and by node.

- The olive production per tree.

The collected data showed a significant variability for the followed parameters between the varieties and between the years. Some tendencies are to be noticed. The tendency towards production alternation such as for Koroneiki and Chetoui. Other varieties have both generative and vegetative behaviour each year such as Chemlali Sfax and Zalmati. The table and dual use varieties (Chemchali, Touffehi, Picholine, Manzanille and Meski) have generally weak vegetative, reproductive and productive performances. The two varieties Touffehi and Meski distinguished themselves by the weakest performances at the level of all the parameters. These results confirm those reported for Meski, the most cultivated table olive variety in Tunisia.

## Sensory evaluation of medlar (*Mespilus germanica* L.) cultivars

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### Introduction

The medlar (*Mespilus germanica* L.) is a minor fruit tree cultivated since the antiquity in Europe and presently almost forgotten. Its late autumn fruits are consumed bletted, when the flesh becomes soft and sweet and lacks its astringency. In Italy, the cultivation of medlar has been proposed in last years for fruit production diversification in niche markets. The fruits are becoming quite a common autumnal sight in greengrocers' and are more widely available both for raw consumption and processing.

The genetic diversity within medlar germplasm is not well known. The knowledge of current availability of biotypes and cultivars and the evaluation of their agronomic and qualitative traits represent the basis of information needed for the exploitation of this species as a fruit plant.

Sensory analysis is a good tool to obtain a deep knowledge of old germplasm quality, integrating the conventional characterization and evaluation work. The investigation was aimed at the definition of sensory differences among four medlar cultivars (Comune, Gigante, Goccia and Precoce) available in Italian nurseries in order to evaluate their fruit quality and the possibility of a commercial exploitation.

### Materials and Methods

Immature fruits were picked from seven-year old plants in the medlar germplasm collection established in Viterbo, at the experimental farm of Università della Tuscia. At the beginning of November anonymous samples of bletted flesh were submitted to eleven panelists previously trained for a better knowledge of the meaning of the chosen attributes. The following characteristics of the flesh were considered: smell intensity, fermented, alcoholic; sweetness, acidity, astringency, aroma, firmness, dryness, fibrousness, grittiness; the level of taste and smell appreciation was also requested. To avoid influences on the evaluation of flesh sensory attributes, the external appearance of the fruits at early ripening and at bletted stages was evaluated by the panelists only after the tasting session. Sensory score sheets with a 11 cm unstructured line scale were used for descriptive terms. Analysis of variance was performed on all the collected data.

### Results and Discussion

Sensory analysis of bletted flesh revealed a good appreciation of Goccia and a negative judgement on Gigante. Sensory profile showed big differences among cultivars. Gigante was perceived as characterized by scarcely sweet, dry and fibrous flesh; Goccia was considered sweet, with low acidity, high aroma and with absence of fibrousness. Goccia and Comune were the most appreciated cultivars for their taste, whereas Gigante was rated the lowest. Comune was the most appreciated cultivar for the external fruit appearance both at early ripening stage, when the fruits are generally sold, and at bletting. The good size (30-40 g) and the regular round shape are probably the traits making Comune more appealing than the other cultivars. The big size (70-80 g) was not sufficient to make the cv Gigante appealing, also because of the frequent presence of cracking.



The introduction of medlar into fruit growing for the commercial exploitation needs an accurate cultivar choice. The differences of exterior and inner quality were clearly perceived and could positively or negatively influence the potential consumer. High sweetness, low acidity and fine texture contribute to the appreciation of this fruit, whereas dry and fibrous flesh and low sweetness negatively affect cultivar judgement. Furthermore, sensory attributes such as sweetness, acidity, smell and texture can influence the quality of processed products, such as jams, jellies, schnaps and liqueurs, which represent an interesting use of medlar fruits.

### **References**

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## **Morphological divergency of garlic ecotypes in Serbia**

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The aim was to study six ecotypes of spring garlic at the Faculty of Agriculture, Belgrade University as part of the national collection of the plant gene bank. The investigated garlic ecotypes coded in the gene bank were: SG-1, SG-2, SG-3, SG-4, SG-5 and SG-6. Field trials were conducted in order to study the morphological properties of the garlic ecotypes. The following was monitored: bulb weight, number of cloves in the bulb, clove weight and the number of leaves enwrapping the bulb. The most significant differences ranging from 17.21g (SG-1) to 31.84g (SG-4) were obtained for bulb weight. These differences were statistically very significant. The number of cloves in the bulb varied from 7.75 to 12.25 (SG-4 and SG-1). The differences in the number of cloves in the bulb were statistically significant. The weight of individual cloves varied from 16.00g (SG-1) to 31.30g (SG-4). These differences were statistically very significant. The values for the number of enwrapping leaves ranged from 4.7 (SG-4) to 6.7 (SG-2). The differences were statistically significant. Based on the results obtained the conclusion which tends to emerge is the considerable impact of both climatic and soil conditions on the morphological properties of the examined garlic ecotypes. In Serbia the use of both in situ and ex situ for the description and maintenance of garlic is known to contribute to objectivity with regard to the results of the garlic ecotypes.

## **Utilization of the local genetic resources in breeding new valuable cultivars for *Malus* and *Pyrus* species**

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### **Introduction**

The genetic resources from Romanian „ex situ” and „in situ” collections for *Malus* and *Pyrus* include 262 autochthonous old genotypes, 162 pear local varieties, respectively (Braniste N., Butac M., 2006). For many years, since 1949, when the first cross pollinations with apple were done within a breeding program at Voinești Station, Dambovita, several local varieties (with traits like: rusticity, late blooming, good storage) adapted to the pedoclimatic conditions of the area have been employed. Identically with pear since 1951 at Voinești Station and since 1969 at Pitesti, many local and breded varieties to improve the fruit quality (skin and flesh colour, size, resistance to major pests and diseases) were used.

## Results

At the Fruit Research Station Voinesti (1949-2007) there were several stages in breeding, using both local old varieties and also bred cultivars from the previous generations. There were performed in all, over 800 hybrid combinations, utilizing 25 Romanian old and new cvs., as well as 58 foreign cvs. as male and female parents. In the first stage (1949-1957), one can see a great deal of hybrid combinations owing to an enthusiastic beginning in breeding but at the same time the same crosses were done one year after another due to less diverse genetic resources. Among the local varieties, the following: Cretesc auriu, Domnesc, Patul, Calugaresc, Cretesc de Valcea, Salciu, Cretesc salbatic, Cretesc rosu, Verzisoare, characterized by rusticity and a remarkable adaptation were employed. For improving the fruit quality as look, size and texture, a wide range of foreign cvs. presently in collections or orchards was used in hybridizations: Jonathan, Golden Delicious, Red Delicious, Kalter Bohmer, Gravenstein, Cox Orange, London Pepping, Wagner, Wealthy, Parmain d'or, Winter Banana, Reinette Bauman .

The pear breeding program was started in 1951 at Voinesti. Unlike the apple, in the first stage the number of cultivars employed as genitors was small, 4 local cvs.: Busuioace, Dulci de vară, Pietrose, Rosior pietros and 6 foreign cvs.: Williams, Clapp's Favourite, M. Marrilat, B. Clairgeau, D. d'hiver, M. Levavasseur. That is why, the number of hybrid combinations was limited, from 1 to 14 and the same crosses were yearly repeated until 1970. The breeding objective was focused on the fruit size and quality (texture and taste).

After 1981, better genotypes were introduced as genitors: Timpurii de Dambovita (Busuioace x Clapp's Favourite), Republica (D. d'hiver x M. Lavavasseur), Euras (P. serotina x Olivier de Serres) x D. d'hiver, Argessis (Napoxa x B.p. Morettini), Napoca (Dr. J. Guyot x pollen mixture), Monica (Santa Maria x Principe de Gonzaga).

In Pitesti, the objective of the first breeding stage (1969 – 1979) was to obtain early varieties with big fruit and no internal breakdown. So, local varieties with early ripening season but with small fruit, scab susceptible were chosen (Codita, Orzaticce, Santiliesti, Cu miezul rosu) and crossed with B. Giffard, B.p. Morettini, Morettini 113, Williams and Santa Maria. There were obtained a few commercial cultivars with early ripening season: Trivale, Triumf, Daciana, Argessis, Getica and Carpica recommended to the farmers.

## Conclusions

To conclude, the utilization of the local varieties in the Romanian breeding programs for a rather long period (1949 – 2007) was a continuous concern due to a small number of varieties with poor quality fruit at the beginning and thereafter for improving the disease resistance and fruit quality characteristics to meet the internal and external market demands. In Voinesti and Pitesti, local genetic resources for apple and pear were involved: 25 old and bred apple cvs., 30 pear cvs., getting over 800 apple hybrid combinations and 113 pear ones. One can see that the genetic advances were achieved along the hybrid generations obtained, which gradually became valuable selections and varieties, some of them being also propagated to day.

## Micropropagation and ex situ conservation of pear germoplasm: the role of exogenous carbohydrates

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Type and concentration of sugars in micropropagation affect quality and morphology of the explants and their survival to storage. In the present work the quality of the *in vitro* grown material and the response to storage was optimised on pear, evaluating the effect of various carbohydrates on the shoot proliferation, the "slow growth" conservation and the crioconservation. Micro shoot of some pear genotypes were cultured on a medium containing LP macro and micro-elements, 6 g l<sup>-1</sup> agar and sucrose, glucose, fructose or maltose at 2 concentrations (2 or 3 %). Benzyladenine (1.8 μM) or indole-3-butyric acid (9.8 μM) were supplied for inducing shoot proliferation or rooting, Multiplication rates, rooting response, water and chlorophyll content of the microshoots, pH and osmolarity of the medium were evaluated. Sucrose and glucose gave the

best results in terms of multiplication rate, rooting percentage, chlorophyll content and low hyperhydricity of the explants. Their effect on “slow growth” conservation of pear, performed on a hormone free medium in darkness at 4° C, and on the cryopreservation, with the encapsulation-dehydration method, was also investigated.

## **GENE SAVE – An interregional project for protection of genetic resources and traditional knowledge in the Alpine region**

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The Interreg III A-project GENE SAVE was started in 2003 as a common initiative between the Research Centre Laimburg of the Autonomous Province of South Tyrol (Italy), the Chamber of Agriculture of the Austrian County of Tyrol and the Agrar-Group of the Tyrolean Government Office. One of the primary intentions of the project was the inventarisation of local genetic resources including old apple varieties, different vegetable species as well as cereal landraces. In the initial phase the project was characterised by a close cooperation between the researchers and the public, in order to retrieve and collect as much as possible of the remaining local genetic resources. In addition to the collection of seed and plant material, memory banking was used to prevent extinction of traditional knowledge. All the plant material included in the seed bank or in the *ex situ* collections was described botanically and characterised agronomically. For apple and landraces of wheat, rye and oat the germplasm collection was additionally investigated by the application of molecular genetic markers. The project, which brought together experts of different backgrounds created a large amount of data, which shall be made available in a common database between South Tyrol and Tyrol. Apart from the moral aspect of saving the genetic variability and traditional knowledge for future generations, the project shall also fulfil a short-term goal of providing new possibilities for niche production and stimulation of sustainable agricultural cultivation in the sensitive Alpine region.

## **Bioagronomic evaluation of local almond cultivars in Apulia region (southern Italy)**

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### ***Introduction***

Almond (*Prunus amygdalus* Batsch) is one of the oldest and most traditional crops widely present in southern Italy and in several countries all around the Mediterranean basin. Due to the particular pedo-climatic and geographical conditions of this area it has been the centre of origin of several autochthon cultivars. Currently large numbers of almond varieties with a range of genetic variability exist. Many have good qualitative fruit characteristics, which, if studied and evaluated, could be of interest for variety selection and improvement. In Italy the cultivation of almond is mainly concentrated in the southern regions. Together with Sicily, Apulia is a region with a natural predisposition for the cultivation of almond and the wealth of varieties found here due to a long selection process is most interesting, but also subject to a high risk of genetic erosion. The research unit, based in Bari, has a long history in the study of almond and is continually involved in the identification, recovery and evaluation of the autochthon varieties still be found in the area.

The aim of this research is to preserve the autochthon cultivars at risk of extinction and to evaluate them in order to identify those that have the best characteristics in terms of productivity and adaptability to location.

### **Material and methods**

The establishment of the experimental field of almond germplasm was accomplished in 1967 and is located 16 km from Bari on plains in the area of Bitetto (southern Italy). The varieties collection, cultivate on dry soil, consists of 225 cultivars which can be sub-divided up into 109 Italian varieties (predominantly Apulian in origin), 43 foreign types of various provenience and 73 new cultivars created by the Institute as part of a programme of genetic improvement begun in 1948. Currently 392 new selections are being evaluated among which several can be considered very interesting. Data is gathered for every plant of every cultivar recording the flowering period, the kernel yield and the main carpological parameters (shelling, double kernel, kernel weight).

### **Results and conclusions**

Table 1. Average values of the productive & carpological parameters of the most productive autochthon almond cultivars in the three-year period 2004-2006

Amongst the autochthon cultivars evaluated in the field of almond germplasm a group of ten stood out in terms of productivity over the period in question. The most productive of all were Filippo Ceo and Barlettana which reached values of over 3 kg tree<sup>-1</sup> with good levels of shelling and kernel weight. Both showed a normal incidence of double kernels. These were followed by Montrone, Cristomorto and Santoro which all had good production levels, only slightly below those of the first group. Santoro also had good carpological characteristics (shelling, kernel weight and double kernels). Our research, recovery and evaluation of the genetically autochthon material has saved from extinction many cultivars which are proving to be of great use in the current initiative to relaunch almond cultivation in southern Italy and is also showing positive effects throughout the entire almond area of the Mediterranean Basin. The genetic variability conserved in the germplasm field represents enormous potential for future studies of genetic improvement.

<b>CULTIVARS</b>	<b>Yield kernel (kg tree<sup>-1</sup>)</b>	<b>Shell-ing (%)</b>	<b>Kernel weight (g)</b>	<b>Double kernel (%)</b>
Filippo Ceo	3.21	33,70	1,44	26
Barlettana	3,18	42,00	1,51	21
Montrone	2.92	33.21	1.41	12
Cristomorto	2,89	29,28	1,18	10
Santoro	2,87	36,51	1,65	3
Catuccia	1,78	32,75	1,40	30
Tenente	1,73	28.22	1,29	4
Piangente	1,66	28,12	1,12	15
Pidocchioso	1.61	25,50	1,22	36
Tuono	1,57	34,62	1,36	32

tana which reached values of over 3 kg tree<sup>-1</sup> with good levels of shelling and kernel weight. Both showed a normal incidence of double kernels. These were followed by Montrone, Cristomorto and Santoro which all had good production levels, only slightly below those of the first group. Santoro also had good carpological characteristics (shelling, kernel weight and double kernels). Our research, recovery and evaluation of the genetically autochthon material has saved from extinction many cultivars which are proving to be of great use in the current initiative to relaunch almond cultivation in southern Italy and is also showing positive effects throughout the entire almond area of the Mediterranean

Basin. The genetic variability conserved in the germplasm field represents enormous potential for future studies of genetic improvement.

## **Recovery, preservation and analysis of fruit quality of the apple “Mela rosa” a biotype from Campania region (Italy)**

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The denomination “Mela Rosa” is referred to an old population of apple trees cultivated in Apennines elevation. The fruits have a pink/red-pinkish over colour and a yellow/green ground colour. In the past they were appreciated mainly for the long shelf-life. Unfortunately, due to the depopulation of rural territory and to the spreading of commercial varieties, their cultivation was nearly completely abandoned: only few old trees survived in kitchen gardens. Nowadays these varieties excite an increasing interest because of ecological and healthy reasons. Since few years Campania Region (Italy) is promoting recovery and preservation of own genetic resources. The Se.S.I.R.C.A. (Experimental Sector, Information, Research and Consultancy in Agriculture) of Agriculture Regional Councillorship recovered many apple biotypes from hilly and moun-

tain areas of the region (Santangelo I. et al., 2002). ML03 recovered from Valva (Salerno), is one of these biotypes named “Mela Rosa”. In the CRA-Fruit Tree Research Institute laboratories of Caserta, was achieved *in vitro* establishment of this biotype, in order to set up rapid propagation methods and to obtain plants disease-free.

In autumn one year old cuttings have been taken from mother plant. Meristems have been excised from nodal buds and sterilized according to the following procedure: manual washing with lysofrom® soap; 20 min immersion in lysofrom® soap; 20 min immersion in 1% sodium hypochlorite water solution; rinse with sterile water; 2 min immersion in 70% ethanol; rinse with sterile water; 10 min immersion in sterile solution of ascorbic acid 250 mg/l. The meristems have been placed in tubes with MS medium supplemented with ascorbic acid 100 mg/l, IBA 0,05 mg/l, BAP 0,5 mg/l, sucrose 30 g/l, agar 6 g/l the pH was adjusted to 5,6. Some meristems (15,6%) started to develop showing hyperhydricity of shoots, they were recovered by seven weekly-subcultures on fresh medium containing MS salts, MS vitamins without glicine, BAP 0,3 mg/l; IBA 0,1 mg/l; sorbitol 20 g/l, sucrose 10 g/l, agar 4,5 g/l the pH was adjusted to 5,6. Afterwards, subculturing every month in the medium described above, the culture had a  $3,77 \pm 0,46$  multiplication rate without showing hyperhydricity anymore. Rooting trials were undertaken to examine the effects of two kind of macrosalts LP/2 and MS/2 (the basal medium containing MS microsalts and vitamins without glicine, sucrose 20g/l, agar 4,5g/l and pH adjusted to 5,6). Ten shoots 2 cm long were used per treatment and the experiment was repeated three times. After 30 days rooting frequency and number of roots/rooted plant was recorded. Plantlets showed very high rooting efficiency on medium with macrosalts LP/2 (100% of rooting frequency;  $5,1 \pm 0,21$  roots/rooted plant) compared to the lower efficiency on medium with macrosalts MS/2 (40% of rooting frequency;  $2,1 \pm 0,35$  roots/rooted plant). Rooted plants are able to acclimatize on a substrate with brown peat at pH 5,8.

Fresh fruits harvested on 31<sup>st</sup> August 2007 were analysed for weight ( $187,25 \pm 14,18$  g), flesh firmness ( $3,33 \pm 0,11$  Kg/0,5cm<sup>2</sup>) and soluble solids content ( $12,5 \pm 0,31$  °Brix). Total content of proteins were extracted and purified from flesh using a brand new protocol. An average of 120 µg of proteins were extracted from 1g of dry sample. The proteins were separated with 2-dimensional electrophoresis technique. In this gel each protein was represented by a spot, specified by two coordinates (isoelectric point and molecular weight). They were compared to most common allergenic proteins (Lipid-transfer proteins and profilin) in apple fruits (Garcia-Sellés et al.). ML03 turned out to be those allergenic proteins-free.

In conclusion we obtained a good micropropagation method for an interesting biotype of “Mela Rosa” from Campania in order to allow a large scale multiplication of disease-free material suitable to its new spreading. We hope to be able to confirm the absence of the most common allergens that would make ML03 more interesting to consumers and producers. This procedure can be used to revalue other old fruit varieties.

### **Acknowledgements**

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## **Biodiversity of genetic resources of *Maloideae (Pomoideae)* and *Prunoideae* subfamilies in Latvia**

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### **Introduction**

Since 2003 every season regular expeditions for sampling of Latvian plant genetic resources are carried out. A broad biodiversity of potentially useful plants in horticulture was found. The real problem is several spots of infection of fireblight *Erwinia amylovora* first time found in Latvia in 2007. The sampling of plants of *Pomoideae* subfamilie was abruptly terminated in this season because of this disease.

### *Accessions sampled*

The biggest part of accessions sampled belongs to genus *Malus*. There are only two native wild species in Latvia – *M. sylvestris* and *M. praecox*. *M. sylvestris* is rare and endangered because of easy natural hybridisation with everywhere wide distributed *M. domestica*; only one morphologically identical accession was found. A few trees of probably *M. praecox* were found near Kandava, but no more in places mentioned in reports of earliest botanical expeditions. *M. domestica* group apples are common in wild conditions as open pollination seedlings and sometimes as natural inter-species hybrids with *M. sylvestris*, *M. baccata* and *M. x prunifolia*. They are interesting for horticultural breeding due to winter hardiness and good disease resistance. Some of them could be registered in future as ornamental, processing or dessert cultivar.

The same problem is stated for *Pyrus communis* – only one morphologically typical plant was found near Koknese. Accessions of genus *Pyrus* are wide common in wild conditions (mostly located in West part of Latvia), but only as seedlings of pear cultivars or natural interspecies hybrids with *P. communis*.

Significant horticultural potential in Latvia have mountain ash. Large natural variability for *S. aucuparia* and ability to easy inter-species and inter-generic hybridization in wild environments can be important source to enrich traditionally cultivated assortment for rowans. Till now it was considered that in Latvia in wild grow only two mountain ash species: *Sorbus aucuparia* and *S. intermedia*. In expeditions were sampled also *S. rupicola* (very rare), *S. aria* (only apomictic plants), *S. hybrida*, *S. x Meinichii*, different natural inter-species and inter-generic hybrids. From collected accessions of *S. aucuparia* some interesting samples were found: with deeply divided pinnate leaves (promising as ornamental), accession with seven months long storability of fresh fruits, two different *pendula* type mutations, with yellow coloration of fruits, with rust resistance, with globe-shaped fruit clusters, and accession without tannins. *S. x Meinichii* and *S. hybrida* accessions showed high sugar content in fruits. Natural inter-generic hybrid *Sorbus x Crataegus*, already named 'Saule', has fruits suitable for long storage. Distribution and biodiversity of Latvian wild rowans need to be evaluated additionally. Unfortunately they are endangered in wild ecosystems at present because of increasing recreative load on forests near the beach zone and timber felling. Therefore it is advisable to maintain samples in germplasm collections.

Sweet cherries *Cerasus avium* are most common stone fruits in wild forests. There were sampled accessions of different fruit colour and size suitable for cultivation. Since 19-th century sweet cherries were escaped from old manors gardens in forests. The biggest part of wild populations is located in west part of Latvia, but at present they become common also in eastern part with rigorously climate conditions, possibly because of introduction of new high winterhardy varieties in cultivation and global climatic changes. In contrary, the distribution of Latvian local sour cherries *Cerasus x vulgaris*, (Kazdangas, Daugmales Stikla) is significantly decreased, in some places cherry trees are even completely lost already, due to spread of hard fungal diseases during past 20-30 years.

*Prunus* diversity in wild is scanty. There is not any native wild *Prunus* specie in Latvia, but during expeditions we found that three species are starting to escalate in forests from cultivated gardens. *P. spinosa* are spread out in few places, and also often we found different tree habit and leave coloration *P. cerasifera* var. *divaricata* and *P. domestica* open pollination seedlings from cultivated varieties. There were several wild plums accessions sampled with good ability to be as pollinators. The first trials showed that these plums can be used instead of traditionally planted, but not winterhardy enough pollinators for popular in Baltic region commercial cultivar 'Yellow Egg Plum'.

*Amelanchier*, *Crataegus*, *Padus* accessions selected from wild conditions were polymorphic also.

All sampled accessions are planted in the collection of genetic resources.

## Proof of hybrids in interspecific *Cyclamen*-crossbreeds – a comparison of methods

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Cyclamen, i.e. *Cyclamen persicum* has a great importance in the European pot plant production. The sale of approximately 31 million pieces in the year 2004 is entitled in place three after Orchids and Poinsetia. However stagnation and/or a slight drop of the number of sold cyclamen plants has been observed in recent years. Different causes of this phenomenon are discussed. In spite of massive marketing actions and intense breeding work, this trend could not be ended up to now. A representative consumer questioning formulates goals in the direction of breeding (stress and disease tolerances, an extended use spectrum as well as new colours and forms). These tasks can not be realized within the species *Cyclamen persicum*. Therefore we performed interspecific crosses between cultivars of *C. persicum* and different cyclamen species using special In-vitro-techniques.

After the successful combination of *C. persicum* and *C. purpurascens* we could introduce the new cultivar group Odorella into the market in cooperation with a tissue culture laboratory and a horticultural company already in 2004. Further interspecific crossbreeding was performed to extend the cultivar spectrum.

For the early inspection of the success of the crossbreeding, two different procedures were available to us. The first method was RAPD PCR, which allows a very particular hybrid detection. However, whether this would be applicable for the great number of expected hybrids with a justifiable expenditure remained to be tested.

The second method was the determination of the relative DNA content of the cells by flowcytometric measurements. For the moment this method seemed to be more efficient and more economical but the statement safety as well as the application restrictions had to be clarified (assumption is the presence of detectable differences of the DNA content of the crossbreeding partners).

Both methods are universally useable for tested combinations. They differ in time and expense.

Both modern methods are compared with classical microscopical procedures. Of special importance are: the safety of the results, the possibility of early investigations and the applicability as broad as possible.

## Studies on Strawberry Germoplasm in Italy

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The growing strawberry (*Fragaria x ananassa*) is an octoploid hybrid obtained almost three ages ago from a cross between two wild species coming from America, *F. chiloensis* (L.) Duch and *F. virginiana* Duch. Since the beginning of 1800 this species was used in breeding activity with the aim to obtain new strawberry varieties. Recent investigations have shown that in less than 200 years, 2000 strawberry varieties have been released. The strawberry germoplasm represents a genetic inheritance of big interest for biodiversity. In Italy, the germoplasm conservation is made in our Institute (Forlì-Po Valley). Some old varieties were used in specific cross combination in order to introduce high fruit taste and plant reblooming capacity.

Some *F. chiloensis* clones collected in Chile were crossed with some strawberry varieties (*Fragaria x ananassa*) in order to transfer plant rusticity linked to plant diseases tolerance and good fruit quality. Also, some old varieties as Fracunda and Liberation d'Orleans with very good fruit flavour have been used.

The hexaploid *Fragaria moschata* is grown in the Center and North of Europe. In Italy, in Tortona area (Piedmont Region), the old variety Profumata of Tortona, popular in 1600, is grown again. It has fruits bigger than *Fragaria vesca*'s characterized by a particular muscat flavour. Profumata of Tortona variety is

dioecious and both male and female plants have to be grown for having fruits. Capron is another *F. moschata* clone with hermaphrodite flowers, but the aroma of its fruit is less strong than Profumata of Tortona's. A specific Project, with the aim to promote and develop Profumata di Tortona variety, to improve plant yield and resistance, fruit size and firmness, maintaining strong muscat aroma, started 3 years ago. Some specific crosses between female and male plants, selected in populations of Profumata of Tortona and Capron, with good behaviour and resistance to soil borne pathogenes, and big fruit size and strong aroma, have been made. Also crosses between *F. moschata* clones and *F. x ananassa* have been made to obtain some fertile F1 hybrids, but several backcrosses are necessary to find again large fruit size and high firmness.

Some molecular studies to identify molecular markers or genes linked to sexual determination have been started. Profumata of Tortona, dioic, with staminate (male) and pistillate (female) flowers, and Capron, monoecious, with hermaphrodite flowers, have been analyzed.

The discrimination between Capron and Profumata of Tortona occurred with all the tested primers, instead the discrimination among the Profumata of Tortona plants of the two different sexes occurred only with few pairs of primers. More studies are necessary to confirm these data.

## Fruit quality traits of six ancient apple Sicilian cultivars.

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### Introduction

Identification and characterization of the regional fruit germoplasm represents an important strategy to collect genetic resources of great value for the local environment (Fideghelli, 2007). Those genetic resources are also critical to identify the most important quality traits for future breeding programs including sustainable issues and landscape conservation. Some of those local cultivars in fact present interesting quality characteristics (Bounous et al., 2006) and disease resistance along with good adaptation to climate and soil conditions. In Sicily many apple trees have been grown since ancient times. Most of these varieties are of local origin or were acquired a very long time ago (De Michele, 1992) and are being replaced by new, more selected ones. The aim of this work was to characterize six ancient Sicilian apple cultivars in order to determine their quality and aroma composition. 'Golden Delicious' was included in the trial to appreciate differences between old and new varieties selected for strictly commercial traits.

### Materials and methods

Four trees of the cultivars Bommino, Canonico, Granatino, Rotolari, San Giuseppe, Virchiata and Golden delicious (clone B) were grown on M.9 rootstock, planted in single rows, spaced at 4 m between rows and 1.5 m within the row, trained to a central leader, and received conventional cultural cares. The six local varieties were collected from different locations in the Palermo and Catania provinces and planted near Caltavuturo (37° 49' N and 850 m above sea level), Sicily, in 1998. Trees. Fruits were harvested at full maturation (11-21 October 2006) and a sub-sample of 15 fruits per tree was taken to the laboratory to determine average fresh weight, size, flesh firmness, total soluble solids, pH and acidity, percentage and intensity of peel red colour, and starch index. Peel colour and flesh staining for starch quantification were determined by digital image analysis using an algorithm that quantifies colour characteristics as the weighed distance (CIE L\*a\*b\* space) of each pixel in the image from a reference sample. The output is a colour index and a starch index ranging from 0 (no red colour or staining) to 1 (max red colour or staining). Volatile compounds were determined in fruit peel and flesh by Solid Phase Micro-Extraction technique in Head Space followed by Gas Chromatography/Mass Spectrometry.



## Results and Conclusions

Our observations showed significant differences in fruit quality among the cultivars. Among the local cultivars, Canonico exhibited the largest fruits (272 g), followed by Rotolari (260 g) and Granatino (165 g), and with Golden Delicious averaging 166 g; Virchiata (136 g) and Zitello (138 g) reported intermediate fruit size, whereas S. Giuseppe (105 g) and Bommino (79 g) produced the smallest fruits. S. Giuseppe, Bommino and Canonico showed the highest percentage of cover color extension and intensity of red colour. Firmness ranged from 6 (Rotolari) to 11 (S. Giuseppe) kg cm<sup>-2</sup>, with 7.7 kg cm<sup>-2</sup> of Golden Delicious. Soluble solids were highest (above 15 °Brix) in Bommino and Rotolari and lowest (less than 13.0 °Brix) in S. Giuseppe, Virchiata and Zitello, with Golden reporting 14.6 °Brix. Titratable acidity was relatively low in Bommino and Rotolari (0.7-0.9 g L<sup>-1</sup>) and consequently the optimal sugar/acid ratio was reported by Canonico and Granatino. The volatile fraction was always more concentrated in the fruit peel than in the flesh, regardless of cultivar. Aroma composition generally showed great variability among cultivars. Some of the old varieties (Canonico, Rotolari, S. Giuseppe) exhibited a higher number and concentration of compounds with greatest volatility compared to Golden Delicious, and for this reason their aroma is more rapidly perceived by the consumer. Overall, the evaluation of these ancient cultivars showed great variability of quality characteristics and evidenced specific interesting traits for genetic improvement or for a revaluation of these fruits in niche markets.

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## Grapevine goes “retro”: A Survey of LTR-Retrotransposons in the Grapevine Genome

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Retrotransposons are abundant in plant genomes and contribute to genome structure, evolution and genetic control architecture. They possess the capability to change their genomic locations and increase their copy number whereby they cause mutations. The copy number, the abundance and the insertion sites of retrotransposons within most investigated plant genomes are considered to be a promising basis for the development of genetic marker systems and the general understanding on genomic stability in vegetatively propagated grapevine.

The recent availability of the grapevine genome sequences made possible to extensively search *in silico* for new LTR-retrotransposon based sequences. We used the software LTR\_Struc to find *de novo* full length elements as well as the updated algorithm of the FastPCR program to find putative LTR sequences. Analyses were done on the genome shotgun sequences published in the NCBI database in January 2007. Using LTR\_Struc software we found 1,009 putative full length LTR elements which could be grouped in more than 100 different families. The analysis with FastPCR software was based on 22 sequences designed from conserved PBS regions of different families of retrotransposons found in different plants species. We found 33,125 putative LTR fragments which were clustered in 3,712 groups.

Information on the abundance and relationships among elements found is given and potential applications will be discussed. Fingerprinting studies employing sequence information of conserved LTR regions may serve as tools (basing on molecular marker systems e.g. S-SAP) for the identification and differentiation of

grapevine clones. Furthermore, knowledge on the occurrence of LTR elements within and among historic clonal grapevine lineages may provide information on genomic evolution. Tracking breeding records of grapevine hybrids will add information on the transmission of these elements.

## **Description of an *ex situ* *Vitis vinifera* cv. *sylvestris* Gmelin Germplasm Collection from the Upper Rhine Valley by means of Genotypes and Phenotypes**

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Wild, European grapevine *V. vinifera* ssp. *sylvestris* V. species are endangered in their natural, riverine habitats and thus there is need of collection and preservation of these species in *ex situ* collections or the conservation *in situ*. The already preserved genetic variation in *ex situ* collections has to be evaluated and the need for further measures identified. In this study thirty three wild *Vitis vinifera* ssp. *sylvestris* accessions from the Upper Rhine Valley including a defined subpopulation are described and compared with seven accessions originating from the East European gene centre (former Yugoslavia) stored in an *ex situ* collection. The accessions were described by means of ampelographic descriptors and molecular methods employing six microsatellite loci. Nei's genetic diversity indices were used and a possible population structure was determined.

## **Old and local tomato varieties: agronomic, analytical and sensory quality**

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### ***Introduction***

Since a few years, the interest for "old tomatoes" has increased. Indeed, some consumers look after fleshy and round fruits evoking tomatoes from their garden. Moreover, the diversity of the products remains an important factor of commercial development. The old tomato characterization compared to various improved varieties of old or modern types enable to better inform the consumers.

### ***Experimentation***

The trial was carried out in a non heated tunnel with a surface of 270 m<sup>2</sup>. Twelve old tomato varieties (no hybrids) belonging to various types of fruits forms were compared with five improved varieties (F1-hybrids). The aim of these studies was to appreciate the agronomic and quality behaviour of these varieties as non grafted plants.

### ***Results***

The agronomic examination shows a significant increase in productivity of the old types thanks to recent improvement (F1 hybrids). The fruits have more homogeneous size, a better firmness and the plants are more resistant to diseases. The results of analyses and testing by semi-professional tasters determined five most appreciated varieties: Sapho F1. Rose of Berne, Baselbieter Röteli, Climberley F1 and Pomodoro di

Catenna. These varieties had high soluble solid content (°Brix) and a citric acid content over 3,5 g/l. The ten other varieties, including other Ox-heart Varieties, were less appreciated than the variety Climberley F1, the modern savour reference variety. Old varieties cannot be distinguished from modern ones by content of vitamin C and carotenoids (lycopene and beta-carotene). It is the colour of tomato which mainly determines its carotenoids content (antioxidants).

### **Conclusions**

Old tomato varieties or improved one's are interesting for diversification: other form and colour. There is a place for these varieties on our markets.

## **The use of symbiotic fungi to propagate Hungarian native orchids**

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About 60 orchid species are native in Hungary, all of them belong to the protected plant species, and several are under strict protection. The Hungarian terrestrial orchids have very specific demands with respect to the habitat, and live in symbiosis with fungi. A possible way to conserve the endangered species is the artificial propagation of the orchids, followed by replanting into their natural habitat to increase population density. Co-cultivation with symbiotic fungi during this period may enhance the survival rate after replanting in nature. The aim of our experiments was to establish the optimum conditions for seed germination in the presence of mycorrhizal fungi. Seeds of 11 orchid species were collected to analyse symbiotic germination and to optimise culture conditions. In contrast to most experiments described in literature we only used mycorrhizal fungi, which have been taxonomically identified by molecular biological methods. The seeds have been sowed on 3 different media. In each case the plates were inoculated in parallel with five different fungi isolated from various orchids. The germination percentage was determined. The presence of mycorrhizal fungi strongly stimulated the germination of *Dactylorhiza incarnata* and *Ophrys sphegodes*, while *Anacamptis pyramidalis* germinated better under asymbiotic conditions. Germination of *Ophrys scolopax* ssp. *scolopax* and *Orchis laxiflora* ssp. *palustris* seeds was dependent on the media. No effect of mycorrhizal fungi on germination was detectable in the case of *Orchis coriophora*. Despite the relatively high sugar content of the medium most fungal lines coexisted with the seedlings, however, the *Thanatephorus* isolate 5DI1/6A parasitised and finally killed all seedlings except those of *Anacamptis pyramidalis*.

Our results show that the germination stimulating activity of mycorrhizal fungi isolated from orchids is species dependent. Ongoing studies shall clarify, whether the advantageous influence of the symbiont is also prevalent during nurturing.

## Breeding of plums and prunes resistant to PPV

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Sharka is one of the most serious virus disease in fruit growing. Multiple factors can influence the expression of symptoms on trees and fruits. Economic damages can be avoided by prevention of PPV-infection or by cultivation of tolerant or resistant varieties. Such varieties are in most regions the only way for a further cultivation of plums and prunes. The terms tolerance and resistance must be used in a correct way. There are two different kinds of resistances, the quantitative and the hypersensitive resistance.

For breeding purpose it is very important to have a source for tolerance or resistance. In variety testing there must be taken into consideration some parameters like the virus isolate, the inoculation method, the estimation of symptoms and others more. Different varieties of European plums for resistance breeding are called. Some of them were used as donors in the resistance breeding in Hohenheim. Tolerant and resistant new varieties from our breeding work are presented. Such varieties are only relative resistant, they can be infected by PPV, but symptoms are small. Depended from the stress of a plant the symptom expression may vary. Because of the change of climatic conditions the trees suffers in the last years more and more under stress resulting in insufficient resistance. For this reason breeders have to look for a solution. Because there were found no immune varieties or wild forms, the only way is the hypersensitive resistance, found firstly by KEGLER et al. in 1985.

Such hybrids were discovered also in our breeding material in Hohenheim. With 'Jojo', the first absolute sharka resistant variety world wide was introduced in 1999. 'Jojo' can not be infected by aphids; it is completely field resistant and is growing now more than 20 years healthy in different heavy infected orchards. 'Jojo' was taken as donor for hypersensitivity in a new breeding programme started in 1995. A sister of her, reacting also hypersensitive, was used already some years earlier. From more than 2,500 seedlings were till now selected 36 genotypes of high pomological value and reacting hypersensitive for a second selection trial in different regions. With the utilisation of hypersensitivity we hope to solve the problem of sharka.

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## Characterization of phenotypic diversity in offspring of *Malus sieversii* (Ledeb.) Roem.

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Offspring from 35 wild *Malus sieversii* (Ledeb.) Roem. trees primarily collected as seeds in 10 geographic collecting areas, mainly in Kazakhstan, were germinated and planted for evaluation at the Fruit Gene bank of the Institute of Fruit Breeding, Dresden-Pillnitz. The Central Asian wild species is the main progenitor of the domesticated apple. The collection areas of *M. sieversii* are characterized by mountain ranges with elevations between 600 and 1685 m and a West to East latitude of 2.000 km. The seeds were originally collected during a series of four collection trips made by USDA-ARS, Plant Genetic Resources Unit, Geneva, USA.

Fruit size is one of the critical characteristics in selecting of apple seedlings for further breeding. Therefore the evaluation of seedlings was mainly focused on fruit traits in addition to resistance traits. Only 60 % of 994 *Malus sieversii* individuals fruited at least once until the age of a 10-year-old seedling. The results of

the evaluation based on 19 fruit parameters and two parameters of frost will be presented and discussed in relation to diversity of seedlings within offspring and within the collection area. High variability of fruit size, between 1,5 x 2 cm and 5,2 x 6,4 cm (comparable to the size of the cultivated apple) was estimated. The same variability was found for the five inner parameters of the fruit investigated. The diversity ranged from fruits containing strong bitter substances to very tasty qualities. Evaluation of frost injury by estimating the percentage of surviving flowers demonstrated single seedlings with a high level of surviving flowers and a dependence on the collecting area. Seedlings with 60 - 80 % surviving flowers were estimated whereas the date of full flowering was four to six days after the frost nights.

On the basis of all evaluation data obtained concerning resistance, frost injury, fruit quality and genetic diversity (Wiedow 2006), 94 seedlings were selected to establish a *Malus sieversii* core collection at the Fruit Gene bank in Dresden-Pillnitz.

## Evaluation of native hybrids of *Prunus fruticosa* Pall. as cherry interstocks

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### Introduction

Searching for growth reducing cherry rootstocks, researchers produced and tested numerous hybrids of *Prunus fruticosa* Pall. Some of them proved to be promising dwarfing rootstocks in cherry growing. The native flora of Hungary is rich on native hybrids of *Prunus fruticosa* Pall. identified and described under different names, but this rich genetic diversity has never been evaluated and used as cherry rootstocks. 25 native hybrids were identified in different locations of Hungary, clonally propagated and planted in the repository of our research station. Based on their phenotypical characteristics they were identified as *Prunus x mohácsyana* Kárp., *Prunus eminens*, *Prunus x jávorkae* Kárp. (*P. fruticosa* x *P. mahaleb*) and *Prunus fruticosa* f. *aucta* Borb.

### Materials and methods

Using the clones of the above mentioned hybrids as interstems, cherry trees of cultivar 'Sunburst' were raised for field testing and planted into an orchard trial in spring 2000. The root of the trees is *Prunus mahaleb*. As control, 'Sunburst' trees with Gisela 5 interstems are also planted. Trees were trained to spindle at spacing 4x2 m. Growth of trees (scion and interstem trunk girth, canopy size), yield, fruit size were measured every year. Health status and incompatibility symptoms on trees were also evaluated by rating as general tree condition.

### Results and discussion

Some interstem trees showed yellowish leaves, stunting growth, their tree condition rating ranged between 0 to 3. Only those interstem combinations were evaluated as promising, of which the general tree condition resulted a minimum rating 4 or higher. Based on their trunk and canopy size trees with various interstems were compared to those grafted with Gisela 5 as interstem. Most of promising interstems resulted larger trunk cross sectional area (TCSA cm<sup>2</sup>) than those grafted with Gisela 5, but their canopy fits well into the spacing 4x2 m. Although the scion trunk is thicker, there is no considerable difference in canopy size of trees compared to Gisela 5 interstem trees. Majority of interstem part of the trunk showed shrinkage (Table 1), the girdling effect of interstem reached the 45%.

Based on their growth, productivity (calculated yield t/ha) and compatibility characters four clones of *P. x mohácsyana* Kárp. (KV2, 3H, PZ2, PZ5), and three clones of *P. eminens* (3H and FV 1 and KV 2) are selected for further evaluation as cherry rootstocks. We are aware, that the selected interstems used later as rootstocks may differ in certain characters, but this method as a pre-selection seems to be useful tool for selection in a screening stage of hybrids.

Table 1 - Main characteristics of promising cherry hybrids as interstems

Interstem	Tree condition 1-5	TCSA		Yield kg/tree in average of four years	Fruit weight 2007		Yield t/ha		
		cm <sup>2</sup>	Interstem girdling %						
Prob	2,1	51,49	a	67	8,07	a	10,2	ab	10,0
Gisela 5	3,6	87,02	abc	85	10,58	ab	11,6	abc	13,2
P. x moh. KV 2	4,4	186,47	h	50	19,67	de	10,9	abc	24,6
P. x moh. 3H	4,9	150,84	e-h	76	20,27	de	11,2	abc	25,3
P. x moh. PZ 5	3,9	128,56	c-g	45	20,44	de	11,8	bc	25,5
P. x moh. PZ 2	4,4	157,68	fgh	58	23,28	e	10,6	ab	29,1
P. x emin. 3H	4,3	152,03	e-h	75	19,14	de	10,3	ab	23,9
P. x emin. KV 2	4,4	187,63	h	52	20,50	de	12,1	bc	25,6
P. x emin. FV 1	4,9	168,79	gh	94	20,69	de	13,3	c	25,9

## Evaluation of local olive varieties from Slovene Istria

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Olive (*Olea europaea* L.) is a characteristic and economically important agricultural plant in the Mediterranean part of Slovenia. Preliminary surveys based on morphological data of olives in Slovene Istria showed the presence of different local varieties of which only a few biological, agronomical and economical reports are available. The variety structure of olives in the region was shaped over several centuries and is also the result of a number of attempts to revitalise olive growing. Old local varieties still grow in extensive or abandoned olive groves, but they are being replaced by introduced varieties, mainly from Italy and Spain. Old local varieties are very important, since their characteristics could contribute to the typicality of olive oil produced in the region. To preserve their cultivation, evaluation of olive genetic resources and chemical composition of oil produced from important local varieties is taking place. Some varieties have already been described, but there are still unclassified or even unknown ones. The identification of varieties is hindered and uncertain due to the long juvenile stage of the olive tree, the presence of different types and clones and the use of many synonyms and homonyms. Morphological description could be substantially improved by the aid of molecular techniques, nowadays widely used for genetic characterization and assessment, studies of origin and dispersal route elucidation, for paternity analysis and for identifying certified planting material in nurseries.

In the present study we aim to identify some of the old local olive genotypes and to evaluate their chemical and sensorial characteristics. Confirmation of identity of six olive varieties (Komuna, Štorta, Zmartel, Mata, Črnica, Buga, Žižula) from old collection orchard in Seča was performed by microsatellite markers. DNA fingerprints of trees included in the analysis were compared by previously genotyped nineteen reference olive varieties from national collection. To obtain information on the quality of olive oil produced from some local varieties, the composition of fatty acids was determined and sensory evaluation was performed.

# Ecological establishment and maintenance of perennial borders in urban areas and selection of hardy perennials in Finland.

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Economic and ecologic landscaping of urban environments is a big challenge for landscape gardeners. In Finland, woody plants are most often used in public areas. Perennial plants could offer more variation than woody plants and an economic alternative for annuals in parks and cemeteries. However, perennials are not currently widely used in public areas because of the lack of guiding principles for modern low-maintenance techniques and choice of species.

This study aims at examining the suitability of different perennial species to economically sustainable maintenance techniques in which treatments take place only a few times during the growing season and detailed care of plants is avoided. The ecological basis for combining specific species is investigated. The study is composed of field trials carried out at the research stations of Agrifood Research Finland and trial areas in six towns and three cemeteries in different climatic conditions around the country. The emphasis is, on one hand, on large perennials suitable for broad perennial plantations and, on the other hand, on low ground covering species. Guidelines and principles based on plant ecology and sociology are prepared for the use of 50-100 most important perennials.

In some perennial genera and species, e.g. *Aconogonon*, *Asarum*, *Aster*, *Eupatorium*, *Iris*, *Monarda*, *Nepeta*, *Salvia* and *Thymus*, nursery products are varying and/or nomenclature is non-uniform. In these species and genera, a clone selection is done at one or two sites in different climatic conditions. From the approximately 20 genera in selection, more than 350 clones have been collected for trial purposes. Most of the clones have been in cultivation in different regions of Finland, some for decades, some only for a short while. Consequently, they are, to a varying extent, adjusted to the northern climate, even though some originate from very remote locations on the globe. The best clones will be renamed and they will be issued the trademark FinE<sup>®</sup>. The trademark can be issued to plants which have been studied in Finland and which are of high genetic quality, healthy, hardy in Finland and without problems in propagation.

## Characterisation of Gooseberry Genetic Resources in Latvia

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### Introduction

Gooseberries (*R. grossularia* L.) has been widely grown in home gardens of Latvia, but commercial cultivation was limited by lack of powdery mildew (*Sphaerotheca mors-uvae*) resistant cultivars with high fruit quality and productivity. Interest for gooseberries in market is increasing, because of their good storage possibilities. In order to fulfil this demand, we need to develop new cultivars. The aim of this research is to evaluate Latvian gooseberry genetic resources for further breeding and possible commercial production.

### Material and Methods

Since 1998, more than seventy genotypes of gooseberry, originated in Latvia, were collected at the Latvian Institute of Fruit Growing, including local cultivars ('Koknese', 'Kursu Dzintars', and 'Anna Viksne') and breeding lines of Latvian breeders. This material was evaluated since 1999, but at the 2006, after acceptance of specific IPGRI descriptor, the data can be supplemented and completed according to the international standards. The genotypes were evaluated for morphological traits, yield components, fruit quality, disease and pest resistance, and winter and spring frost hardiness. Genotypes, potentially useful for both commercial

growing and hardiness breeding were recognized and described, characterized with spinelessness, winter and spring frost hardiness and pest and disease resistance, and high berry quality.

### **Results**

Genotypes with traits, valuable for breeding programs, were found. Differences between ripening times of early-ripening accessions (5-14, 6-4 etc.) and late-ripening ones (Koknese 55, 445-10, 536-9, 433-3, 323, 250-F<sub>2</sub>-60 etc.) were up to a month, depending on season. Accessions with erect plant habit (5-2 etc.) and very few prickles (424, 445-10, Koknese 55 etc.) were found, however, very few had fruit weight over 4 g (484-18, 39 etc.) in 2007. The most productive were accessions 4-14, 3-10, 410-15, 435-1, 5-2, Anna Viksne, Koknese 55 etc., and the highest soluble solid contents in 2007 had accessions 424 (16.1 %), 445-10 (15.3 %), etc. In total there were not significant differences among accessions in susceptibility to powdery mildew, leafspot, winter damages, and spring frost damages, though there were noticeable differences in separate years. Accessions with uniform fruit ripening time ('Kursu Dzintars', N-17-15 etc.) and fruit size (410-15, 433-26, 6-4, Citronu etc.) were found.

## **Epidemiological investigations on Cherry leaf roll virus**

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### **Introduction**

The *Cherry leaf roll nepovirus* (CLRV) is a world-wide pathogen occurring primarily on deciduous and fruit trees from at least 17 genera, but also on ornamental and vegetable plants. Reports are from Europe, North America, Turkey, New Zealand, Japan, Chile and the countries of the former Soviet Union. In these countries CLRV could be proved consistently, isolated in the forest, in public greense, plantations or in nurseries. In producing areas of South and Eastern Europe and of the USA economic losses in cherry and walnut plantations were sporadically reported. Since 2002, in Finland an increasing occurrence of characteristic CLRV symptoms in birch populations is observed. The strong symptom expression and degeneration of affected trees may become a serious problem for the paper industry.

Main investigations concerning natural CLRV incidence are from Germany. Since 2000, occurrence of CLRV is examined. At 25 out of 37 locations CLRV-infected groves were found in parks, gardens, lawns, forests and street plantations (Rebenstorf et al. 2006).

### **Methods and Results**

The bipartite genome organisation of CLRV is according to other members of the genus, especially the long 3' non coding region (3'NCR) of about 1600 nt on both RNAs is a typical feature of the nepovirus subgroup 3.

The wide host range and geographical distribution of the virus indicates a fast adaptability to different hosts and therefore a genetic heterogeneity among CLRV-isolates for different origins. This was confirmed by sequence analysis of 375 nucleotides of the 3' proximal region of the 3'NCR including 56 CLRV-isolates from 19 host species. The phylogenetic affiliations were strongly correlated with host plant species. This was also approved by analyses of the coat protein-coding region (1539/1542 nt, 512/513 aa) of 12 different CLRV-isolates and the complete 3'NCR of six isolates. Sequence alignments revealed up to 25 % nucleotide sequence diversities in the coat protein whereas a maximum of only 16% were found in the 3'NCR. Thus the 3'NCR is more conserved referring to its high functional impact for the virus, whereas genetic variability in the coat protein correlates with different serological reactions of CLRV-isolates.

CLRV is generally transmitted through pollen and seeds (Johansen et al., 1994) requiring a high degree of host specificity of CLRV-isolates as aforementioned and substantiated in a homogenous phylogenetic walnut group. However, this is not a strict principle as many CLRV-isolates clustered in different phylogenetic groups, i.e. one elderberry-isolate into the cherry group, another into the rhubarb group.



Within the present investigations on CLRV this study therefore focuses on the epidemiological relevance of mechanical transmission of CLRV between host plant species. Three CLRV-isolates (elderberry-isolate E603, walnut-isolate E326, rhubarb-isolate E395) from different phylogenetic groups were selected and mechanically inoculated on five natural woody host plants by stem slashing. One year after inoculation it could be proved that a CLRV-isolate from black elderberry was able to infect four out of five plant species: (*Sambucus nigra*, *Sorbus aucuparia*, *Juglans regia*, *Betula pendula*, but not *Prunus avium*). This suggests that for this isolate host adaption is not stringent and/ or transmission barriers between host species are developed differentially. Mechanical inoculation of virus-isolates originating from walnut and rhubarb resulted in very low infection rates; therefore to date no interpretation concerning its host specificity is possible.

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## **'GiSela' Cherry Rootstocks in Tests for Virus Tolerance**

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### **Introduction**

Cherry rootstocks developed by the Giessen breeding programme were already screened for response to Prune Dwarf Virus (PDV) and Prunus Necrotic Ringspot Virus (PNRSV) in previous studies (Lankes, 2003). Under heavy virus pressure applied to the 1-year-old ungrafted plants forced in the greenhouse 'GiSela 5' proved to be virus tolerant, whereas 'GiSela 3' and 'GiSela 6' showed reductions in shoot growth.

The investigations were continued to gain data on the expression of these response types in a commercially-important sweet cherry cultivar.

### **Material and Methods**

For this, 'Regina' was grafted onto the 'GiSela' rootstocks mentioned above as well as onto 'Alkavo', an invigorating commercially-important sweet cherry rootstock used as a reference. For phytosanitary reasons, the plants, together with 'Skeena' as a pollinator, were arranged in an aphid-proof screen house without root contact by planting them individually in containers (40 l) filled with sterilized soil-substrate. Half of the plants were inoculated with PDV and PNRSV by bark grafting in 2005 at the time of leafing-out and in addition to that by budding in the summer of the same vegetative period.

Vegetative and generative performance of the trees was evaluated by measuring stem diameter and shoot length, counting numbers of flower clusters and fruits and weighing the fruit yield per tree.

### **Results and Discussion**

For the grafted trees the incubation period was more than one year, and only in May 2006 first leaf symptoms were observed. Symptom expression was similar in 'Regina' trees on the three 'GiSela' rootstocks. In trees on 'Alkavo', virus symptoms were less expressed and retarded by four to five weeks. When serologically tested by DAS-ELISA in June 2006 all the inoculated trees proved to be infected by both viruses.

In 2006, no impact of virus infection on the performance of the trees was observed. However, a clear difference between trees on 'Alkavo' and the three 'GiSela' rootstocks was noted with 'Alkavo' enhancing shoot growth and impairing flowering and fruiting.

In 2007, the invigorating effects of 'Alkavo' could no longer be observed. This was probably due to the limited space for root development in the containers used. Also, there was no significant impact of virus infection on yield parameters, neither in trees on 'Alkavo' nor on the three GiSela clones tested. However, vege-

tative growth of 'Regina' trees on 'GiSelA 3' was much stronger without virus infection, thus indicating virus sensitivity of trees on 'GiSelA 3'. Trees on 'GiSelA 5' and 'GiSelA 6' did not show any growth response to the virus infection after three years of observation. Therefore, they are regarded as virus tolerant. For 'GiSelA 6' this is in some contrast to earlier findings with ungrafted plants when much stronger virus pressure was applied (Lankes, 2003). However, it is in accordance with the results of other investigations using various sweet cherry cultivars grafted on GiSelA rootstock clones.

### **Literature**

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## **Are heirloom *Lactuca* varieties suitable for market gardening and commercialization?**

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In a pilot project, we examine the chance of maintaining plant genetic resources by commercial utilization on a local scale. We chose *Lactuca sativa* as a model plant because in comparison to other crops it is easy to grow and has a short period of cultivation. Further more, a huge number of various accessions is available from genebanks.

In preliminary field trials we examined genebank material and identified several varieties with attractive attributes like a special shape, color or name. In the next step, we tested their suitability for market gardening in co-operation with several local gardeners in the region of Berlin and Brandenburg, Germany. The market gardens cultivated a selected subset of varieties at four times in spring and summer to test field performance, yield and quality attributes and supplied the products to the market in their manner customary (farmer's market, farmer's shop, direct delivery to the customers as 'Abokisten', health food shop) to test marketing success.

The pilot project also focuses on the promotion of consumer's awareness for the conservation of crop genetic resources and aims to stimulate consumer's demand for crop diversity. We prepared information and advertising material to attract consumer's interest for rare crop varieties and to stimulate a consumer behaviour that contributes to the conservation of genetic resources. Complementary, we interviewed customers, wholesale and retail staff to analyse purchase patterns and to develop new incentives.

The pilot project is funded by the Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (BMELV/BLE) FKZ: 05BM007/2).

## The effect of soil type on the growth of three organic seed potato varieties

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Field experiments on certified organic potato seeds (*Solanum tuberosum* L.) of three different varieties (Blue Salat, Pink Fir Apple and Roseval) were conducted on three certified organic farms with heterogeneous soils (Sandy Clay Loam/SCL/L, Sandy Clay/SC, Loam/L), in order to study the capability of producing organic potato seeds. The potato planting took place on 31st March 2004 and tubers were harvested on the 112th, 117th and 118th day after planting on the farms (SCL/L), (SC) and (L) respectively. During the plant elongation period, consecutive measurements of the quantitative characteristics were made. In the Loam Soil, the three varieties had the smallest number of above ground parts with lowest number of side buds, the maximum number of flowers per plant, the highest above ground parts and the maximum dry weight of stolons. The Sandy (SCL/L, SC) soils had maximum number and weight of tubers. After planting, the Blue Salat variety initiated the development of tubers on all soils, its maximum above ground part was of minimum height, had more stolons and the weight of tubers per plant exceeded substantially over the other two varieties. The mean maximum height (A) of the above ground part was 60cm for the Blue Salat variety, 81cm for the Pink Fir Apple and 90cm for the Roseval. The duration of elongation (D) of the above ground part lasted on average 59, 67 and 92 days respectively. The mean relative growth rate of the three varieties differed, following a reverse order than that of the maximum length and the elongation period of the plants: Blue Salat (0.06 day<sup>-1</sup>), Pink Fir Apple (0.05 day<sup>-1</sup>), Roseval (0.04 day<sup>-1</sup>). The mean maximum weight (A) of tubers per plant was 614g for the Blue Salat variety, 553g for the Pink Fir Apple and 485g for the Roseval. Their mean growth duration (D) was 44, 37 and 36 days respectively. The variety with the lowest mean maximum weight (A) and shortest growth duration (D) had the maximum mean relative growth rate (R: 0.13 day<sup>-1</sup>).

## Seeds pre-treatment effects on the germination potential of *Vaccinium corymbosum* L. and interspecific hybrid genotypes

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*Vaccinium corymbosum* L. and interspecific hybrid genotypes are being studied in the sphere of the Interberry project with the aim to characterize a wide genotype collection, utilize it for breeding purposes and to obtain adapted genotypes to the local environment. Due to its condition of being genetically heterozygous, *Vaccinium* species do not reproduce individuals from seeds that are similar to the seed parent. Sowing is the natural reproduction technique for producing plantlets from hybrids so understanding its behaviour is highly useful for crossbreeding programs. Although there are some reports about seeds germination in various species of the *Vaccinium* genus, additional specific information is necessary to understand the most effective method for seedlings germination and further implantation of *V. corymbosum*. Dormancy problems, strongly interacts with the evaluation of germination behaviour masking the final germination percentage, so it makes necessary to break this mechanism in order to obtain a proper characterization of this trait.

This work has the aim to characterize germination potential of *V. corymbosum* and hybrids genotypes under indoor conditions; to understand the main dormancy mechanisms acting in *V. corymbosum*, to find the optimal treatment to bring up dormancy in the studied genotypes and finally to set up a proper system to establish and grow plantlets in our productive conditions.

Germination potential of 23 genotypes of highbush blueberry *Vaccinium corymbosum* L. and hybrids was tested under seeds pre-treatment for dormancy breaking in two year cycles. The plant genetic material included 'Blue Crop', 'Brigitta', 'Chandler', 'Duke', 'Elliott', 'Jubilee', 'Legacy', 'Ozarkblue', 'Rubel'; 'Fol35', 'Misty', 'Nui', 'Reka', 'RH38', 'RH48', 'RH52', 'Star'. After extraction by maceration, seeds were manually classified under stereoscopic microscope. The applied tests included: A- fresh seeds seeded as soon as extracted from fruit; (CH2) were cold stratified 2°C; (CH3) were cold stratified and imbibed in gibberillic acid previous to seedling; (CH4) were cold stratified and scarified with sulphuric acid for 15 minutes. Furthermore, a test was added, fresh seeds were extracted from 100 days stratified fruits and treated with gibberillic acid for two clones of 'Blue Crop', 'Chandler', and the hybrid 'Fol 35'. The experiment has been carried up under indoor environmental conditions with natural illumination and temperature was near 18-20°C during the first year and in a second year, indoor growing lamps were used for plantlets establishment. Individual seedlings per genotype and treatment were seeded on a substrate composed of (1:1:1) perlite, vermiculite and sand in the first year and in the second year, individual pots filled with peat and perlite were used in order to avoid transplanting in early stage. Irrigation was manually done with a spray applicator the first year while in the second was combined with flooding system using acidified water (pH 4-5,5). Records were registered weekly.

Observed germination rates compared to non germination, analysed with  $\chi^2$  test, indicate significant differences ( $p < 0,001$ ) between different genotypes out of treatments effects and between treatments independently. Total germination performance varies from 0 to 100% germination, on an average of 31%. A group of genotypes (10 in 23) germinated from 60% up to 100%. Positive effects were observed with gibberillic acid imbibition, cold stratification for 33 days or interaction between both factors for most genotypes. 90 days of stratification tend to reduce germination values. For the pre-treated seeds with stratification for 33 days and GA<sub>3</sub> addition, the phase of major rates of germination, lasted around 3-4 weeks, compared with the ones of A test which lasted around 5 weeks. Sulphuric acid treatments showed low and uneven results. Results suggest that seedcoat or external dormancy should not be the predominant mechanism for *V. corymbosum*. The positive effect of gibberellins and stratification (pre-chilling), remarks in most cases, the possibility of embryo or internal dormancy occurring in this species. For most of the analysed genotypes, the factors that seem to have major effects in breaking dormancy are gibberellins and cold stratification for a maximum of 33 days. Germination behaviour increased with cold stratification until 33 days and strengthened with hormones addition and also promoted precocity and homogeneity. Comparing independently hormonal to pre-chilling treatment, gibberellins tend to show stronger effect, even if some genotypes behave better with only pre-chilling as 'Star', 'Rubel', 'Misty' and 'Brigitta'<sup>2</sup> and 'Brigitta'<sup>3</sup>. Using indoor growing lamps ensured plant establishment, increased growing rates and plantlets vigour making it possible also in winter periods.

## **Recovery and characterisation of old cultivars and accessions of long storage tomatoes**

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### **Introduction**

The tomato (*Lycopersium esculentum*, Mill) is a vegetable widely used in the "Mediterranean diet" and is well known for its health properties. Indeed, the tomato has assumed the status of a functional food, considering the overwhelming epidemiological evidence that it reduces the risk of certain types of disease. It is a source of numerous antioxidant compounds such as ascorbic acid, vitamin E, carotenoids, flavonoids, and phenolic acids. The disease-preventing potential of a food is a consequence of several constituents which may interact synergistically. Lycopene, a biomolecule of great interest, has been shown to have strong antioxidant properties; its dietary intake is correlated with diminished risk of prostate cancer and with the inhibition of cell proliferation in various human epithelial cancer cell lines. Recently, there has been increasing interest in the recovery of old tomato cultivars grown on non-irrigated marginal land. In this

context, the Botanical Garden of the Salento University (Lecce, Italy) is involved in the recovery of old tomato species cultivated in the past in Southern Puglia (Italy). The aim of the present work was to recover and characterise nine cultivars and/or accessions of long storage “winter” tomatoes.

### **Materials and Methods**

Nine different cultivars and/or accessions of winter tomatoes were selected: “*Minervino*”, “*Tricase*”, “*Botrugno*”, “*Brindisi Pirunella*”, “*Brindisi Rosso*”, “*Morciano*”, “*Palmariggi*”, “*Serranova Giallo*” and “*Serranova Rosso*”. These plants were cultivated in the Botanical Garden and characterised in terms of their morphological properties, yields and biochemical composition, quantifying the carotenoids, flavonoids, and phenolic components by HPLC/DAD. The total antioxidant activity (AA) was determined by DPPH assay on the lipophilic and hydrophilic fractions, and by ABTS assay on the hydrophilic fraction.

### **Results**

The yield varied between 0.5-1.5 and 1-2.5 kg/plant for red and yellow tomatoes respectively; shelf-life was higher for red fruits and was inversely correlated with the size of the fruits. The dry matter content of the plant material varied between 8.5 and 9.6%. The biochemical characterisation showed that the quantity of carotene varied between 9.6 and 36.6 mg/kg fresh weigh (FW). The lycopene content ranged between 8.9 and 106.5 mg/kg FW while the lutein content varied between 1.7 and 5.1 mg/kg. When compared with commercial cherry tomatoes, the cultivars analysed showed high amounts of lutein (up to three times higher). Total phenol content ranged between about 68 and 290 mg/kg FW; specifically, the chlorogenic and ferulic acid content varied from 3 to 16, and from 1.5 to 6.3 mg/kg FW respectively, while caffeic acid was detected only in the “*Minervino*” and “*Brindisi rosso*” cultivars, which contained about 1.5 and 6.3 mg/kg FW respectively. The rutin content varied between 56 and 109 mg/kg FW. The results obtained were similar to those observed for the commercial tomato cultivars.

The antioxidant activity (AA) of the hydrophilic fraction evaluated by DPPH assay was significantly higher than the AA of the lipophilic fraction, corresponding, on average, to about 40%. The ABTS assay, performed only on the hydrophilic fraction, found high AA in the winter tomatoes, in accordance with the literature, varying between about 30 and 90%.

These ancient winter tomato varieties are rich in numerous compounds characterised by high biological activity; for this reason, they should be protected and valued as a natural source of phytochemicals.

## **A survey of small fruit European germplasm**

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COST ACTION 863-Euroberry Research from Genomics to Sustainable Production. Quality and Health -by its Working Group 1 named “From genom to berry fruit” has as major task an inventory of the berries genetic resources in Europe. The goal of this work is to establish a gene data base for all berry species under study in the “ex situ” locations. This study will provide a helpful tool for genebanks. Breeders and other users in order to ensure safety of germplasm and to build up an European representative berries collection. A first overview of the small fruits collections included 2090 entries of 13 species of the genera: *Ribes*, *Rubus*, *Vaccinium*, *Sambucus*, *Hippophae*, *Lonicera*, *Cornus* and *Rosa* from 15 sites of 10 countries. Of these. 850 black. Red and white currant accessions are preserved in 7 cetnters. 292 gooseberry ac. in 5 centers. 394 raspberry ac.in 7 centers. 71 blackberry ac. in 4 centers and 62 ac. in 2 centers. Also. a number of 277 entries of minor small fruits : seabuckthorn .(46 ac.), elderberry (95 ac.) bluehoneysuckle (38). cornelian cherry (66 ac.)are maintained in 2 locations. Of total entries cultivars represent the largest part (82.7%), followed by clones, hybrids. lines (16.8%) and wild species (0.5%).Nearly half of the listed accessions are

grown only on one site. Our paper will present a classification of genotypes, their pedigree, Geographic origin, major strong traits and variability of some characteristics within some species such as disease and pest resistance and biochemical fruit content.

## **The use of molecular markers for germplasm management in an Italian olive collection**

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Olive (*Olea europaea* L.) is a species of great economic importance in the Mediterranean basin, where 95% of world production is concentrated. Among Mediterranean countries, Italy occupies a very important place in the olive industry. Italy is the main exported of olive oil in the world. The genetic patrimony of this country is very rich and is characterized by the abundance of varieties, most of them landraces vegetatively propagated at the farm level since ancient times. The existence of many varieties maintained by vegetative propagation reinforces the need of a reliable identification of varieties, for nurserymen and growers benefit as plant cost represents the major investment in the new orchards. At the same time, it is important to improve the *ex-situ* plant germplasm collection and adequately to characterize all accessions and develop future breeding programs.

The Italian olive germplasm is estimated to include over 650 varieties and over 1300 synonyms, most of which are landraces vegetatively propagated at the farm level since ancient times. For years, the Consiglio per la Ricerca e Sperimentazione in Agricoltura - Centro di Ricerca per l'Olivicoltura e l'Industria Oleria (CRA-OLI) of Rende in Cosenza, Italy has made significant efforts in the individuation and collection of olive germplasms, generally within Italy. For each species, cuttings have been collected with the aid of local experts for successive propagation. During the second year following grafting, the plants were numbered using a unique code and placed in the varietal collection area. The plants were then evaluated by the CRA-OLI at Mirto-Crosia in Cosenza. To date, over 450 Italian accessions and about 50 accessions from other Mediterranean countries have been collected. With more than 450 olive accessions, the CRA-OLI olive collection includes a major part of the Italian germplasm. We used molecular markers to characterise all accessions and to study genetic relationships between cultivars. The olive trees were genotyped using nine nuclear SSR loci: GAPI59, GAPI71A, GAPI71B, GAPI103A, UDO01, UDO03, UDO12, UDO28 and UDO39.

This study allowed us to construct a molecular data-base for the reference collection and to analyse genetic diversity for further prospecting, and for introducing new olive accessions.

### ***Acknowledgments***

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## **Properties of promising Oblačinska sour cherry clones D4 and D10**

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Oblačinska sour cherry is an autochthonous variety, heterogeneous in properties, present in a large number of various genotypes in commercial orchards of Serbia. To select trees of the best economical-technological properties, clone selection of this variety is done all the time. Clone selection of Oblačinska sour cherry has

been in progress for many years at the Faculty of Agriculture in Belgrade. The result of this activity are clones D4 and D10 selected as the most promising, which are compared in the present paper with a standard (non-selected Oblačinska sour cherry). During the 2000-2006 period the studied pomological-technological properties in the selected clones and a standard were as follows: fruit shape, skin color, flesh color, juice color, taste, ripening time, yield, fruit size, fruit weight, stone weight, percentage of flesh, fruit stalk length, contents of soluble dry matter, total sugars, invert sugars, and total acids. The results indicated that both promising clones and a standard had a fairly round fruit shape, dark red color of fruit skin, intensive red color of flesh and juice, and sour taste of fruit. With respect to more prominent components of fruit yield and quality, the studied clones were better or at the standard level. Clone D4 stands out for high fruit yield and largeness, while clone D10 for fruit quality.

## **New Acorn Squash (*Cucurbita pepo*) having sweet fruit flesh**

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### ***Introduction***

*Cucurbita pepo* (squash, pumpkin and gourd) is an economically important species containing eight edible-fruited cultivar-groups. One of these groups is acorn squash, which is characterized by turbinate (top-shaped) fruits having ten longitudinal ridges and furrows. Acorn squash has been appreciated over the centuries for its mature fruits, which externally usually are dark green. Generally, they are prepared by cutting in half, removing the seeds and placenta, and baking in an oven. Nonetheless, their soluble solids content (SSC) is usually only around 7% and, therefore, honey, brown sugar, and/or other sweeteners or flavors are added during culinary preparation. A breeding program was begun many years ago to improve the culinary quality of acorn squash. Specifically, it was desired to enhance the exterior color to black-green, to improve the sweetness and soluble solids content, and to provide resistance to powdery mildew, a disease that is difficult to control and that decreases fruit quality. Herein are described, in brief, the results of these efforts.

### ***Materials and Methods***

The source of the black-green exterior fruit color, conferred by the dominant allele *L-1*, was the zucchini (*C. pepo*) squash 'Fordhook Zucchini'. *L-1* was backcrossed into acorn squash germplasm. A small, yellow, sweet acorn squash breeding line designated 'OSU 19' and provided by J. R. Baggett of Oregon State University (USA) was the source of improved fruit sweetness. This was used as a donor parent in a backcross-pedigree program to obtain a sweet, black-green acorn squash. Selection was conducted subjectively by tasting a microwaved fruit sample from each self-pollinated plant in each generation. Curiously, these newly developed black-green, sweet acorn squash possessed a flavor that was not detected in any of the parental germplasm. This flavor, to many who sampled the microwaved fruit samples, was reminiscent of roasted chestnuts. Lastly, the source of powdery mildew resistance, conferred by gene *Pm-0*, was a straightneck (*C. pepo*) squash, and this gene was backcrossed into the already developed best-tasting, black-green-fruited acorn squash germplasm. Eventually two inbreds that were powdery mildew-resistant, black-green-fruited, and outstanding for flavor were selected as parents for a hybrid that was given the series number 1700.

### ***Results and Discussion***

'Hybrid 1700' is a new acorn squash having bushy plants that are resistant to powdery mildew bearing small (average 400 g), turbinate, ridged and furrowed, black-green fruits having orange-yellow flesh and a sweet flavor reminiscent of roasted chestnuts. 'Hybrid 1700' and its parents were tested against 'OSU 19' as well as other acorn squash, some of which were reported to be sweet. Results from the Spring 2005 season were that 'Hybrid 1700' was significantly preferred for taste over 'Honey Boat', 'Sugar Loaf', 'Table Queen', 'Sweet Dumpling' as well as 'OSU 19'. In fall 2005, in a test against 'OSU 19', it was highly preferred, with an average SSC of 12.3 as compared with 6.5 of 'OSU 19'. In Fall 2006, 'Hybrid 1700' averaged 15.6 SSC, which was significantly higher than the next best, 11.0, for 'Bush Delicata' and SSCs of under 10.0 for the remaining five acorn squash cultivars tested. In spring 2007, 'Hybrid 1700' had 13.3 and 'OSU 19' 13.0

SSC, with 'Bush Delicata' and the remaining four others having significantly less. Hence, 'Hybrid 1700' was consistently preferred for fruit flesh quality over all of the other acorn squash cultivars tested. First commercialized in Israel in 2007, 'Hybrid 1700' was produced on 2 hectares. Over 40 metric tonnes (100,000 fruits), which had been cleaned and bulk-packaged in crates by the farmer, were sold by a major supermarket chain in Israel at the price of 10 sheqalim/kg (approx. US\$1.00 per fruit). The fruits stored well, even though they were given minimal care, and were received well by consumers. 'Hybrid 1700' fruits are expected to be exported to Europe in 2008.

### **Acknowledgement**

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## **Research on phytoplasma persistence in pear and apricot trees under protected environment**

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Among the objectives of two national projects, coordinated by the Unit of Research in Fruitculture, the evolution of infection by phytoplasmas in pear and apricot trees growing in field and protected by anti-aphid tissue was studied.

The first research was carried out on pear decline in the Caserta province. Twelve trees planted in open field, belonging to seven rootstock/varieties combinations and survived to a pear decline epidemic, done to 16SrX-C phytoplasmas ('*Candidatus* Phytoplasma pyri'), transmitted by *Cacopsilla pyri*, were covered with anti-aphid tissue and tested during 7 years for phytoplasma presence twice a year, to control the evolution of the infection. All phytoplasma positive plants died, except three. This experiment shows that after six years from inoculation the phytoplasma can still be detected and this evidence induces us:

- to trust reliability of molecular methods employed, even if all infected plants resulted negative to molecular tests for at least one year out three (dead plants), and for two years out six (surviving plants);
- to underline the high risk that an infected plant represents because it is reservoir for long time of 16SrX-C phytoplasmas, without contact with insect vector.

The second research was carried out on apricot trees, planted in soil inside a greenhouse covered with anti-aphid tissue, in Caserta. The forty-five plants, belonging to 11 varieties, were patch-grafted with plum tissue infected by 16SrX-B phytoplasmas ('*Candidatus* Phytoplasma prunorum') (the same infecting plum and apricot species). Many varieties resulted infected after the second grafting but there aren't symptoms associated to phytoplasma presence in the whole plant. The presence of these prokaryotes was not permanent in this case because it could be detected at the most for three years. This experiment shows that 16SrX-B phytoplasmas transmitted to apricot by grafting are:

- detectable from three months to three years after patch-grafting;
- not permanent;
- not dangerous for the whole plant but only for the grafted branches.

These results are very interesting because obtained under protected environment and contribute to confirm the persistence of 16SrX-C phytoplasma, associated to pear decline after transmission in open field by *Cacopsilla pyri* and indicate the poor performance of infection by 16SrX-B phytoplasma, associated to apricot chlorotic leaf roll and plum leptonecrosis, when transmitted by patch-grafting of infected plant tissue. The protected environment could have positively influenced the plants to react to pathogen presence: the plants infected by insects, because the presence of the pathogen couldn't be detected at least during one year, and the plants infected by grafting, because they resulted entirely free from phytoplasma presence.



# Soil Fungal Diversity and the Sustainability of the Viticultural Soils

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Considering that the “*natural state of the soil*” could be similar to “*soil health state*” and “*soil quality*” (chemical and biological), it could be accepted as necessary to define the state quality of a soil as a results of the environmental factors influences (soil, climate) and anthropic factors (maintenance systems, cultivation practices), through the identification of the needs and opportunities for identifying and promoting guiding principles and good practices to enhance soil biodiversity and its functions and to realise the benefits of biological management of soil ecosystems in terms of enhanced productivity. This paper reffers not only to soil biodiversity, but to the wider concept of soil health, which includes the ecological attributes of the soil, with implications beyond its quality. These attributes are chefly those associated with the soil microflora: its diversity, food web structure, activity and the range of functions it performs. The authors considered that trying to correlate the chemical manifestattions with the biological ones from the viticultural exploitations is a needed task on the requirements of practicing an ecological viticulture in Europe. To evaluate the environmental value and benefits from applying different soil maintenance systems on a viticultural area – Experimental Didactic Station of the University of Craiova – Romania - this paper is presenting the status of chemical parameters (N, P, K, pH, rH, macro- and microelements, humidity, ashes – organic matter, conductivity) reflected on the soil biodiversity and functions, through the isolated fungi biomass (as “ecological engineers” of the soil) and some aspects concerning the kinetics of their enzimatic activity (phosphatase, urease), so the impact on soil biodiversity and the ecological processes and ecosystem services they provide. These kind of informations could be taken as examples that can be selected and used by different end-users (farmers, extension workers, scientists) to provide a set of indicators of *soil healt status*.

## Molecular characterization of pear (*Pyrus communis* L.) using microsatellite markers

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In the last decade the major European Institutions involved in germplasm preservation (ECP/GR, UPOV, IPGRI) have recognized the need of implementing classical descriptive tools (morphological, agronomical and chemical) with the molecular ones.

The purpose is to establish, for each species, a database containing all the morpho-agronomic and physiologic information and a distinctive molecular pattern for each accession.

The aim of the present work was to molecularly characterize several *P. communis* accessions collected from the ‘Centre of Germplasm Conservation’ (CRA-Centro di Ricerca per la Frutticoltura, Rome) to resolve cases of synonymy and homonymy and to provide insights on the genetic variability in *P. communis*.

The genetic analysis was carried out on about 150 accessions using microsatellite markers developed from a genomic enriched library of the pear cultivar ‘Barlett’, by a capillary electrophoresis apparatus. The analyses were performed with NTSYS-pc 2.11X. A similarity matrix was constructed according to Dice’s index and the dendrogram was obtained by the UPGMA method.

The cluster analysis showed that 80% of the accessions were discriminated and displayed a very high degree of intraspecific variability as reported in literature.

This preliminary investigation has pointed out a few cases of synonymy and homonymy. One case was that of two accessions collected in the little town of Arce (southern Lazio) and considered different. In our analysis they were not separated probably because they are the same genotype.

Another case regards the 'Pero Fico' and 'Fico di Udine' accessions that were classified by morphological descriptors as synonymous. The analysis with the molecular markers showed that these two accessions have different genetic profiles.

The present research is still in progress with the objective of characterizing those accessions that were not separated by increasing the number of molecular markers. The number of the accessions to analyse will be also enlarged with 250 more genotypes *in situ* maintained in the 'Centre of Germplasm Conservation'.

## **Evaluation of genetic diversity among and within local populations of common bean (*Phaseolus vulgaris* L.) from central Italy.**

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Common bean (*Phaseolus vulgaris* L.) is one of the most important crop species and represents a cheap source of dietary proteins in several countries. In Italy, after its introduction in 1532, common bean promptly reached a wide diffusion in all the regions of the peninsula. The microclimate of the cultivation areas and the selection made by farmers played a strong influence on the evolution of this crop, resulting in the differentiation of a multitude of landraces. In the last years local populations have been gradually replaced by modern cultivars, more uniform and productive, causing a conspicuous erosion of the genetic resources of the species. The persistence of local varieties is necessary to preserve a source of genes useful for breeding programs.

In the present work several *P. vulgaris* landraces from central Italy (Viterbo-Lazio) have been genetically analysed by using RAPD and SSR markers.

The percentage of polymorphism detected was quite high both for RAPD and SSR markers.

Genetic similarity among landraces was investigated by a cluster analysis. All landraces were separated showing appreciable level of genetic dissimilarity. Two main clusters, with a high divergence index, were obtained which may reflect a different genetic pool of the landraces analysed: Andean or Mesoamerican origin.

Genetic similarity within landraces was also investigated by a cluster analysis. All the genotypes of each population were separated, except for two 'Giallo' samples. Hierarchical grouping followed the results of the inter-population analysis. Individuals belonging to the same population were grouped together with just few exceptions. A level of intra-population divergence was observed for all the landraces studied.

The codominant nature of SSR markers has also allowed an analysis of the genetic structure of the landraces. Though the percentage of homozygotes was high in all the populations, heterozygous loci were observed in the most of them. Genetic diversity within ( $H_S$ ) and among ( $H_T$ ) populations was also calculated showing quite high values of heterogeneity.

This study may provide a starting base for elucidating and further understanding those aspects concerning the genetic origin and its associated characters of the landraces analysed.

## Collection and identification of local quince genotypes (*Cydonia oblonga* Mill.) in Iran

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Quince (*Cydonia oblonga* Mill.) is a small deciduous temperate tree related to apples and pears. Iran is known as a centre of origin for this species. Selection of superior local genotypes is the first step in any fruit breeding program. In Iran, no study was performed to identify and evaluate these valuable fruits. Therefore, research was carried out to identify and evaluate local quince genotypes in Isfahan region in 1997-1999. Nine private orchards with mature trees were selected and a morphological and phenological study was carried out during successive growth and developmental phases. Identification of local genotypes was based on 109 morphological characters for different developmental phases such as: onset of winter dormancy, flowering, development of mature leaf and fruit ripening stages. Vegetative and reproductive characters included leaf and tree morphology (form, height, size), flower morphology (size, colour, shape) and fruit characteristics (morphological characters such as shape, size, colour, weight and chemical attributes such as sugar, acidity, pectin, fibre content). These factors were studied for 1000 quince trees considered as different genotypes at the start of this study. Genotypes were grouped based on measured morphological characters using multivariate analysis. Finally, the economical important genotypes "Bagh Koomeh" (MM1), "Gol Sefid" (CC3), "Kewiji" (BB6), "Golabi" (GG5), "Koosehei" (LL9), "Beh Torsheh" (LL6) and "Gourtoon" (ZZ1) could be identified based on this cluster analysis.

## Evaluation of variability in Japanese wild azaleas and application of lime-tolerant genetic resources for breeding

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Evergreen azaleas (genus *Rhododendron* L. section Tsutsusi Sweet) are horticulturally important ornamental plants, with Asian origins and a long breeding history. Especially in Japan, wild evergreen azalea species are distributed widely and abundantly. *Rhododendron* species grow in nature in humus soils with low pH values. Azalea cultivars, performing best when the pH of the medium is between 4.5 and 6.0 (acidic), often show strong iron-deficiency chlorosis symptoms if they are grown on calcareous soil. This stress is one of the most serious difficulties in their cultivation. At present, no evergreen azalea cultivar has complete resistance to iron deficiency chlorosis. However, genetic variability, which permits improvement by plant breeding, exists even within species. In this study we investigated the biodiversity of Japanese wild azaleas during the flowering season looking for genetic resources tolerant to abiotic stresses. In particular, in order to select lime-tolerant species, we analysed the soil taken from their root zone in natural habitats. Wild azaleas showed different adaptability to soil pH, according to the species and habitat environment. The soil pH from the root zone of *R. kaempferi* and *R. macrosepalum* populations, growing in edges of secondary forest and hillside, generally ranged between 4.2 and 5.7. The lowest pH value (3.9) was found for *R. kiusianum*, present in active volcanic area, while the highest pH value (7.6) was detected in the soil of some *R. ripense* populations located in stony river areas.

Results on germination and seedling growth of these species, carried out at different pH conditions, also reflected this different aptitude. *R. ripense* could be therefore identified as a precious genetic resource, showing not only lime tolerance but also cold, drought and water resistance. Subsequently, starting from the collection of *R. ripense* seeds and cuttings in the wild, field and *in vitro* trials were established for screening and selecting lime-tolerant individuals to be used in future breeding programs. However, traditional breeding strategies are often limited by the complexity of stress tolerance traits, low genetic variance of ornamental components under stress conditions and the lack of efficient selection techniques. Thus, genetic studies of stress-resistant mechanisms are needed. Placing lime-tolerance genes on a linkage map of *Rhododendron* could allow marker-assisted selection of lime-tolerant genotypes.

## Seed germination in five autochthonous campanula species of Northern Italy

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The *Campanulaceae* (the “hare bell” or “bellflower” family) are a nearly cosmopolitan angiosperm family consisting of 600 to 950 species sorted in 35 to 55 genera.

The genus *Campanula* includes about 300 herbaceous species distributed exclusively in the boreal hemisphere, with their epicentre in the Mediterranean lands, particularly Greece and Turkey and their islands, and in the Caucasus. They are strongly represented also in Italy (about 50 species), where the Alps in particular harbour a considerable number of species. In this genus annuals, biennials, monocarpics and perennials, which can be both long or short-lived, are present. The perennials represent the genetic bases of the cultivated species, showing a remarkable range of forms and dimensions.

In the past, several bellflowers were cultivated and appreciated for alimentary and officinal uses. From the 17<sup>th</sup> century, some *Campanula* species (*C. carpatica* Jacq., *C. isophylla* Moretti and *C. persicifolia* L.) have acquired a considerable interest as ornamental garden and potted plants and, more recently, as cut flowers (*C. medium* L.), mainly thanks to their typical colour ranging from a deep violet to the very pale milky blue. Anyway, despite the numerous species characterizing the genus, only a few are now available on the market. Wild bellflowers can represent therefore a very important genetic resource for finding new ornamental characteristics, diversifying plant and flower production.

In the present study, at first we selected five autochthonous bellflower species of Northern Italy showing aesthetic traits potentially interesting for flower market as garden plant or cut flower. Subsequently, in order to propagate and evaluate this germplasm for selection and breeding activities, we collected their seeds in the wild and we carried out different experiments to evaluate and promote seed germination.

In total, we collected seeds in the wild from 24 ecotypes belonging to the following autochthonous bellflower species of the Piedmont area (Northern Italy): *C. barbata* L., *C. latifolia* L., *C. rapunculoides* L., *C. spicata* L. and *C. trachelium* L.. To evaluate and enhance seed germination, three temperature conditions (14°C for 8 weeks, 18°C for 8 weeks, and 24°C for 2 weeks followed by 20°C for 6 weeks) and two photoperiodic regimes (12h light/12h darkness; 16h light/8h darkness) were tested.

Temperature of 14°C and photoperiod of 16h light/8h darkness allowed to obtain the best results in terms of germination percentages for all the species, except *C. latifolia*. At these controlled environmental conditions, the average germination percentages ranged between 20% and 70%, according to the different species. In particular, the highest levels of germination were obtained in *C. spicata* and *C. rapunculoides* (70% and 60% of seeds germinated, respectively). In *C. trachelium* and *C. barbata* the germination percentage was much lower, about 20%. Anyway, large differences were detected not only among species, but also among ecotypes. In several cases, some ecotypes could outperform others two or three times. This points out a great natural variation in campanula seed germination within species. Therefore, the geographical locations and environmental conditions in which plants grow influence very much the seed germination. Concerning *C. latifolia*, at all tested conditions almost no seed germinated (1%).

With the aim of evaluating these wild campanula species for selection and breeding activities, further investigations on seed germination are thus needed.

## **Research on the biodiversity of the local bean cultivars (*Phaseolus vulgaris*) present in the Banat area in the view of their utilization in breeding programs**

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The food security, the life quality and the integrity of the environment are tightly connected to the genetic resources and the biodiversity of the local vegetal world.

Bean (*Phaseolus vulgaris*) represents an important source of protein in the diet of the population of Banat (western part of Romania). It is cultivated through intensive farming systems but especially on small surfaces in people's gardens. Due to this fact in Banat there is a large biodiversity of genetic variations for beans. The bean varieties created in Romania originate in the local variety 'Alba de Banat'.

Objectives: the exploitation and identification of the types of green beans and obtaining a representative collection of local varieties from the Banat area. The purpose of our research was to identify some genotypes with a good tolerance to osmotic stress and with a high level of protein.

Seeds of 24 bean cultivars (*Phaseolus vulgaris* L.) were submitted to four levels of osmotic tension (0, -1, -2, -4 MPa), induced by polyethylene glycol-6000 (PEG-6000). The experiment was carried out in a germinator with temperature and relative humidity control, making use of a neutral pH paper towel as a substrate. The results obtained have shown that the percentage of germinated seed was very different between 5% on stressed seeds (-4 MPa) and 100% (0 MPa). Also we made some observation regarding the percent of dry matter of the seeds and plants which was germinated in osmotic tension. The total protein content was obtained with Kjeldahl method and the result presented differences between 13-27%.

From the results obtained regarding the germination potential measured at various time intervals, it has been noticed that Sacu, Dudeștii Noi and Tincova local land race showed the higher germination rate on V3. The best results regarding protein content was observed at Bocșa Română (21,69%), Sacu (21,16%), Cireșu (20,90%).

For the proline content accumulation in stressed variant the best results was noticed in Dudeștii Noi (3,2478 mg/g f.w.), Sacu (3,0002 mg/g f.w.) and Sântana (2,9791 mg/g f.w.)

## Putative endogenous dsRNA-viruses in *Capsicum* species

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The presence of an endornavirus and a cryptovirus in *Capsicum annuum* has been reported previously. We investigated whether these endogenous dsRNA-viruses also occur in other *Capsicum* species. Samples were taken from a collection of eight pepper species (*Capsicum annuum*, *C. baccatum*, *C. chacoense*, *C. chinense*, *C. eximium*, *C. frutescens*, *C. praetermissum*, *C. pubescens*) kept at ABC in Gödöllő. High-molecular weight dsRNA (>10 kbp), which may represent the genomic dsRNA of endornavirus was detected in every/almost every cultivar of *C. annuum*, *C. chinense*, *C. frutescens*, *C. praetermissum*, *C. baccatum* var. *baccatum* and var. *pendulum* species; in *C. chacoense* the molecular weight of dsRNA was lower than in the other species. No dsRNAs could be detected in *C. eximium* and *C. pubescens*. For cryptic viruses, a segmented genome consisting of 2-3 dsRNA-species in the size range 1-3 kbp is characteristic. Such dsRNA-pattern was observed only in *C. chinense*, *C. frutescens*, *C. chacoense* and *C. annuum*. In the literature only one sequence, the complete sequence of the RNA-dependent RNA-polymerase (RdRp) of a *Capsicum annuum* cv. 'Jalapeno M' cryptovirus was published up to now. Based upon this sequence information primer sequences were designed to investigate the sequence similarity of dsRNA species by Northern hybridisation with labelled oligonucleotids and by RT-PCR with primer sequences derived from the published sequence. In parallel, dsRNA was purified from three *C. chinense* species for cDNA-cloning and for molecular characterisation of putative cryptoviral dsRNs. The results of molecular analysis will be presented. This project was financed by grants OTKA T032393, GVOP-3.1.1.-2004-05-0163/3.0 and Ferenc Deák Scholarship of Ministry of Education and Culture.

## Cereal landraces from the Alpine region of Italy and Austria as a potential source of genetic variability for the future

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Landraces have an important task for local biodiversity as being a result of farmers' selection and adaptation to different habitat conditions. Due to enhanced breeding programs starting at the end of the 19<sup>th</sup> century, in Europe cereal landraces have been increasingly replaced by improved varieties of higher yield, with the consequence of a stealthy loss of local genetic resources. However, there is consensus that landraces might represent an important reservoir of genetic traits, such as pest and disease resistance, for future improvement of breeding germplasm. Therefore, cereal landraces were collected in Tyrol (Austria) and in South Tyrol (Italy) and included in the seed bank of the County Tyrol. In addition to phenotypic and agronomical characterisation, the landraces of wheat, oat and rye were investigated by the use of microsatellite markers in order to determine the inter- and intra-genetic variability and the genetic relatedness. The first results of wheat demonstrate not only a high genetic differentiation between different landraces but in several cases also within them. About 50 % of the total number of alleles in wheat was considered to be rare alleles, with a frequency < 5%. With only a few exceptions, the wheat landraces showed no relationship to commercial wheat cultivars. Our data provide an important support for the efficient management of the germplasm collection and contribute to the conservation of the local genetic variability.

# Genetic relationships of wild and cultivated roses and their potential for breeding

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Within the framework of the European research project GENEROSE “Genetic Evaluation of European Rose Resources for Conservation and Horticultural use”, more than 1000 individuals belonging to 28 wild roses species were sampled in Belgium, France, Germany, the Netherlands and Scandinavia. Molecular genetic analyses, based on both AFLP and STMS markers, were performed. Selected individuals were also morphologically investigated in order to gain insight in the taxonomical structure. In Flanders (Belgium) a detailed morphological study was performed on individuals of eight species of the sections *Synstylae* (*R. arvensis* Huds.) and *Caninae* (*R. tomentosa* Sm., *R. rubiginosa* L., *R. agrestis* Savi, *R. micrantha* Borrer ex Sm., *R. balsamica* Besser (syn: *R. tomentella* Léman ex Cass), *R. canina* L., and *R. corymbifera* Borkh.). In this study, the diagnostically most decisive characteristics were assessed and compared with the existing species descriptions. Different taxonomical groups could be identified combining morphometric and descriptive characteristics. However, no clear boundaries between the sections, subsections or species were found. Within the section *Caninae*, a combined variation in pubescence, presence or absence of glands on leaflets, pedicels and hips discriminated between the four subsections, but lacking the presence of clear boundaries. Similarly, at a lower level, it is hard to distinguish in between the analyzed species within the subsections. Complementary, to these morphological data, AFLP analyses and STMS data confirmed the subdivision of the European subgenus *Rosa* in several sections. The sections *Pimpinellifolia*, *Cinnamomeae*, *Synstylae* and *Rosa* (*Gallicanae* (DC.) Ser. 1825) formed concise groups, clearly differentiated from each other and from the very large and dense section *Caninae*. Neither the genetic nor the morphological analyses were able to clearly discriminate between the different subsections of the section *Caninae*, or between the species within one subsection.

Besides the study of these genetic relationship, the collected wild genotypes were screened for resistance to powdery mildew and black spot. Therefore standardized infection protocols were used. A concern while using species for ameliorating disease resistance is that these traits within the species are often genotype specific. Therefore, resistance for a specific pathogen should be tested on individual plant genotypes with specific pathotypes. Although most collected European rose species were found to be susceptible some interesting genotypes showing resistance to either powdery mildew or blackspot could finally be identified.

The possibilities to introgress genes from these (diploid or pentaploid) species into tetraploid cultivars was also investigated. Testcrosses between different European wild species and selected tetraploid culture roses were made to evaluate cross efficiency. Significant differences were observed among the tested combinations. In general the same crossing efficiency was observed between crosses with the tetraploid roses used as seed parent compared to the reciprocal combinations. The average number of seeds obtained per hip was significantly higher when wild species were used as seed parent. However when individual cross combinations are analysed differences could be observed in both efficiency and germination capacity. The F1 progenies, with both the wild species as seed or as pollen parent, had some dominant characteristics of the wild parent: no flowers were formed during the year of germination and growth habitus was in Theme 6 more wild.

## Acknowledgements

This study has been carried out with financial from the Commission of the European Communities, specific Research programme “Quality of Life and Management of Living Resources”, QLRT-2001-01278 “Genetic evaluation of European rose resources for conservation and horticultural use”. It does not necessarily reflect the Commissions views and in no way anticipates the Commission’ s future policy in this area

# Genetic variability of phytopathogenic *Fusarium proliferatum* associated with crown rot in *Asparagus officinalis*

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## Introduction

*Fusarium proliferatum* (Matsushima) Nirenberg (teleomorph: *Gibberella intermedia* (Kuhlman) Samuels) is an important pathogen involved in the destructive crown and root rot of asparagus (*Asparagus officinalis*). Infection of plants with *F. proliferatum* leads to asparagus decline, yielding unprofitable plantations in long-term cultivation of this perennial plant on a single location. This species as well as *F. oxysporum*, *F. redolens*, *F. subglutinans* and *F. verticillioides* are described as producers of the fumonisin mycotoxin. Fungal isolates could not only be isolated from roots and crowns but were also found in asparagus spears obtained within the main harvest period, which does not show symptoms of an infection with *Fusarium* spp. (Goßmann et al. 2005).

## Methods and Results

*F. proliferatum* isolates obtained from perennial asparagus plantations from Austria and Germany were included in a study of genetic variability and detectability of two essential genes of the fumonisin-gene cluster and compared with *Fusarium* species infecting crops. Genetic fingerprinting of 45 isolates by RAPD- and DAF-PCR revealed genetic heterogeneity of *F. proliferatum* by establishment of fourteen different fingerprint groups. Most isolates differentiated into three main fingerprint clusters, but no association was found between fingerprint group and origin of the isolates. By gene specific PCR it was shown, that in all isolates tested both initial genes of the fumonisin biosynthetic pathway - *FUM1*, encoding a polyketide synthase as well as *FUM8*, an aminoacyltransferase gene - were detectable. In *F. oxysporum*, *F. redolens*, *F. subglutinans* and *F. verticillioides* isolates these genes were sporadically detected. The fumonisin biosynthetic gene cluster is thought to be exclusively present in isolates of the genus *Fusarium* with fumonisin producing potential (Waalwijk et al. 2004). Verification of *FUM*-genes suggests that these fungal strains are able to produce fumonisins and therefore responsible of contamination of asparagus spears with this mycotoxin. Fumonisin B production *in vitro* and *ad planta* has been confirmed for two *F. proliferatum* strains. Amplified *FUM*-gene fragments exhibited nucleotide polymorphisms as was shown by sequencing, but were not discriminated by PCR-RFLP. Sequence variability of *FUM1* and *FUM8* gene fragments of the *F. proliferatum* isolates was below 1%. *FUM8* fragments exhibited overall higher interspecific sequence divergences than *FUM1* genes. Fragments from *F. verticillioides* shared 84% (*FUM1*) and 77% (*FUM8*) sequence identity with *F. proliferatum* sequences.

## Conclusion

Genome wide variability of *F. proliferatum* occurring in asparagus obtained by RAPD- and DAF markers is mirrored by *FUM1* and *FUM8* gene diversity, but obviously not in the presence or absence of the fumonisin biosynthetic gene cluster within this species.

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## **Fruit quality of raspberry genotypes: a multidisciplinary approach**

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Fruit quality was investigated on raspberry cultivars through several approaches: horticultural evaluations, volatile analysis by SPME GC-MS and PTR-MS, and molecular studies were used to characterize top varieties. Primocane fruiting ('Autumn Bliss', 'Heritage', 'Polka', 'Josephine', 'Caroline', 'Himbotop', 'Pokusa', 'Opal', 'Benefis') and floricanne fruiting varieties ('Tulameen', 'Glen Ample', 'Laszka') were analysed for three growing seasons in soilless conditions and screened for phenotypical characteristics of the plants and fruits. The parameters investigated were related to horticultural aspects, to shelf life and tolerance to the main diseases of this crop. Two-spotted spider mite (*Tetranychus urticae* Koch) infestation was recorded on the raspberry accessions and *Resseliella theobaldi* (Raspberry Cane Midge – RCM) tolerance was also investigated for two years. Attacks of aphids were also screened. The volatiles emitted by raspberry genotypes have been evaluated by means SPME GC-MS and by Proton Transfer Reaction-Mass Spectrometry (PTR-MS). While GC analysis allows the identification of the main compounds of the volatile profiles of the raspberries, PTR-MS spectra can serve as sample fingerprints for the varieties characterization/discrimination. Among the different compounds identified, terpenes seem to separate samples in two main classes allowing a first important cultivar screening. The study has also progressed on the genetic identification of the *Rubus* genotypes comparing the chemical evaluation with analysis based on molecular markers. Amplifiable DNA was recoverable from *Rubus* leaves and a set of Single Nucleotide Polymorphism (SNP) markers on enzyme sequences involved in the specific biochemical pathways was started to be developed and used in the study of genetic diversity and in the marker-assisted selection (MAS). This research was financed by InterBerry (Fondo Unico per la Ricerca).

## **Morphological characterisation of selected old local apricot cultivars from Eastern Austria under organic farming conditions**

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In Eastern Austria, the main producing area of apricots in Austria, many old and well proved apricot cultivars like "Ungarische Beste" and "Klosterneuburger Marille" are still cultivated. Aim of this work was to compare 35 selections of these old cultivars, with 7 control cultivars, regarding essential criteria like yield, tree vitality and fruit quality characteristics. The trees were planted in autumn 2001 on rootstock "Torinel" with 3 replications per cultivar in the orchard of the Institute of Horticulture and Viticulture in the north-east of Vienna. The enquiry period has been 5 years. In 2006 also data about vegetative and generative characteristics like fruit quality, susceptibility to diseases, fruit- and stone weight, color intensity, fruit firmness, dis-solvable dry matter, pH value and subjective appreciation of the flavor were collected. Obtained data were calculated stastically with spss 12.0 (anova, significancy with S-N-K test at 5 % level), manageable processed, user-friendly compared and interpreted. In spite of little signifancies in statistic data some differences between the selections could be observed. The differences are interesting and important for pomological and commercial relevance. This applies for example to the average fruit-weight, the stone-percentage of the fruit, the crop-volume and the percentage of waste fruits per tree. On the base of this data conclusions for the further use and preservation of the tested selections were given. More observations, e.g. sensory tests (degustation of fruits before and after processing) and the description of the selections with genetical fingerprints are planned.

# Vegetative propagation of different *Cyclamen* species via adventitious shoot formation from seedling tissue

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## Introduction

*Cyclamen persicum* is an important ornamental plant worldwide. Only a few of the other nineteen species of the genus *Cyclamen* with a lot of interesting traits have reached commercial relevance. To examine these traits under in vitro culture and greenhouse conditions an efficient vegetative in vitro propagation method of different cyclamen species should be established. Moreover, micropropagation would be an interesting tool to preserve some of the endangered cyclamen species.

## Materials and Methods

Seeds of *Cyclamen mirabile*, *Cyclamen coum*, *Cyclamen graecum* and *Cyclamen hederifolium* were surface sterilised with ethanol (70%) for two minutes and 30-45 minutes with sodium hypochlorite (1%) and Tween 20 (0.005%). Seeds were washed three times with sterile water and germinated on Murashige and Skoog (1962) based medium like prescribed by Schwenkel and Winkelmann (1998) without additional growth regulators. Seedlings were divided in cotyledon, tuber and roots and cut in 1 mm to 4 mm thick explants. Shoot induction was obtained on Murashige and Skoog (1962) based medium like prescribed above containing 0.5 mgL<sup>-1</sup> IAA and 1 mgL<sup>-1</sup> BAP (Shoot Induction Medium I) and on medium containing 0.5 mgL<sup>-1</sup> IAA, 1 mgL<sup>-1</sup> BAP, 1 mgL<sup>-1</sup> 2iP and 1 mgL<sup>-1</sup> kinetin (Shoot Induction Medium II) as growth regulators.

In a second set of experiments, the effect of explant position on shoot regeneration was tested for *C. graecum* and *C. hederifolium*. Explants were prepared from petioles of in vitro grown shoots obtained from the former experiments.

## Results

In all cases sterile seedlings without endogenous contamination were obtained and used for the experiments. The most suitable tissues for shoot induction were tubers and cotyledons depending on the species. From roots as starting tissue less or no shoots were obtained, but root formation was found in high frequency. In most species the Shoot Induction Medium II showed the highest shoot formation except in *C. graecum*, which showed a better response on Shoot Induction Medium I. *C. graecum* was with up to 100 % of explants with shoot formation (from cotyledons as explants) the best regenerating species. There were pronounced genotypic differences in the reaction to the different media within one species. Media with lower content of cytokinins showed a high formation of roots possibly due to a high endogenous auxin content in the tissue. For *C. graecum* and *C. hederifolium* stable in vitro cultures with a further propagation protocol could be established.

The results of the second set of experiments clearly demonstrated that adventitious shoots arose mainly at the apical cut surface of petiole segments. The most regenerative part of the petioles were the sections from the upper part next to the leaf blade.

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## **Fruit genetic resources rediscovery and management in Northern Italy natural areas: Capanne di Marcarolo Regional Park and Val Grande National Park**

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The loss of valuable fruit and nut germplasm is a worldwide concern and the need to preserve and protect this precious heritage from erosion is fundamental.

The research was carried on in 2 protected areas of Piemonte (Northern Italy), rich in wilderness and biodiversity, in order to explore the local fruit genetic diversity, witness of the fruit culture of the past.

Accessions of chestnut (*Castanea sativa* Miller), apple (*Malus x domestica* Borkh.), pear (*Pyrus communis* L.), plum (*Prunus domestica* L.) have been described *in situ* and, most of them, collected *ex situ*.

DNA analysis was applied to characterize and identify the sampled material and to evidence eventual synonymies and homonymies. Furthermore the biometric and quality traits of the fruit were determined.

The study allowed the rediscovery of a group of accessions particularly appreciated for valuable traits, suitable for the reintroduction in culture and the sustainable development of the territory.

This ancient germplasm represents an opportunity to use and exploit it in terms of fruit production, but also as a traditional food in ethnic gastronomy, and as an ecological component in the frame of the restoration of the traditional landscapes and sustainable rural development.

## **Repellency and Toxicity of Calneem oil to *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae) in stored products**

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The wide spread use of pesticides to control stored-product pests is posing serious environmental and health problems. Native to India and Burma, the neem tree, *Azadirachata indica* A. Juss is a member of the mahogany family, Meliaceae. It was introduced to Africa earlier this century and is now well established in at least 30 countries, including Ghana where it has become an important source of fuel, lumber and bioinsecticide. Calneem oil is a biopesticide produced and marketed in Ghana by AQUA AGRIC Community Projects (AACP). It is an in-house prepared, cold pressed, double filtered, pure and natural oil derived from high quality neem seeds. Calneem oil contains about 0.3% azadirachtin as its major active ingredient. It is a broad-spectrum insecticide which is effective against several pests of vegetables, food crops, fruit and other tree crops and durable stored products. Toxicity and protectant potential of Calneem against *Tribolium castaneum* was investigated in the laboratory using contact toxicity, grain treatment and repellency assays. The Calneem oil was applied as concentrates in which the oil was dissolved in water using soap as emulsifier. It was applied at six concentrations (0.1%, 0.2%, 0.5%, 1.0%, 2.0% and 3.0%). Calneem oil applied topically, impregnated on Whatman filter papers of weight, 0.58 g and 90 mm diameter or mixed with whole grains was highly toxic to *T. castaneum*. Calneem oil was more effective on grain and on filter paper discs [since the lowest dosage of 0.1% killed more than 50% of the beetles within 24 h. The effectiveness of Calneem oil was significantly reduced by the length of storage after application. Calneem oil was also highly repellent to *T. castaneum* tested with overall repellency in the range of 80-100%. The development of eggs and immature stages inside cracked wheat was completely inhibited by Calneem oil treatment. The implications of these results with respect to the suitability of Calneem oil for grain protection against insect pest infestation are discussed.

# **The plum psyllid *Cacopsylla pruni* as vector of the European stone fruit yellows phytoplasma (EFSY)**

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The European stone fruit yellows is an important quarantine disease which is caused by phytoplasmas (Apricot chlorotic leafroll phytoplasma). This disease is widely distributed in Austrian apricot producing areas. Infested propagating material or transmission by insects (vectors) are the reasons for spreading. The plum psyllid *Cacopsylla pruni* (Psyllidae, Homoptera) has been proved as vector for this quarantine disease in several countries.

Due to the fact that there are only rare data about the presence of the plum psyllid in Austria a monitoring program was carried out in apricot producing areas by the Institute of Plant Health (AGES) in 2005. The monitoring took place at 10 investigation areas in Lower Austria (Wachau) and in Burgenland. Beating samples from apricot trees were taken for the monitoring from spring to summer in 2005.

Results demonstrated not only the occurrence of *Cacopsylla pruni* in Austria, but also its importance as possible vector of this quarantine disease in Austria.

## **Characteristics of selected grapevine hybrids from the progeny of direct and reciprocal crossing between Smederevka and Red Traminer cultivars**

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Creating new grapevine cultivars has been done for many years at the Experimental Station “Radmilovac”, the property of the Faculty of Agriculture, Belgrade. The methods most applied for the purpose are hybridization and clone selection. The progeny of F<sub>1</sub> generation of the cultivars Smederevka and Red Traminer (128 hybrids) was also produced by sexual hybridization method. The aim of crossing was to improve yield components in Red Traminer cultivar but to retain its good quality. After detailed studies, hybrid 25773 was selected from the progeny of direct crossing between Smederevka x Red Traminer, and hybrids 27665, 27715, 27722, 27726 and 27785 were selected from the progeny of reciprocal crossing between Red Traminer x Smederevka. The present paper gives their more important morphological, agrobiological and technological characteristics as compared to a standard cultivar Red Traminer. It was found that all studied hybrids had hermaphrodite flower type and aromatic berry taste. Two hybrids possessed white and four hybrids red berry skin. All hybrids were characterized by fairly late ripening time, higher yield level, and larger bunches and berries than standard cultivar. Contents of sugars and total acids in must were nearly at the same or slightly lower level than in Red Traminer cultivar. Based on demonstrated characteristics, all selected hybrids are recommendable for registration procedure or further breeding work in creating new grapevine cultivars.

# Measurement of Holistic Quality Parameters of Pumpkins

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Several kinds of pumpkins were investigated by the holistic methods of biophoton-counting and P-value. The aim was to determine additional holistic quality parameters of the pumpkins related to their reap time. Intermediate results seem to show significant (?) differences in biophoton emission and P-values representing the holistic vitality of the pumpkins dependent on the cultivation of the plants and the kind of treating the soil.

## *Intermediate report*

From biophysical point of view electromagnetic interaction is dominant in biological systems described as non-equilibrium thermodynamic organisation of biochemical matter. Therefore, living systems are emitting and absorbing electromagnetic fields whose quanta are called photons. This emission of light fields of bio-systems is known as "biophoton emission", sometimes called "low level bioluminescence" or "ultra weak bioluminescence".

Each living cell is producing a certain type of non-equilibrium, ultra weak photon emission for the aim of self-organisation the cell union. Since biophotons have physiological importance and implications, the emission intensity reflects the physiological state of cells respectively organisms. Thus biophoton counting opens up a new field of holistic food quality research in comparative studies. Growing processes and stress, for instance, change the emission of biophotons of certain wavelengths. There is a close connection to delayed luminescence which corresponds to excited states of the coherent photon field. If excited states are kept longer, fewer photons are emitted due to hyperbolic relaxation function thus indicating high biophysical quality of a biosystem. Spontaneous biophoton emission and the connected photoluminescence which occurs after light irradiation have been applied to distinguish foods of different cultivation and processing systems.

The P-value of a living system represents an integrative value calculated from three parameters, namely redox potential, pH-value and electrical conductivity indicating the activity of electrons. This means the lower the P-value, the higher the reductive power of a biosystem. The effectiveness of a range of mechanisms protecting the organism against free radicals is reduced by aging, environmental contaminants, unhealthy life style and stress. As an instrument of holistic medicine it has gained essential meaning as for preventing and treating by products of organic cultures. During the last decades it has also been used to determine electrochemical quality aspects of food. In addition to traditional chemical analysis P-Values show the vitality of foods by defining their ability to promote life. The underlying hypothesis states that the reductive potential of food counteracts oxidative stress. In this respect freshly reaped food from organic agriculture shows a considerable advantage.

The used measuring methods taken together have essential indications. They complete each other and yield a new extended view concerning the notion of quality. The final results of the investigation of pumpkins will be determined after finished harvest.

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## Safenut: an European project for the valorization of autochthonous almond and hazelnut genetic resources

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The general interest for genetic resources is based on the opportunities offered by their utilization (Maxet *et al.*, 2002). Genetic resources not only provide the required raw material for sustainable genetic improvement of crops, but offer a unique gene combination, as naturally occurring co-adapted gene background, to ensure adaptability and productivity. Therefore, their conservation is of paramount importance to achieve sustainable production and food security for future generations (Kassar & Lasserre, 2004). This is reflected in the objectives of the Convention on Biological Biodiversity (CDB) and the FAO International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA). However the conservation of agro-diversity is of critical importance, because of the direct benefits to humanity that can rise from its exploitation in improving agricultural and horticultural crops as well as the potential for developing new medicinal and other products. Up to now, the management of genetic resources is based on conservation, evaluation, utilization with the conservation function played by *ex situ* and *in situ* collections (Berthauld, 1997). For a few years now, this concept has evolved (Brush, 1995, Franks, 1999). The farmer becomes one of the actors of the creation and maintenance of the present diversity (Maxed *et al.*, 2002). The problem of continuously expanding the number of accessions in gene banks introduces the concept of 'core collection': a selected and smaller collection representative of species' diversity (Brown 1989). Designing of core collections involves an appropriate use of diversity, offering breeders an opportunity to work with a number of accessions quite manageable. Within the Council Regulation (EC) N. 870/2004 AGRI GEN RES, which established a Community programme on the conservation, characterisation and utilization of genetic resources in agriculture, the project 'SAFENUT' : 'Safeguard of almond and hazelnut genetic resources from traditional uses to modern agroindustrial opportunities' represents an example of a resourceful strategy for well-organizing the use of genetic resources through the fulfilment of the core collections and gene banks. The present work summarizes the main objectives and the expected results of the in progress project discussing its potential impact on the possibility to recognise and share in a more efficient manner the genetic resources of these species upgrading the knowledge on their value and uses. Moreover, the economic importance of the predicted results will be related to the requirements of developing Countries of Africa, Asia and South America that can consider this project a starting-point for a wider collaboration, enhancing the sharing of genetic resources.

## S-genotyping of Hungarian and Eastern European almond [*Prunus dulcis* (Mill.) D.A. Webb] cultivars

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Almond is a typically outcrossing species. Its flower self-incompatibility is of the gametophytic type and is controlled by the highly variable *S*-locus. Allele diversity and frequency of this locus basically determine the availability of mating partners. It has also considerable implications for cultivation and breeding as diploid cultivars sharing both of their *S*-alleles cannot fertilize each other and consequently, fruit set will not be adequate if only these cultivars are grown in the same orchard. Therefore, cultivars of identical *S*-genotypes form cross incompatibility groups (CIGs). In almond, more than 150 cultivars were assigned to 23 CIGs and to the group 0, which encompasses cultivars mutually compatible and with all other groups. Although *S*-genotyping studies were restricted mainly to countries (USA, Spain, Italy etc.) with considerable almond production, future will emphasize the need of such an experimental work also in the screening of large germplasms in other areas in order to assess the genetic resources of subsequent breeding programs.

In the present study, 26 almond cultivars of different geographic origin (Hungary, Ukraine and Moldavia, France and Italy) were analysed with PCR amplification of the first and second intron regions of the *S*-RNase gene. The size of the 2<sup>nd</sup> intron PCR products was estimated by comparison with the 1-kb + DNA ladder in agarose gels. To determine the exact size of the *S*-RNase first intron region fragments, fluorescently labelled products were run in an automated sequencer. Some 2<sup>nd</sup> intron fragments were cloned into a pGEM-T Easy plasmid vector and sequenced.

Altogether, 20 alleles could be detected in the tested 26 almond cultivars indicating a great diversity of the almond *S*-locus. Only half of the alleles detected could be corresponded to alleles previously described in American, Spanish and Italian cultivars. The remaining 10 *S*-alleles seem to be new, previously non-identified alleles; however, sequence analysis will be needed to confirm the novelty of some of these alleles. *S*<sub>9</sub> turned out to be the most frequent allele as it was shown to be carried by 8 cultivars involving French, Ukrainian and Hungarian accessions.

Determining genomic sequences of the region C2 to C3 (including the 2<sup>nd</sup> intron) of the *S*-ribonuclease gene allowed us to identify the full or at least partial *S*-genotypes in case of 8 and 11 cultivars, respectively. All of these cultivars belong to the CIG 0 and therefore can be proposed as potent pollinizers for any other cultivars. Our analysis confirmed the previously determined *S*-genotype of 4 Italian self-compatible cultivars. Putative *S*-genotypes could be proposed for further 3 cultivars, although test crosses or DNA sequencing is required to confirm these genotypes. As expected, cross-incompatibility was confirmed between several clones of the same cultivars.

The *S*<sub>f</sub>-allele rendering self-compatibility in almond was amplified in 4 Italian cultivars known to be self-compatible, but it cannot be assigned to any of the remaining 22 cultivars indicating that these are all self-incompatible. This result seems to confirm the previous hypothesis that self-compatibility has arisen in the Italian region of Apulia as *S*<sub>f</sub> could not be detected in cultivars native to regions that are located east from Italy.

Our experiments revealed a great genetic diversity at the *S*-locus of Eastern European almond cultivars, which can be applied for theoretical studies to elucidate the molecular basis and evolution of self-incompatibility in almond and other *Prunus* species as well as to assist almond breeding programs.

This work was funded by the NKTH-OTKA K68921 grant.

## Testing protein and DNA-based methods to clarify the presence of *S*-ribonucleases in quince (*Cydonia oblonga* Mill.) cultivars

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Species in the *Maloideae* subfamily of the *Rosaceae* family are characterized by gametophytic self-incompatibility (GSI), a trait controlled by the highly variable *S*-locus. This locus encodes for a ribonuclease (*S*-RNase) enzyme that is expressed in pistils. The pollen *S*-gene candidates are the recently identified *S*-haplotype-specific F-box brothers indicating that the 26S proteasome system may take place in the recognition and differentiation of self and non-self pollen tubes. Self-pollen is rejected to prevent inbreeding. However, self-compatibility (SC) may also occur in these species due to genetic mutations. In Japanese pear (a species belonging to the *Maloideae* subfamily), SC was confirmed to be the consequence of the absence of an *S*-RNase protein.

Although quince (*Cydonia oblonga* Mill.) is a minor fruit crop, some regions and countries have sizeable production, most of which is devoted to processing and sold as jam or marmalade. Quince cultivars are regarded self-compatible since single trees of home gardens yield well but monovarietal orchards are unfruitful or very poor yielding. Fruit set studies revealed many contradictions; the five most investigated cultivars were sometimes evaluated as being SC, while other studies described the same cultivars as SI. As quince belongs to the *Maloideae* subfamily, its self- and mutual compatibility properties are hypothesized to be under the control of a GSI system. Our analyses were started to confirm or reject the presence of the *S*-locus encoded ribonucleases in quince pistils.

In the present study, 8 quince cultivars were analysed. Initially protein extracts of pistils were separated with non-equilibrium pH-gradient electrofocusing and stained for ribonuclease activity. Subsequently, degenerate PCR primers were designed from a set of maloideous *S*-RNase sequences to amplify several regions of the gene. The size of the 2<sup>nd</sup> intron PCR products was estimated by comparison with the 1-kb + DNA ladder in agarose gels. Evaluation of the results was carried out through reconciling the protein and DNA-based information.

All known *S*-ribonucleases are glycoproteins that are characterised by a high isoelectric point. Our *S*-RNase electropherograms revealed that all tested cultivars had one or two RNase isoenzymes with isoelectric points that fell within the pH range of 8-10. This indicates that *S*-RNases are putatively present in quince pistil tissue. Correlation between the previously reported compatibility phenotypes and the *S*-RNase zymograms was not complete; therefore, DNA-based analyses were required.

We have designed two forward and three reverse consensus PCR primers amplifying different regions of the maloideous *S*-RNase gene. Four primer combinations were tested until now. The first primer pair amplified a fragment from the signal peptide to the C2 conserved region, and gave a product of approx. 180–195 bp. The second primer pair amplifies nearly the whole gene from the signal peptide to the C5 region. Since introns may show considerable size variability, sizes of the amplified fragments could not be anticipated, but they must exceed 570 bp. The third and fourth primer combinations amplified fragments from C2 to C5 conserved regions in the *S*-RNase gene, their size exceeds 450 bp in all cases. Several cultivars showed similar *S*-RNase zymograms and accordingly similar fragment sizes, indicating that the designed and adapted methods are able to predict *S*-genotypes of quince cultivars.

DNA sequencing of the PCR amplified fragments may help to confirm putative *S*-genotypes and improve the newly elaborated methods. These may provide reliable and easy to apply techniques to detect compatibility properties.

This work was funded by the NKTH-OTKA K68921 grant.



# **Posters of General Themes**

# Water management and productivity of olive trees (*Olea europaea* L.) in Southern Italy: results from a four-year field study

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## Introduction

In the Mediterranean region, water availability for agriculture has declined due to the increase in urban and industrial use, and because of climate and land use changes. In these conditions the efficient management of limited water resources requires irrigation strategies aimed to improve water productivity. Field trials have been carried out to test regulated deficit irrigation strategies reducing water supply in phenological stages less sensitive to yield reduction. The aim of the present four-year study was to test the hypothesis that mild water stress during fruit growth allows a good crop yield, while controlling excessive vegetative vigour in a typical hilly area of olive growing in Southern Italy.

## Materials and Methods

The trial was carried out on an olive orchard at the experimental station of CNR-ISAFoM located near Benevento, Italy (41°06' N, 14°43' E). The plantation started in 1992 and several cultivars have been subjected to different water management treatments since 2003, using a drip irrigation system: a) rainfed; b) deficit irrigation - 33, 66 and 100% of ET<sub>c</sub> (crop evapotranspiration) - from the beginning of pit hardening to early fruit veraison; Treatments were arranged in a completely randomised block design replicated four times.

The climatic pattern of this sub-humid Mediterranean zone is characterized by mean annual precipitation of 736 mm (1984 - 2006 average) mainly occurring in fall and in spring months, while scarce or no rainfall are detected from mid-June to mid September. The yearly mean reference evapotranspiration is about 1.233 mm.

## Results

The interaction between cultivars and irrigation levels was not significant for vegetative plant development. Trunk section area, crown volume and pruned wood clearly showed an increase with the amount of water applied, and differences between treatment 0 and 66% of ET<sub>c</sub> were always significant. Only a minor increasing trend was detected comparing treatment 66 and 100% of ET<sub>c</sub>. These results imply that a water supply of 66% of ET<sub>c</sub> had to be applied during the period from pit hardening to harvest beginning, before an evident vegetative tree development could be obtained, at least in this experimental area. In addition, a water amount completely replenishing ET<sub>c</sub> could be applied during this period without an excessive vegetative growth.

Fruit and oil yield per plant showed to increase with the amount of water supplied, reaching the highest yield in treatments irrigated with 100% of ET<sub>c</sub>. Treatment 33% of ET<sub>c</sub> resulted in no-significant yield increase when compared with rainfed control, while 66% of ET<sub>c</sub> showed an intermediate effect. The yield increase was primarily due to a higher fruit weight that was generally determined by the dry matter accumulated. This behaviour was more evident for mesocarp mass than for endocarp mass.

The fatty acid profile was not modified by irrigation treatments, while the polyphenol contents decreased in treatment 100% of ET<sub>c</sub>. Percentage of oil content, oil acidity and peroxide number were not affected by the irrigation regime and all quality parameters were within limits fixed by the Commission Regulation for extra virgin olive oils.

In conclusion, water supply from pit hardening to early fruit veraison may be a feasible strategy in this area to reduce seasonal irrigation volume without significant yield and quality losses and may control excessive canopy development reducing the high pruning cost that generally account for 20-30% of total expenses.

## **Multi-criterial approaches for the inventory and the evaluation of traditional cultural landscapes**

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The Mediterranean environment is characterized by an high variability in ecological features and by a rich biodiversity, whose interaction has generated complex agro-forestry systems. The resulting cultural landscapes can represent a remarkable trait of Italian landscape. Nonetheless, since several decades they are at risk mainly owing to the consequences of cultural intensification, that has turn out in new cultural models, i.e. specialized high density agronomic plantation, or in a progressive abandonment of agricultural land. In order to prevent the degradation or loss of these particular ecosystems, it becomes a priority to adopt measures for their preservation and promotion. Regardless of this necessity, up to now it lacks in Italy an exhaustive classification of the traditional landscapes that necessitate to be preserved. The inventory is the first step to be considered in order to elaborate a successive global evaluation of their function and conservation requirements.

The present study reports on an holistic multidisciplinary methodology formulated and proposed for the classification and the analysis of cultural landscapes. As far the inventory is concerned, it has been evaluated the concomitant presence of a plurality of characterizing descriptors, represented and implemented in a GIS system, such as geomorphological parameters, pedoclimatic features, land use typology and history of the territory. As far as the evaluation of cultural landscape significance is concerned, it has been proposed a global value obtained by integrating particular values, like the biological, historical, socio-economic, artistic, emotional ones.

## **A biophysical model describing carbon and water fluxes in tomato fruit – a good candidate for the future virtual fruit**

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### ***Introduction***

In order to progress in our understanding of fruit quality build-up, we need to integrate the numerous processes involved and to describe the complex interactions and feedback mechanisms among these processes occurring at different scales. A virtual fruit model has been recently designed by Génard et al. (2007) which proposes a hierarchical organization of different fruit levels (from cells to organ) and intends to integrate process-based models describing the main processes involved in fruit growth and composition. Cell expansion, xylem and phloem fluxes, transpiration and respiration should be integrated in the virtual fruit. The Fishman and Génard (1998) model proposes an integrative approach of these processes involved in fruit growth and carbon and water accumulation in peach fruit. This model assumes that the fruit mesocarp behaves as a single large cell and relies on a biophysical description of water and carbohydrates transport coupled with the stimulation of cell wall extension driven by the turgor pressure (Lockart, 1965). In this study we showed that the peach model could describe growth of tomato fruit in relation to carbon and water fluxes with minor modifications.

### ***Results***

Main modifications of the model relied on the literature on tomato and mainly concerned: (i) the decrease in cell wall extensibility coefficient during fruit development. (ii) The increase in the membrane reflection coefficient to solute from 0 to 1, which accounted for the switch from symplasmic to apoplasmic phloem

unloading. (iii) The negative influence of the initial fruit weight on the maximum rate of active carbon uptake based on the assumption of higher competition for carbon among cells in large fruits containing more cells. The sensitivity analysis indicated that there were no strong interactions among parameters and that the model was mostly sensitive to the phloem sucrose concentration. This parameter was assumed to be constant and it was independently estimated on 17 experimental datasets ( $0.11 \text{ g g}^{-1}$ ). Finally the model was calibrated and evaluated with satisfaction on 17 experimental datasets obtained under contrasted environmental (temperature, air vapour pressure deficit) and plant (plant fruit load and fruit position) factors. The main source of errors might be the estimation of the initial fruit weight. Then the model was used to analyze the variations in the main fluxes and factors involved in the control of fruit growth (phloem and xylem fluxes, transpiration and respiration rates, carbon transport pathways, osmotic and turgor pressures) involved in tomato fruit growth and accumulation of carbon in response to virtual carbon and water stresses.

### ***Discussion***

The present adaptation to tomato fruit confirmed the genericness of the Fishman and Génard (1998) model and its suitability for fleshy berry fruit. This model integrating simple biophysical laws was able to mimic the fruit behaviour and to analyze the interactions and feedback regulations among the fruit system components, for instance between fruit osmotic and turgor regulation and water and phloem fluxes in response to environmental and plant factors. Thus it may be fruitfully integrated in the future virtual fruit.

## **Detached tomato fruit grown on various sucrose solutions and comparison with fruit grown on plant**

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### ***Introduction***

In vitro fruit culture should be very interesting to study the influences of various external and endogenous factors in order to better understand the fruit growth mechanisms. For that, it seems that two conditions have to be met: the first is that the main growth processes which occur in the fruit grown on the plant exist also in the in vitro fruit; the second is to understand the possible differences if mechanisms are lacking or super-added in order to be able to extrapolate to the fruit growth on the plant. In this view, effects of sucrose concentration and osmotic pressure of the solution entering the fruit on parameters of several important fruit growth processes (cell division and expansion, dry matter, sugars and water accumulations, maturation) were studied in vitro and some comparisons with the in planta fruit were made.

### ***Materials and methods***

Fruits of Cherry tomatoes were sampled some days after anthesis from plants grown under greenhouse. These fruits were transferred in glass containers with nutrient solutions of different sucrose concentrations and osmotic pressures, in which fruit pedicel was immersed. Fruit diameter was measured through the glass container at various times over the growth period up to maturity. In addition, fruit samples were taken out at four times to measure a number of parameters: fruit weight, dry matter mass and water mass, Brix, pericarp cell number and volume. These parameters were also measured on fruits grown on the plant.

### ***Results***

In vitro, a fraction of the fruits did not grow, likely due to contaminations. In healthy fruits, cell number increased with sucrose concentration between 2 and 8%. In the in planta fruit, this number was in the middle of this range. The volume of one pericarp cell increased with sucrose concentration up to 4 - 8% and decreased when PEG was added. In the in planta fruit, this volume was much higher. Also fruit diameter increased with sucrose concentration up to 4 - 8% and decreased at higher concentration or when mannitol or PEG was added. Fruit expansion rate was null when osmotic potential of the solution was close to  $-2.5 \text{ MPa}$ , which coincides with the value predicted by a model of water import in fruit. In planta fruit was much

greater than in vitro fruit. Dry matter content and Brix increased with sucrose concentration and osmotic potential; at 8% sucrose concentration they were close to those of in planta fruit and changed similarly over growth period.

### ***Discussion and conclusion***

Despite fruit and cell expansions were strongly lowered in vitro, there were many similarities between fruit grown on the plant and in vitro fruit. Therefore, the experiences suggested that increased sucrose concentration may increase carbon supply, cell division and fruit and cell expansions but that very high concentration may decrease these expansions through osmotic potential. The causes of the low expansions in vitro need to be identified. The results indicated that experiences made with in vitro fruits may be very useful to study and to better understand the fruit growth, especially cell division and expansion.

## **Studies Concerning the Behaviour of some *Gladiolus* Cultivars for Improving the Romanian Floral Assortment**

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In Romania *Gladiolus* is grown extensively in the field as are commercial crop, as cut flowers and also are used as landscape plants.

Researching activity for diversification of *Gladiolus* assortment by introducing of the most competitive foreign floral cultivars is one of the objectives of the Floricultural Department at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania. Introducing the most competitive foreign cultivars in the *Gladiolus* culture is the main way of increasing the *Gladiolus* production (Cantor Maria at all, 2000).

The new *Gladiolus* varieties subject to research in our collection during 2006-2007 periods, were received from abroad 10 *Gladiolus* varieties: 'Break a Dawn', 'Jester', 'My love', 'Mon Amour', 'Madonna', 'Princess Marg. Rose', 'Tradehorn', 'Blue Isle', 'Fiorentina' and 'Expresso'.

These were observed in our Transylvanian behaviours and recorded for the following characteristics: blooming time, colour of florets, plant height, spike length, number of florets per spike, media florets diameter and number of florets open in the same time.

The varieties were compared with the Romanian cultivar Speranta. The observations were made for 20 plants from each variety.

Data obtained were statistical interpretation. The results were calculated and analyzed, using the standard deviation and the coefficient of variability for each characteristic of the plants. The coefficient of variability shows the modality of transmission of these characters to descendants in order to use the best cultivars as genitors in our breeding researches.

The majority of *Gladiolus* cultivars need for blooming over 70 days. Bloom very early the cultivar 'Break a Dawn' (60 days) and varieties 'My Love' and 'Jester' blooming late, after 80 days.

Plants height of the varieties were over 100 cm, the most vigorous was 'Traderhorn' and with less vigorous was 'My Love'.

The spike length has between 78.6 cm ('Princess Marg. Rose') and 96.6 cm ('Mon Amour').

The cultivars under study show between 15-22 florets/spike. The averages of florets diameter were between 7.2 cm ('Princess Marg. Rose') to 12.1 cm ('Traderhorn').

The cultivars studied have a rich range of colours, are vigorous with great number of florets per spike, and some blooming earliness, have good health and more corms, and so can be used as special cut flower or for landscape design.

## Horticulture in Bosnia and Herzegovina

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All of the aspects of horticulture in Bosnia and Herzegovina (such as production, research, education) nowadays meet numerous limitations. As a part of former Yugoslavia, Bosnia and Herzegovina had significant horticultural capacities in the areas of production, research and education. One of the factors that contributed to this is certainly different, but very favourable ecological conditions for this production. The main emphasis in production was on fruit, vine, flowers and vegetables. Due to the war at the area of former Yugoslavia there was not only back set in this area, but very significant regression as well, which consequently resulted that all of the participants in this sector are facing with important consequences and problems even years after the war was finished and after establishing and developing of new social and territorial structure. War devastation and change of social structure, as two main causes for problems in horticulture in B&H separately have great impact, and having in mind that they were happening concurrently gives higher emphasize on their intensified synergistic effect. Today, B&H is consisted of two entities, and defying of whole agricultural policy, according to the constitution, is at entity levels. Important advancements in horticulture are noticeable in the last 5-6 years, and there are evidential in all sections: organization of production set on different ownership relations and more contemporary technologies, enabling of human and infrastructural research capacities and advisory services, as well as creating of new university syllabus that are adjusted to main principals of Bologna process. One of the most important advancements is for sure starting of integrated production of fruit, vine and vegetables, first throughout small scale projects, and than later trough preparation of harmonized legislations at the area of whole B&H. The biggest improvements are made in restoring fruit and vine production. Reconstruction of vegetable production, mainly in protected area, is progressing a bit slower. Capacities in part of floriculture and ornamental plants are nowadays way behind than it was before, and that is why we need to use similar approach as we did in formerly mentioned areas. MAP is nowadays area of horticulture with much bigger importance than it had before. But most of the activities are based on collecting, and special emphasize are given on problems of inadequate education and low responsibility of collectors from the aspect of protection and conservation of biodiversity. It is also important to mention activities in introduction of organic production, and in the last few years there are strengthen activities in conservation and sustainable use of plant genetic resources.

## Plant genetic resources in Republica Srpska (BiH)

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Activities on establishing of the Program on plant genetic resources of Republika Srpska have been started in 2004. Inventory, collection and evaluation are done through the established crops' specific national working groups. Conservation is done through *in situ* and *ex situ* methods. Gene bank is established in 2005 and

it is responsible for the seed collection. Seed collection is now consisted of next accessions: *Zea mays* L. (18), *Secale cereale* L. (7), *Avena sativa* L. (15), *Hordeum vulgare* L. (48), *Triticum aestivum* L. (184), × *Triticosecale* Witmack ex A. Camus (27), *Hypericum perforatum* L. (3), *Thymus vulgaris* L. (2), *Salvia officinalis* L. (1), *Juniperus communis* L. (1), *Gentiana lutea* L. (1), *Phleum pratense* L. (2), *Dactylis glomerata* L. (1), *Trifolium pratense* L. (4), *Lotus corniculatus* L. (3), *Nicotiana tabacum* L. (3) and *Linum usitatissimum* L. (1). Gene bank also recognizes field gene bank as well as botanical garden of agricultural and horticultural plants. Field gene bank is consisted of next accessions: *Malus × domestica* Borkh. (30), *Pyrus communis* L. (20) and *Prunus avium* L. (13). Database for PGR activities is created and all accessions are registered by Multi Crop Passport Descriptors (MCPDs) and collecting forms. Very important achievement related to the plant genetic resources of Republika Srpska is Programme on Genetic Resources of Agricultural and Horticultural crops drafted by National Working Group for this programme under Ministry of Agriculture, Forestry and Watermanagement of Republika Srpska.

Rising awareness is done on next levels: governmental, scientific and public. Plant genetic resources activities are supported by the SEEDNet Project and Ministry of Agriculture, Forestry and Water management of Republika Srpska.

## **Reproductive biology: pistil development in some olive cultivars in central Italy**

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### ***Introduction***

The olive, like other monoecious species, is characterized by an abundant flower differentiation, but by a low fructification. The abundant flower differentiation is accompanied by the production of “imperfect or stamiferous” flowers, in which it is possible to identify a well formed perianth and androecium, but a small, precociously degenerated pistil (Reale et al., 2006), that results to limit the final production. In this research, in order to investigate the mechanisms determining the production of staminate flowers in olive, we studied the morphological and cytological changes related to differentiation of this kind of flowers and the possible correlation between flower or pistil abortion and reserve content.

### ***Materials and methods***

Field observation and experiments were carried out in Olive-Growing Experimental Centre, located in Castel Ritaldi (Spoleto). Coeval plants in good phytological conditions and of certain genetic origins, belonging to Frantoio, Leccino, Moraiolo, Dolce Agogia and San Felice cultivars, were chosen. For each cultivar three plants were considered and for each plant ten inflorescences were collected. In each inflorescence we distinguished, under stereoscopic observation, between hermaphroditic and stamiferous flowers. In order to determine the cytological characteristics of pistils at different degeneration levels, one part of plant material was embedded in paraffin and cut into sections of 8-10 µm thick. The sections were stained by Safranin Fast-green and iodine-iodide solutions and observed at the light microscope (DMLB, Leica, Wetzlar, Germany).

### ***Results and conclusions***

As a result of our observations, in hermaphroditic flowers a perfectly conformed pistil and ovules containing developed embryo sac were observed. In stamiferous flowers just before the anthesis the style and the stigma are not completely differentiated and sometimes these parts are necrotic. Paraffin sections of stamiferous and hermaphroditic flowers were also stained with iodine-iodide solution and showed the presence of starch grains. In hermaphroditic flowers a lot of starch grains were observed in the peduncle/receptacle zone, in the ovary wall and in the ovule, in the style and stigma, in the anther wall. In the ovule, the starch grains were observed mostly in the chalazal and micropyle portion. In stamiferous flowers, the staining with iodine-iodide solution outlined the presence of starch grains only in the pedun-

cle/receptacle zone and in the anther wall. In the style and stigma, in the ovary wall and in the ovule no presence of starch grains was detected. The differences observed in starch content between staminiferous and hermaphroditic flowers suggest that the nutrients stored in flowers could be an additional factor superimposed to pollination/fertilization and involved in fruit set. A more detailed investigation could be useful to elucidate the origin of starch and the regulation of its accumulation in order to identify the mechanisms that regulate staminiferous flower differentiation.

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## Detection of *Rhizoctonia* diseases

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The fungus *Rhizoctonia solani* Kühn, the anamorph of *Thanatephorus cucumeris* (Frank) Donk is a ubiquitous soil-borne pathogen comprising plant parasites and saprophytes. The species *R. solani* affects many economic important agricultural and horticultural crops and is composed of genetically isolated groups, so called anastomosis groups (AGs). To date, 12 anastomosis groups (AGs), AG 1 to AG 12 are recognised. Many of these AGs have been subdivided on the basis of host range, cultural morphology, biochemical, or molecular characteristics. The AGs show a certain degree of host specificity. Economical losses caused by *R. solani* are important especially on sugar beet, potato and lettuce in Germany. The AGs causing diseases are not well documented for each crop. So the causal pathogen of late sugar beet rot was identified as *R. solani* AG 2-2IIIB. However, the occurrence of AGs and subgroups causing bottom rot in field-grown lettuce in Germany has not been documented. Bottom rot on lettuce is a problem especially in South of Germany. One objective of our study was to characterise isolates of *R. solani* causing bottom rot in field-grown lettuce. Isolates were collected from various commercial lettuce production regions and identified as *R. solani* AG 1-IB based on anastomosis behaviour and pectic zymograms.

The aim of this study was to develop a specific and sensitive identification method for *R. solani* AG 1-IB isolates based on phylogenetic relationships of *R. solani* AG-1 subgroups using rDNA-internal transcribed spacer (rDNA-ITS) sequence analysis. A neighbour joining tree analysis of 40 rDNA-ITS sequences demonstrated that *R. solani* AG-1 isolates cluster separately in six subgroups IA, IB, IC, ID, IE and IF. A molecular marker was generated from a random amplified polymorphic DNA fragment (RAPD). After conversion into a sequence-characterized amplified region (SCAR), a specific primer set for identification of subgroup AG 1-IB was designed for use in a polymerase chain reaction (PCR). The primer pair amplified a single DNA product of 324 bp. *R. solani* AG-1 subgroups were discriminated by sequence analysis of the ITS region. The designed SCAR primer pair allowed an unequivocal and rapid detection of *R. solani* AG 1-IB in plant and soil samples. Sequence analysis of the rDNA-ITS region can be used for differentiation of subgroups within AG-1. The use of the developed SCAR primer set allowed a reliable and fast identification of *R. solani* AG 1-IB and provides a powerful tool for disease diagnosis.



# Quantification of calcium amount deposited on tomato fruit cuticles and apple seedling leaves by means of energy dispersive x-ray analysis

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The energy dispersive x-ray microanalysis (EDX) is a well known technique to determine semi-quantitatively the elemental composition of samples' surface. Despite of its lower sensitivity in comparison to classical analytical procedures, the EDX has the great advantage to give a spatial resolution of elements when used jointly with a scanning electron microscope (SEM). One promising application field for this technique in agricultural sciences is the quantification of fertilisers and selected agrochemicals deposited on plant surfaces. However, prerequisite for an effective quantification is a standardised sampling area, which should comprise the whole active ingredient (a.i.) deposit (e.g. the spread area of one individual droplet). As a rule, an applied solution droplet shows a circular footprint on a horizontal plant surface, so that the quadrangular area to be analysed in the SEM-EDX system is constituted by a three-dimensional deposit residue covering a smooth or structured plant surface, and the plant surface itself, which is visible at the borders of the picture. These lateral and vertical heterogeneities in micromorphology and elemental composition are responsible for an unequal penetration deep of the primary electron beam and the consequent emission of x-rays, constituting the peculiarity of such analysis. Even though, we hypothesise that a semi-quantitative evaluation of selected organic chemicals or inorganic foliar fertilisers deposited on samples' surface is possible. In this study using calcium chloride as model substance, selected parameters of the EDX analysis, the droplet spread area, and the area effectively covered by active ingredient (Ca and Cl) were evaluated. In a first experiment using smooth, enzymatically isolated tomato fruit cuticles as substrate we evaluated the effect of a  $\text{CaCl}_2$  concentration series on the SEM-EDX parameters 'Net Intensity', 'Peak/Background', and standardised '% Weight'. Moreover, the ratio of Ca/Cl (% Weight) was calculated, the droplet spread area and area effectively covered with the elements Ca and Cl determined. Calcium chloride droplets (0.5 and 1  $\mu\text{l}$ ) were applied with a microsyringe and left to dry for 1h before performing analysis. The calcium amount of single droplets ranged from 0.05 to 50  $\mu\text{g}$  and from 0.10 to 100  $\mu\text{g}$ , respectively for 0.5 and 1.0- $\mu\text{l}$  droplets. On each cuticle a reference measurement of the substrate only was taken. Values of the applied calcium amount were log-transformed before statistical analyses. As our results show, all evaluated parameters were influenced by both droplet volume and calcium concentration, while the Pearson's analysis revealed a strong correlation between 'Net Intensity' and area covered by Ca. Simple linear regressions for 'Net Intensity', 'Peak/Background' and Ca/Cl ratio showed variable determination coefficients ( $R^2$ ) ranging between 0.49 and 0.79. Multiple regression equations comprising 'Net Intensity', Ca/Cl ratio, and deposit area were established to estimate the amount of calcium present on the cuticles and showed determination coefficients of 0.89 and 0.81 for 0.5 and 1.0- $\mu\text{l}$  droplets, respectively. The importance of the physicochemical properties of the spray solution was explored in a second study where a rather hydrophobic or a rather hydrophilic adjuvant was added to a 10  $\text{g l}^{-1}$   $\text{CaCl}_2$  solution, which was applied to apple seedling leaves. The addition of adjuvants increased values of 'Net Intensity' and 'Peak/Background', which correlated significantly with the area covered by calcium. In another study, the cuticular penetration of calcium through tomato fruit cuticles was evaluated adopting the finite-dose system. After a period of 4h, the penetrated Ca was measured by atomic absorption spectrometry and the cuticles subjected to SEM-EDX analyses. Here, the correlation between 'Net Intensity' and remaining deposit area was very strong and positive ( $r = 0.99$ ), whereas the correlation between 'Net Intensity' and penetrated calcium was strong and negative ( $r = -0.70$ ). Considering the results presented here, we conclude that the energy dispersive x-ray analysis is a suitable technique for semi-quantitative determination of calcium deposited on leaf or fruit surfaces. However, characteristics of the target surface and of the applied solution have to be considered when establishing a calibration curve, which has to be made for each EDX system. Moreover, the use of this technique will facilitate the understanding of some complex interactions between micromorphology and chemical composition of plant surfaces and the physicochemical characteristics of spray solutions.

## The effect of storage temperature on antioxidant activity in bell pepper during prolonged storage

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Red sweet pepper (*Capsicum annum L.*) is a vegetable known for its antioxidant contents which are highly important for its nutritional values. Therefore the goal of this research was to evaluate the total antioxidant activity during prolonged storage and shelf life simulation.

The total antioxidant activity (TAA) of pepper fruits was measured by TEAC (Trolox equivalent antioxidant capacity). This assay measures both the hydrophilic (vitamin C) and lipophilic (carotenoids and vitamin E) contents based on the total radical scavenging capacity, and the ability of a scavenger to scavenge the stable ABTS radical (ABTS<sup>+</sup>) described by *Vinocur & Rodov* (2006). Sweet red pepper fruit (cv. Silica) were harvested during the winter season from commercial plastic house in the desert region in Israel. Fruit were treated with hot water rinsing and brushing (HWRB) at 55°C as described by *Fallik et al.* (1999). Tap water wash was served as control. Fruit were stored at 2 or 7°C during 3 weeks plus 3 days at 20°C (shelf life simulation).

TAA in pepper immediately after harvest was 4.3 (0.7 lipophilic and 3.6 hydrophilic) µmol TE/g fw (fresh weight). After 3 weeks storage at 2°C, TAA in control fruit was 4.14 compared to 3.97 TEAC µmol TE/g fw in HWRB treatment. After 3 additional days at 20°C, TAA slowly increased and reached 5.24 in control and 5.16 TEAC µmol TE/g fw in HWRB. This was mainly due to changes in the lipophilic activity (treatment with cold water-1.79 and 1.81 µmol TE/g fr.wt. in HWRB, comparing with 0.74 µmol TE/g fr.wt. on beginning of storage). Hydrophilic antioxidant activity remains practically unchanged.

In fruit stored at 7°C pepper ripeness has been associated with carotenoids accumulation especially after shelf life, TAA was 5.33 TEAC (lipophilic 2.03) µmol TE/g fr.wt.

This study confirmed that pepper contains significant amounts of antioxidant activity, which may preserve during prolonged storage (3 weeks + 3 days shelf life). Storage at 2°C with HWRB treatment increased antioxidant lipophilic activity, prevents chilling injury, resulted in less weight loss and less percent of diseases.

## Possibilities to save Energy in Winter Production of potted Herb Species

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Not only *Ocimum basilicum*, other potted herb species as well got an important all year round culture in the last years. The winter production of herb species especially of the thermophile mediterranean ones like *Salvia officinalis* causes high heating costs, constantly raising by increasing energy prices.

In winter 2005/2006 and 2006/2007 the influence of different cultivation methods on energy uptake as well as on quality and growth of herb species as *Petroselinum crispum*, *Anethum graveolens*, *Salvia officinalis*, *Melissa officinalis*, *Satureja hortensis*, *Origanum majorana* and *Thymus vulgaris* was examined. Temperature, daylength and sun irradiation of September should be utilised for an energy saving winter precultivation. So compared with a continuous charge production with sowing and further cultivation at constant temperatures of average day/night 18/16°C, all charges for the selling period December to March were sown at two respectively three September dates (beginning, middle or end depending on herb species) and cultivated for 3-4 weeks at the usual 18/16°C temperatures. Afterwards the plants were spaced close together and kept at 6°C. In the period December to March the plants were cultivated charge by charge at 18/16°C again just the last 7 to 14 production days to reach the necessary selling size and quality.

The herb species showed different reactions. Especially the quality of *Petroselinum crispum* and *Melissa officinalis* could be improved decisively. With the crop calculation program KUKA (developed in Weihenstephan) the individual heating costs per pot for every cultivation period and charge could be calculated and compared, using not average, but actual weather figures. For herb species like *Petroselinum crispum* and *Satureja hortensis* the precultivation method allowed a massive reduction of at least 50% of the heating costs. Though not for all herb species warm precultivation with following cooling period brought positive effects. For the slowly growing *Salvia officinalis* heating costs could not be reduced.

With the results of two years the precultivation method could be adapted to the individual needs of each herb species. Besides energy saving effects further economic parameters are in discussion.

## **Biology of Downy mildew (*Peronospora* sp.) of basil (*Ocimum basilicum* L.)**

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*Ocimum basilicum* L. (*Lamiaceae*) is cultivated worldwide under different climatic conditions, from temperate zones over the subtropics to the tropics. Besides *O. basilicum* further species are grown as spice or medical plant. Taxonomy divides *Ocimum* into 3 subgenera: *Ocimum*, *Nautochilos* und *Gymnocinum*. Species and hybrids of the section *Ocimum* are economically important: *O. basilicum*, *O. basilicum* var. *purpurascens*, *O. basilicum* var. *difforme*, *O. minimum*, *O. x citriodorum* and *O. basilicum* var. *basilicum* interm. *O. basilicum* var. *purpurascens*. Selections of *O. basilicum* differ in habitus and flavours.

Downy mildew of basil was first described in Uganda in 1932. Significant losses occur in european growing areas since some years: Switzerland 2001, Germany 2002, Italy 2003 and France 2004. Total losses are reported especially under greenhouse conditions. Primarily, the downy mildew of basil was identified as *Peronospora lamii*, but new results indicate a new *Peronospora* species. Information regarding biology and epidemiology of the new pathogen are quiet marginal. No studies are available concerning differences in susceptibility of cultivars and selections of *Ocimum basilicum*.

Aim of our project are basic studies concerning biology and epidemiology of *Peronospora* sp. of basil for the development of a screening for resistance and for the development of a control strategy. First results indicate that the germination of conidia of *Peronospora* sp. depends on temperature and time under optimum humidity conditions. Low temperatures accelerate the germination process, but the germination rate reaches an equal level at temperatures of 5°C to 25°C after incubation for 24 h. Higher temperatures reduce the germination rate. Maximum infection rates occur under temperatures of 10-25°C. Again higher temperatures reduce the rate of infection. The inoculum concentration influences the infection rate, the duration of the latent period and the intensity of sporulation. Sporulation intensity depends on the temperature during infection and sporulation, inoculum concentration und duration of the latent period.

# Adaptation of spray solutions to weed surface characteristics: improvement of glyphosate deposit characteristics and biological efficacy

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Herbicide applications play an important role in horticultural practices whereas world-wide restrictions demand a minimization of pesticide inputs into the environment. In the last years great advances in terms of reducing active ingredient (a.i.) load were achieved by using appropriate adjuvants, which also have the potential of reducing losses due to drift or droplet bouncing. Moreover, adjuvants added as built-in or tank-mix products influence droplet spread and deposit formation on the target surface, which may have a bearing on the biological efficacy of the agrochemical. Previous studies have shown that ethoxylated rapeseed oil (RSO) surfactants can alter spray solution characteristics and biological efficacy of glyphosate, whereas results depended on the interaction between leaf surface characteristics and physicochemical properties of these adjuvants. As a rule, weeds with hydrophobic surfaces were better controlled when a rather hydrophobic adjuvant (RSO 5) was added to the spray solution. On the other hand, the addition of rather hydrophilic adjuvants (RSO 60) increased the biological efficacy of glyphosate on weeds characterized by an easy-to-wet leaf surface. In the current study we hypothesize that the above cited results are related to the herbicide deposition patterns on the target surface, and that there is a significant correlation between glyphosate biological efficacy, droplet spread area, and area ultimately covered by the active ingredient. Experiments were conducted with the difficult-to-wet weed species *Chenopodium album* and *Setaria viridis* as well as the easy-to-wet species *Stellaria media* and *Viola arvensis*. Biological efficacy of the glyphosate treatments (dry biomass) was evaluated 8-10 days after application of herbicide solutions as single droplets (0.5  $\mu\text{L}$  or 1  $\mu\text{L}$ ), whereas the a.i. load was maintained constant within a weed species. Moreover, herbicide-treated leaf surfaces were studied in a scanning electron microscope with integrated energy dispersive x-ray microanalysis (EDX) 1 h after application in order to characterize and quantify the droplet spreading area and the area ultimately covered with phosphorus as a characteristic element of the glyphosate molecule.

Results showed an increased droplet spreading (glyphosate + RSO 5) up to sixfold for the difficult-to-wet species *C. album* and *S. viridis*, whereas enhancement for the easy-to-wet species only reached twice of the amount (with respect to the unformulated glyphosate solution). The addition of RSO 60 enhanced the droplet spreading area twice as much within the easy-to-wet species *S. media* and *V. arvensis*. However, only the 1  $\mu\text{L}$  droplets showed this enhancement, whereas on the difficult-to-wet species a slight enhanced spread area within both droplet volumes after addition of RSO 60 was observed. Additionally, both adjuvants altered glyphosate distribution within the droplet residues on the different plant surfaces. The unformulated glyphosate solutions induced non-uniform, small droplet residues on all leaf surfaces tested. The addition of RSO 5 resulted in larger glyphosate residue areas, whereas these were aggregated in small patches on three of the weed species. On the other hand, *V. arvensis* showed one small residue area within the dried droplet. In contrast, addition of RSO 60 resulted in one patch of glyphosate residue within the difficult-to-wet species, whereas easy-to-wet species showed more distributed patches. Both adjuvants led to a higher biological efficacy in easy-to-wet species. However, only addition of RSO 5 reduced dry biomass from *S. viridis*, while *C. album* was not affected from adjuvants. In general no influence of droplet volume was observed. Reduction in dry biomass ranged from 70 % (in relation to untreated control; treatment: glyphosate + RSO 5, *S. media*) to 30% (treatment: glyphosate solely, *V. arvensis*). Irrespective of weed species, a Pearson's analysis showed significant correlations between area ultimately covered with glyphosate and biological efficacy for the unformulated glyphosate treatment, and for glyphosate + RSO 60, respectively.

## Biotechnological approaches to contain virus diseases in woody crop plants

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Virus and phytoplasma infections seriously limit stone fruit production in Europe and the Mediterranean region. The most important viral pathogen is Plum pox virus (PPV), however, other viruses, e.g. Prune dwarf virus (PDV) and *Prunus* necrotic ringspot virus (PNRSV), and European stone fruit yellows phytoplasma (ESFY) also represent major threats (Laimer 2003). To produce pathogen-free plant material, in vitro techniques combining thermotherapy and meristem preparation were adapted (Balla et al. 2002).

Genetic transformation is a key technology to enhance the potential of existing cultivars, by taking advantage of the increasing knowledge available in fruit tree genetics. Different traits have been modified in transgenic fruit trees, comprising altered processing and storage qualities, modified nutritional properties, i.e. the influence of desirable/ undesirable components, modified growth habit and vigor, resistance to biotic and abiotic stresses, e.g. drought, low temperature. The breeding and cultivation of virus resistant plants is a major contribution to the control of viral diseases (Laimer 2006), since there no chemical control strategies exist.

However, beyond technical feasibility, efficiency of resistance, environmental safety and IPRs, also public acceptance needs to be considered. Efforts are required to create public understanding and acceptance and to build public confidence for these crop plants. In the frame of the project “Characterisation of transgenic fruit trees and analyses of direct and indirect biological interactions” long-term stability of the introduced transgenes was considered a major issue and carefully analysed (Maghuly et al. 2007). (<http://www.boku.ac.at/sicherheitsforschung/open-e.htm>)

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## Nutraceutical value of local fruit species germplasm in agroecosystems of the Alps

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The aim of the research was to determine the nutraceutical value and the antioxidant power of traditional fruits, once grown in Piemonte (Northern Italy) in order to put in evidence their value as functional food. We studied old apple cultivars now still cultivated for local niche markets and for their adaptability to mountain environments, some accessions of mulberry (*Morus* spp.), and other minor fruits. The content in phenolics was analyzed according to the method of Slinkard and Singleton (1977), the vitamin C amount was measured with spectrophotometric method by enzymatic kit (Boehringer Mannheim R-Biopharm) and double reading to 578 nm.

The traditional apple cultivars showed a stronger antioxidant activity compared to Golden Delicious and other standard apple cultivars.

Fruit culture in the mountainous areas of the Alps can't compete in terms of costs with the intensive orchard systems.

The nutraceutical and sensorial characters of the local fruits can be valuable elements to distinguish this produce, and a tool of differentiation to obtain label certifications to attract the consumers.

## **Effects of cultivation temperature, chilling and inoculation with endophytes on photosynthetic parameters of different pepper (*Capsicum annuum*) varieties**

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### **Introduction**

Vegetables originating from subtropical regions like peppers can suffer from low temperatures (already above freezing temperatures). Thus pepper is an excellent model plant to study effects of low temperature on the photosynthetic apparatus.

In recent years it became more and more evident, that endophytic bacteria (= bacteria living inside the plant) can interact with the physiological status of the plant, which is dependent on the environmental situation. In this project we investigated the influence of chilling temperatures during the night in combination with inoculation of a selected endophytic strain on photosynthetic parameters during plant development and on fruit quality.

### **Materials and Methods**

Ten different pepper varieties were cultivated at three temperature levels (15, 25 and 35°C). Photosynthesis and chlorophyll fluorescence parameters were measured regularly to identify temperature sensitive and insensitive varieties. The variety Milder Spiral turned out to be a cold-sensitive and Ziegenhorn Bello an insensitive variety.

These two varieties were then cultivated in a climate chamber (18-23°C) and half of the plants were inoculated with an endophyte (*Arthrobacter* sp. "ZB04" isolated from Ziegenhorn Bello). One group of the plants was exposed to chilling temperatures during the night at two occasions, the first time at 6°C and the second time to 4°C. Photosynthesis parameters ( $A_{\text{sat}}$ ,  $C_i$ , Cond), chlorophyll fluorescence parameters ( $F_0$ ,  $F_m$ ,  $F_v$ ,  $F_v/F_m$ ,  $F_s$ ,  $Tf_{\text{max}}$  and Area) and chlorophyll content were measured during chilling and recovery at regular intervals.

### **Results**

Among the ten tested pepper varieties significant differences in the sensitivity to low temperatures could be detected based on the measurement of photosynthetic parameters. Plants inoculated with endophytes showed a significant reduction in the ground fluorescence ( $F_0$ ) compared to non-inoculated plants throughout the whole experiment. Chilling in the dark showed an influence on most of the measured parameters.

### **Conclusion**

Our results show that chilling temperatures can have negative effects on the photosynthetic apparatus independent of simultaneous exposure to light. Inoculation with endophytic bacteria obviously can have an effect on the photosynthetic apparatus of leaves.

## Differences in light (PAR) and their effect on the physiology and morphology of *Allium ursinum* L.

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*Allium ursinum* L. is having a Renaissance in the kitchens around Europe in the last years. It is distributed mainly as the fresh green leaf on the markets and during its season used in a lot of products. The estimated usage in the Switzerland in 2004 was 40 t. alone as fresh-leaf selling.

But *Allium ursinum* L. also referred to as wild garlic is known as a spice- and medicinal plant for about 500 years right now. It is not yet licensed to be a pharmaceutical product but at least as a dietary supplement.

As a perennial bulb-geophyt belonging to the family of the *Alliaceae* *Allium ursinum* L. is closely related to garlic (*Allium sativum*) and can mostly be found under beech forest.

To understand the plant and its requirements it is indispensable to describe the habitat the plant's growing on. For *Allium ursinum* it is not easy to find a habitat which is typical for the plant because of its ability to colonize a wide range of places. In the woods the conditions of different factors concerning the plants often varying widely in plots next to each other. One of these factors is the amount of incoming light at ground level which differs widely because of the irregular stand of the trees and their uneven allocation of leaf in the treetops.

So it is important to find out which amount of incoming light (PAR 400 – 720 nm as well as UV-A 320 – 400 nm and UV-B 280 – 320 nm) provides best conditions for growth of the plants. Aim of this study is to determine the factor light under natural conditions in the woods which seems to be necessary because of the existing inconsistent statements on this topic.

In 2006 Bonn University started an experiment concerning the factor light and its influence on *A. ursinum*. The trials took place in the woods of the Eifel next to a small village called Schloßtal on 395 – 410 m above sea-level with an average gradient around 10 °.

To describe the factor light repeating measurements of the amount of incoming light were done on plots with apparent differences of the allocation of the trees. The measurements were done by using three identical optometers with triple-head-gauges from gigahertz-optik® on plots next to each other at the same time compared with the measurements on a free field as a reference in 100 m distance from the two other plots. The measurements took place on the 12. of May 2006 at the best date for harvesting (before opening of the blossoms) when the treetops were not fully closed but the plots were already shaded.

Further on the LFS Marhof started experiments with two shading nets ("PH 55 open" & "PH 77 open") from TGU® in order to simulate a reduction of the incoming light comparable to the conditions in the woods. These two nets provide the best compromise between the absolute shading effects in a possible model compared to the increasing shade over the time by the growing leaf in the treetops on the natural habitat. These nets are supposed to reduce the PAR 55 % (PH 55 open) and 77 % (PH 77 open) which could be verified. It could also be found that the reduction of the UV-A and UV-B radiation was lowered in the same way so that the shading nets are feasible to imitate the conditions in the described wood besides the total of the radiation. For that reason heavier nets (also available) had to be used.

Tab. 1: Relative incoming PAR, UV-A and UV-B radiation in % in different plots in the woods (Eifel) compared to an unshaded plot (= 100 % of the light at ground level) in May 2006

	PAR	UV-A	UV-B
free area	100%	100%	100%
wider tree distances	2,50%	7,68%	7,99%
closer tree distances	1,35%	3,81%	3,39%

It could be found that in plots next to each other in the woods there were significant differences in the incoming light.

In both plots there was a higher reduction of the PAR compared to UV-A and UV-B but the decline of both of the UV-radiations was very consistent.

# Research concerning greenhouse tomatoes grown on organic substratum in Transilvania-Romania

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In the great majority of the countries with great greenhouses surfaces "soiless systems" were extended. The most used "soiless systems" are NFT, rockwool systems or organic substrata system, with a rigorous control of water supply and nutrition. (Choux Cl., Cl.Foury, 1994, Lemaire F., 1991). Since both hydroponics system and culture on synthetic substrata are expansive and require special equipment. The research team of the vegetable growing department, from USAMV Cluj-Napoca, Romania, focused their work on organic substrata system, using cheap and available materials easy to find in the region. A mixture of brown peat with other organic or inorganic materials was used like substrata for tomatoes culture in polyethylene bags with nutrient supply.

In Romania the greenhouses are cultivated in classical system (in soil), in two culture cycles, winter-summer and summer –autumn. Because of this, yields are relatively small, late and they are obtained with high expenses due to high energy and materials cost, with appreciably economic efficiency diminish of greenhouse cultures. The systems on organic substratum in mat polyethylene bags are simple, cheap, no polluting and can represent an alternative for soil culture systems, for Romania. The main advantages of "soiless systems" on organic substrata in polyethylene bags are: high decrease of substrate volume (about 4-5%), of water requirement (about 8-10%) and nutrients used (about 3-8%) comparing with classical system, in soil, requirements (Sonnenveld C., 1981).

The aim of the research team of the vegetable growing department was to establish the best substrate composition and volume for a plant, as well as the fertilization way. Partial peat replacement possibilities with other materials, as perlite or re-use peat were studied.

Increase of organic substrate volume from 6 to 12 l/plant had favorable effects and determines an improvement of yield and yield indicators. The volume of 8 l/plant substrate ensured the best efficiency both for early yield and for total yield too. Re-used of old substrate, steam disinfected, over fertilized, as well as a perlite mixed with new substrate can represent an efficient way for increased volume of substrate/plant when cost decrease of substrate is considerable. Growth and plant fructification were influenced both by composition and volume of substrata used. When new mixture and brown peat + perlite were used as a substrate, height of plants varied between 271.7 and 280.8 cm depending on volume of substrate used for each plant. The research undertook in the frame of Vegetable growing Department at USAMV Cluj-Napoca for greenhouse tomatoes cultivated on organic substrate realized a significant increase in early yield while for total yield 15-16 kg/mp were obtained, in a culture cycle beginning from January till July (Apahidean Al.S., and. col). Commercial quality of tomatoes fruit, recorded for all types of used substrate, was the best for a medium volume of substrate/plant( 8 l) comparing with the volume of 6 l /plant and with insignificant differences when the maximum volume 12 l/plant was used.

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# Effects of Intercropping-systems on Red Radish

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## **Introduction**

This research was conducted at the horticultural experimental site in Jedlersdorf (VZJ Gerasdorferstrasse, Vienna. Sandy loam above Danube's gravel, 162 m above sea level, continental climate: 9,8°C avg. temp, 1800 hrs avg. annual sunshine, ) in the frame of an interdisciplinary students' research project in horticulture.

## **Implementation**

Intercropping systems were set up as follows: 1) Radish/Radish (*Raphanus sativa* L., Brassicaceae) 2) Radish/Salad (*Lactuca sativa*, Asteraceae) 3) Radish/Carrot (*Daucus carota*, Umbelliferae) 4) Radish/Spinach (*Spinacia oleracea*, Chenopodiaceae) 5) Radish/Onion (*Allium cepa*, Alliaceae) 6) Radish/Pea (*Pisum sativum* convar. *medulare*, Leguminosae). Four repetitions per intercropping system were arranged in a randomized block trial (24 fields in total). Red radish and intercropping partners were sown in parallel rows on open air trial fields (25 m<sup>2</sup>) and cultivated (fertilization, irrigation) according to Austrian IP good-practice (integrated production). Emphasis was put on red-radish, which was regarded as the 'main crop' - and effects of intercropping partners on quality and harvest parameters were investigated. Weed management was done manually.

Red radish was harvested at optimum harvest stage, washed, green leaves were removed and discarded. Total fresh weights of red radish and intercropping partner were taken per field.

Laboratory investigations were conducted the following day: Individual fresh weight of all single radishes. Samples of 5 individual radishes for measures were taken: Two diameters (mm, electronic caliper), fresh weight (g, Merck electronic precision balance), colour of skin and flesh (CIE L\*a\*b; Minolta CR 200), tissue resistance (Mecmesin Penetrometer), dry weight (105 °C 2h, 85 °C 48 hours; Ehret aerated drying oven; Merck precision balance). Other samples of 5 radishes were homogenized and a press saps were used for taking measures of NO<sub>3</sub> concentration (semiquantitative method, Merck RQflex), P-value (electrochemical holistic quality measurement, BE-T-A Vincent) and Brix value (SSC-soluble solids content, digital Brix-meter, Atago). Calculations of dry matter and water contents were done as well as statistical analysis. Results are presented in tables and graphs.

## **Results**

Considering the results concerning the harvest parameters, the intercropping system with spinach shows negativ effects on radish, resulting in lower fresh weight and size. But considering quality parameters, the results show (weak) differences between the single intercropping systems. There may be further studies helpful to give clear evidence about which intercropping system is effective to manage red radish intercropping systems for the benefit of radish.

## Flavonoid and ascorbic acid content in a red-pigmented *Lactuca sativa* variety as affected by different light conditions

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Light serves as a source of energy and information in plant life and thus represents a major determinant of metabolism and development. At the same time, short wavelength UV-B radiation may have damaging effects on molecular structures and therefore activates various plant defence mechanisms. Protection is provided for example by the accumulation of metabolites with UV-B screening and possibly antioxidative capacity, e.g. flavonoids. The objective of this study was to determine qualitative and quantitative differences in the flavonoid pattern as well as the ascorbic acid content in *L. sativa* 'Bughatti' cultivated under green house foils and glasses differing in the rate of light transmission particularly in the UV-B region. An elevated content in UV-B screening pigments and antioxidants may facilitate adaptation to outdoor conditions when transferring plants to the field.

Experiments were performed in six green houses covered with three different materials which provide a UV-B transmission of 16% (Floatglass), 37% (MM-AR glass) and 74% (ETFE foil), respectively. The abbreviation 'MM-AR' describes the microstructured surface of the glass which is coated with an antireflex layer; 'ETFE' is a fluorized polymer. The red-pigmented lettuce cultivar was grown from seed in trays containing 100 small press pots. In order to examine preadaptation to outdoor conditions, a part of the seedlings was transplanted to the field 22 days after sowing. The greenhouse cultivar was sampled approximately every 4 days from the 15<sup>th</sup> to the 30<sup>th</sup> day and the field cultivar once a week from the 22<sup>nd</sup> to the 36<sup>th</sup> day. Samples were analysed for flavonoid and ascorbic acid content. Determination of flavonoids in plant extracts was performed by HPLC-MWD. Additionally, anthocyanidine concentration was measured spectrophotometrically at 535 nm. Ascorbic acid content was determined following the enzymatic method of Boehringer-Mannheim GmbH, FRG. Experiments were repeated three times from April to June 2007. Throughout the whole experimental period radiation intensities were monitored in the greenhouses and in the field.

The results indicated a positive correlation between flavonoid content in *L. sativa* and the degree of UV-B transmission of the covering materials. Anthocyanidine as well as quercetin levels were significantly elevated in the order of increasing UV-B exposition of the plants. Consequently, plants grown under Floatglass showed the lowest anthocyanidine content, under MM-AR glass and ETFE foil values were 27% and 47% higher, respectively. Quercetin was increased by 15% and 32% under MM-AR glass and ETFE foil. Kaempferol, only detected in trace amounts, showed the same trend. After transfer of plants to the field, differences in the flavonoid content among treatments were completely compensated within 24 h. Thus a possible advantage provided by a higher flavonoid content of plants grown under a UV-B transmissive material lasts for only one day. Role and function of ascorbic acid are currently under evaluation. As light conditions, especially UV-B intensity, may influence plant morphology and physiology in various manners, potential impact of UV-B transmissive materials on other aspects of plant fitness (e.g. growth and pathogen resistance) remains to be elucidated.

# **Multimedia support in the problem-based and interdisciplinary lecture on 'Agricultural Marketing and Quality Management'**

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## ***E-Learning and Multimedia***

Within the last few years increasing technical possibilities for processing and provision of information stimulate the discussion about potentials and tasks of these techniques in the context of teaching and learning processes. The requirements to graduates of universities and universities of applied sciences are increasing constantly. Collaborative learning offers opportunities, to increase the share of self-organized, problem-based learning. Such modules can be developed also in the context of e-learning concepts. In the project funded by the multimedia centre of the Humboldt University such elements will be implemented into the BSc module *Agricultural Marketing and Quality Management*. Besides a scientifically sound arrangement of the subject by lectures to provide basic theoretical and methodical knowledge also the ability to solve complex problems shall be promoted. The ability to solve complex problems shall be specially supported by problem based learning activities. The representation of the problems to be worked out by students is supported by multimedia. This shall help to overcome the problem of so-called tacit knowledge. And in addition economic aspects belong certainly to the advantages of e-learning. E-learning can happen independently of personal presence. Students are spatially and temporally independent and thus they can repeat the subject in the same way as often as they want to.

## ***Anchored Instruction***

The project counts on the concept of the Anchored Instruction. This represents the attempt to improve the applicability of theoretical knowledge. Anchored instruction arose from the problem cited in education literature, suggesting students' knowledge often remains "inert" and cannot be used in response to many different changing situations or problems. The innovation was to situate learning in realistic and professional field. A central feature is a narrative anchor to arouse the interest and to focus the attention on main features of the problems. Findings show that the use of media, particularly videos, create suitable conditions to work successfully on realistic problems to discover new situations and to acquire new skills and abilities. Besides it motivates to find solutions, equalises complexity and it strengthens the abilities to get involved with the problem.

## ***Virtual Field Trips***

The given problem situations represent complex but comprehensible contexts in narrative form. Case studies videos of approx. 10-15 min., so-called Virtual Field Trips offer the basis for the problem solving process. The videos to be made are assigned to the individual topics of the module, tied by further teaching materials into the learning platform *Moodle*. In these videos the students get the state of knowledge adapted to problem situations represented in cooperation with agricultural enterprises. Lecture material is also embedded in the videos and completes the theoretical background knowledge. Students have to answer questions, develop solutions, extract key issues, facts and data individually or in groups. The results are discussed online in Moodle and in the lecture.

## ***Potentials***

Those problem-based learning units shall be developed gradually. On the long run the experiences from this project are supposed to improve effective planning processes for designing collaborative learning and to be transferred to modules of the Master Program. The generated films are also designed for integration into teaching at other Faculties of Agriculture and Horticulture. In addition, they can easily be used as base for Marketing lectures by completing with individual materials and interested Faculties can get hints and suggestions for creating Virtual Field Trips on their own.

# A process oriented and stochastic model for asparagus spear growth

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## *Model*

A process oriented simulation model for the prediction of asparagus spear yield is presented. The main environmental information used is the soil temperature at different depths. Apart from classical approaches a population of asparagus plants is considered. For each plant, there are independently growing bud clusters which produce one spear after another, i.e. there is a strong apical dominance of the growing spear over the resting spears within a bud cluster. A new spear can only grow, if the precursor was removed through harvest. Length growth of spears is described by exponential growth, but the actual relative growth rate depends both on spear tip temperature and spear length. These relations were established from independent experiments. The bud cluster number per plant is simulated as binomial distributed variable as indicated by experimental results. The dynamics of spear diameter was described by a simple empirical relation to spear order number within a bud cluster. The higher the spear order, the smaller is the mean spear diameter. Distribution of spear diameter can be well represented by a two-parameter beta distribution, where parameters are derived from simple relations to the mean diameter. The initiation time for individual bud cluster varies by a normal distribution around a mean starting day for the population. Spear growth is simulated using a daily time step, while efficient algorithmic corrections are introduced for the effects of diurnal temperature and spear diameter variations. Assuming one harvest per day, all spears attained a length greater 37 cm within 24 hours prior daily harvest start were summed up for spear yield prediction.

## *Results*

Very different spear growth dynamics were observed during an experiment with the application of diverse plastic coverings (7) including the coldest and warmest possible modes (e.g. white plastic versus antitrop plastic coverings). The simulation considered a population of 500 asparagus plants which matched exactly the conditions of the validation experiment. Measured daily soil temperatures at 5, 20 and 40 cm below ridge top and daily spear yields were available for each used covering treatment during 60 days in 2002. Only the mean starting date of bud clusters initiation was treated as a treatment (plastic) specific parameter. Using one overall parameter set for all treatments, predicted and measured daily spear yields showed a very good agreement, which could not be obtained by a concurrently tested empirical model approach. It can be shown theoretically, that a combined approach of apical dominance and population dynamics can explain the growth periodicity of asparagus spear populations. There is a strong correspondence between the mean duration between the harvest of two consecutive spears and the time period of spear yield variation. Such a periodicity was also established by the empirical model.

Compared to the empirical model approach a process oriented and stochastic approach had two advantages:

- (1) Spear yield of all temperature treatments could be well simulated with one parameter set.
- (2) Periodicity of the simulated spear yield is an intrinsic feature of the model.

These results seem to favour process oriented and population based models of spear growth in basic and applied model applications.

# Effects of mechanically induced stress on growth and development of tomato (*Lycopersicon esculentum* Mill.) seedlings

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## Introduction

The objectives of tomato seedling production are short and stocky plants that are resistant to damage and quickly resume growth after transplanting into field or greenhouse. Plant growth retardants are excluded from use on all food crop plants, making alternative ways of growth control necessary. Tomato seedlings (*Lycopersicon esculentum* Mill.) were mechanically stressed to study thigmotress induced changes and to gain information on the consistency of induced effects while on-going culturing under greenhouse conditions.

## Materials and Methods

Two on commercial scale important cultivars 'Vanessa' and 'Loreto' were cultured in greenhouse at the experimental station for horticulture, University of Hohenheim (Germany). Optimal growth conditions were ensured by providing sufficient water, fertilizer and plant protection management almost without mechanical impacts apart from treatments.

Treatment started three weeks after germination, i.e. when the seedlings reached the four leaf stage. The treatments were wind treatment by ventilator (1), brushing 10x (2), brushing 20x (3), brushing 30x (4), music (5), re-position (6) and untreated control (7). The tomato seedlings were treated twice a day for four weeks. Measurements on plant height, leaf and inflorescence development (taken in first and second trial) were taken until week 12 after start of the treatments. Stem diameter, fresh and dry matter, and leaf area were measured specific leaf area, and specific leaf weight were calculated (only in second trial).

## Results

The results show that stem elongation of tomato seedlings was significantly reduced in all mechanically stressed treatments. Brushing 30x resulted in the most evident reduction in plant height (33% compared to untreated control). This effect was still consistent for several weeks after stopping the treatments and differences in plant height were significant. Caused through brushing leaf area was only reduced in the highest brushing treatment (30x). Specific leaf area was significantly higher in all brushing treatments than in control plants which means that brushed leaves were thinner. Stem diameter, dry and fresh matter of leaves which were developed while treatment were not affected.

For yield important factors like number of leaves as well as number and appearance of inflorescences the data from present experiments mostly revealed no influence.

Regarding plant height there were no differences between ventilator and music treated plants but those plants were significantly higher than brushed plants. During monitoring period ventilator treated plants initially grew faster than music treated plants. Plants of ventilator, music, and re-position treatments almost reached height of untreated tomato seedlings.

The two cultivars 'Vanessa' and 'Loreto' exhibited differing results to different forms of thigmotress. While 'Loreto', a cultivar with distinctively high growth rates, was proportionally less influenced through brushing treatments 'Vanessa' showed growth reduction to a greater extent. Ventilator, music, and re-position nearly had identical influence on the cultivars.

## Conclusion

Mechanically induced stress is an effective means of growth control with mainly no negative influence on further development. The differences in cultivar responses and in responses to different forms of thigmotress have to be further defined to give growers exact instructions for their production.

# Application of manure fertilizer on the basis of sheep's wool in the horticulture

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Within the framework of a research and development project together with the Institut für Neuwirtschaft Anwenderzentrum GmbH Lauchhammer (Germany) we developed a new manure fertilizer based on sheep's wool. In cooperation with the Institute of Horticultural Sciences at the Humboldt University of Berlin various fertilizer experiments with ornamental plants and vegetables were performed.

The aim of the experiments was to test the agronomical effect of the new developed pelleted fertilizer made from sheep's wool in greenhouses and outdoors.

Fertilization experiments were carried out with *Euphorbia pulcherrima* (variety 'Tosca' and variety 'Primerio') and *Lycopersicon lycopersicum* (variety 'Alkassar') in a greenhouse. It was shown that the pelleted fertilizer has a very high, long-term fertilizing effect for poinsettias as well as for tomatoes.

The application of pelleted fertilizer was varied between 1 and 10 g/l substrate by poinsettias. For comparison a control application was not fertilized. The best plant growth was obtained for poinsettias with a fertilizer application of 10 g/l substrate. At the end of this experiment the average fresh weight of plants with a fertilizer application of 10 g/l was up to 2/3 higher than the control. The life duration of poinsettias fertilized with new pellets was also much higher than control.

The cultivation of tomatoes was carried out on two substrates: perlite and bark compost. 100 g pelleted fertilizer per plant was mixed into the substrate. Again plants without pelleted fertilizer were used as a control. The same nutrient solution was added for all plants. As a result of the experiment it was found, that the plant growth could be increased by applying pelleted sheep's wool. Due to the application of the new fertilizer in perlite the yield of tomatoes was by 20 % higher than that of the control, in bark compost the yield increase of tomatoes amounted 14 %.

We performed new fertilizer experiments outdoors with petunia, iceberg lettuce and kohlrabi. The final results haven't come in yet.

## **Introducing e-learning to horticulture teaching and training**

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### ***Introducing e-learning to horticulture teaching and training***

LVG Heidelberg is a state horticultural college and a research station for horticulture in the federal state of Baden-Württemberg, Germany. Currently, about 150 professionals are being trained per year. Since 2002 LVG Heidelberg is working on a blended learning concept for horticultural training.

Blended learning represents a combination of traditional face-to-face teaching in the classroom and computer-based e-learning at home. This combination reflects the actual working conditions where flexibility and one's own initiative are essential qualities for having success in business. For a lot of our students this is the only way to reconcile family and business life with continuing education. During the e-learning part the student is given a possibility to arrange the time of learning independent of time and place. Autonomous learning processes can be initiated and establish a good starting point for lifelong learning.

In contrast to usual e-learning programs focusing on a wide variety of end-users the e-learning programs of LVG Heidelberg are specialized on applied horticultural subjects. A lot of learning content can be presented more efficiently by multimedia than by traditional techniques. Pictures, movies and animations can be included and thus improve learning efforts. Interactivity is the basic tool to maintain the interest of e-learning students. Video, audio and all other parts of the e-learning courses are designed, produced and coded on-site, based on the screenplays written by our teachers. Due to the teacher's large part in authoring we keep a close connection between face-to-face teaching and computer-based learning.

The open-source learning-platform OLAT (Online Learning And Training) is the main tool for accessing and using the learning material (<http://www.gartenbauschule.de>). A wide collection of supplemental training material from the teachers is stored on the platform. Cooperative learning processes can be intensified by the use of this learning platform with interactive functions like e-mail, forum, chat and wiki.

By developing multimedia learning software LVG Heidelberg experience shows that students have to be conducted very closely through the learning modules. The teachers lead the students through the e-learning part and give assistance in all their problems. So every learner receives contemporary answers to his questions.

In 2007 LVG Heidelberg started a Leonardo-da-Vinci e-learning-pilot-project with the European partners France, Great Britain, the Netherlands, Sweden, Hungary and Latvia. This e-learning course will be provided for European arboriculturists on an internet-platform. Following 5 successful years in developing e-learning LVG Heidelberg conducts technical coordination, support and advice of all partners. We train our partners in blended learning concepts, in writing screenplays which are coded into a multimedia integrated course in English language.

In addition to that we also develop other e-learning modules for several organizations in horticulture. Modules for economics have already been produced while others are in progress. We would be glad giving you information and support in all needs of e-learning concepts and establishing computer-based learning environments.

# Effect of a new “silver” PE greenhouse covering film on microclimate and production of a tomato crop

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## *Introduction*

High temperature levels characterise the greenhouse microclimate during summer in Mediterranean areas, if no measures are taken to control the greenhouse microclimate; and yield and quality of greenhouse crops grown under such conditions may be suppressed. Accordingly, greenhouse cooling is a matter of increasing interest in these regions. Various methods for cooling the greenhouse atmosphere may be used, with natural ventilation and whitening the cover material being the normal practice. A new technique for greenhouse cooling is the use of films that exclude excessive heat during day-time by reflecting and/or absorbing the Near Infra-Red (NIR) radiation, the part of solar spectrum carrying most of the heat entering a greenhouse in day-time and which is otherwise useless for plants growth. Aim of this work was to study the effects of a new polyethylene (PE) "silver" film (with high reflection and absorption in NIR radiation) on greenhouse microclimate and on growth and production of a hydroponic tomato crop.

## *Materials and Methods*

The experiments were carried out from November to July of 2005 in two similar greenhouses covered by different PE films made by Plastika Kritis S.A.: one covered by a PE film with high reflection and absorption to NIR radiation (NIR-PE); and the second covered by a common PE film (control greenhouse, C-PE). The tomato crop (cv. Belladona) was planted on 15 November 2004 (winter season), and on 15 April 2005, (summer season), in perlite slabs with a plant density of 2.4 plants·m<sup>-2</sup>. The spectral properties of the cover materials were measured by a portable spectroradiometer and an integrated sphere. Greenhouse and the outside microclimate parameters such as air temperature, vapour pressure deficit and solar radiation were recorded. Furthermore, crop growth as long as production and quality measurements were also carried out.

## *Results and Discussion*

The reflectance and absorptance of the films to radiation from 700 nm to 1100 nm were found to be 0.20 and 0.16 for the NIR-PE and 0.12 and 0.05 for the C-PE, respectively. However, the results of the microclimate measurements showed that the air temperature and vapour pressure deficit levels were similar in both greenhouses. It has to be noted that both greenhouses were heated and ventilated when the air temperature was lower than 14°C and higher than 23°C, respectively. The mean height of the tomato plants measured during winter was higher in the C-PE greenhouse while during summer was higher in the NIR-PE greenhouse. No differences were found in the number of nodes of plants. The leaf area index of the tomato crop was higher under the NIR-PE greenhouse during the summer period. The total crop production was similar between the two greenhouses for both winter and summer period. However, marketable tomato production was higher in the NIR-PE greenhouse.

## *Conclusion*

In conclusion, it seems that under the conditions created during the experiments, no temperature and vapour pressure differences were induced by the use of the NIR-PE film. The lower solar radiation levels observed under the NIR-PE covered greenhouses did not affected the growth and production of tomato plants but increased slightly the marketable production of the crop.



# Comparing of yield and nutrient status of greenhouse tomato grown in drainage and recirculating nutrient systems

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There is important interest to introduce for the horticultural practice a new method of plant cultivations which could be friendly to environment. One of them is the application of closed fertilization systems, mainly with the recirculating nutrient solution.

The main purpose of the study was to compare the yield and nutrient status in leaves in the cultivation of greenhouse tomato with the application of drip fertigation which functioned in the drainage system (without recirculation) with the recirculating nutrient system.

The experiments were done in 2005 – 2006 under greenhouse condition with the growing of tomato cv. 'Emotion F<sub>1</sub>' in the rockwool. Experiments were conducting from the middle of April to the end of October each year. In the drainage system about 26.3 – 57.1 % of nutrient solution (drainage water) was flooded from the rockwool slabs and collected in a tank. Next it was used to fertilization other cultures (vegetables and turfgrass). In the drainage system nutrient solution was prepared from stock solutions gathered in tanks A and B and 100 times diluted by Dosatron apparatus. In the recirculating nutrient system 36.1 – 42.7 % of the drainage water, after disinfection with application UV lamp, and mixing with the tap water was applied again. Nutrient solution was prepared by mixer Scangrow from the stock solution in tanks A, B and C (nitric acid) after 100 times dilution.

Tomato fruit yields with sorting on different classes were registered during the cultivation period. In the monthly intervals differences in nutrient concentration in rhizosphere both in the drainage and recirculating nutrient systems were determined. Also the status of macro and microelements in the leaves were analyzed.

It was find out, there were not significant differences in the total and marketable tomato fruit yield between the drainage and recirculation nutrient systems. Content of potassium and copper in the index plant part (8-9 leaf from the top) was significant higher in the drainage system but calcium, magnesium, sulphur and zinc in the recirculation one. In the recirculating nutrient system there was more intensive accumulation of calcium, sodium, chlorides, sulphates and zinc in the nutrient solution collected from the rockwool slabs than in the drainage system.

In the recirculating nutrient system it was saved average 45.7 % of water in comparison to the drainage system and the following amounts of nutrients (in %): 36.3 N-NO<sub>3</sub>, 15.6 P, 41.2 K, 41.8 Ca, 30.1 Mg, 29.9 S-SO<sub>4</sub>, 43.4 Fe, 35.5 Zn, 10.4 Mn, 43.4 Cu, 32.1 B. There were no visible symptoms of root diseases in the both tested systems.

The study shown that high yield of plants and significant saving of water and nutrients and protection of natural environment allow recommending wider application the recirculating nutrient system to the horticultural practice.

# Development of techniques for rapid production of rattan seedlings

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## Introduction

Rattan industry in Africa has bright prospect for expansion. However, this is hampered by the scarcity of raw materials of rattan (Zoro Bi and Kouakou 2004; Zoro Bi and Kouassi 2004). The low supply of rattan has been attributed to the destruction of natural forest, lack of program for rattan regeneration and plantation allowing the production of planting materials at a large scale in large quantities. Germination of rattan seeds is generally poor and seeds, if available, have erratic germination. Manokaran (1978), Mori and Rahman (1980) found that seeds of various rattan species require 4 to 41 weeks to germinate and percentage of germination varies from 0.02 to 79%. The aim of this work is to develop a strategy which can speed up seed germination in order to develop an efficient seedlings production for rattan.

## Material and methods

Seeds of *Laccosperma secundiflorum* and *Eremospatha macrocarpa* were collected in December and January 2007. *E. macrocarpa* (cane diameter <2cm) is known to be the best source of cane in Africa. *L. secundiflorum* (cane diameter ≥3cm) is highly prized as source of cane and used predominantly for furniture frames. Fresh seeds were washed in tap water, treated with detergent solution and washed in sterile water. Excised embryos were washed in sterile water and planted on the following media: medium (M1) made of Murashige and Skoog salt (1962) supplemented with *myo*-inositol (100mg/l), casein hydrolysate (500mg/l); M2 and M3 consist of M1 supplemented with Gibberellins 10<sup>-6</sup>M and 10<sup>-5</sup>M respectively and M4 Gamborg basal medium. The embryos were incubated in growth chamber at 28 ± 2°C in total darkness. As for the nursery, the cleaned seeds (after scarification) were subjected to various tests of breaking dormancy (pre-chilling to 0°C during 4 days, seeds imbibed in GA<sub>3</sub> 10<sup>-6</sup> to 10<sup>-5</sup>M during 4 days; in KNO<sub>3</sub> 10<sup>-3</sup>M to 10<sup>-4</sup>M during 4 days and in H<sub>2</sub>O<sub>2</sub> 5%, 10% and 20% during 1 hour).

## Results and Discussion

Germination is noted when the radicle emerged and the young conical shoot took shape. A significant difference was observed between the different media used. The media M2 and M3 gave 29% and 100% of germination rate after respectively 6 and 20 days of incubation for *E. macrocarpa*; 50% and 100% of germination rate after respectively 10 and 24 days of incubation for *L. secundiflorum*. Media M1 and M4 gave 75 to 85% of germination rate after 30 days of inoculation. In the nursery, the first germination was observed 36 days after sowing for *E. macrocarpa* and 60 days for *L. secundiflorum*. Seeds imbibed in GA<sub>3</sub> 10<sup>-5</sup> and 10<sup>-6</sup>M gave 92% and 57% germination rate after 245 days of sowing for *E. macrocarpa* against 24% and 20% for *L. secundiflorum*. Excised embryos germinate *in vitro* during few days in presence of GA<sub>3</sub>. These results suggest that seeds of the two rattan species may have exogenous and/or endogenous dormancy. Indeed, rattan seeds were covered by an impermeable testa which causes the restriction of gaseous and water uptake by embryo (Bradbeer 1988). The dormancy could also result from the fact that the radicle was unable to develop sufficient thrust to cause testa rupture.

Besides maturity and freshness of seeds, the addition of GA<sub>3</sub> in the culture media and imbibed seed in GA<sub>3</sub> solution is adequate to speed up rattan seed germination.

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## **Productive and vegetative responses of 'Gala' and 'Fuji' apple trees under controlled irrigation strategies**

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### ***Introduction***

Maximizing fruit production and quality with minimum irrigation inputs, i.e., increasing plant water use efficiency is essential in the dry climate of southern Mediterranean regions. Regulated deficit irrigation (RDI) was developed to minimize irrigation inputs for fruit production. It consists in the withholding of water during certain periods to produce a moderate drought stress and obtain beneficial consequences on fruit quality and reductions of vegetative growth. Although results of RDI experiments have been promising for some fruit crops, in species like apple and grapes with concurrent fruit and shoot growth, RDI reduces fruit size and yields (Lötter et al., 1985; Ebel et al., 1995). Further efforts toward improving irrigation efficiency of grapes in Australia has led to the development of a novel technique, partial rootzone drying (PRD), where only one half of the rootzone is irrigated, whereas the other half is not, alternating the two sides at specific intervals (Dry et al., 1995). The physiological basis for PRD is that roots in drying soil produce abscisic acid (ABA) which is translocated to the leaves, inducing partial stomatal closure, reduced transpiration and increased water use efficiency. Since the other half of the rootzone is kept well watered, the effect on plant water potential is minimal and most metabolic and physiological processes associated to water stress are not affected (Dry et al., 1995; Dry et al., 2000). The objective of this work was to investigate the effect of PRD on yields and fruit quality of 'Gala' and 'Fuji' apple trees in the dry climate of central Sicily.

### ***Materials and Methods***

The experiment was conducted near Caltavuturo, in central Sicily, using 72 six-year-old apple trees (36 'Gala' and 36 'Fuji') grown on M.9 rootstock and arranged in a randomized block design. Three treatments were imposed: Conventional irrigation (CI), where 104 mm of water were distributed by a drip system in 17 events to maintain soil moisture above 80% of field capacity; PRD irrigation, where only one side of the rootzone (alternated every 2-3 weeks) received 50% of the CI water; and continuous deficit irrigation (DI), where 50% of the CI irrigation water was applied to both sides of the rootzone. Soil water tension (SWT), stomatal conductance ( $g_s$ ), fruit growth, and shoot extension were recorded weekly, yield and fruit quality after harvest.

### ***Results and Conclusions***

In CI trees and on the wet side of PRD trees, SWT remained generally above -50 kPa (about 80% field capacity) and exhibited fluctuations due to irrigation cycles. In DI and the dry side of PRD trees, SWT progressively decreased to values around -200 kPa. In PRD trees, wet and dry sides were alternated when SWT of dry side reached -100 kPa. In both 'Gala' and 'Fuji',  $g_s$  of DI trees was significantly lower than that of CI trees (52% reductions) during the entire irrigation period, while  $g_s$  of PRD trees exhibited intermediate levels (24% reductions). In both cultivars, fruit and shoot growth rates were similar for all treatments although 'Gala' fruits of PRD trees tended to be larger than those of CI and DI at harvest, and 'Fuji' shoots stopped growing earlier than 'Gala' shoots. In both cultivars, trees of the three treatments had similar yields, number of fruits, yield efficiency, although PRD tended to reduce yields of 'Gala' trees. In 'Gala', fruit quality was similar for all treatments; in 'Fuji', fruits of PRD and DI trees were firmer and better colored than those of CI trees. This first year of observations suggests that, in 'Fuji', increases in water use efficiency may be attrib-

uted to a simple reduction of irrigation volumes (DI), while fruit quality improvements seem to be more consistent under the PRD regime. In 'Gala', both deficit irrigation strategies could lead to significant yield reductions over the years.

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## **Study regarding to the application of some cultural techniques with the view to obtain "reduced size plants" - Establishing of some methods of reducing of size plants at some flower species decorative by leaves**

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### **Introduction**

The diversity that characterises the floriculture is also manifested in the appearance, during the last years, of a growing interest from the consumers and producers, for the group of reduced size plants (RSP). They can be used in small places: flats, miniature gardens in containers, balconies and terraces, places that are highly appreciated nowadays. (Vidalie H., 2004) The origin of this group of plants can be specific (plants with reduced size or slow growing plants in the early years), genetic (newly created cultivars with reduced sizes) and cultural (reduction of the quantity of soil, hydric, thermic stress, applying retardants.) (M. Manda and al, 2007)

This paper presents the results of the research that had as purpose to obtain reduced size plants at two ornamental species *Syngonium podophyllum* and *Anthurium andreanum*, grown in pots, by application of different cultural methods (reducing the nutrition space, hydric stress), results that are part of a research project that implies the formation and studying of a reduced size plants collection.

### **Material and methods**

The biologic material consisted of *Syngonium* and *Anthurium* plants, which can be found in the collection of the Floriculture Department of the Faculty of Horticulture in Craiova City, Romania. The material obtained from cuttings, and bush separation was planted in a mixture of peat and perlite (1:1). In case of *Syngonium*, the rooted cuttings were planted in pots of different dimensions (diameter of 12 cm-V1, 10 cm-V2 and 8 cm-V3). In case of *Anthurium*, the plants which resulted from the bush separation were planted in pots with the diameter of 10 cm. After 1 month, they were watered with different quantities of water (50 ml-V1, 30 ml-V2, 15 ml-V3). By repeated determinations, we followed: the evolution of vegetative growth, the morphologic characters and the active photosynthetic radiation (determined with Mini-Pam portable Chlorophyll Fluorometer) according to the substrate volume and the quantity of administered water.

### **Results and discussions**

In *Syngonium*, the data which was registered in about 4 months after the cutting rooting shows that the height of the plants cultivated in pots with a diameter of 10 cm (V1) increased with 89% than the plants cultivated in pots with a diameter of 6 cm (V3). Following the decrease of nutrition area, the average length of the internodes at the end of the experimenting period significantly decreased from 15 cm when the plants benefited from an optimal nutrition area (V1) to 1.8 cm when the plants grew with a minimum substrate volume (V3). The average number of leaves per plant proportionally decreased with the available substrate

volume of the plants; therefore, at last determination, the values were between 11,6 leaves (V3) and 18 leaves (V2). In *Anthurium*, applying the hydric stress determined, 6 months after the plants were put in the pots, a decrease of the plant height with 26% for the case when we administered 15 ml of water per pot, as compared to the case when we administered 50 ml water per pot. All the analyzed morphologic characters (plants' height, number and length of leaves, number of flowers at a plant), registered the lowest values when we administered a minimum quantity of water (V3-15 ml). In both species, we noticed a decrease of the values of the active photosynthetic radiation with the reduction the substrate volume respectively of the water quantity used.

### **Conclusions**

The results we obtained show that the decrease of the sublayer volume, as well as the hydric stress can be used as methods in getting RSP in *Syngonium* and *Anthurium*.

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## **The BBCH Scale - Codification and description of phenological growth stages of plants and there international use in horticultural and agricultural research**

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As all branches of science, the individual disciplines in horticultural plant research also work more closely together, and, in addition, have become more international. The exchange of new findings and joint work on projects presuppose, however, that all those involved have the same understanding of the terms they use. This calls the need for an extensively standardised description of plant development stages in order of their phenological characteristics and their coding.

The extended BBCH-scale is a two and three digit coding system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species. The phenological development stages of plants are used in horticultural and agricultural practice and science, meteorology, climatology and insurances, each with its own varying individual objectives. The BBCH coding system is an integral part of the EPPO guidelines.

### **The basic principles of the BBCH scale**

- The general scale forms the framework within which the individual scales are developed. It can also be used for those plant species for which no special scale is currently available.
- Similar phenological development stages of each plant species are given the same code.
- For each code, a description is given.
- For the description of the phenological development stages, clear and easily recognised (external) morphological characteristics are used.
- Except where stated otherwise, only the development of the main stem is taken into consideration.
- The growth stages refer to representative individual plants within the crop stand.
- Relative values relating to species- and/or variety-specific ultimate sizes are used for the indication of sizes.
- The secondary growth stages 0 to 8 correspond to the respective ordinal numbers or percentage values. For example stage 3 could represent: 3rd true leaf, 3rd tiller, 3rd node or 30 % of the flowers open.
- Post harvest or storage treatment is coded 99.
- Seed treatment before planting is coded 00.

BBCH Codes in horticulture plants: Bulb, Root and stem vegetables, Leaf vegetables, Pea, Brussels sprout, Cauliflower, Cucurbits, Beans, Solanaceous, Ginseng, Pome- and Stonefruit trees, currants, strawberries, Olive, Loquat tree, Apricot tree, Quince tree, Pomegranate tree and Raspberry.

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## **Restricted Irrigation Effects on Peach and Nectarine Fruit Growth, Quality and Storage Ability**

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Peaches require fair amounts of irrigation water for shoot and fruit growth. Restriction of water availability in a fresh market peach cv. Royal Glory and nectarine cv. Caldesi 2000 orchard was studied in 2006 as a means of saving water resources, but also possibly altering fruit quality and storage ability. Each eight years' old control tree received irrigation water from two drippers at 0,5 m<sup>3</sup>/wk, while each tree with restricted irrigation received 75% of the above during the last 20-25 days before harvest (end of June – early July) and 50% during postharvest. Fruit growth was negatively affected mainly in nectarines from restricted irrigation. Fruit quality was followed at harvest and after 2, 4 and 6 wks cold storage of fruit harvested from the upper and the lower part of the canopy. Fruit skin color, flesh firmness, titratable acidity and specific conductivity were not affected by restricted irrigation but low water availability increased fruit final conductivity, total phenols and, mainly in nectarines, fruit flesh dry matter. Thus, besides smaller fruit size, restricted irrigation seemed to positively affect fruit quality without altering fruit maturity at harvest. Quality changes during storage were as expected but, in particular, skin red color improved and flesh firmness and specific conductivity showed similar changes with storage time in each cv.. The appearance of internal breakdown for the two cultivars and treatments will be discussed. Finally, fruit from the upper part of the canopy were of superior quality and advanced maturity because of redder skin color (mainly in peaches), higher soluble solids and dry matter content, higher phenols and specific conductivity and lower flesh firmness.

## **Optimized nitrogen management in the production of chicory roots with respect to chicon quality**

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### **Introduction**

In Switzerland, in connection with modernization of the chicory production, the question of an optimized fertilization strategy in the field with respect to the quality of the chicons arises. Former investigations revealed that availability of potassium in the soil might affect both the root development in the field and the

formation of the chicons at forcing to a high extent (Neuweiler and Flisch, 2005). Hence the importance of fertilization with nitrogen (N) in the chicory fields should be investigated.

### ***Material and methods***

The field experiments were established in the north-eastern part of Switzerland in 2004 and 2005. The soils were sandy loams. In 2004, after a first application at sowing, which was 30 kg N/ha for all treatments, increasing rates of nitrogen applied 3 times between the 5<sup>th</sup> and the 11<sup>th</sup> week of the crop development were investigated. Procedures were as follows: 30 + 0, 30 + 3x15, 30 + 3x30 and 30 + 3x60 kg N/ha. In 2005, nitrogen fertilization was varied both at sowing and during the proceeding development of the crop. Procedures with totally 4 applications included 3 applications to the developing crop in the 5<sup>th</sup>, 8<sup>th</sup> and 11<sup>th</sup> week after sowing. In procedures with 5 applications a further application followed in the 15<sup>th</sup> week. The following strategies were investigated: 0 (no N fertilization), 0 + 3x15, 0 + 3x30, 0 + 3x60, 30 + 3x15, 30 + 3x30, 18 + 4x18, 36 + 4x36 kg N/ha. During the crop development concentrations of available mineral nitrogen in the soil and of nitrate in the petioles were measured. The chicory roots were harvested in late October. Before roots were cold-stored their diameter was calibrated and samples were collected, which thereafter were dried and analyzed for their concentration of total N. The cold-stored roots were divided into two lots, which were forced in a hydroponic procedure during 21 days in February and October of the following years. At harvest, the chicon yield and quality parameters such as shape and solidity, which determine marketability, were assessed.

### ***Results and discussion***

In both experiments, the concentration of available nitrogen ( $N_{\min}$ ) in the soil layer 0 to 60 cm decreased with proceeding crop development. High rates of N fertilization did not lead to a significantly increased level of  $N_{\min}$  in the 11<sup>th</sup> week after sowing any longer. However, chemical analyses performed on samples of petioles in the 11<sup>th</sup> week indicated an increasing uptake of nitrate by the chicory plant with progressive rates of N fertilization. In addition, concentration of total N measured in the harvested roots significantly rose with increasing N fertilization. It can be assumed that higher rates of nitrogen stimulated the chicory crop to absorb nitrogen from the soil to a higher extent, leading to an increased accumulation of nitrogen in the roots. In both experimentation periods root diameter did not respond to the strategy of N fertilization. Moderate rates of N fertilization positively affected yield and quality of the chicons. A strategy including a first application of 30 kg N/ha at sowing followed by 3 splits of 15 kg N/ha, spread between the 5<sup>th</sup> and the 11<sup>th</sup> week, mostly led to a significantly higher yield of marketable chicons as compared to the procedure without N fertilization. However, higher amounts of applied nitrogen tended to decrease the marketable yield. Shape and solidity were negatively affected by excessive N fertilization, high rates of nitrogen leading to an increased formation of loose chicons with half-open tips.

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## **Fate of thiacloprid applied in nutrient solution to a tomato crop grown in a closed hydroponic system**

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The neonicotinoid insecticide thiacloprid was applied at two different ways, via the nutrient solution and foliar spray, to a tomato crop grown on rockwool, in a closed hydroponic system. The concentration of insecticide was 4.2 mg l<sup>-1</sup> (14 mg per plant) and 0.15 ml l<sup>-1</sup> of active ingredient, via the nutrient solution and foliar spray respectively. The experiment was conducted in a glasshouse located in Arta (lt. 39° 7' N, long. 20° 56' E.), Greece. The application of thiacloprid via the nutrient solution and of the first foliar spray took place at the stage of five true leaves of tomato. A second foliar spray application took place fifteen day later. The fate of thiacloprid residues were traced in the plants by regularly collecting samples of drainage water, leaves and tomato fruits during a period of 70 days, and analysing them by liquid chromatography system (HPLC).

The results obtained from the analysis of drainage solutions samples, showed that thiacloprid was rapidly dissipating from the recycling nutrient solution after its application via the root system to tomato grown. As a result, only a small amount of 0,5 mg l<sup>-1</sup> was detected 4 days after application while a lower value 0.07 mg l<sup>-1</sup> was still present 24 days after application. The amount of thiacloprid transported to the leaves was appreciable high and reached a level of approximately 7.7 mg/kg fresh wt. (1/3 of the total amount supplied to the crop) 24 days after application. In the case of application by foliar spray the residue concentrations was maintained at considerably high levels for 32 days, at least. However, the residue levels were very higher comparing with the corresponding in plants that thiacloprid was applied in nutrient solution, for all sampling.

The amount of thiacloprid found in tomato fruits was also relatively high up to 20 days after applications to both treatments (3.11 and 2.42 mg/kg fresh wt., in the foliage and root application, respectively) but dropped considerable to much lower levels 0.42 and 0.23 mg/kg, respectively, at the 32<sup>nd</sup> date. The MRL for thiacloprid in tomato is 3 mg/kg.

The above results indicate that the application of thiacloprid via the nutrient solution to a tomato crop grown in a closed hydroponic system, is not only a safe way of application but provides a long-time protection against pests, if this application takes place at early growing stage, nearly four weeks before commencement of harvesting.

## **Behaviour of some high valuable ornamental species/varieties within propagation, growther and fortification process for a better quality and impact of green spaces on the environment**

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The ornamental species and varieties of wood deciduous plants are of great decorative interest being utilized in landscape arrangements as simple samples or in together with others. The propagation of these ornamental varieties is usually difficult due to their specific biological features. The commercial extension of these varieties was done into a less extent due to a low rate of propagation although there is a great demand among the growers. Having in view the importance of these species in the landscape field as well as their difficult propagation on studies will be focused on: 1. Behaviour of the ornamental varieties during the mul-



tiplication process; 2. Behaviour of the ornamental species/varieties during the growth stage and obtaining of planting stock un the field and pots. 3. New modern systems for utilization the studied species/varieties in the landscape decorations and enrichment with other valuable types. Achievements of our objectives will go to prove a better development of the ornamental varieties in the special nurseries binging about a better improvement and balance of the environment, a major factor in the social life.

## Formation of honey guides in *Rudbeckia hirta*

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Honey guides are part of the pigmentation patterning in flowers. Their physiological function is to guide pollinators to the center, where the sex organs and nectar are present. They may take the form of colored dots or lines in particular areas of the petals (Kevan and Mulligan, 1973). Often honey guides are invisible to the human eye, but can be detected by UV-sensitive insects due to their intense absorption in the UV (Eisner et al., 1969, Thompson et al., 1972). The prime example for the formation of UV-honey guides is *Rudbeckia hirta* (Abrahamson and McCrea, 1977). In daylight, the petals of these flowers are uniformly yellow (Figure, left), but in UV light, the outer parts of the ray are UV-reflecting and bright, while the inner parts are dark-absorbing (Figure, right).



Figure: *Rudbeckia hirta*, left: photo in the daylight, right: UV-photo

We investigated the UV-honey guides of *Rudbeckia hirta* by UV-photography, reflection spectroscopy, LC-MS analysis and studies of the enzymes involved in the formation of the UV-absorbing flavonols present in the petals. We showed that the typical UV-pattern of *R. hirta* is already established in the youngest petals and that the underlying chemical basis is not a simple +/- system of flavonol formation as originally reported. The enzymes involved in their formation were demonstrated and characterized for the first time.

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## Flavonoid metabolism in apple leaves after infection with different strains of *Erwinia amylovora*

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Fire blight, caused by the bacterium *Erwinia amylovora*, is a serious disease of pome fruit trees and severe economic losses are reported worldwide. The progressive spread of the disease imposes great demands for new protection strategies. Flavonoids, important secondary plant metabolites, are known to be related to resistance against phytopathogens (Mayr et al. 1997, Snyder and Nicholson, 1990, Feucht et al., 1996). We investigated the involvement of flavonoid enzymes in the defence reaction of apple leaves after infection with fire blight. Two different strains of *Erwinia amylovora* were used showing different pathogenicity.

Interestingly, apple leaves seemed to react in different ways to the pathogenic and the apathogenic *Erwinia* strain. For some enzymes of the flavonoid pathway e.g. chalcone synthase/chalcone isomerase (CHS/CHI), flavanone 3-hydroxylase (FHT) or dihydroflavonol 4-reductase (DFR), leaves infected with the apathogenic strain showed an earlier induction of the enzyme activities than the control, whereas the leaves infected with the pathogenic strain showed a distinct delay.

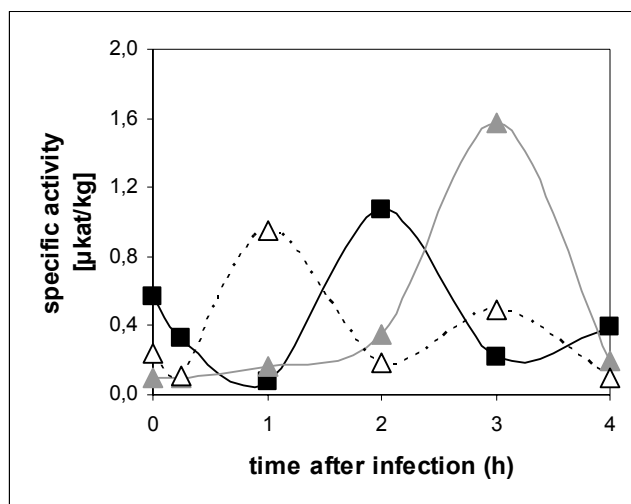


Figure: Specific DFR activity [ $\mu\text{kat}/\text{kg}$  protein] in apple leaves at different times after infection with a pathogenic (grey triangles) and an apathogenic (white triangles) *Erwinia* strain compared to a control (black squares)

However, phenylalanine ammonia lyase (PAL), which is the key enzyme located at the interface between primary and secondary metabolism, showed a contrary behaviour. A parallel PAL activity of control and the apathogenic strain was observed, whereas the pathogenic strain showed an earlier induction. Up to now, it remained an open question whether the observed effects were variety-specific and whether the effects are similar when other pathogenic strains are used. Further work on this topic is in progress.

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## Does Flavonoid 3'-hydroxylase accept also chalcones as substrates?

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Some important functions of flavonoids and related compounds - such as flower and plant coloration, disease defence, antioxidative capacity and UV-protection - are highly influenced by the hydroxylation pattern of flavonoids [Harborne, 1988 b, 1994]. Thus, the elucidation of the hydroxylation reactions is of particular interest. Whereas the biochemical establishment of the flavonoid hydroxylation pattern is well investigated, the knowledge on the hydroxylation of chalcones is still limited. Hydroxylation of various flavonoid classes in position 3' is catalyzed by the well known flavonoid 3'hydroxylase (F3'H), which accepts flavanones, flavones, flavonols and dihydroflavonols as substrates (Forkmann and Heller, 1999). Hydroxylation of chalcones in position 3 corresponds to position 3' of flavonoids (see figure below). Microsomal enzyme preparation of many plants, which show high enzyme F3'H activity, are not able to catalyze the hydroxylation of 6'-deoxychalcones, whereas many Compositae e.g. *Dahlia variabilis* are able to convert both, flavonoids and 6'-deoxychalcones to a high extent [Wimmer et al., 1998].

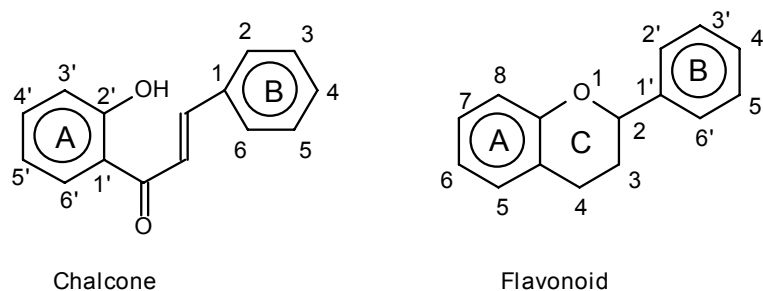


Figure: The basic structures of chalcone and flavonoid. Please note the divergent ring numbering of chalcones.

Recombinant F3'H from *Arabidopsis thaliana* (Schönbohm et al., 2000) was able to catalyze the hydroxylation of 6'-deoxychalcones, although the enzyme preparations from *Arabidopsis* plants did not show any conversion. However, hydroxylation of flavonoids was much more effective. Similar results were observed with recombinant F3'H from *Dahlia variabilis*. As enzyme preparations from *Dahlia* showed a much higher hydroxylation rate of 6'-deoxychalcones compared to the recombinant enzyme, it seems to be likely that further enzymes are involved in the hydroxylation of chalcones.

Further work on this topic is in progress.

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# Pest Risk Analysis for *Metcalfa pruinosa* Say, an introduced planthopper to Austria

STRAUSS GUDRUN

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At the end of the 1970s the polyphagous planthopper *Metcalfa pruinosa* (Hemiptera: Flatidae) was accidentally introduced to Italy from its native habitat in the United States. It then spread to South, West and East Europe, mainly by human activities. Due to the absence of specialized enemies in the new areas high population densities rapidly built up, which caused important quality losses in the wine and fruit production.

In Austria the first mass occurrence of *M. pruinosa* was detected 2003 in Vienna in a public park adjacent to a nursery, leading to an infestation of several sites in 2006. To assess the relevance of *M. pruinosa* for the agriculture and horticulture in Austria a Pest risk analysis (PRA) was conducted according to the EPPO decision support scheme PM 5/3 (2) (2006). The pathways of introduction, the probability of establishment and spread in Austria as well as the economic consequences will be presented.

There are two important pathways for introducing *M. pruinosa* into Austria: trading of deciduous trees and shrubs for planting infested with eggs and the transfer of individuals by traffic from neighbouring states of Austria where *M. pruinosa* is common. The probability of entry is high because official control measures for *M. pruinosa* are not obligatory within the European Community. It is very likely that *M. pruinosa* will disperse because the imported plants are distributed to many different end users and it easily finds new host plants.

In Austria *M. pruinosa* has formed stable populations in the field but without significant damages so far. The computer programme CLIMEX<sup>®</sup> was used to determine the potential occurrence of *M. pruinosa* in Austria under present climatic and climate change conditions (temperature increase of +3°C). The environmental requirements (temperature, moisture) of *M. pruinosa* were estimated through its distribution in North America and were then used to generate a distribution map for Europe. The exotic planthopper will be able to develop very well in the eastern and southern part of Austria with its milder climate compared to the alpine region and where host plants are very common. In this region the important fruit and wine producing areas are located. Therefore orchards, vineyards and nurseries in Vienna, Lower Austria, Burgenland and Styria are endangered, especially when they are close to broadleaf forests where the pest finds outstanding conditions for its development. In Austria the following fruits have a high risk to be damaged: Apple, pear, apricot, plum, cherry, strawberry, raspberry and blackberry as well as red and black current. On some of these crops larvae of *M. pruinosa* were already recorded.

During the PRA gaps in the knowledge of *M. pruinosa* became apparent. There is a need to investigate its complete distribution in Europe for estimating the prevalence of this pest at a certain origin, its potential of active and passive spread as well as its role to act as a vector of plant pathogens.

In summary, the PRA shows that *M. pruinosa* has a high probability of entry to, establishment and spread in Austria. It is likely that *M. pruinosa* will cause damage in vineyards and orchards and lead to economic

losses in the horticulture and agriculture in Austria. Risk management options should be taken rapidly with the aim to prevent the introduction and of containment of *M. pruinosa*.

## **Comparison of the genetic diversity of Hungarian apricot cultivars and group of cultivars from Middle Asia, North America and Mediterranean area**

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Considering some morphological and agronomical traits the Hungarian apricot germplasm is assumed to be relatively narrow. The aim of this study was to make comparison of the genetic diversity of 17 cultivars from Hungary with the polymorphism observed among 35 cultivars from Middle Asia, North America and Mediterranean area. The genetic diversity was studied with six polymorphic microsatellite markers developed in apricot by Vilanova et al (2006). The primer set was designed to investigate microsatellite regions in the first linkage group of apricot. Altogether 51 alleles were identified among all the apricot genotypes assayed. Number of alleles occurred in Hungarian cultivars was limited to 15. There were identified alleles characteristic just for cultivars with the specific origin. Alleles of the Hungarian are rather common in the group of cultivars from Middle Asia than in the others. The results obtained confirmed earlier assumption concerning the relatively low genetic diversity of the Hungarian cultivars. According to that despite of the number of desirable agronomical traits utilization of these cultivars in breeding programmes must be reevaluated.

This work was supported by the Péter Pázmány programme RET 04./2006.

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## **A new conception for the hypothetical infection mechanism of blossom blight [*Monilia laxa* (Ehr.)] on apricot**

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Blossom blight caused by *Monilia laxa* (Ehr.) is the most important fungal disease in Hungarian apricot orchards. In order to be able to select resistant genotypes, it is necessary to clarify the precise mechanisms of the infection. It is widely accepted that conidia can enter pistils across stigmata, similarly to pollen tubes. In our experimental station, we have observed that emasculated and pollinated flowers were not susceptible to the infection. It was hypothesized that the failure of the infection is attributable to the consequence of pollination. Natural and artificial infections indicated that penetration of the pathogen never occurs through the stigmata. Instead, resistance of the forced pollinated and emasculated flowers may be due to the removal of petals and anthers. This assumption was partly confirmed by the tissue structure of floral organs as it was demonstrated from electromicroscopic studies. Our analyses suggest that the putative site of flower resistance correspond to the susceptible parts involving petals and anthers.

This work was supported by the Ányos Jedlik programme NKFP-A2-2006-0084.

## First Report of Potato Spindle Tuber Viroid (PSTVd) on Ornamental Plants in Austria

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PSTVd is an important pathogen affecting mainly the potato (*Solanum tuberosum* L.), but also tomatoes and other plants from the Solanaceae family including ornamentals plants. It is regulated in Europe (RL 2000/29/EG, A 1) and an EPPO A2 quarantine pest (OEPP/EPPO, 1978). To date the described infection pathways for this viroid are mainly mechanically. However an association with the aphid transmissible Potato Leafroll Virus (PLRV) through encapsidation by the capsid protein and a following vector transmission is described (Marczewski 2001).

PSTVd infection on potato and tomato plants often leads to visible symptoms, albeit infections on ornamental plants proceed without symptoms and have to be detected e.g. by PCR. Findings of PSTVd on ornamental Solanaceae were recently reported from different European countries.

A monitoring of described PSTVd ornamental hosts started 2007 in Austria. Meanwhile two PSTVd positive samples (*Solanum jasminoides* and *Brugmansia* sp.) from garden centers could be determined. This is the first report of PSTVd on Austrian territory. The infected plants were imported from EU countries for final sale.

RNA extraction from the leaves was done according to the instructions of the RNA Plant Mini Kit (Qiagen) with a previous maceration of the plant material using a Bioreba Homex 6 homogenizer. RT-PCR has been carried out with the Roche Titan One Tube RT-PCR Kit using the primers developed by Weidemann and Buchta (1998). PCR products were subjected to electrophoresis in 2% agarose gels and visualized by ultraviolet light after staining with ethidium bromide. Lyophilized PSTVd infected plant material from the German Collection of Microorganisms and Cell Cultures (DSMZ PV0064) was used as a positive control.

The possibility for the transmission of PSTVd from ornamental plants to potato or tomato plants in Austria should be considered. Smaller market gardens working with ornamental and crop Solanaceae could be potential transmission places for this plant pathogen. Furthermore the relatively small farming structures, the proximity of ornamental pot plants and crop plants from the Solanaceae family, especially in the climatic more adequate eastern part of the country, could lead to a cross over of the viroid. All so far described host plants should be part of routine monitoring surveillances to avoid the spreading of this plant disease through these or other eventually latent infection pathways.

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## Occurrence and spread of *Scaphoideus titanus* in Austria

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Grapevine yellows are widespread in many viticultural areas of the world. Flavescence dorée (FD) causes crop losses, a decrease in the lifespan of grapevines and finally the death of the affected plant. FD is transmitted specifically by the cicadellid leafhopper *Scaphoideus titanus*. This species was first introduced to Southern France in the 1950s from North America and it is now expanding its range to the north. In the summer of 2004 the first *S. titanus*-specimen reached the vineyards around Bad Radkersburg near the Slovenian border in Styria.

Since then the Austrian Agency for Health and Food Safety carried out intense monitoring surveys with yellow sticky traps in selected Austrian vineyards along the Hungarian and Slovenian border. During the last 3 years, in the middle of August, passive spread by north-westerly winds along the valley of rivers from Balkan States brought hundreds of specimens of *S. titanus* over the border to Austria, while further inland the abundance of the vector was much lower. The results of the monitoring surveys show, that the distribution of this vector in Austria is limited to a restricted area in Southern Styria and so far the distribution of *S. titanus* does not present a phytosanitary problem.

## **Molecular Pathways for Detection of *Xanthomonas axonopodis* pv. *poinsettiicola*, the Bacterial Leaf Spot Pathogen on *Euphorbia pulcherrima***

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In August 2007 we received diseased *Euphorbia pulcherrima* plants from an Austrian market garden. These plants showed typical chocolate brown to rust coloured leaf spots, which were surrounded by a pale green to yellow halo. The brown spots and haloes enlarged rapidly and coalesced into irregular, yellow or brown, dry, dead areas on the leaf. These symptoms strongly indicated that the plants were infected with the bacterium *Xanthomonas axonopodis* pv. *poinsettiicola*. Isolations from diseased leaves were done on Yeast-Agar (YPGA) and King's B Agar. The colonies, which could be isolated, were more or less yellow and glossy. Six colonies were selected for further molecular characterisation. DNA was also extracted direct from the infected plant material with the Qiagen Plant Mini Kit.

No specific molecular or serological detection method for *Xanthomonas axonopodis* pv. *poinsettiicola* could be found in the literature. For molecular characterisation, two semi qualitative approaches were chosen, which combined allowed precise evidence about the pathogen. Three pairs of oligonucleotide primers specific for different *hrp* gene regions of several pathovars from the *Xanthomonas campestris/axonopodis* group were used for identification on species level (Leite et al. 1994). From the six isolates that were screened, one (K1D) could be identified as a bacterium from the *Xanthomonas campestris/axonopodis* group. In addition the 16S rDNA of these strains was amplified by PCR and restricted with *AluI* only the same single isolate (K1D) showed a restriction pattern matching the *Xanthomonas campestris/axonopodis* group, respectively. This was also confirmed by sequencing the 16S rDNA fragment of the K1D isolate.

Subsequently one of the three *hrp* gene PCR products was also sequenced and restricted *in silico* with the endonucleases *HaeIII* and *Sau3AI* (performed with VectorNTI<sup>®</sup> programm). This procedure narrowed the identified pathovars to pv. *vesicatoria* and pv. *poinsettiicola* (Leite et al. 1994). A following PCR specific for *Xanthomonas campestris* pv. *vesicatoria* designed on the *rhs* family gene (Park et al. 2007, in press) was negative. We therefore concluded to have the molecular identification of a *Xanthomonas axonopodis* pv. *poinsettiicola* strain in the examined *Euphorbia pulcherrima* plants. As controls we used a *Xanthomonas axonopodis* pv. *poinsettiicola* strain (LMG849) from the Belgian Coordinates Collection of Microorganisms and a *Xanthomonas campestris* pv. *vesicatoria* strain (DSMZ 50861) from the German Collection of Microorganisms and Cell Cultures.

This procedure enables the detection of *Xanthomonas axonopodis* pv. *poinsettiicola* from isolates or directly from infected plant material with PCR and following RFLP and is an important tool for confirmation of this disease on *Euphorbia pulcherrima*.

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# Grapevine phytoplasma diseases – possible risks for the Austrian viticulture

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A survey for the presence of phytoplasmas in grapevines, different alternative host plants and planthopper vectors was conducted by AGES in Austrian vineyards over three years. At different sampling sites in Burgenland, Lower Austria, Styria symptomatic plants and vector specimens were sampled and analysed for the presence of Stolbur-group, Elm Yellow-group and Aster Yellow-group phytoplasma. In grapevines and different herbaceous host plants (*Convolvulus arvensis*, *Trifolium alba*, *Glechoma hederacea*, *Fragaria viridis*, *Rumex acetosa*, *Rumex acetosella*, *Setaria glauca*, *Ranunculus repens*, *Polygonum minor*, *Potentilla repans*) only stolbur phytoplasma could be identified by molecular genetic analysis. In Austrian vineyards Elm-Yellow-group and Aster-Yellow-group phytoplasmas were not detected up to the present. The population density of the Cixiid planthopper *Hyalesthes obsoletus*, as only known vector for the stolbur-group phytoplasmas, was low at all investigation sites. About 25% of these planthoppers were carrier of stolbur phytoplasma. A total number of 773 specimens of *Scaphoideus titanus*, the vector for grapevine flavescence dorée, were examined, but Elm-Yellow-group phytoplasmas were not detected. Based on the monitoring data a pest risk analysis was conducted by following the EPPO decision support scheme.

High risks of introduction of grapevine flavescence dorée evolve from the extensive trade in rootstocks to Austrian vine growing areas where the vector is already established, currently South-East Styria. Such areas are highly endangered, since any introduction of grapevine planting material latently contaminated with the phytoplasma, might result in serious epidemics. Another risk of introduction is the passive or active spread of infested vector populations from Slovenia/Serbia and/or Friuli-Venezia-Giulia along traffic routes and waterways. The recently observed north- and eastward spread and establishment of vector populations to vine growing areas in Hungary suggests that the climate will not limit the establishment of the vector in the large northern vine growing regions of Lower Austria and Burgenland, in particular the warm microclimate of the vine growing area around Lake Neusiedl would be favourable for an establishment. An introduction of the disease would have serious impacts on Austrian viticulture. The Institute for Plant Health, AGES, monitors disease and vector occurrence and coordinates measures to prevent disease-entry and to minimise impacts of established vector-populations.

## Influence of Effective Microorganisms as a means of improving the quality of tomatoes in protected cultivation

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In 1968 a Japanese researcher found a link between the strength and quality of citrus which is biochemical tested and photosynthetic bacteria. The addition of effective micro-organisms "EM" (named after their discovery) to the substrate (soil or hydroponics) allows to reduce different plant damages by increasing plant productivity both in quality and quantity. To test the application of "EM" as a means to improve tomato-production, a two year survey was conducted in a greenhouse tunnel film in the experimental garden of the



University of Natural Resources and Applied Life Sciences Vienna. The tomato varieties "CASSIOPEIA" and "MERCEDES" were used in 2006 and 2007 growing seasons, respectively. A Completely Randomized Block Design (RCBD) with 2 factors, 2 treatments (EM and tap water) and 2 planting patterns (line and ellipse shape) was established in 5 replications. There were 8 plants per replication, i.e. 80 plants in each treatment. "EM A" with appropriate dose was applied to all plants using an effective automatic drip irrigation system with a low water pressure. The proportion was 1:200 (5 ml "EM A" per 1 litre of water). Thus, different types of tests and methods were carried on, among others, analysis of chlorophyll, leaves and substrates nutrient analyses, Biophotons of fruits and leaves, an allergic analysis of tomato fruits, and potential of fruit yield (productivity). The chlorophyll measurements were made by the acetone method. Inorganic nitrogen in the substrate ( $N_{in}$ ) was measured with the ÖNORM L 1091 method, microbial biomass nitrogen ( $N_{mic}$ ) with the fumigation-extraction method (Brookes et al., 1985). Nutrient analyses were arranged in different methods: analysis system CNS-2000 of LECO, Atom absorptions spectrophotometer and ÖNORM L methods. For the allergic analysis, SDS-PAGE and Western blot analyses were used. For biophotons, a single photon counting method was employed. Statistical analysis of data was made with SPSS-15.0 (ANOVA, t-Test,  $\alpha=0.05$ ). In both years, the following results were obtained in the "EM" treated pots in comparison to the untreated control: a significantly higher yield of marketable fruits (6,63 kg par plant in EM treatment and 5,89 kg in control in 2006, and 4,97 kg par plant in EM treatment and 3,58 kg in control in 2007, respectively), less fruits with necrosis (3% in "EM" treated and 31% in untreated control in 2007), a significantly higher chlorophyll "ab" (10,25 mg) and "a" (0,41 mg) content, in 2007 a significantly lower  $N_{in}$  content in the substrate was measured in the EM-treated plants ( $235 \mu\text{g } N_{in} \text{ g}^{-1}$ ) compared to the untreated control plants ( $668 \mu\text{g } N_{in} \text{ g}^{-1}$ ), corresponding to a significantly higher microbial biomass nitrogen content in the EM-variant ( $234 \mu\text{g } N_{mic} \text{ g}^{-1}$ ) compared to the control ( $161 \mu\text{g } N_{mic} \text{ g}^{-1}$ ), higher nutrient contents in plant leaves (Fe: 839,77 ppm in the "EM" compared with untreated control: 182,90 ppm) than the untreated plants. The "LTP" allergy was found in water treated tomatoes but not in 'EM' treated ones. The number of biophotons was higher in 'EM' treated tomatoes than in the control. The P-value of fruits which indicates a higher vital quality showed no statistically significant differences. The tomatoes of the untreated control showed a significantly lower redox potential in 2007, which indicates less oxidative stress. The pH-value was not significantly different in both years. The electric resistance of fruits in treated plants was higher in both years, but statistically significant differences could only be found in 2007.

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## Quarantine Insects on Imported Orchids: A Modified Realtime PCR Identification Method for *Thrips palmi* Larvae

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*Thrips palmi* (Karny 1925; Thysanoptera) is a polyphagous pest damaging leaves and blossoms of plants of several different families, such as Cucurbitaceae, Solanaceae, Asteraceae and Orchidaceae. It has developed high levels of resistance to many insecticides. Hence, effective control of the pest is difficult to achieve and resulting economic costs are high. Moreover, eggs deposited inside the plant tissue do not get in contact with insecticides and usually survive such treatments unharmed. *T. palmi* is widespread in tropical areas and cannot survive in Europe under field conditions. Nevertheless, it is able to establish in greenhouses and is therefore listed in the Annex I of the EU Council Directive 2000/29/EG as a quarantine pest.

Larvae and adults of *T. palmi* have often been detected on orchids illegally introduced by tourists arriving from Southeast Asia. In regularly imported orchids, the decision of permitting or rejecting the import of a shipment must be reached quickly, in order to minimize economic losses. Therefore, a fast and reliable method to identify any developmental stage of *T. palmi* found alive on orchid blossoms during import inspections is very important. Whereas adults are identifiable by morphological characters according to scien-

tific keys or EPPO protocols, no such identification keys are available for larvae. Several PCR based methods have thus been developed to ensure rapid identification of *T. palmi* larvae.

In order to obtain a very quick and reliable result, a Taqman Realtime PCR method (Kox et al. 2005) was chosen for identification of *T. palmi*. DNA extraction of single individuals was performed according to an adapted protocol originally drafted for marine midges (Chironomidae) (courtesy of G. Schöfl, Max Planck Institute for Chemical Ecology, Jena).

The Taqman Realtime PCR protocol as described did not provide satisfactory results and had to be adapted to our conditions. An additional extension step (72°C for 20 seconds) was added and the number of cycles was increased from 40 to 50 cycles to optimize the sensitivity. The Taqman probe concentration was doubled in the assay to obtain an improved fluorescence signal. Offspring from a morphologically identified adult *T. palmi* were used as positive controls. To confirm specificity of the modified analyses, DNA from *Frankliniella occidentalis* extracted with the same protocol was used as a negative control. With these adaptations of the published Taqman Realtime PCR, we were able to detect *T. palmi* larvae on imported plant material within a few hours.

A further DNA extraction method will be established to detect *T. palmi* eggs directly in the plant tissue. A combined quantification of DNA from eggs in relation to general plant DNA would allow a semi-quantitative determination of eggs per plant material and a conclusion about the original infection level before possible control measures were taken.

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## Current Fire Blight Situation in Austria, Control Strategies and Measurements

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Fire blight, caused by *Erwinia amylovora* is a potentially devastating disease of pome fruits and ornamentals belonging to the family of Rosaceae. In Austria first outbreaks of the disease were detected in 1993 in Vorarlberg. Since then fire blight has spread from west to east of the country. Whereas the disease is established in Vorarlberg, Tirol (except the district of Lienz), Salzburg and Upper Austria, the rest of Austria is listed as a protected zone since *Erwinia amylovora* is regarded to be not endemic in these areas. In 2007 most severe outbreaks were observed due to favourable weather conditions during the blooming period of pome fruits. Commercial orchards were affected as well as traditional orchards, private and public gardens. More than 3000 samples were tested in the laboratory, in 71% *Erwinia amylovora* was detected.

As a consequence of the heavy losses a renewed concept for control strategy has been developed.

Using resistant or less susceptible host plants respectively varieties must be one main aim besides eradication and sanitation activities. The use of plant protection products should come second if preventive measures fail. Their appropriate use (timing, purpose), mode of action and possible risks should be communicated to the growers to optimize their application. Scientific research on fire blight completes the tasks of the strategy.

Public relations on different fire blight – topics will be intensified and edited for several markets.

## Inhibitory Effect of Some Medicinal Plants Essential Oils on Post-Harvest Fungal Disease of Greenhouse Tomato Fruits

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The consequence in misuse of chemical biocides for controlling pest and disease has drawn the attention of policy makers in development of methods potentially available in nature for the purpose. One of the new and safe methods of controlling pest and disease is the usage of essential oils from medicinal plants. In the present investigation, inhibition of spore germination of post-harvest Gray Mold Rot and *Rhizopus stolonifer* exposed to the different concentration of some medicinal plants essential oils (*Thymus vulgaris*, *Mentha piperita*, *Carum caraway*, *Rosmarinus officinalis* and *Foeniculum vulgare*) were studied. Essential oils were examined at 0.5 % and 1 % in comparison to the control (without any treatments). All the data statistically analyzed. Results shown that growth of gray mold and *Rhizopus* completely inhibited by *Carum caraway* at both levels until first week. *M. piperita* and *Rosmarinus officinalis* oils (at both levels) and *Thymus vulgaris* oil (at 1%) had suitable effects until third days, after this time the fungal diseases start to decaying of the fruits from the blossom end point. The fruits that treated with fennel oil start to decaying from the second days after treatment. Therefore inhibitory potency of essential oils on the post-harvest disease of greenhouse tomato fruits was as *Carum caraway* > *M. piperita* > *Rosmarinus officinalis* > *Foeniculum vulgare* > *Thymus vulgaris* and the extent of inhibition of fungal growth was dependent on the concentration of essential oils usage. These results clearly indicated that it is necessary to focus on practical application of the essential oils for inhibition of post-harvest pathogen growth and these compounds could be used as a substitute for chemical fungicides since they are natural, and none-toxic to humans

## Multiple Cropping for *Myopardalis pardalino* Control of *Cucumis melo* var. *inodorus*

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An investigation was conducted on control of melon fly, *Myopardalis pardalino*. *Cucumis melo* var. *inodorus* is an economic vegetable species widely planted in northeast of IRAN (Provinces of Khorasan and Semnan) and the pesticides application for control of the pests is a major problem. In regard to this, the main aim of this research was to study the influence of intercropping of *Cucumis melo* var. *inodorus*, as main crop and *Thymus vulgaris* and *Mentha piperita*, as intercrop plants on pest population and fruits damaged with the pest. The check (control) treatment was a field of *Cucumis melo* var. *inodorus* without intercrop plants but with pesticide application, Zolonand Diazinon, for pest control (4 times during the growth period). *Cucumis melo* var. *inodorus* cultivated with intercropping and without pesticides, gave the best results, while the check field showed many fruits that damaged by the pest or its larve. The study of repellent behavior of thyme and peppermint to melon flies reveals new opportunities for decrease of chemical use, especially in vegetable growing and organic production of many horticultural crops

## **Ferula assa-foetida as a Natural Pesticide for Aphids and Spider Mite Control in Peach, Plum and Sweet Cherry Orchards**

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Many plants have natural substances as essential oils, latex and oil in different parts and organs that can repel (anti feedents) and / or attract insects. In this study the effect of pesticide activity of *Ferula assa-foetida* (8 times at 0.05%) was investigated for aphid and spider-mite control of peach, plum and sweet cherry and compared with trees that treated by pesticide (Zolone / 8 times at 0.05%) application as control. The results showed that in some cases there were significant different between natural and synthetic control, and in some cases there were not any significant different between natural and pesticide control of the pests. Finally, results reveals, new opportunities for decrease of chemical use for pests control and ability of natural substances for pesticides substitution and organic production of fruits.