Nematode-resistant rootstocks as a major component of the management alternatives for GFLV control in grapes

Daniel Esmenjaud, Gerard Demangeat, Maarten van Helden, Nathalie Ollat

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Hosted by the University of Natural Resources and Life Sciences (BOKU), Vienna (AT), and held in conjunction with the 30th International ESN Symposium.

This 5th International Phylloxera Symposium is held under the ISHS umbrella to fulfill its mission to maintain a global network of horticultural scientists.

The conference language (presentations, administration etc.) is English.

Date: 2010 September 19–21

Venue:

BOKU Universität für Bodenkultur Wien
(University of Natural Resources and Life Sciences)
Wien (Vienna), 19. Bezirk (District)
Street: Peter-Jordan-Straße 82 | Building: Wilhelm Exner Haus

Two additional workshops will be held in conjunction to the symposium (both also in English language):

Phylloxera Genome Workshop
chaired by Denis Tagu (INRA Rennes, FR), hosted by Astrid Forneck (BOKU, AT)

Phylloxera Quarantine Workshop
Information exchange on phytosanitary regulations referring to Phylloxera
chaired by Christoph Hoffmann (JKI Siebeldingen, DE), hosted by Michaela Griesser

Symposium Organisers:

Prof. Dr. Astrid Forneck
Dipl.-Ing. Dr. Michaela Griesser

Postal Address:
BOKU Universität für Bodenkultur Wien,
Dept. of Applied Plant Sciences and Plant Biotechnology
Institute of Horticulture and Viticulture (IGOW)
Peter-Jordan-Str. 82, 1190 Wien, Austria

Detailed info → www.viticulture-research.com

Scientific Committee:

Astrid Forneck
László Kocsis
Kevin Powell

Organization Committee:

Astrid Forneck
Michaela Griesser
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Monika Pribil
Katharina Schödl

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Program

Sunday, Sept. 19

16:00-20:00 Registration and Welcome Reception at BOKU

14:00-17:00 **Phylloxera Genome Workshop**
Chair: Denis Tagu
@ Seminar Room SH3

Monday, Sept. 20

08:30-12:00 Phylloxera Symposium Registration & Poster Set up

09:00-12:15 **Opening Session**
Keynote Lectures:
Heribert Hirt,
Jonathan Hodgin

12:15-14:00 – Lunch Break –

14:00-15:40 **Session 1 & 2: Biology, Ecology, Genomics of Grape Phylloxera**
Chairs: Hugh Loxdale / Astrid Forneck
@ Lecture Room EH5

14:00-14:15 Introductory Lecture ISHS
By L. Koscis

14:15-14:30 Grape Phylloxera – The Movie
By Urs Wyss; presented by N. Lawo & A. Forneck

14:30-14:50 Sun, Q.H., Chen, Y.-C., DU, Y.-P., Zhai, H.
Genetic structure of Grape Phylloxera in China

14:50-15:10 Loxdale, H.D.
Aphids: the ultimate asex maniacs...or are they?

15:10-15:30 Lawo, J.P., Kolberg, R.; Forneck, A., Lawo, N.C.
Misconceptions about the comparison of intrinsic rates of natural increase

15:40-16:10 – Coffee Break –

Monday, Sept. 20

16:10-16:30 Kolberg, R., Lawo, N.C., Forneck, A.
Insights on the interaction of Grape Phylloxera (D. vitifoliae), associated Pantoea agglomerance and the Grapevine (Vitis sp.)

16:30-16:50 Lawo, N.C., Griesser, M., Forneck, A.
Involvement of expansins in Phylloxera induced Nodosities

16:50-17:10 Zhao, Q., Du, Y.P., Zhai, H.
Influence of Phylloxera infestation on secondary metabolites content, their regulative enzyme in primary roots and comparison on secondary metabolites content in tertiary roots of different Vitis species.

17:10-17:30 Weingart, G., Lawo, N.C., Schuhmacher, R., Forneck, A.
Metabolomic profiles of phylloxerated gall tissues of root tips

20.00-23.00 **Reception** at Vienna City Hall

Tuesday, Sept. 21

08:00-10:10 **Session 4: Pest Management Strategies in Viticulture against soil-borne Pests**
Chair: Kevin Powell
@ Lecture Room EH5

08:00-08:20 Johnson, D.T., Sleezer, S.M., Lewis, C., Rothrock, C., Goggin, F., Savin, M.
Grape Phylloxera seasonal biology, predictive model and management in the Ozarks

08:20-08:40 Hoffmann, M., Rühl, E., Eisenbeis, G., Huber, L.
A quantitative assessment of root system infesting Grape Phylloxera population in field

08:40-09:00 Kocsis, L., Szalanski, W.
Grape Phylloxera develepment on potted grapevine treated with arbuscular mycorrhiza

09:00-09:20 Bruce, R.J., Robinson, S.A., Morng, S., Powell, K.S.
Grapevine leaf pigment response to root infestation by Phylloxera

09:20-09:40 Blenheim, D., Robertson, E., Potter, I., Rochfort, S., Powell, K.S.
Protecting vines in stress environments: Early detection of Grape Phylloxera (D. vitifoliae) infestation through identification of chemical biomarkers

09:40-10:00 Kocsis, L., Lönhard, T., Kocsisne, M.G.
Grape Phylloxera (D. vitifoliae) threat in grafted vineyards

Tuesday, Sept. 21

10:00-10:40 **Poster Session I**
Lecture Room EH5

10:10-10:45 – Coffee Break –

10:45-12:25 **Session 3: Rootstock Breeding for Phylloxera & Nematode Resistance**
Chair: Laszlo Koscsis
@ Lecture Room EH5

10:45-11:05 Esmenjaud, D., Bouquet, A., Demangeat, G., van Helden, M., Ollat, N.
Nematode-resistant rootstocks as a major component of the management alternatives for GFLV control in grape.

11:05-11:25 Korosi, G., Carmody, B.M., Hoffmann, A.A., Powell, K.S.
The importance of genetic characterisation in rootstock resistance screening for Phylloxera resistance under controlled conditions

11:25-11:45 Hausmann, L., Eibach, R., Zyprian, E., Töpfer, R.
Genetic analysis of phylloxera root resistance in cultivar „Börner"

11:45-12:05 Schwappach, P.
How tolerant are rootstock varieties against Grape Phylloxera?

12:05-15:00 **Poster Session II**
@ Lecture Room EH5

12:00-15:00 **Grape Phylloxera Quarantine Workshop**
Chair: Christoph Hoffmann
@ Seminar Room SH3

12:25-14:00 – Lunch Buffet –

15:00-18:00 **Excursion**
Meeting poimt: in front of Wilhelm Exner Building

18:30-... Casual Conference Dinner at a " Heuriger "
Weinhof Zimmermann, Mitterwurzergasse 20, 1190 Wien
Tel: +43 (0)1 44012070

Abstracts

Genetic Structure of grape phylloxera in china

SUN Qing-Hua, CHEN Ying-Chun, Du Yuan-Peng, ZHAI Heng

(College of Horticulture Science and Engineering, Shandong Agricultural University/ State Key Lab of Crop Biology, Tai'an 271018, Shandong, P. R. China)

The genetic structure of four chinese populations of grape phylloxra was analyzed using seven polymorphic microsatellite markers. The mean effective number of allele (Ne) was ranged from 1.7143 to 2.0077, while expected heterozygosity (He) from 0.2435 to 0.2580. In addition, the mean Shannon values were all less than 1. All those data showed that genetic variations of 4 grape phylloxera populations were fairly low. The Nei's genetic distances between SHJ and each of other three populations was larger, ranged from 0.4531 to 0.4694, than those between two of three populations (0.0028-0.0114). The UPGMA cluster analysis indicated that the four phylloxera populations were clustered into two groups based on the dendrogram on the basis of Nei's genetic distances. All samples from SHJ formed one cluster, and the other samples from SXX, HNH and LNX were grouped into another one.

Keywords: grape phylloxera; SSR marker; genetic diversity

Aphids: the ultimate asex maniacs…or are they?

Hugh D. Loxdale

Institute of Ecology, Friedrich Schiller University, Dornburger Strasse 159, 07743, Jena, Germany. E-mail: Hugh.Loxdale@uni-jena.de

Aphids are renowned for their performance as global pests of a wide variety of plants of agricultural, horticultural and forestry importance, including grape root stocks, causing damage physically and in many plant species, by transmission of pathogenic plant viruses. Their role is exacerbated by their prodigious reproductive abilities, involving asexual (parthenogenetic) propagation, with, in many species, a single sexual phase in the autumn. Thus these small animals are able to switch between an r-type to a K-type strategy during the course of a single growing season. Sexual reproduction has many advantages: recombination of new favourable alleles, crossing over and the elimination of deleterious alleles. However there are disadvantages too, mainly the breaking up of existing favourable allele associations, which may be locally adapted, and difficulties of finding a mate. Asexual reproduction, the main reproductive mode for most of the growing season, allows quick exploitation of fleeting food resources, but it is assumed aphids are not so adaptive during this reproductive mode due to the nature of clonality, i.e. that offspring have an essentially fixed genome (non-recombinant) and are largely genetically identical. In this talk, I suggest that clonality is 'not all it is cracked up to be' and that there are indeed costs to being clonal. I will present data showing that in semi-natural habitats at least, aphid clones are in the minority, possibly being continually eliminated by natural selection and competition for limited resources. If this is true, then maybe the Red Queen holds sway and that ultimately variation is all in a changing world, more especially to keep pace with evolving predators, parasitoids and pathogens, locked with the aphid hosts in an ongoing life and death struggle, a so-called 'arms race'. I further suggest that this may have implications for control of aphid pests in IPM scenarios.

Misconceptions about the Comparison of Intrinsic Rates of Natural Increase

Lawo, J.-P.[1], Kolberg, R[2], Forneck, A.[2], Lawo, N. C.[2]

[1] Global Biopharm Preclinical Research and Development, Baxter Innovations GmbH, Wagramer Strasse 17-19 1220 Vienna, Austria

[2] University of Natural Resources and Life Sciences, Department of Applied Plant Sciences and Plant Biotechnology, Institute of Horticulture, Fruit-Growing and Viticulture, Peter Jordan Str. 82, 1190, Vienna, Austria

The intrinsic rate of natural increase (rm) is a common measurement in entomology to describe and evaluate growth and adaptation of a population of arthropods to certain environmental conditions. It can be therefore used for performance measurements of phylloxera (Daktulospharia vitifoliae Fitch). Following the method of Birch (1948) the rm is calculated by an exponential equation which is depending on the whole life cycle of each female and her survival time. A simplification of this equation was provided by Wyatt and White (1977) which allows the study to be shorten since it does not depend on any survival times and only part of the life cycle of a female. Therefore, this method has become quite popular among entomologists and is of major interest when assessing life-table parameters for phylloxera due to its long life cycle. As the rm is a population parameter it lacks any variance and thus a statistical valid comparison of rms for different populations is not straightforward. Thus, many approaches for comparisons of rms include statistical misconceptions. We briefly discuss the drawbacks of the others methods and present an easy to implement and consistent method for comparison of rms. The procedure will be demonstrated with a data set of phylloxera.

References:

Birch, L. C. 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. 17: 15–26

Wyatt, I. J., and P. F. White. 1977. Simple estimation of intrinsic increase rates for aphids and tetranychid mites. J. Appl. Ecol. 14: 757–766

Insights on the Interaction of Grape Phylloxera (Daktulosphaira vitifoliae), associated Pantoea agglomerans and the Grapevine (Vitis sp.)

Kolberg, R., Lawo, N. C., Forneck, A.

University of Natural Resources and Life Sciences, Department of Applied Plant Sciences and Plant Biotechnology, Institute of Horticulture, Fruit-Growing and Viticulture, Peter Jordan Str. 82, 1190 Vienna, Austria

Daktulosphaira vitifoliae Fitch, the grape phylloxera, is responsible for the development of leaf galls and root nodosities, this last the most relevant for the damage in vineyards, especially on the susceptible European V. vinifera. Until recently no bacteria were known to be associated with grape phylloxera until the identification of Pantoea agglomerans in adults and eggs of several leaf galling populations. P. agglomerans species can be found as free-living saprophytes, as plant pathogens, and are frequently members of other insects gut microbiota. In this last situation, they often must be acquired via the food source and show positive (e.g. protection against pathogens) as well as negative effects (e.g. reduction of the fertility). So far, however, no information of the transmission of P. agglomerans nor regarding their role for phylloxera itself are available. Establishing highly sensitive methods P. agglomerans could be detected in very low concentrations (up to 1-100 cfu/mL) in insect and plant samples, allowing both the investigation of maternal transmission and the presence of the bacterium inside the plant. The results suggest that P. agglomerans colonizes the aphid surface but is not able to grow inside grape phylloxera neither it is transmitted via the mother aphid to the progeny. Isolating P. agglomerans from non-surface sterilized as well as surface sterilized vine leaves and plant sap suggests that phylloxera can be uptaking it via the plant or even spreading it. These are the first insights on the relationship between P. agglomerans, the grape phylloxera and the host plant Vitis spp. and further studies are needed to ensure our findings.

Involvement of Expansins in Phylloxera induced Nodosities

Lawo, N. C., Griesser, M., Forneck, A.

University of Natural Resources and Life Sciences, Department of Applied Plant Sciences and Plant Biotechnology, Institute of Horticulture and Viticulture,
Peter Jordan Str. 82, A-1190 Vienna

Phylloxera (Daktulosphaira vitifoliae Fitch), a threat in viticulture, has successfully been suppressed by grafting European V. vinifera cultivars on tolerant North American rootstocks. This strategy has been working well for over 100 years, however, in the past more aggressive phylloxera biotypes appeared in different regions throughout the world and therefore this topic gains actuality again. As sedentary feeders phylloxera requires a gall to feed and reproduce. The aphids induce their feeding site within the meristematic zone of the root tip, where they stay attached to the root, feeding intra- and intercellularly. Besides several changes in cell structures and composition, an increased cell division was reported, resulting in tissue swelling opposite to the feeding site to form a nodosity.

As expansins are involved in cell enlargement and cell wall loosening via non-enzymatic mechanisms in many plant tissues e.g. during fruit softening, root hair growth, and nematode attack we hypothese that an involvement in the nodosity formation occurs.

To identify different expansin genes in Vitis spp. in silico studies based on the protein sequences of Arabidopsis thaliana were performed identifying eleven putative expansins affirmed to be present in the rootstock Teleki 5C via PCR followed by sequencing. To evaluate the putative expansin gene expression, quantitative real time PCR (qRT-PCR) studies were conducted comparing young and old nodosities to uninfested root tips. Two putative expansin genes were up-regulated (2.4-20.7 fold) and three where down-regulated (3-36.7 fold).

We assume that the up-regulated putative expansins were responsible for gall growth and down-regulation could either be caused by phylloxera itself causing the inhibition of root tip growth or a defense strategy of the plant inhibiting tissue expansion.

Influence of phylloxera infestation on secondary metabolites content, their regulative enzyme in primary roots and comparision on secondary metabolites content in tertiary roots of different Vitis species

Zhao Qing, Du Yuanpeng, Zhai Heng

Secondary metabolites and their regulative enzyme in primary roots infested by phylloxera and the backgroud value of secondary metabolites in tertiary grape roots were tested, and the results showed that: secondary metabolites and their enzyme increased in primary roots after infested by phylloxera, the accumulation of total phenols, lignin and the increased activity of PAL, PPO and SOD in infested roots was highly correlative with grape phylloxera resistance, and their interrelating coefficient were 0.987, 0.841, 0.965, 0.909 and 0.782, respectively. The content of total phenols and lignin in 140Ru primary roots infested by phylloxera were 33.68%, 43.01% and 23.49% higher than that of Tyoho, Tamina and Crimson. The increased activity of PAL, PPO and SOD in 140Ru primary roots infested by phylloxera were higher than the other three varieties including Tyoho, Tamina and Crimson. The correlation between grape root phylloxera resistance level and the content of total phenols, flavonoids, lignin and pectin in tertiary roots was positively significant, whose interrelating coefficient were 0.797, 0.881, 0.885 and 0.919 respectively. In descending order, the 4 secondry metabolites quantity in grape roots were: rootstocks > HPDs > (V. vinifera, V. vinifera-V. labrusca and wild grapes).

Metabolomic Profiles of phylloxerated Gall Tissues of Root Tips

Weingart, G. J. F., Lawo, N. C., Schuhmacher, R., Forneck, A.

It is well known that many plant species respond to herbivory attack by an increased emission of volatile organic compounds (VOCs). Most of the studies, which investigated the chemical nature and biological functions of VOCs have been described for damaged aerial parts of the affected plants, especially green leaves. Further, it was proven that these metabolites also play a substantial role in the defence of plants against root herbivores. However, to our knowledge only little is known about the production of VOCs by grapevine plants infected by grape phylloxera (Daktulosphaira vitifoliae Fitch).

Therefore, the presented study investigates any changes in the profile of volatile metabolites produced by grapevine roots upon phylloxera infestation. Bioassays were performed in the glasshouse in 2009. Root galls, so called nodosities hosting either the 2nd nymphal instar or young adults (≤ 5 eggs), as well as uninfected root tips of infected plants and uninfected root tips of uninfected plants were collected after removing the aphids over a period of 6 months (June - November). After homogenizing the samples via a pestle a fully automated non-targeted gas chromatography-mass spectrometry (GC-MS) based approach was performed for the identification and profiling of VOCs in root tips of grape plants.

Differences in VOC emission were observed between young and old nodosities and uninfected root tips of uninfected and infected plants. Around 50 different metabolites could be identified. The volatiles were assigned to the major classes of C6-compounds, mono- and sesquiterpenes as well as aromatic substances, some of which have already been described to be involved in plant defence.

Grape phylloxera seasonal biology, predictive model, and management in the Ozarks

Johnson, D.T., S.M. Sleezer, B. Lewis, C. Rothrock, F. Goggin, M. Savin.

Grape phylloxera (GP), Daktulosphaira vitifoliae (Fitch), is a serious pest of wine grapes in USA. Studies were conducted on seasonal biology of GP to develop monitoring and degree day model for foliar GP, and test efficacy of insecticides and biopesticides against GP crawlers. Numbers of crawlers and alates were recorded weekly from sticky tape traps on canes and trunks and soil emergence traps. Reported cumulative degree-day (DD) value for development of foliar GP from oviposition to adult was 303 DD (base 6.4C). Our field study noted that second and third generation crawlers appeared in leaf galls by early May (305 DD accumulated since first leaf = biofix), 30 May (580 DD), respectively, and first alates by 7 Aug. (2,013 DD). Insecticide efficacy studies were conducted against foliar crawlers. In 2006, one application against second generation crawlers on 11 May (454 DD) of Danitol (fenpropathrin) and two applications of Assail (acetamiprid) on 11 and 25 May (637 DD) allowed < 3 shoots per vine with galled leaves which was significantly less than 6.4 galled shoots per vine for soil drench by Admire (imidacloprid) on 11 May or two applications of BAS 320 (metaflumizone) (11 and 25 May) compared to the check with 15.8 galled shoots. In 2008, Movento (spirotetramat) and Danitol applied to foliage on 30 May (580 DD) and a soil drench treatment of Admire (imidacloprid) applied on 17 March all had significantly fewer galled leaves per shoot than did foliar sprays of Assail and Esteem (pyriproxyfen) and all had significantly fewer galls than the check. In 2009 and 2010, foliar sprays of Danitol, Movento and white-washing foliage with Surround (kaolin clay) against third generation crawlers all prevented damaging levels of leaf galling.

A Quantitative Assessment of Root System Infesting Grape Phylloxera Population in Field.

Mark Hoffmann and Ernst Rühl
Department of Breeding and Grafting
Geisenheim Research Center
65366 Geisenheim
Germany

Gerhardt Eisenbeis
Institute of Zoology
University of Mainz
55098 Mainz
Germany

Lars Huber
SCC – Scientific Consulting Company
55234 Wendelsheim
Germany

Keywords: grape phylloxera, nodosities, Vitis berlandieri x Vitis riparia, rootstocks, root system development, Win Rhizo Pro

Below ground host-parasite interactions are connected to many soil ecological and plant physiological processes. Quantitative field assessment methods of parasite populations are necessary to identify possible interactions and to verify the efficiency of control strategies. The aim of the study was: (i) to give a general quantitative description of the dynamics of grape phylloxera population under consideration of the fine root system dynamics of grafted vines, and (ii) to investigate the relations between the appearance/characteristics of nodosities and grape phylloxera abundances on phylloxera tolerant rootstocks in field. The present data based on a long-term investigation (2006-2009) of a commercial used vineyard in Rheingau/Germany with Riesling grafted on two V. berlanieri x V. riparia rootstocks (5C, 125AA). Roots of the top 20 cm soil under foliage wall were investigated periodically. A scanner based root analysis (Win Rhizo Pro, Regent Inst.) was used for quantifying fine roots and to classify coloration of nodosities. Nodosities and phylloxera instars were counted and classified manually. Grape phylloxera abundances (different larval stages) as well as nodosity amounts were calculated per cm root length. Mean nodosity occupation rates were calculated. (i): Significant ($p<0.001$) increases of juvenile and adult virginopara abundances could be recorded in summer. A significant ($p<0.001$) peak of juvenile winged sexupara (nymphal instars) was verified for July and August. Differences between rootstocks were found. (ii): Grape phylloxera abundances were related moderately to nodosity occupation rates under field conditions (max. $R2 = 0.35$). Further it was demonstrated that sole nodosity classes showed similarly development trends as instars, but a directly relation of general nodosity appearance to grape phylloxera abundances was not possible.

Grape phylloxera development on potted grapevine treated with arbuscular mycorrhiza

L. Kocsis[1] – Włodzimierz Szałański[2]

[1]University of Pannonia, Georgikon Faculty, Department of Horticulture, 8360 Keszthely Deak F. St. 16. kocsis-l@georgikon.hu

[2]MYKOFLOR, Rudy 84, 24-130 Końskowola, Poland

Grape phylloxera is the most destructive insect of vineyards in Europe since it's inadvertent introduction at the end of the 19th century. Grape rootstocks are the only effective solutions to protect and maintain our vineyard more than a century ago. The key is if phylloxera could establish a feeding site on the root or not. If the insect was able to establish a feeding site, the next step would be if it could develop and reproduce on the root or not. It is known that arbuscular mycorrhizal symbiosis affects the community and diversity of other organisms in the soil (Marschner and Timonen, 2004). The idea for an experiment was that arbuscular mycorrhiza could be a natural protector against phylloxera with blocking to establish feeding site or influence the nutrition sources for further development of the insect.

Four different VAM treatments were applied, provided by the laboratory MYKOFLOR and control plants were maintained under glasshouse conditions. 7 plants in each treatment were inoculated with 50eggs per plant. The numbers of nodozity, the number of the insect in different development stages were counted. Reproduction ratio and multiplication ratio were calculated by treatments. The multiplication ratio was higher on VAM treated plants compare to the control however Vitis IIAK treatment showed positive effect to reduce the reproduction ratio. Because we were not so satisfied with the given results next year the treatment was repeated. The differences were compared to the previous year experiments that the mycorrhiza inoculation was made in the MYKOFLOR lab and the phylloxera inoculation in Hungary. The VAM increased the biomass production of the plants compare to the non VAM inoculated plants. 50 eggs were placed on each root and over one season the biomass production was measured and the number of phylloxera counted. There were significant differences among biomass production both of the VAM inoculated plants were 5-6 times bigger that none inoculated. Phylloxera was able to establish on all the three treated plants. There were differences in the number of the insects, highest was on Vitis, the lowest on non VAM inoculated plants. Our results show that VAM will help to develop the shoot and root system of the plants, but on the sensitive genotypes makes no control on number of phylloxera. Arbuscular mycorrhiza could not be an alternative solution to control the pest.

Literature:

Marschner, P., Timonen, S. (2004). "Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere". Applied Soil Ecology 28: 23–36

Grapevine Leaf Pigment Response to Root Infestation by Phylloxera

RJ Bruce[1], SA Robinson[2] S Norng[3] and KS Powell[1]

[1] Department of Primary Industries - Victoria, Rutherglen Centre, Biosciences Research Division, RMB 1145 Chiltern Valley Road, Rutherglen, Victoria 3685, AUSTRALIA

[2] Institute of Conservation Biology and Environmental Management, University of Wollongong, Wollongong, New South Wales 2522, AUSTRALIA

[3] Department of Primary Industries, Private Bag 15, Ferntree Gully Delivery Centre, Knoxfield, Victoria 3180, AUSTRALIA

Grapevine phylloxera (Daktulosphaira vitifoliae Fitch) is a root-feeding insect that establishes populations on the root system of grapevines, causing the formation of galls on Vitis vinifera and ultimately resulting in plant death. As phylloxera populations build-up on the grapevine root system and vine health declines, the chemical composition of the foliage, in the form of pigments, changes with the resulting leaf chlorosis often being the initial visual indication of potential infestation. Once chlorotic leaves are obvious, the infestation is already well established, and has generally spread to a broader area within the vineyard. Being able to detect pre-visual phylloxera-specific chemical changes as an indicator of infestation would be a significant step in the direction of early phylloxera detection, and may aid in the containment of new infestations and minimise the potential for spread.

A field trial was conducted in a newly detected phylloxera-infested vineyard in the Yarra Valley region of Victoria, Australia in 2007. Duplicate leaf samples were collected from phylloxera-infested and uninfested Vitis vinifera L. 'Cabernet Sauvignon', and analysed using high performance liquid chromatography for a variety of photosynthetic and photoprotective pigments. Pigments quantified included neoxanthin, chlorophyll a+b, ß-carotene, violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z). Statistical analysis of the results indicated changes in the ratios and concentration of some leaf pigments appeared to be correlated with the relative abundance of phylloxera emerging from the root system. In particular the proportion of the pool of xanthophyll photoprotective forms (AZ/VAZ) increased exponentially (with a steep rate and levelling off at about 250 crawlers) as the abundance of phylloxera increases. With further investigation pigment fingerprinting may prove to be useful for either a stand alone or an integrated (linked to spectral analysis) rapid, non-invasive, phylloxera detection system.

Protecting vines in stress environments: Early detection of grape phylloxera (Daktulosphaira vitifoliae Fitch) infestation through identification of chemical biomarkers

D. Benheim[1,2], E. Robertson[2], I. Potter[2], S. Rochfort[1] & K.S. Powell[1]

[1]Department of Primary Industries, Biosciences Division, Rutherglen Centre, RMB 1145 Chiltern Valley Road, Rutherglen, Victoria, Australia 3685.
[2]Department of Molecular Sciences, La Trobe University, Kingsbury Drive, Bundoora, Victoria, Australia 3086.

Early detection of grapevine phylloxera (Daktulosphaira vitifoliae Fitch) has become an issue of paramount importance to ensure sustainability and profitability of the viticulture industry the world over, with late detections causing significant economic damage to affected vineyards.The economic implications stem from the high costs, AUS$20,000 - $25,000 ha, associated with replanting vineyards with Phylloxera resistant rootstocks.

Detection methods under development, within Australia, include a soil DNA probel, hyperspectral leaf-level reflectance spectrometry, aerial imaging of vineyards and chemical fingerprinting (biomarkers and pigments).

Initial biomarker early detection studies have shown metabolic shifts in the leaves of infested vines both in glasshouse and field trials, with a number of potential biomarkers detected of a transient nature; their presence being as a function of seasonal vine growth stage. A decrease in relative concentrations of linoleic acid to linoleic acid within the triglyceride component of the leaf extract is postulated as a potential indicator of phylloxera infestation.

Challenges to overcome, in developing a robust chemical detection system, involve accurately discriminating between biomarkers and metabolic profile changes attributed by phylloxera infestation when compared to other stresses. The metabolic profile of phylloxera infested vines more closely approaches that of nitrogen deficiency rather than reduced water availability.

Field studies in the Yarra Valley, Victoria indicated a strong discrimination between flavonols within leaves between uninfested and infested vines, in addition to discriminatory non-identified metabolites.

Our study highlights the development of a novel early detection method built from foundation metabolomics conducted on the nature of vine material, leaves and berries, aimed at discovering chemical biomarkers indicative of phylloxera infestation. These biomarkers may be of insect origin or past of the host plants' immune response.

These studies aim to determine the viability of chemical fingerprinting techniques using NMR, LC-MS and FT-IR spectroscopy,). Data collected will be used to develop robust statistical models to discriminate between infested and non-infested vines, and between other plant stressors, such as nutrient or water stress. These models will further the effectiveness of biomarker discovery through identification of one or a sequence of chemical markers attributed to phylloxera infestation, leading to the possible development of routine field or laboratory analysis.

Grape phylloxera (Daktulosphaira vitifoliae Fitch) threat in grafted vineyards

L. Kocsis – T. Lönhárd - M.G. Kocsisné

University of Pannonia, Georgikon Faculty
H-8360 Keszthely, Deak F. u. 16.
kocsis-l@georgikon.hu

Grapevine phylloxera (Daktulosphaira vitifoliae, Fitch) is present in Hungary closely one and a half centuries ago. The insect could form the whole lifecycle under our circumstances because of the suitable environment. Earlier studies show that virulent phylloxera strains were present in Hungary feeding on rootstock roots (Kocsis et al. 1998). Other studies have shown that leaf galling forms appeared in Vitis vinifera L. cultivars (Györffyné Molnár et al. 2003; 2009). In our study we would like to examine whether phylloxera could cause quantity and quality yield loss in grafted vinifera vineyards or not.

We have surveyed root and leaf galling phylloxera populations in three locations. The V. vinifera was cv Cserszegi fuszeres. The rootstocks were Teleki 5C, Georgikon 28 and Fercal K25 respectively. The number of leaf galling forms were counted and as well as the number of individual insects per gall. Root samples were taken and the number of insects was recorded. The yield quantity, the sugar content of the yield, the acid content of the yield was measured, the length of the internodes of the canes and the weight also. Yield/cane weight ratio was calculated as well.

The results show that phylloxera causes damage on the quantity and the quality as well if the population builds up in grafted vineyard. Therefore cultural techniques need to be applied to maintain low population and rootstock selection will be extremely important in the future.

Nematode-resistant rootstocks as a major component of the management alternative for GFLV control in grape

Esmenjaud, D.[1]; Bouquet, A.[2]; Demangeat, G.[3]; Van Helden, M.[4]; Ollat, N.[5];.

[1]Inra, UMR Interactions Biotiques et Santé Végétale, 06903 Sophia-Antipolis, France. E-mail: Daniel.Esmenjaud@sophia.inra.fr;
[2]Inra, UMR-Diversité et Adaptation des Plantes cultivées, 34060 Montpellier, France;
[3]Inra, UMR Santé de la Vigne et Qualité du Vin, 68021 Colmar, France. E-mail: Gerard.Demangeat@colmar.inra.fr;
[4]ENITAB, Equipe de Zoologie, 33175 Gradignan, France.
E-mail : m-vanhelden@enitab.fr;
[5]Inra,UMR Ecophysiologie et Génomique Fonctionnelle de la Vigne, 33883 Villenave d'Ornon, France. E-mail: Nathalie.Ollat@bordeaux.inra.fr.

The ectoparasitic dagger nematode Xiphinema index specifically transmits Grapevine fanleaf virus (GFLV) which is responsible for a progressive degeneration of grapevines occurring in most vineyards worldwide. Because of the ban on nematicides and of the economically unacceptable interval (>7 years) required for nematode eradication between two successive vines, alternative nematode management strategies must be developed. These alternatives will have to face two major constraints: the nematode location in deep soil layers and the long retention time of the virus by the nematode in the soil between two successive vines. In this context, natural resistance appears a promising control alternative even though the long developmental cycle of the crop highly increases the risk for resistance breaking. A high resistance to X. index has been detected in the muscadine grape Muscadinia rotundifolia and attempts to introduce this resistance into rootstocks to obtain agronomically suitable candidate rootstocks have been difficult. A few M. rotundifolia x Vitis F1 hybrids and one BC1 individual (designated RPG1) were shown to express a good level of resistance. Resistance of these accessions has been challenged to a range of isofemale lines created from representative populations from the world nematode dispersal area. No interactions between nematode lines and accessions were observed but significant pathogenicity differences between lines have been shown. RPG1 shows a delayed viral contamination under field conditions. Nevertheless, under controlled conditions, a progressive adaptation of the nematode to the plant resistance factors seems to occur and better performing nematode-resistant hybrid material is being selected to cumulate muscadine resistance and the best partial Vitis resistance. In an integrated control strategy, this resistance strategy will be associated to the cultivation of non host or antagonistic crops between two vines.

New hybrid rootstock resistance screening for phylloxera under laboratory conditions

Korosi GA.[1], J Wood[2], B. Smith[3], R. Walker[3], P. Clingeleffer[3] & KS. Powell[1]

[1]Department of Primary Industries, Biosciences Division, RMB 1145 Chiltern Valley Road, Rutherglen, Victoria 3685, Australia.
[2]Department of Primary Industries, Farm Services Victoria, 255 Ferguson Rd, Tatura, Victoria 3616, Australia.
[3]CSIRO Plant Industry, Merbein, Victoria 3505, Australia.

Corresponding author: ginger.korosi@dpi.vic.gov.au

Grafting Vitis vinifera L., European grapevine onto rootstocks (hybrids of American Vitis species) has been a successful management tool for both phylloxera resistance and other important traits for grapegrowers worldwide including Australia There are many different commercially available rootstocks to choose from. Grapevine rootstock improvement and breeding for Australian environment conditions is very important for the viticulture industry. However, most of the commercially available rootstocks were bred outside Australia. Some of the most important desirable features for rootstocks under Australian conditions include nematode resistance, salt tolerance, good scion rootstock compatibility and phylloxera resistance.

A laboratory rootstock screening method has been developed to test the resistance of newly developed rootstocks in Australia against selected phylloxera genetic strains. This rootstock screening is conducted on excised roots under controlled laboratory conditions.

The development and survival of two phylloxera clones over an 8-week trial period was compared on 3 newly released rootstocks (two V. berlandieri Planch x and one V. cinerea Engelm x) and ungrafted V. vinifera. Relative resistance levels from the screening process are presented.

Genetic analysis of phylloxera root resistance in cultivar 'Börner'

Hausmann L., Eibach R., Zyprian E., and Töpfer R.

Phylloxera (Daktulosphaira vitifoliae Fitch) is one of the three most important viticultural pathogens introduced from North America to Europe in the middle of the 19th century. Since Vitis vinifera is highly susceptible for phylloxera on the roots the grapevines died soon after root infection and huge areas of viticulture were devastated in the following decades. In the end the problem has been overcome by breeding phylloxera-tolerant rootstocks to be used for grafting. Nearly all rootstocks currently used in Germany show to a certain degree nodosity symptoms indicating that phylloxera is present ubiquitously and can easily multiply in the soil. The recently released rootstock cultivar 'Börner' is an exception showing hardly any nodosity formation after phylloxera infection. 'Börner' is an interspecific cross of V. riparia Gm 183 and V. cinerea Arnold and therefore differs in the genetic background considerably from the majority of the rootstock cultivars descending from V. berlandieri x V. riparia crosses. A classical map-based cloning approach was used to identify the genetic determinants of the phylloxera root resistance in 'Börner'. The roots of the progeny of a cross of V3125 ('Schiava grossa' x 'Riesling') x 'Börner' were artificially infected with phylloxera leaf galls and phenotyped qualitatively and quantitatively concerning nodosity formation. After establishing a genetic map of the parents a single significant locus named Rdv1 (Resistance Daktulosphaira vitifoliae) was localised on chromosome 13 on the map of 'Börner'. SSR marker comparison between 'Börner' and the parents V. riparia Gm 183 and V. cinerea Arnold confirmed the later one as the origin of Rdv1. Detailed analysis of individual plants with a recombination break point near the Rdv1 locus delimited the locus further. The paternal haplotype sequence (360 kb) of Rdv1 was determined from V. cinerea Arnold using BACmids of 'Börner'. The entire Rdv1 sequence shows overall sequence similarity to the corresponding V. vinifera PN40024 reference genome sequence except for the middle part where a small resistance gene (RGA) cluster is located. Although the genes of this cluster are mixed with truncated transposable elements which makes an annotation more difficult some candidate genes for Rdv1 can be defined.

How tolerant are rootstock varieties against grape phylloxera?

Peter Schwappach, Bavarian State Research Center for Viticulture and Horticulture, Veitshoechheim, Germany

Since grape phylloxera (Dactylosphhaira vitifoliae FITCH) is present in vineyard soils of northern Italy in 2000 a rootstock-vineyard was planted near Lake Garda to check their resistance against infection of grape phylloxera. For this 170 different varieties have been planted with three vines each per replication. Spread over a surface of 5.000 m2 two replications per cultivar were planted in a distance of 2.5m between and 1.15m within the rows. Beginning in 2005 every year the development of the vines was monitored. By digging out the roots with a mini-bagger occurence of typical symptoms of phylloxera infestation at roots like tuberosities, nodosities, presence of phylloxera at the roots and root decay was registered for each variety.

There are distinct differences between the varieties tested. Some of them do not show any or at least little root decay while being surrounded by plants heavily infested. The same is true the other way around: There are varieties suffering heavily under the attack of grape phylloxera while growing in vicinity of almost healthy vines. This clearly can be explained by a specific reaction of the single variety.

There is no problem for the rootstock varieties that are most common in Germany. Varieties like 125AA, 5BB and SO4, but also less common varieties like 41B or 3309C may still be regarded as tolerant even under high infection pressure of phylloxeras. In contrast to this result are highly susceptible varieties like Sorisil and 26G. Those are infected by root destroying micro-organisms as a consequence of the injuries caused by sucking of phylloxeras. This leads to decay of roots and furtheron to weak growth and a lack of vigorness of the vines.

Only few varieties showed such a strong tolerance like Boerner and Cina. Unluckily both rootstocks are unsuitable for calcareous soils that are typical for many vineyards like e.g. in Franconia.

Once the resistance to root phylloxera is broken it will be very difficult to find other rootstock varieties within short time performing a high tolerance against grape phylloxera like that ones we can use at present. Also because of this it seems highly important to prevent spreading of grape phylloxera in German vineyards as far as possible.

Poster

A stepwise Assessment of Daktulosphaira vitifoliae infested Grapevines in a Viennese Vineyard Site

Aigner, C., Koennecke, T., Specht, S., Lawo, N. C., Forneck, A.

University of Natural Resources and Life Sciences, Department of Applied Plant Sciences and Plant Biotechnology, Institute of Horticulture, Fruit-Growing and Viticulture, Peter Jordan Str. 82, 1190 Vienna, Austria

Around 1863 the grape phylloxera, Daktulosphaira vitifoliae Fitch, was introduced from North America to Europe. In Austria phylloxera firstly appeared in 1872 in Klosterneuburg and quickly became known as a devastating vine pest. The major damage is caused by their sucking activity on the roots resulting in nodosities and tuberosities, causing the eventual death of the vine.

Plant protection may only be achieved by grafting European Vitis vinifera cultivars on tolerant American rootstocks so far.

The appearance of aggressive phylloxera biotypes has been reported in the last decades. Phylloxera successfully propagates on leaf forming rootstocks, escaped from cultivated vineyards. Here the natural habitats for the aphid are provided accelerating both abundance and migration into economic vineyards eventually causing the vine's decline

To examine the abundance of phylloxera on leaf forming rootstocks, selected areas of Viennese vineyards were observed over the vegetation period in 2010. Each leaf forming rootstock was examined for any phylloxera infestation on the leaves – starting in May with the first appearance of leaf galling phylloxera. In addition of mapping the current infestation, the individual habitat was recorded.

First observations have shown that, there is a very high chance to find phylloxera on escaped leaf forming rootstocks on sunny sites that are sheltered from the wind. Further, a considerable number of phylloxera infested wild rootstocks have been made and some immigration to cultivated vineyards in the first half of July.

The importance of genetic characterisation in root-stock resistance screening for phylloxera resistance under controlled conditions

Korosi, GA.[1], BM. Carmody[1], AA. Hoffmann[2] & KS. Powell[1]

[1]Department of Primary Industries, Biosciences Division, RMB 1145 Chiltern Valley Road, Rutherglen, Victoria 3685, Australia.
[2]Centre for Environmental Stress and Adaptation Research, 30 Flemington Road, Parkville, University of Melbourne, Victoria 3010, Australia.

The primary long-term grape phylloxera (Daktulosphaira vitifoliae Fitch) management option worldwide has been to graft varieties of Vitis vinifera L., European grapevine, onto resistant rootstocks. These resistant rootstocks are hybrids of American Vitis species including V. berlandieri Planch, V. riparia Michx, V. rupestris Scheele, V. cinerea Engelm and V. candicans Engelm. In Australia grape phylloxera is a quarantine pest and is restricted to phylloxera infested zones located in only two states (Victoria and New South Wales). So far 83 genotypic clones of phylloxera have been identified in Australia of which six genetic strains have been selected for screening, based on genetic diversity and geographical distribution, against conventional and newly developed rootstock hybrids for resistance traits.

A three-tiered rootstock screening approach has been developed in Australia to test the resistance of commonly used rootstocks in Australia against selected phylloxera genetic strains. Rootstock screening is conducted initially in the laboratory on excised roots and under glasshouse conditions on potted grapevines and finally in the field on commercially-grown grapevines.

The development and survival of six phylloxera clones and associated root damage over an 8-10 week trial period was compared on 5 commercially available rootstocks belonging to five hybrid crosses: 5BB Kober (V. berlandieri x V. riparia), Schwarzmann (V. riparia x V. rupestris), Borner (V. cinerea x V. riparia), Ramsey (V. rupestis x V. candicans) and 140 Ruggeri (V. berlandieri x V. rupestris). Results from laboratory and glasshouse rootstock screening are presented which highlight the importance of developing a robust screening program for phylloxera resistance based on genetic traits of both host plant and target pest.

Presence of Pantoea spp. in Leaf and Root-galling Phylloxera

Lawo, N. C., Forneck, A.

University of Natural Resources and Life Sciences, Department of Applied Plant Sciences and Plant Biotechnology, Institute of Horticulture and Viticulture, Peter Jordan Str. 82, A-1190 Vienna, Austria

Previous studies could show that grape phylloxera (Daktulosphaira vitifoliae Fitch) lack endosymbionts such as Buchnera spp. but the presence of Pantoea agglomerans on/in leaf-galling forms was reported. So far, however, no information regarding their role for phylloxera itself are available. Different studies in the literature report that P. agglomerans is involved in varying biological impacts in different insects, such as nitrogen fixation or antifungal phenols production. These statements allow the assumption that P. agglomerans might also be of some importance for phylloxera.

Thus, we aim in a first step to analyze if Pantoea spp. is always associated with leaf-galling grape phylloxera and further, if it can also be detected in/on root-galling phylloxera. Different populations of leaf- and root-galling phylloxera were sampled throughout Europe (Austria, France, Alsatia, Tyrol, Spain, Hungary, and Germany) and Australia and screened for bacterial association by a nested PCR technique.

Further, studies of the location and viability of P. agglomerans in different leaf galling populations (Austria, France, Italy and Spain) were conducted.

Towards improved early detection of Grapevine Phylloxera (Daktulosphaira vitifoliae Fitch)

R. Bruce[1], D.W. Lamb[2], A.A. Hoffmann[3], J. Runting[4] & K.S. Powell[1]

[1]Department of Primary Industries, Biosciences Division, Rutherglen Centre, RMB 1145 Chiltern Valley Road, Rutherglen, Victoria, 3685.
[2]Precision Agriculture Research Group, School of Science & Technology, University of New England, Armidale, NSW, 2351.
[3]Centre for Environmental Stress and Adaptation Research, University of Melbourne, 30 Flemington Rd, Parkville, Victoria, 3010.
[4]Grape Advice Pty Ltd, PO Box 413,Balnarring, Victoria, 3926.

Grapevine phylloxera (Daktulosphaira vitifoliae Fitch) is a significant threat to the Australian viticulture industry, with over 80% of grapevines planted on highly susceptible, ungrafted Vitis vinifera L. Early detection of phylloxera is critical as it can spread unnoticed in the early years of infestation when vine foliar symptoms may not reflect subterranean root damage. Management strategies such as phylloxera exclusion zones (PEZ) and a number of quarantine protocols relating to machinery and movement of grape materials are in place within Australia in an attempt to contain proliferation throughout viticultural regions. Such measures are particularly important for regions like the Hunter Valley in New South Wales and the Barossa in South Australia, which are phylloxera-free and sustain a high volume of viticultural production.

Currently, early detection relies on systematic sampling of plant roots to inspect for the presence of phylloxera, and has included multispectral aerial imagery to identify potentially stressed vines. Whilst the former is labour intensive, aerially identified vineyard weak spots may not necessarily be due to phylloxera infestation, rather the expression of non-specific water or nutrient related symptoms. Healthy canopy vigour may also disguise the expression of above ground signs of root degradation.

The delayed appearance of visible symptoms, coupled with the dynamics of phylloxera infestation means current detection methods would be improved through development of an approach based on biophysical descriptors that directly indicate the potential susceptibility of vineyards to phylloxera infestation. This paper reports on the integration of spatially-registered measurements in a more targeted phylloxera detection system. Measurements include photosynthetically-active biomass (PAB) in the vine canopy (GreenseekerTM), soil electrical conductivity (ECa) derived using EM38, and direct measures of phylloxera incidence including a soil-based phylloxera-specific DNA probe, root surveying and emergence trapping. Preliminary observations from a trial conducted in phylloxera-infested vineyards in the Yarra Valley Region (Victoria, Australia) over two consecutive growing seasons are described.

Biological compatibility between the entomopathogenic fungus Beauveria bassiana and fertilizers products used in organic viticulture

Oana Cristina Popa*, Cristina Fatu**, Maria Gheorghe***, Silvia Cazacu***, Ioan Rosca*, Ana Maria Andrei**

* University of Agricultural Sciences and Veterinary Medicine-Bucharest,
** Research-Development Institute for Plant Protection,
*** Institute of Research-Development for Vineyard and Vinification -Valea Calugareasca

Assuming that the use of organic fertilizers and biological control agents make to increase the soil's repressive effect against the development of phylloxera and other pests in the vineyard, it was conducted laboratory experiments, in order to assess the effect of farm manure and compost on biological parameters of some Beauveria bassiana strains selected for obtaining biological insecticides. It was tested three B. bassiana strains belonging to the entomopathogenic microorganisms collection of the Romanian Research-Development Institute for Plant Protection. Barkley kernels colonised by fungal strains was incorporated in soil fertilizers; after a six months incubation period at 240 C, the fungal strains were re-isolated from test fertilizers and it was quantified the following biological parameters of single-conidium isolates: vegetative growth, conidiogenesis, viability and virulence. To test the virulence, aqueous spray of conidia were applied on Plodia interpunctella larva, used as test insect. B. bassiana strains colonized organic substrates, the saprophytic development was abundant, the vegetative multiplication and the sporulation were not inhibited in any of the experimental variants. The average size of fungal colonies and their daily average growth rates were close to the control variants. Estimated conidia viability showed a mean percent germination up to 91%. The conidia was high virulent, it was registered 89-93% P. interpunctella mortality. This study shows that the organic fertilizers farm manure and compost are compatible with B. bassiana, the romanian fungal strains can be used for a B. bassiana inoculum conservation strategy in organic viticulture based on preliminary trials with good results in control of phylloxera.

Phylloxera extension through: National Phylloxera Management and Identification Workshops

PT Mee, GA Korosi & KS Powell

Department of Primary Industries, Rutherglen centre, Biosciences Division, Bioprotection Platform, RMB 1145 Chiltern Valley Road, Rutherglen, Victoria, 3685. Corresponding author email: kevin.powell@dpi.vic.gov.au

Agricultural extension programs are an effective means of communication to raise productivity, assist farmers in identifying problems and engage industry to change practices. Evidence of the effectiveness of extension programs has been highlighted in recent evaluations of National Phylloxera Management and Identification Workshops which have been conducted annually in Australia, for the past sixteenth years. Grapevine phylloxera (Daktulosphaira vitifoliae Fitch) is a significant biosecurity issue to the Australian viticulture industry, due to the predominance of ungrafted Vitis vinifera L. plantings, with the insect being contained by legislation within designated quarantine boundaries. New infestations both within and outside quarantine boundaries are primarily identified by vineyard staff, noticing changes in vine canopy health, which highlights the need for effective informative extension programs. Phylloxera workshops cover a range of core topics including phylloxera biology and genotypic diversity, geographical distribution, detection methods, quarantine protocols and rootstock selection. Workshops provide a unique learning environment engaging participants from every aspect of the viticultural industry, to learn from, interact with and question, phylloxera research scientists, as well as engage with affected growers currently managing this pest. Phylloxera workshops create a progressive learning environment bringing together the latest information on modifications to quarantine protocols, existing infestations and scientific advancements in rootstock selection and surveillance techniques. Hosted by the Victorian Department of Primary Industries-Rutherglen, workshops include practical components conducted in quarantine laboratories and on infested commercial vineyards, enabling theoretical knowledge on detection and surveillance techniques acquired at the workshop to be applied. Over recent years all workshops have been evaluated using Keepad technology and revised accordingly where necessary. In addition supplementary audio visual and reference material has been developed. Successful completion of this program not only increases phylloxera awareness within the industry but also enables participants to receive accredited units towards a Certificate IV in Agriculture. Recent phylloxera outbreaks in Australia highlight the need for increased awareness of this significant pest across all viticulture regions. This can be and has been achieved in Australia, through the delivery of National Phylloxera Management and Identification Workshops, assisting in the prevention of phylloxera's spread and ensuring the security of the whole viticultural industry.

Do Root-feeding Phylloxera influence the Leaf Spectrum of Vitis vinifera cv. Cabernet Sauvignon?

Scholz, M., Lawo, N. C., Schoedl, K., Forneck, A.

University of Natural Resources and Life Sciences, Department of Applied Plant Sciences and Plant Biotechnology, Institute of Horticulture and Viticulture, Peter Jordan Str. 82, A-1190 Vienna

Phylloxera (Daktulospharia vitifoliae Fitch) causes major damage by root feeding ba forming root galls (nodosities and tuberosities) and plants respond to serious root damage with reduced growth, wilting and fading.

We aim to identify significant parameters that distinguish among infested and uninfested vines by monitoring reflectance spectra in order to devleop a tool for monitoring the plant's response.

Measurements of leaf reflectance spectra provide a useful and fast screening tool to assess the physiological status of a plant. The spectra are based on leaf surface properties, internal structures plus the concentration and distribution of biochemical compounds. In the past Australian studies have shown that there is a variation in the leaf reflectance spectra and the caroteinoid / chlorophyll content between uninfested and root-feeding phylloxera infested vines. To confirm this observation for Austrian phylloxera and to determine at which state the physiology of the plant will be influenced we will compare the leaf spectra of uninfested Vitis vinifera cv. Cabernet Sauvignon vines with the one of vines having a strong phylloxera infested root system. Spectra are recorded from 300 to 1100 nm using a Unispec - DC Spectral Analysis System (PPSystems, Amesbury, MA, USA) and are monitored from twelve vines per treatment before infestation and three, six, ten, 13, 20 and 27 days after infestation under controlled greenhouse conditions.

The aim of the study is to find any differences in the monitored spectral regions between the treatments, and thus a way to determined root-feeding phylloxera early enough before the vine is seriously damaged.

Participants

❖ **AUSTRALIA**		
Dr. Kevin POWELL	Department of Primary Industries Victoria Rutherglen, Victoria 3685 kevin.powell@dpi.vic.gov.au	P
❖ **AUSTRIA**		
Mr. Clemens AIGNER	BOKU University Wien (Vienna) ClemensAigner@gmx.at	P
Professor Astrid FORNECK	BOKU University Wien (Vienna) astrid.forneck@boku.ac.at	P
Dr. Michaela GRIESSER	BOKU University Wien (Vienna) michaela.griesser@boku.ac.at	P
Ms. Roberta KOLBERG	BOKU University Wien (Vienna) parasitoidegryon@yahoo.com.br	P
Mr. Thore KÖNNECKE	BOKU University Wien (Vienna) thorekoennecke@aol.de	P
Mr. John-Philip LAWO	Baxter Innovations GmbH Wien (Vienna) john-philip.lawo@gmx.de	P
Dr. Nora LAWO	BOKU University Wien (Vienna) nora.lawo@boku.ac.at	P
Ms. Helga REISENZEIN	AGES Wien (Vienna) helga.reisenzein@ages.at	P
Ms. Katharina SCHÖDL	BOKU University Wien (Vienna) katharina.schoedl@boku.ac.at	P
Mr. Markus SCHOLZ	BOKU University Wien (Vienna) h-m.scholz@aon.at	P

Dr. Rainer SCHUHMACHER	BOKU University Wien (Vienna) rainer.schuhmacher@boku.ac.at	P
Mr. Soeren SPECHT	BOKU University Wien (Vienna) soeren.specht@web.de	P
Mr. Georg WEINGART	BOKU University Wien (Vienna) georg.weingart@boku.ac.at	P
❖ **BOSNIA AND HERZEGOVINA**		
Mr. Branimir NJEZIC	University of Banja Luka Banja Luka branimirnjezic@yahoo.co.uk	P+N
❖ **CHINA**		
Mrs. Yuanpeng DU	Shandong Agricultural University Tai'an 001duzi@sina.com	P
Mrs. Qinghua SUN	Shandong Agricultural University Tai'an qhsun@sdau.edu.cn	P
❖ **CROATIA**		
Mrs. Ivana PAJAC	University of Zagreb Zagreb	P
❖ **FRANCE**		
Mr. Patrice COLL	IRD Montpellier patrice.coll@ird.fr	P+N
Mr. Francois DELMOTTE	INRA Bordeaux Villenave d'Ornon Cedex delmotte@bordeaux.inra.fr	P
Dr. Daniel ESMENJAUD	INRA Sophia-Antipolis Daniel.Esmenjaud@sophia.inra.fr	P+N
Ms. Nathalie OLLAT	INRA Villenave d'Ornon ollat@bordeaux.inra.fr	P
Ms. Daciana PAPURA	INRA Bordeaux Villenave d'Ornon Cedex dpapura@bordeaux.inra.fr	P

❖ **GERMANY**		
Dr. Joachim Eder	Dienstl'zentrum Ländlicher Raum Rheinpfalz Neustadt/Wstr. joachim.eder@dlr.rlp.de	P
Dr. Ludger HAUSMANN	Julius Kuhn-Institute Siebeldingen ludger.hausmann@jki.bund.de	P
Dr. Christoph HOFFMANN	Julius Kuhn-Institut Siebeldingen christoph.hoffmann@jki.bund.de	P
Mr. Mark HOFFMANN	Geisenheim Research Center Geisenheim hoffmanm@uni-mainz.de	P
Prof. Hugh LOXDALE	Friedrich Schiller University Jena Hugh.Loxdale@uni-jena.de	P
Mr. Niels MÜLLER	Staatliches Weinbauinstitut Freiburg Freiburg nielsm5@aol.com	P
❖ **HUNGARY**		
Ms. Sharareh GHASEMI	Corvinus University Budapest ghsharare@gmail.com	
Prof. Laszlo KOCSIS	Pannon University Keszthely kocsis-l@georgikon.hu	P
❖ **IRAN**		
Ms. Fatemeh MOHAMMADI	Tehran Institution of Public Health Tehran shabnamm_2005@yahoo.com	P
❖ **NETHERLANDS**		
Mr. Hans HELDER	Wageningen University Wageningen Hans.Helder@wur.nl	P
❖ **POLAND**		
Mrs. Aneta CHALANSKA	Research Instit. of Pomology and Floriculture Skierniewice aneta.chalanska@insad.pl	P+N
Prof. Gabriel LABANOWSKI	Research Instit. of Pomology and Floriculture Skierniewice glabanow@insad.pl	P+N

Dr. Miroslaw SOBCZAK	Warsaw University of Life Sciences (SGGW) Warsaw miroslaw_sobczak@sggw.pl	P+N
❖ **PORTUGAL**		
Ms. Mónica ZUZARTE	University of Coimbra, Portugal Coimbra mzuzarte@hotmail.com	P+N
❖ **ROMANIA**		
Prof. Ioan ROSCA	Univ. of Agricultural Sciences and Veterinary Medicine, Bucharest ioanrosca_usamv@yahoo.com	
❖ **USA**		
Dr. Donn JOHNSON	University of Arkansas Fayetteville dtjohnso@uark.edu	
Prof. Isgouhi KALOSHIAN	University of California Riverside isgouhi.kaloshian@ucr.edu	P+N
Mrs. Sandra SLEEZER	University of Arkansas Fayetteville ssleezer@uark.edu	
Dr. Robert VAN STEENWYK	University of California Berkeley rvsfam21@yahoo.com	

P = attending Phylloxera Symposium
P+N = attending Phylloxera and Nematodes Symposium

Participants

Phylloxera Genome Workshop

D. Coll (FR)

A. Forneck (AT)

T. Gabaldon (ES)

L. Hausmann (DE)

N. Lawo (AT)

D. Kreil (AT)

K. Powell (AU)

C. Stauffer (AT)

D. Tagu (FR) - Chair

D. Yuanpeng (CN)

Grape Phylloxera Quarantine Workshop

J. Eder (DE)

A. Forneck (DE)

M. Griesser (AT)

C. Hoffmann (DE) - Chair

R. Kolberg (BR)

N. Lawo (AT)

N. Müller (DE)

C. Pajac (HR)

K. Powell (AU)

P. Schwappach (DE)

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