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Allelopathy in a changing world

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BOOK OF ABSTRACTS

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Book of abstracts

Allelopathy in Mediterranean Forest Ecosystems: Insights from 20 Years of Research

By Catherine Fernandez and all the DFME team

The Mediterranean region, one of the world's major biodiversity hotspots, is heavily impacted by reforestation, which directly threatens the mosaic of open areas. Recent economic and social changes in the northern Mediterranean regions have led to significant rural depopulation since the early twentieth century. As a result, meadows that developed on abandoned agricultural lands, known for their high species richness, are now undergoing reforestation. Although the different stages of successional dynamics leading to the formation of forest ecosystems are well described, the functional mechanisms involved are poorly documented or unknown. In particular, allelopathic mechanisms are believed to play an important role in the replacement of plant species during succession. In North Mediterranean forests, *Pinus halepensis* Miller, a pioneer and expansionist species, colonizes abandoned agricultural land characterized by high biodiversity. The result is an almost monospecific young forest, which leads to paraclimaxes that delay the establishment of oak forest ecosystems. Due to their richness in specialized metabolites, the various species involved in the succession, particularly *P. halepensis*, could play a role in ecosystem functioning through several processes (e.g. biotic intercations, decomposition). During this conference, we will summarize the numerous laboratory, greenhouse, and/or in situ experiments carried out at all stages of Mediterranean secondary succession, demonstrating that allelopathy is one of the drivers of this secondary succession.

Applications of Metabolomics and Metabolite Profiling for Characterisation of Plant, Microbial and Herbivore Interactions - Methods, Bioinformatics and Data Processing

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Plant secondary products are the drivers of many plant interactions, both above and below ground, with other plants, herbivores and soil-borne macro- and microbiota. During plant growth and cellular ontogeny, secondary plant products are biosynthesized and often accumulate in a highly regulated manner in specialized tissues and organelles. Metabolites released by plants and their residues can play important roles in rhizosphere signalling, plant defence and responses to abiotic stresses. Plants employ a variety of sequestration and transport mechanisms to store and release bioactive metabolites and export them selectively into the surrounding environment. Metabolites can be released by leaching, volatilisation or exudation into the environment where further transformation by microbiota may take place. Herbivores also ingest plant tissues and metabolites can be transported to various organs through the blood stream and further transformed following digestion.

Here, we focus on the production, localization and release of plant-produced metabolites by combining visual growth observations and bioassay results with metabolomics and metabolic profiling. Targeted and non-targeted metabolite profiling has successfully identified novel metabolites involved in plant-plant, plant-microbe and plant-herbivore interactions and biosynthetic pathways of interest, as well as microbial transformation products. We describe case studies using metabolite profiling to discuss experimental methods employed to evaluate interactions or treatment differences. We present techniques to minimise chemical, electronic and biological noise in the resulting profile, and also discuss chemometric and data processing approaches for model development and data presentation.

Keywords: metabolomics, metabolite profiling, methods, data processing, chemometrics approaches.

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Metabolites from Allelopathic Plants to Control Weeds

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Weeds infest economically important crops causing noteworthy losses in agrarian production, and represent serious damage to the forestry and natural heritage as well as to historic gardens. Weed pests, including parasitic plants, have always being considered as one of the most serious agricultural and environmental problems due to competition with the growth of agrarian crops and forest and ornamental plants by subtraction of water, nutrients, light and by the serious obstacles they represent for agronomic activities.[1-3] A number of weed management strategies have been followed in agriculture production, including mechanical, cultural, chemical, and biological strategies. Among them the use of soil solarisation, hand pulling, crop rotation, delay in the sowing date, catch and trap crops, soil amendments and crop genotypes with better competitive and allelopathic ability do not provide a satisfactory solution to weed problems. On the other hand, the use of chemical pesticides differing widely in respect to spectrum, unit activity, crop safety, toxicology, and environmental effects has increased herbicidal resistance and environmental and toxicological concerns raise a question mark over their large scale use. Consequently, many efforts were devoted to the development of alternative strategies based on the use of natural products and in particular on the use of fungal and allopathic plant phytotoxins alone, as natural herbicides, or in a more efficient, integrated management.[1,5] The most efficay formulation of potential bioherbicide is another important goal to be reached in view of a practical application of these bioactive compounds.[6,7]

Keywords: weeds, parasitic plant, chemistry and biological propertis, formulation

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ADVANCES OF PLANT METABOLOMICS IN THE STUDY OF SELF-DNA INHIBITION

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Extracellular DNA (exDNA) widely occurs in the environment due to release by either cell lysis or active secretion. The role of exDNA in plant-soil interactions has been investigated in the past years. An inhibitory effect on the growth of conspecific individuals by their self-DNA has been recently reported.[1,2] This effect was first discovered in plants and then demonstrated on the model organisms: *Arabidopsis thaliana* (plants),[3] *Coenorabtidis elegans* and *Drosophila melanogaster* (animals). [4,5]

Transcriptome analysis in the model plant *A. thaliana* showed a clear recognition by the plant roots of self- and nonself-exDNA, with inhibition occurring only after exposure to the former.[3] Untargeted NMR and LC-MS metabolomics followed by chemometrics were used to assess at molecular level the plant reactions to self and nonself-exDNA exposure.[6] Results evidenced that self-DNA significantly induces the accumulation of RNA constituents (nucleobases, ribonucleosides, dinucleotide and trinucleotide oligomers). Interestingly, AMP and GMP are found along with their cyclic analogues cAMP and cGMP, and in form of cyclic dimers (c-di-AMP and c-di-GMP). Also methylated adenosine monophosphate (m6AMP) and the dimeric dinucleotide N-methyladenylyl-(3’→5’) cytidine (m6ApC) increased only in the self-DNA treatment. Such striking evidence of self-DNA effects highlights a major role of exDNA in plant sensing of its environment.

This discovery opens a new scenario of pharmacological applications integrating metabolite profile assessment with specific inhibitory effects by self-DNA.

Keywords: self-DNA inhibition, Metabolomics, NMR, LC-MS, RNA constituents

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Can an allelopathic study in Gramineae provide real applications?

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Allelopathy is a biological phenomenon in which an organism produces one or more allelochemicals that influence the growth, survival, and reproduction of other organisms. Allelopathy has been the subject of a great deal of research in chemical ecology since the 1930s.[1]

The main aim of the research carried out to date has been to shed light on the importance of these interactions in agroecosystems, especially in relation to the interactions between crops and weeds. Among the natural products, benzoxazinones and benzoxazinoids arise as promising groups of allelochemicals for use as natural herbicides, both as pure compounds or through plants such as rye, in which they are produced in great quantities (Figure 1).[2] I*n vitro* applications of these compounds have been evaluated and demonstrated through different plant models, but *in vivo* and *in agro* evaluations are limited by poor physicochemical properties, mainly solubility.

Last years, our research has been focused on the synthesis of new analogous compounds whose keep the activity of the natural compounds but improve the properties. Sulphur analogues, as DIMBO derivatives with a thioether group instead of oxygen function, have been synthesized. Halogenated derivatives or carboxylic groupshaven been introduced in the aromatic ring scaffold to enhance bioavailability. In the recent experiments, encapsulation techniques with the natural benzoxazinoids have been applied, by using nanoparticles and micelles.[3]

This main route has carried to out to some final products that we are able to test in crop field with promising results in the protection of wheat against weeds that reduce the production yield. Hence, this objective has aimed to achieve a real use of allelopathy for a sustainable agriculture by using Gramineae as an example of what can we learn from nature.

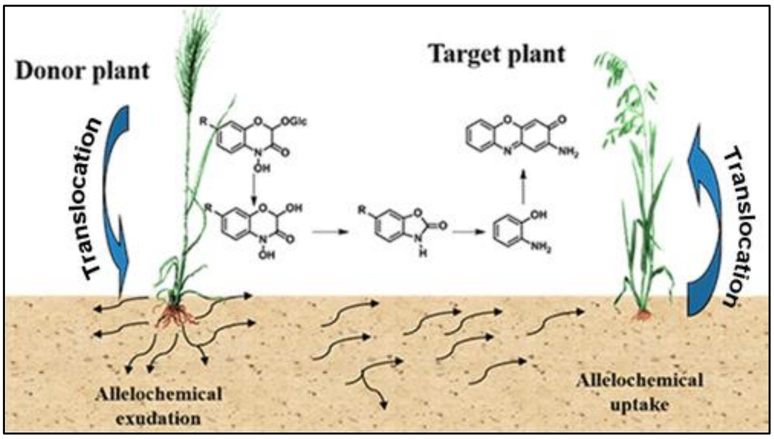

Figure 1. Proposed representation for the allelopathic pathway in the coculture of *S. cereale* and *A. fatua*. Reproduced with permission from [1].

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Methodologies for Ecologically Rigorous Study of Plant-Plant Allelopathic Interactions: The Challenge of Complexity Above- and Belowground

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The complexity of the many direct and indirect interactions between plants and other organisms has hindered progress in understanding allelopathic interactions among plants. Allelochemicals are only one of many factors that can impact plant germination and growth, along with resource limitations, pathogens, herbivores, and microbial interactions. Interaction between interference mechanisms is the norm rather than the exception, and the magnitude of effects will depend on both the biotic and abiotic environment. Belowground, the rhizosphere is a dynamic and complex chemical environment, with the chemical composition varying in both time and space as thousands of potentially bioactive allelochemicals are produced by plants, microorganisms, and soil invertebrates, leached from decaying plant material, and degraded and transformed by soil microorganisms before being taken up by plants, binding to soil components, or leaching from the root zone. This presentation will review recent advances in soil analysis techniques and ecological approaches which have potential to advance our understanding of allelopathic interactions, provided they are applied in ecologically rigorous and meaningful ways.

Screening wheat varieties for being used as allelopathic crops in sustainable agriculture. The role of allelochemical compounds

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This study focuses on the potential benefits of screening allelopathic cultivars for sustainable weed management. The allelopathic potential of 30 wheat cultivars grown with two aggressive and herbicide-resistant weeds, the dicotyledonous common purslane (*Portulaca oleracea* L.) and the monocotyledonous annual ryegrass (*Lolium rigidum* Gaud), was evaluated to see how different varieties could affect each weed differently. After co-culture of wheat cultivars with the weeds, germination rate (GR), plant weight (PW), shoot and root length (SL and RL), and plant height (PH) of the weeds were measured to obtain information about the reduction or stimulation of shoot and root growth, the ability of weed species to colonise and occupy the space through germination and development (Shoot Invasive Capacity (SIC) and Root Invasive Capacity (RIC)) and how weed plants can develop in next generation (Seedling Vigour Index (SVI)). SL, RL and PW of each wheat variety were also measured. Moreover, allelochemicals from shoots, roots, and root exudates from wheat were quantified to evaluate the accumulation or release of these compounds by the different wheat accessions in front of the weeds. A correlation was found between root exudation and weed inhibition. For example, the large amount of root exudates of Ursita resulted in a strong inhibition of both weeds. The same was true for Maurizio and Midas, which exuded higher amounts of benzoxazinoids than other varieties, resulting in weaker and smaller weeds. By contrary, accumulation of phenolic compounds in the plant organs without exudation to the medium suggests a defensive or protecting role of these compounds more than an allelochemical role. Moreover, the weed pressure influences also the amount of compounds exuded by the roots to the media, which supports the idea of promoting biodiversity in agroecological systems. Regarding *L. rigidum* and *P. oleracea* management, the most promising varieties for controlling annual ryegrass are NS Azra, Ludwig, Maurizio, PS Dobromila, Midas and Tobias, while Capo, Glosa, Adesso, Annie, Ludwig, Midas and NS Azra were the most phytotoxic against common purslane. In summary, this study reveals that variety screening is crucial to move towards sustainable weed management and agroecological farming.

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Keywords: allelopathic crops, wheat, biological control, allelochemicals, agroecological farming

NMR-based metabolomics to study allelopathy: where do we stand, where do we go?

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NMR-based metabolomics is a powerful tool to study allelochemicals. This approach is useful to speed up the discovery of chemicals (or their mixtures) able to influence growth and performance of other plants. It allows to study allelopathy from the point of view of both the donor and receiving plant, can assist in the elucidation of the mode of action of allelochemicals and allows to follow the fate of these bioactive compounds in the environment and in the receiving plant. [1-3]

Its potential in this field is shown here by a few case studies focusing on Mediterranean plants. These studies were carried out on plants potentially producing allelochemicals (donor plants). Donor plant extracts were analysed by ^{1}H- and 2D-NMR to define their chemical composition. These extracts were tested for their phytotoxicity on hydroponically grown receiving plants, using a standardized procedure which also includes metabolomics analysis of the treated receiving plants and controls.[2,3] The results were then validated by testing enriched fractions or pure compounds.[3]

The outcomes of these studies included the identification of potential allelochemicals. Furthermore, using metabolomics, it was possible to study the main metabolic pathways affected by the treatments in the receiving plants, while also following the fate of the active compounds.

The studies that will be discussed show how the application of NMR-based metabolomics to the study of allelopathy evolved over time, while also highlighting possible drawbacks and discussing future perspectives and developments.

Keywords: Mediterranean plants, Metabolomics, NMR

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Allelochemical interactions of Sphagnum mosses under climate changes: from physiological responses to peatland functioning

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The genus *Sphagnum* covers 80-100% of peatlands and due to their physiological activity, *Sphagnum* species contribute significantly to carbon (C) productivity and storage (up to 30% of the global soil C). Global increases in temperature, atmospheric concentrations of CO_2 and nitrogen (N), and ultraviolet (UV) radiation, combined with a reduction in water availability, are important major changes currently occurring in peatlands and are predicted to increase.

Here we propose a synthesis of the effect main environmental variables on biological, physiological and metabolites production of *Sphagnum* conducted in laboratory or *in situ* experiments. Allelochemical interactions between *Sphagnum*, their microbiome or surrounding mosses or other plants were then found to be determinant to *Sphagnum* responses under those variables. Results reinforce the interest of such allelochemical investigations in peatland functioning, even if it is complex to make the link between the different biological levels studied and the spatio-temporal variations of such ecosystems.

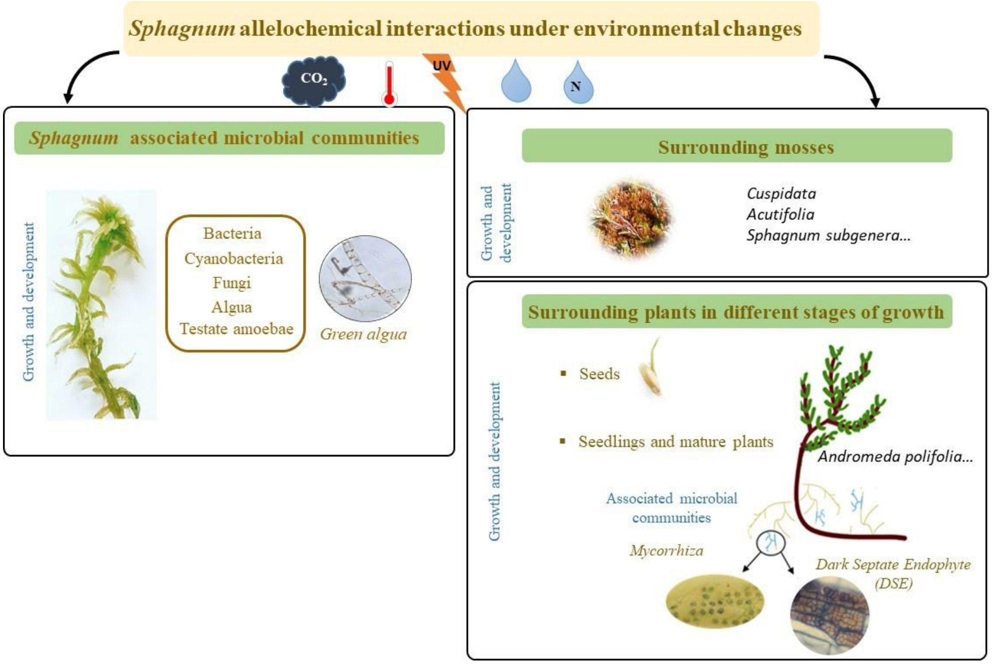

Figure from G. Chiapusio, P. Binet, C Bertheau, P Priault, Aquatic Ecology 2022, 56, 429 – 445. https://doi.org/10.1007/s10452-021-09924

All We Know about the Mode of Action of Azelaic Acid in Plant Metabolism

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In the present study, we investigated the phytotoxic potential of the specialised metabolite azelaic acid (AZA) on *Arabidopsis thaliana* metabolism to elucidate its mode of action. The results revealed that low concentrations of AZA affected root morphology, inhibiting root growth and stimulating adventitious root formation. Furthermore, AZA treatment altered root apical meristem, resulting in reduced meristem size, cell number, length, and width. Molecular docking simulations demonstrated that AZA has high affinity for the auxin receptor TIR1, competing with auxin for the binding site and consequently disrupting the proper functioning of TIR1/AFB complex, which may alter auxin polar transport, inducing auxin accumulation in elongation and maturation zones, and affecting root gravitropic response, as observed in this study. Short-term experiments were conducted to monitor the endomembranes due to AZA's auxin-like effects, revealing alterations in Golgi dynamics (reducing Golgi size and velocity), disruption of the secretory pathway, and induction of ER stress on *A. thaliana* metabolism.

Untargeted metabolomic analysis revealed general accumulation of osmoprotectants induced by AZA, including amino acids (lysine, GABA, threonine, etc.), organic acids (glutaric, shikimic, succinic acid, etc.) and sugars (glucose, mannose, xylose, etc.). Exogenous auxin application has been reported to induce starch accumulation, accompanied to reduction of meristem size, thereby inhibiting auxin polar transport in *Arabidopsis*[1,2]. These findings establish a connection on AZA's mode of action, as the increase of sugar levels is linked to the rise of free auxin in *Arabidopsis* roots due to AZA's competition with auxin for TIR1. Ionomic analysis revealed reduction of K^+ content, aligned with putrescine accumulation, considered a metabolic biomarker of K^+ deficiency. Moreover, AZA caused imbalance in the levels of jasmonates and brassinosteroids. This study provides insights into how AZA affects plant metabolism, suggesting that AZA could act as an auxinic herbicide, interesting for the development of new bioherbicides.

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Phloretin – A Lesser-Known Allelochemical from the Apple Trees and Its Proposed Mode of Action

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Phloretin is a phenolic compound from apple trees that is also found in traces in some other plant species such as *Pieris japonica*, *Hoveniae lignum* and *Loiseleuria procumbens*. The role of phloretin in plants is less known, although it is widely used in pharmaceuticals and cosmetics due to its excellent antioxidant properties, anti-cancer and immunoprotective effects. The autotoxicity of apple trees is known as Apple Replant Disease (ARD), but knowledge about phloretin effect on other plant species is very scarce. Our research team was the first to report on the phytotoxicity of apple hairy root exudates[1]. Our current research is based on the discovery of the molecular and physiological pathways of phloretin allelopathic activity in the model plant *Arabidopsis thaliana* (L.) Heynh. Our studies show that the inhibitory effect of phloretin manifests at different levels with rapid and cumulative effects. Phloretin most likely affects the plant in several ways: on the one hand, it influences the metabolism and distribution of auxin[2] and, on the other hand, affects photosynthesis. The intensive glycosylation of phloretin in *A.thaliana* tissues is one of the earliest activity of plant defense strategies. However, it is insufficient to prevent phloretin early phytotoxic effects that include elevated expression of genes involved in polar auxin transport and auxin accumulation in lateral parts of the root tips, leading to the inhibition of root growth, and impaired gravitropic response. Further exposure to phloretin results in the relocation and swelling of chloroplasts, changes of thylakoid organization and arrest of photosynthesis, all together leading to the depletion of starch reserves, cell starvation, and recruitment of self-preservation micro- and macroautophagic processes resulting in programmed cell death. We propose that the interplay between auxin signaling and cell starvation is a key to the phytotoxic action of phloretin in model plant *A.thaliana.*

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Canavanine - a plant origin nitric oxide synthase inhibitor arrests cell divisions in the root apical meristem

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Canavanine (CAN) is a non-proteinogenic amino acid, an analog of arginine, synthesized by some legumes. Jack bean (*Canavalia ensiformis*) accumulate high amounts of CAN, particularly in the seeds, where it serves as a nitrogen storage compound and protection against herbivorous insects[1]. It has been demonstrated that alfalfa (*Medicago sativa* L.) secretes CAN into the environment through root exudates. CAN enhances the establishment of symbiotic relationships with rhizobia[2].

As an antimetabolite of arginine, CAN can be used as a substrate in reactions utilizing arginine, including aminoacyl-tRNA synthetase. In our studies, we demonstrated that CAN inhibited tomato root growth at micromolar concentrations without decreasing tissue viability. CAN decrased nitric oxide emission by inhibiting the arginine-dependent nitric oxide biosynthesis pathway. The lowered level of nitric oxide was accompanied by the induction of oxidative stress[3]. We have shown that CAN induced tyrosine nitration in antioxidant enzyme decreased their activity[3].

The meristematic region of roots of CAN treated seedling was strongly modified, and all cells underwent premature vacuolization, likely connected to rapid differentiation. In root tips of control seedlings, starch granules were limited to cells in the root cap, epidermis, and cortex of the elongation zone, while in CAN-stressed tissue, starch granules were widely distributed.

Alterations in cell division were noticed shortly after CAN application. In the elongation zone of the roots of seedlings treated with CAN, endocycling cells were observed.

CAN led to the overexpression of genes encoding cyclins, subsequently altering the expression profiles of cyclin-dependent kinases CDKA and CDKB. Furthermore, induction of endoreduplication was confirmed through the upregulation of genes encoding Kip-related proteins (KRP), known as cyclin-dependent kinase inhibitors.

The restriction of root growth induced by CAN, acompanied by alterations in reactive oxygen and nitrogen species metabolism, is linked to premature cell differentiation.

Key words: non-proteinogenic amino acid, endoreduplication, reactive oxygen species

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Evaluation of biodiverse *Sorghum bicolor* and related genotypes for root exudation, sorgoleone production and potential carbon capture

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Many sorghum species express allelopathic properties resulting from the biosynthesis and release of long chain hydroquinone metabolites impacting the growth of plants and certain microbes [1]. Earliest studies on the hydrophobic root exudates from the roots of *Sorghum bicolor* demonstrated their phytotoxicity to broadleaf crop and weed species, suggesting that root exudates play an important role in the growth inhibition of broadleaf weeds. Most recently, multiple long-chain hydroquinones have been identified in exudates, but sorgoleone, 2-hydroxy-5-methoxy-3-[(Z,Z)-80,110,140-pentadecatriene]-p-benzoquinone, is the major constituent. Interestingly, sorgoleone affects multiple molecular targets [1] and has proven to be a potent inhibitor of photosystem II and respiration [2]. Sorgoleone also strongly inhibits the enzyme HPPD (p-hydroxyphenylpyruvate dioxygenase), which is involved in the formation of plastoquinone and also acts as a respiratory inhibitor. Its phytoxic properties coupled with multiple target sites and the quantity of carbon released in the soil over time offer promise for sorgoleone as a natural product alternative to synthetic herbicides and also as a means to effectively capture carbon release into the surrounding soil environment.

The biosynthesis of sorgoleone occurs in sorghum root hairs, and exudate is rapidly released from the root hair in the form of oily yellow droplets [4]. Sorgoleone has been observed as osmiophilic globules in root hair cells, which are deposited between the plasmalemma and cell wall, and are associated with sorgoleone rhizosecretion [1,2]. The biosynthetic pathway leading to the formation of sorgoleone was determined in recent years and was it later demonstrated that the biosynthesis of sorgoleone.

involves the production of an alkylresorcinolic intermediate [1]. *Sorghum bicolour, S. Sudanese, S. halpense and S. leiocladum* have all been found to produce bioactive root exudates containing long chain hyroquinones[3].

As a result of a Bayer Grants4Ag project, we are employing both metabolomics and genomics approaches to evaluate a biodiverse collection of *S. bicolor* plus other unusual native Australian sorghum genotypes for their ability to a) produce significant quantities of root exudates with a focus on carbon capture, b) generate consistent root hair density and extensive primary roots, c) produce bioactive root exudates containing long chain hydroquinones with bioherbicidal and antimicrobial activity influencing nutrient cycling processes d) produce exudates with altered composition both qualitatively and quantitatively and e) express key biosynthetic genes regulating sorgoleone production. We share our research results in the upcoming presentation.

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Mode of Action and Bioherbicidal Potential of the Natural Phytotoxin *Trans*-chalcone

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In recent decades, specialized compounds have emerged as a source of new phytotoxins eventually used for weed control in the field. Among other characteristics, effective and sustainable bioherbicides should be specific against weed species, and employ alternative molecular targets and mode/s of action (MoA) to conventional herbicides. In this dissertation, we studied the bioherbicidal potential of the flavonoid *trans*-chalcone (chalcone) by investigating its MoA on *Arabidopsis thaliana*, and evaluating its suitability for different agricultural systems.

Dose-response assays demonstrated that chalcone inhibited *Arabidopsis* root growth by 50% (IC_{50}) at only 35 µM, and 80% (IC_{80}) at 73 µM. *Arabidopsis* seedlings underwent Programmed Cell Death in roots and bleaching in shoots grown for 7 to 14 days in media with IC_{50} chalcone[1]. Additionally, phytotoxicity assays indicated that the agricultural systems *Oryza sativa* – *Echinochloa crus-galli*, *Zea mays* – *Amaranthus retroflexus*, and *Triticum aestivum* – *Plantago lanceolata* would greatly benefit from the use of chalcone as a post-emergence bioherbicide[2].

To deeply explore chalcone MoA, a number of time-course experiments were carried out on *Arabidopsis* seedlings treated with 0 and IC_{50} chalcone from 0 to 24 hours. Chalcone induced plasma membrane depolarization after only 30 minutes of treatment, and photo-inhibition and physical damage of the antennae complexes after 24 hours[3]. Metabolomics indicated inhibition of photorespiration in shoots and alterations of central metabolism related to TCA and stress adaptation in roots. Transcriptomics demonstrated alteration of numerous genes related to oxidative stress and xenobiotic detoxification already after 1 hour, as well as several potential primary metabolism target sites. However, enzyme inhibition assays did not confirm the proposed *p*-hydroxyphenylpyruvate dioxygenase and tyrosine aminotransferase[4].

This dissertation confirmed chalcone as a potent phytotoxin with multiple MoA suitable to be used as bioherbicide in different agricultural systems. It likely targets a molecular site within photosynthesis distinct from commercial bleaching herbicides.

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Leaf Endophytes of *Hydrangea macrophylla* subsp. *serrata* (Thunb.) Makino and their Reactions to Dihydrocoumarins

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Some *Hydrangea macrophylla*/*Hydrangea macrophylla* subsp. *serrata* cultivars contain high amounts of the dihydroisocoumarins (DHCs) phyllodulcin and hydrangenol predominantly in young leaves. Treatments with methyljasmonate increases the contents as confirmed by studies with plants grown under optimized conditions[1]. The functions of the DHCs for the plant are presently unclear. Since phyllodulcin has weak bactericide and moderate antifungal properties[2], bacterial and fungal endophytes of young leaves were isolated and PCR methods were used for identification. Most of the bacteria belong to the genus *Bacillus.* Other endophytes may belong to the genera *Patulibacter, Rhodococcus, Rhizobium, Agrobacterium, Shouchilla, Priesta, Aeribacillus* and *Peribacillus,* which is presently under conclusive proof, as are the endophytic fungal species belonging to the Ascomycetes. Culturing of several endophytes in presence of the DHCs revealed that some of the bacteria (*Priestia spec.*, *Patulibacter spec.*, *Bacillus thuringiensis*) and two fungi were able to degrade hydrangenol but not phyllodulcin. Ongoing studies with bacterial cultures point to growth modulation of some species by phyllodulcin. It is therefore hypothesized that phyllodulcin may have allelopathic functions in endophyte colonization success, as well as growth and microbial interaction control in the young leaves.

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Investigating allelopathic interactions between wheat genotypes and *Lolium rigidum*: Insights from *in vitro* and greenhouse experiments

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Allelopathy plays a crucial role in agro-ecological interactions and weed management strategies[1]. In this study, we investigated the allelopathic effects of four different wheat accessions (Adesso, Ehogold, Ursita, and Maurizio) on the germination, shoot length, and shoot invasive capacity of the herbicide-resistant weed *Lolium rigidum* Gaud. The experiments were conducted using *in vitro* bioassays and greenhouse trials to compare the effects of wheat genotypes under controlled and semi-natural conditions. Our findings revealed that all wheat genotypes significantly influenced the germination of *L. rigidum* across all treatments, with varying degrees of impact observed on shoot length and shoot invasive capacity. Interestingly, shoot length was found to be less affected compared to shoot invasive capacity, indicating differential allelopathic effects on different growth parameters. Furthermore, chemical analysis of the growth medium (agar in *in vitro* assays and soil in greenhouse trials) revealed variations in the chemical profile of the different wheat plants between controlled and semi-natural conditions, with some variations being attributed to wheat genotype, while other variations are linked to co-culture time and extraction depth. Specifically, soil solution extracted at different time points (7 days and 14 days after co-culture) and from different depths (top and bottom of the pot) under semi-natural conditions, showed distinct chemical composition, highlighting the dynamic nature of allelopathic interactions in soil environments. Overall, our study underscores the importance of considering both genotype-specific and environmental factors in understanding allelopathic interactions between wheat and *L. rigidum*, providing valuable insights for the development of sustainable weed management strategies in agricultural systems.

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Keywords: Allelopathy, Ecological interactions, Wheat,

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The allelopathic effect of invasive weed *Oxalis latifolia* on its surrounding soil

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Oxalis latifolia Kunth, belonging to the genus Oxalis of the Oxalidaceae family, originates from South America. It has been listed as one of the quarantine weeds in China since 20071. It was first found at the Herb Garden of Kunming Botanical Garden in 1996. In 2010, a survey showed that this weed was only distributed in Yunnan province and was common in landscape-green-belt systems and garden areas. However, its spread was reported in farmland in 2017 and seriously influenced the crops of soybean, maize, tobacco and vegetables2. Allelopathy is one of the mechanisms used by alien plants to invade new environments. This study tried to extrapolate the key factors which could influence *O. latifolia* on colonizing, spreading and breaking out by investigating allelopathic effects of *O. latifolia* on main crops and accompanying plants. In the study, using the aqueouse extraction of *O. latifolia's* leaf and stem, the allelopathy capacity of *O. latifolia* was evaluated by challenging different pasture, crops and weeds, including *Trifolium repens* L., *Sorghum sudanense*, Chinese cabbage, cucumber, rape, *Amaranthus tricolor* L., barnyard grass. The physio-chemical characteristics and microbes of the rhizosphere soil of *O. latifolia* and *O. debilis* Kunth var. corymbosa were also analyzed. It was found that the aqueous extract of *O. latifolia* didn't significantly influence the germination of crop seeds at low concentrations. The degree of inhibition enhanced with the increased concentration of aqueouse extraction, and the influence on the root was stronger than on the bud. By analyzing rhizosphere soil samples, *O. latifolia* could raise soil pH, full potassium and acid phosphatase, while decrease soil available potassium. Compared to *O. debilis* Kunth var. corymbosa, there was an increasing trend of α diversity index richness index chao1 and OTU of *O. latifolia*'s rhizosphere soil. In summary, invasive *O. latifolia* may affect physio-chemical traits and microbes through allelopathy, which could be one of the factors causing its colonization, spread and breakout."

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Is litter decomposition of mangrove pioneer species involved in species recruitment by releasing allelochemicals in sediments?

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In the context of plant succession, previous studies addressed the implication of allelopathy in the recruitment of following species through litter decomposition of dominant or pioneer species. While most of them focused on terrestrial ecosystems[a, b], knowledge remains scarce in wetland forests such as mangroves[c]. Our study evaluated the influence of pioneer species leaf litter decomposition on (i) the settlement success of species following them or not in the natural succession in the Red River Delta (Vietnam), and (ii) on soil characteristics. We set-up an *in-situ* experiment in mesocosms that followed survival and growth of three species (*Kandelia obovata, Aegiceras corniculatum* and *Rhizophora stylosa*) planted in soils of dominant *Avicennia marina* or *Sonneratia caseolaris* pioneer species, with or without leaf litter addition for 203 days. At the beginning and end of the experiment, tested soils nutrient contents were determined, and their metabolic fingerprints were compared using an untargeted metabolomic approach, to highlight potential allelochemicals that could explain an effect, when observed. Target species response to litter addition was species-specific and not related to their successional status. *K. obovata* was most affected by litter addition, showing better growth in the presence of both litter types, while its survival rate decreased by 50% with *A. marina* litter only. Metabolic fingerprints of *A. marina* soils planted with *K. obovata* showed no shift in global chemical composition, but 10 features were found significantly more abundant in the presence of litter. Putative annotations revealed sulfur-containing metabolites, which are probable products of organic matter sulfurization, a frequently occurring process in mangrove sediments. We hypothesize that these unusual compounds may act as allelochemicals and should be further assessed. Globally, our findings suggest that allelopathic processes through litter decomposition may be partly at play in mangrove community assembly and could be a tool for restoration purposes.

Integrated weed management through tillage, allelopathic crops and herbicide against invasive weed '*Hypericum triquetrifolium* TURRA' in northern Tunisia

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Abstract

Controlling perennial weeds poses a significant challenge in agriculture. In this context, high infestation of *Hypericum triquetrifolium* TURRA, an invasive perennial weed, has been reported in northern Tunisia. In preliminary screening conducted in laboratory and greenhouse essays, two allelopathic crops namely barley (*Hordeum vulgare* L.) and rape (*Brassica napus* L.) exhibited the highest phytotoxic activity on the development of *H. triquetrifolium*.

Field experiments were carried out during three consecutive growing seasons to evaluate the effect of this two allelopathic crops under two tillage systems (conventional tillage and minimum tillage) and three herbicide treatments with non-treated control. The impact of different combinations of tillage systems, herbicide treatments and allelopathic crops were studied on *H. triquetrifolium* density, coverage and biomass.

Our results revealed that *H. triquetrifolium* density, coverage and total biomass accumulation, were more affected during barley crop. From the second growing season onward, conventional tillage significantly decreased the density, coverage, and biomass of *H. triquetrifolium*. All the herbicide treatments were not effective in controlling this weed. These results showed that the management of invasive population of *H. triquetrifolium* could be achieved by combining allelopathic crops with a conventional tillage.

Keywords: Invasive weed, allelopathy, conventional tillage, minimum tillage, *Brassica napus* L., *Hordeum vulgare* L.

Chemical defense of yacón (*Smallanthus sonchifolius*) against herbivores

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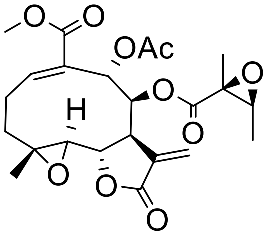
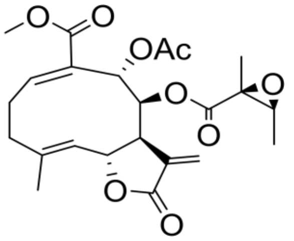
It is well-known that Asteraceae has various sesquiterpene lactones (SLs) as defense chemicals against herbivores. Yacón (*Smallanthus sonchifolius* (Poepp. & Endl.) H. Rob) is a perennial Asteraceae crop. Since this crop has highly tolerance to pests, it is easy to cultivate without pesticides. A large number of glandular trichomes are present on the leaves and have been suggested to function as a pest tolerance factor. The major constituents of glandular trichomes were SLs. In our previous report, these SLs had identified as melampolides, uvedalin and enhydrin and they showed strong insect antifeedant activity against the 3rd instar common cutworms (*Spodoptera litura* F.)[1].



Enhydrin



Uvedalin

As development of yacón the densities of trichomes and glandular trichomes did not chage on leaf surface. So it disclosed that yacón produced SLs for avoidance herbivores damage during young plant. And the unique volatiles did not detect in SPME-GCMS analysis during the wounding by worms feeding. Additionally, the old leaves contained a small amount of SLs compared with young leaves based on GCMS analysis. Thus the young leaves maybe have highly tolerance for herbivore attack. Consequietly, it presumed that these SLs were important source to avoid feeding damage from herbivores in the field.

Keywords: Insect-plant interaction, insect antifeedant, sesquiterpene lactone, yacón, Asteraceae

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Metabolome Variations of Mediterranean Seagrasses

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Plants produce several specialized metabolites (Plant Specialized Metabolites, PSMs) that play a role in intra- and inter-species interactions and between species and their environment[1]. In terrestrial environments, these PSMs are very well documented[2]. PSMs and their ecological role are less well studied in marine environments, particularly benthic ecosystems including the most biodiverse such as seagrasses. Seagrasses are an important component of marine ecosystems due to their valuable ecological services[3]. However, they are extremely sensitive to environmental changes and serve as global biological sentinels[4]. So, increase knowledge of PSMs produced by these ecosystems could lead to a better understanding of ecosystem functioning especially in a context of global change.

The aim of this study is to investigate the leaf metabolomic variability of PSMs of three seagrass species: *Zostera noltei, Zostera marina* and *Cymodocea nodosa* from different Mediterranean coastal lagoons: Berre, Carteau, Ayrolle and Thau lagoons. PSMs were extracted with solvent and studied with a non-targeted UHPLC-QTOF-MS metabolomic approach. Some biomarkers of each species were annotated based on 150 significant ions on heatmaps. The variability among the three species was more pronounced than this between sites for the same species. Phenolics (mainly in the groups of flavonoids and phenolic acids) and sulfur-compound (mainly flavonoid sulfate) were the two main classes of the annotated metabolites. Chicoric acid, rosmarinic acid and diosmetin-7-O-sulfate are the main compounds produced. Numerous studies have shown that chicoric acid helps a plant to defend itself against insects and infection from viruses, bacteria, or fungus[5]. These compounds from seagrasses are known to have an allelopathic potentiality on phytoplankton and could lead to new algicide and better understanding of benthic ecosystems.

Keywords: Mediterranean seagrasses, metabolomic, Allelopathy, UHPLC, ecosystem functioning

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The Ferulic Acid-Induced Loss Of *Arabidopsis thaliana* (L.) Heynh. Gravitropic Response is Mediated by an Alteration of the Starch and Sucrose Metabolism, Auxin Polar Transport and Cortical Microtubule Organisation.

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Ferulic acid is a phenolic compound with well-known allelopathic[1] potential widely produced by plants. The study aimed to understand the phytotoxic potential of this molecule and its mechanism of action using an integrated approach of morphophysiology and metabolomics on *Arabidopsis thaliana* (L.) seedlings cultivated in Petri dishes with increasing concentrations of ferulic acid for obtaining the dose-response curve. Once the dose-response curve allowed to calculate IC_{50} (140 µM), this was the concentration used for the rest of the studies. The bioassays revealed a 75% reduction in lateral root length at 100 µM, and a 75% reduction in the number of roots at 200 µM. However, the number of adventitious roots slightly increased with increasing concentrations. Gravitropic tests showed a random response pattern in treated samples, where the gravitropic stimulus was completely lost after only 3 hours. This seems to be due to an alteration of the PIN proteins, particularly PIN2 and PIN7, and consequently to an altered auxin distribution. Furthermore, analysis of microtubules in treated plants of the MAP4::GFP transgenic line suggested that the alterations observed in microtubule organization could depend on the plant's altered auxin balance. In addition, ferulic acid-treated plants were further characterized by an altered statolith content (which was 50% lower in IC_{50} treated seedlings than in untreated seedlings), and downregulation of the starch and sucrose metabolic pathway as a result of the GC-MS-driven untargeted-metabolomic analysis. Both approaches employed agree that the mechanism of action of ferulic acid would strongly involves auxin, its transport, and its balance within the plant.

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Uncovering the molecular basis of allelopathy in Arabidopsis thaliana using Genome-Wide Association Analysis and non-targeted metabolomics approaches

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Pesticides have been extensively utilized for weed and pest management and increasing agricultural yields, resulting in environmental and health problems. Thus, it is urgently necessary to reduce the overreliance on pesticides. Plant allelopathy is a promising and sustainable solution to reduce the use of pesticides. While research on allelopathy has been mainly focused on the isolation and identification of allelochemicals, there is few knowledges about the genetics underlying allelopathy [1]. Thus, we aim to uncover the genetic architecture of these interactions and new allelochemicals by omics approaches.

Plant-plant interactions involve a complex combination of competition for nutrients, light, and water that can hide the allelopathic processes occurring in the soil. To overcome this, we have recently developed an innovative method (based on the Plant Soil Feedback effect) to estimate the allelopathic phenotype of Arabidopsis accessions using a phenotyping robot [2]. Combining phenotyping of allelopathy for a large collection of sequenced Arabidopsis accessions with genetic approaches (Genome-Wide Association Study (GWAS)), we were able to identify many candidate regions/genes potentially involved in the biosynthesis of allelochemicals. We are currently validating candidates for some of these genes using functional genomics, especially genes involved in glucosinolate metabolism.

In addition, we developed methods for root exudate collection and non-targeted metabolomics to characterize the Arabidopsis accessions for their "metabotype". In a relatively easy approach, root exudates from plants grown in a hydroponic system are collected, and the metabolite composition of root exudates is determined by mass spectrometry. For metabolite identification and analysis of mass spectrometry data from a complex mixture of natural origin, we are using a recent bioinformatics approach called molecular networking.

By combining genetics and metabolomics approaches, we aim to unravel the pathways involved in plant-plant interactions.

Keywords: GWAS, metabolomics, Arabidopsis, plant-soil feedback, phenotyping robot

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GC-MS Based Footprinting Metabolomics: The Case of the Antimicrobial Compound Diplopyrone C causing Metabolomic Alterations in *Candida albicans*

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Metabolomics is an emerging and powerful tool for measuring, studying and comparing metabolites in different samples. By combining high-throughput analytical techniques and statistical data analysis, metabolomics offers a window on metabolic interactions among different organisms/microorganisms or on the metabolic response to different environmental conditions.

In this study we investigated the antibiotic activity of diplopyrone C against some microbial species of medical interest. Diplopyrone C is a new 5,6-dihydropyran-2-one recently isolated and characterized as product of the secondary metabolism of the fungus *Diplodia corticola*[1], one of the most aggressive phytopathogenic species of woody plants.

In particular, our results showed the antimicrobial and antibiofilm activities of this compound against *Candida albicans* which is among the most diffused microorganisms causing diseases in human with various levels of severity and it is able to produce an highly structured biofilm.

Further investigations on the effect of diplopyrone C on *C. albicans* biofilm were performed by a GC-MS based metabolomics footprinting approach which is implemented to investigate changes in microbial metabolism caused by the presence of diplopyrone C. The dataset comprised 52 identified metabolites belonging to different groups of natural products, e.g., amino acids, diketopiperazines, lipids, organic acids and polyalcohols. Statistical analysis of the collected GC-MS data showed that metabolic profiles of cultures of *C. albicans* treated with sub-MIC concentrations (i.e., 0.125 g L^{-1} or 0.250 g L^{-1}) of diplopyrone C are significantly different from metabolic profiles of control cultures (grown in absence of diplopyrone C). In particular, a dramatic decrease in nutrients uptake from the culture medium was observed when biofilm cultures of *C. albicans* are exposed to of diplopyrone C.

Keywords: Phypathogen, Metabolomics, GC-MS, *Diplodia corticola*

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Assessing Allelopathic Activity of *Ailanthus altissima* and *Robinia pseudoacacia* through a Metabolomics Approach

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Invasive alien species (IAS) is a global threat which severely impacts biodiversity and ecosystem processes.[1] IAS produce allelochemicals which promote phytotoxic effects on native species by inhibiting their germination rate and growth thus contributing to their invasiveness.[2] Currently, studies on IAS are focused on their potential as a new source of bioherbicides by harnessing these allelochemicals. *Ailanthus altissima* and *Robinia pseudoacacia* are considered highly invasive species in the Mediterranean basin. In this research, NMR-based metabolomics was utilized to further explore the phytotoxic effect of *A. altissima* and *R. pseudoacacia* to selected receiving plants *Aegilops geniculata* and *Lactuca sativa*. This approach offers simultaneous qualitative and quantitative analysis of crude extracts which require minimal sample preparation and allow high sample throughput.[3] In phytotoxicity bioassays, it was determined that the leaf and root extracts of *A. altissima* and *R. pseudoacacia* inhibited the growth of the receiving plants and caused morphological changes in a dose-dependent manner. NMR-based metabolomics also revealed the upregulation of different metabolites of the receiving plants as it was subjected to *A. altissima* and *R. pseudoacacia* extracts. Pathway enrichment analysis revealed that the upregulation of these affected metabolites caused oxidative and osmotic stress. Various chromatographic techniques have allowed us to isolate the putative allelochemical, and subsequent bioassays suggested their efficacy in inhibiting the growth of the receiving plants. Ad-hoc experiments on *A. altissima* revealed that putative allelochemicals are abundant during spring. However, these allelochemicals were not found in the soil which may be due to rapid degradation by soil microbes. Overall, this study allowed us to identify the putative allelochemicals, study their effects on receiving plants and suggests that metabolomics is a suitable tool in studying phytotoxicity and in speeding up the discovery of bioactive compounds.

KEYWORDS: allelopathy, allelochemicals, NMR-based metabolomics, *Ailanthus altissima*, *Robinia pseudoacacia*

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Toward a molecular understanding of intraspecific plant-plant interactions

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Plants interact with other plants through specialized metabolites also refered to as allelochemicals. Some of these allelochemicals can induce deleterious effects into neighboring plants (a process known as allelopathy), while others can have positive effects. Although the negative impacts of allelochemicals have been widely studied, we know far less about how positive allelochemicals function in interspecific plant-plant interactions. By presenting unpublished data, we unveil the significant role of a major class of maize root-exuded allelochemicals in modulating gene expression and disease severity in neighboring rice plants through a chromatin-based regulatory mechanism.

At the same time, positive interactions are also widespread in intraspecifc plant mixtures, and recent evidence points toward a role for an allelochemical-independent mechanism remaining unknown. By leveraging a multidisciplinary approach that combines forward genetics, metabolomics, transcriptomics and reverse genetics, our team endeavors to unravel the genes and the molecular mechanisms governing intraspecific plant-plant interactions in hexaploid wheat, particularly those bolstering plant immunity against prominent infectious threats. On the long-term, we hope to understand how plants recognize their intraspecific neighbors at the molecular level, which could lead to the development of new biosolutions and the optimization of mixtures at the field to foster agroecological crop farming.

Mode of action of the bioherbicide pelargonic acid on the model species *Arabidopsis thaliana*

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Pelargonic acid (PA) is a saturated fatty acid found in various organisms, known for its phytotoxic activity and used as a bioherbicide in agriculture, although its mode of action is still unknown according to the Herbicide Resistance Action Committee. The aim of this study was therefore to determine the mode of action of this specialised compound on the plant metabolism of the model plant *Arabidopsis thaliana*.

A dose-response curve of PA was established from which the IC_{50} and IC_{80} (concentrations causing 50% and 80% inhibition, respectively) for root growth were calculated, yielding values as low as 30.4 and 61.6 µM, respectively. The PA-treated seedlings showed shorter roots, adventitious roots and twisting phenomena, suggesting PA-induced hormonal imbalance. Bioassays with the anti-auxin PCIB and the synthetic auxin NAA suggested as well the role of auxin imbalance in PA mode of action, as the morphology of PA-treated seedlings was similar to NAA-treated seedlings. Also, PA-treated DR5 and PIN1 transgenic lines showed a reduced signal intensity under confocal microscopy. The microtubules of PA-treated seedlings were also observed by confocal microscopy and were found to be altered and broken.

Molecular docking and quantum chemical calculations were performed to compare the interactions of the natural auxin IAA, the auxin transport blocker NPA, and PA with the auxin transporter PIN1. Computational simulations showed that PA interacts with PIN1 similarly to IAA, although PA-PIN1 interaction is energetically more stable under intracellular conditions than IAA-PIN1. These results suggest that PA could be acting as an auxinic bioherbicide.

Keywords: pelargonic acid, auxin, PIN1, molecular docking.

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Cyclic Isothiocyanate Goitrin Alters *Mesorhizobium loti* Proteomes and Affects *Lotus japonicus* Nodulation

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The continuous release of glucosinolates into the soil by Brassicacea root exudation is a prerequisite to maintain toxic levels of the bioactive, short-lived glucosinolate break down products, such as the isothiocyanates (ITCs). These compounds influence plants, microbial diversities and ecosystems[1,2], in particular, mycorrhizal fungi and bacteria belonging to the Rhizobiaceae are injured. We investigated early effects of the cyclic ITC goitrin on the proteomes of free living and symbiotic *Mesorhizobium loti* using two differentially impaired populations, and of goitrin treated *Lotus japonicus* nodules. In the free-living *M. loti*, goitrin preferentially down regulated abundancies of proteins involved in transcription and translation, of ribosomal proteins, chaperones and ABC transporters, while proteases and glutathione-related proteins are less or not affected. Up regulation of members of the two-component signalling system is one of the earliest responses. Growth of *M. loti* was reduced after 24h when cultured in presence of goitrin. In contrast, symbiotic *M. loti* with its symbiosis adapted proteome is less injured. In the nodules, stress related proteins for ROS detoxification of the plant and bacterial chaperones are among the most upregulated proteins. Nevertheless, the number of nodules and the nodule size are severely affected by goitrin, resulting in yellowish leaves and leaf abscission. Ongoing studies are directed to bacterial goitrin elimination.

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meta-Tyrosine, an intriguing allelochemical of diverse modes of action

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The non-proteinogenic amino acid (NPAA) - *meta*-tyrosine (*m*-Tyr), is a structural analogue of phenylalanine (Phe) and a allelopathic compound secreted as root exudates by plants of the fescue genus (*Festuca* sp.) and myrtle spurge (*Euphorbia myrsinites* L.)[2,3,7]. The toxic effect of *m*-Tyr is the inhibition of the elongation growth of the roots of neighboring acceptor plants[1,2,6]. The direct mode of action of *m*-Tyr is linked to its incorporation into proteins[5] resulting in their improper conformation. In Arabidopsis incorporation of *m*-Tyr into proteins was accompanied by malformations of chloroplasts and mitochondria structure and led to disruption in photosynthesis and respiration[8]. Our data indicate that in plants similarly to animal cells *m*-Tyr may be considered as a marker of oxidative stress and senescence/ageing processes. Concentration of *m*-Tyr increased in artificially aged seeds[4]. To describe the mode of action of *m*-Tyr as the allelopathic compound we determined the impact of *m*-Tyr (50 and 250 μM) on the metabolism of reactive nitrogen species (RNS) in roots of tomato seedlings (*Solanum lycopersicum* L.). *m*-Tyr application resulted in an increased emission and content of RNS (nitric oxide (NO) and peroxynitrite (ONOO-)) in the roots of tomato seedlings. Modification of NO metabolism was observed, mostly as the increase in the expression of the gene encoding nitrosoglutathione reductase (GSNOR), and the increase in the content and activity of this enzyme[1]. In the roots of seedlings treated with *m*-Tyr changes in the post-translationally modified proteins such as carbonylated or nitrated proteins were also found[6,7]. *m*-Tyr impacted the expression of genes encoding enzymes of the cellular antioxidant system: superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GR), with simultaneous alterations in GPX and GR activity[1]. Therefore we believe that induction of nitro-oxidative stress is a secondary mechanism of *m*-Tyr action in roots of acceptor plants.

Key words: nitric oxide, oxidative stress, ageing

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Assessment of induced allelopathy in crop-weed co-culture with rye-pigweed model

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This study evaluates induced allelopathy in a rye-pigweed model driven by rye's (*Secale cereale* L.) allelopathic potential as a cover crop and pigweed`s (*Amaranthus retroflexus* L.) notoriety as a weed. The response of rye towards pigweed's presence in terms of benzoxazinoids (BXs) provides valuable insight into induced allelopathy for crop improvement. In the 2-week plant stage, pigweed experiences a significant reduction in growth in rye's presence, implying allelopathic effects. Rye exhibits increased seedling length and BXs upsurge in response to pigweed presence. These trends persist in the 4-week plant stage, emphasizing robust allelopathic effects and the importance of different co-culture arrangements. Germination experiments show rye's ability to germinate in the presence of pigweed, while pigweed exhibits reduced germination with rye. High-performance liquid chromatography with diode-array detection (HPLC-DAD) analysis identifies allelopathic compounds (BXs), 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) in rye. Rye significantly increases BX production in response to pigweed, age-dependently. Furthermore, pigweed plants are screened for possible BX uptake from the rhizosphere. Results suggest that allelopathy in rye-pigweed co-cultures is influenced by seed timing, and age-dependent dynamics of plants' allelopathic compounds, providing a foundation for further investigations into chemical and ecological processes in crop-weed interactions.

Keywords: allelopathy; benzoxazinoids; rhizosphere

Impact of year and genotype on benzoxazinoids and their microbial metabolites in the rhizosphere of early vigour wheat

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Wheat (*Triticum aestivum*) is grown on more arable acreage than any other food crop and has been well documented to produce allelochemicals. Wheat allelochemicals include numerous benzoxazinoids and their microbially transformed metabolites that can suppress the growth of weed seedlings[1]. However, increased production of these metabolites has not yet been targeted by global breeding programs seeking to breed more competitive crops. Recently, the Commonwealth Scientific and Industrial Organisation (CSIRO), through an extensive recurrent selection program investment, has recently released numerous high vigour wheat genotypes for commercial use, but the basis for their improved vigour is under investigation. In this research project, we evaluated several high vigour genotypes alongside common commercial and historic wheat cultivars to evaluate the impact of improved early vigour on the production and release of benzoxazinoids by field-grown wheat roots over a two year period. Using UPLC coupled with triple quadrupole mass spectrometry (LC-MS QQQ)[1], we quantified common wheat benzoxazinoids and their microbial metabolites (aminophenoxazinones) in soil collected from the rhizosphere and rhizoplane of wheat plants over two growing seasons in the Riverina region of New South Wales, Australia. We found that MBOA and aminophenoxazinones were detected readily in soil samples but concentrations differed greatly between years and among genotypes. In contrast to 2019, the concentration of aminophenoxazinones was significantly elevated in 2020, a year receiving adequate rainfall for optimal wheat growth. Aminophenoxazinones were detected in the rhizosphere of many early vigour genotypes and also over their parental lines exhibiting weed suppression, suggesting that a portion of improved early vigour and competitiveness may be related to increased root exudation. We found both BOA and MBOA in the soil rhizosphere of wheat. Depending on the year and genotype, we also observed enhanced biotransformation of these metabolites to various aminophenoxazinones. Interestingly, a few early vigour wheat genotypes were noted to produce the highest concentrations of aminophenoxazinones. Our research team has also evaluated if other early vigour traits including early canopy closure and biomass accumulation, were also contributing to improved competitive ability.

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Thapsigargin and Dehydrocostuslactone, Guaianolides with Potential Applicability in Developing Innovative Weed Management Solutions

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Natural products provide unique chemical structures with potential applications across various fields, including agronomy. This is the case of guaianolides, a subgroup of sesquiterpene lactones whose research has prompted further investigation into their potential applicability as agrochemicals from the perspective of allelopathy.[1]

Recent research on guaianolides have covered both the isolation from natural sources and the preparation of structural derivatives, with the aim of exploring the effectiveness of novel bioactive structures against specific weed species. *Thapsia garganica* (Mediterranean umbelliferous plant, rich in secondary metabolites) was investigated as source of phytotoxic extracts and metabolites, leading to the discovery of the guaianolide thapsigargin as phytotoxic metabolite against the weed *Lolium perenne* following in vitro and pot bioassays. Research on guaianolides also focused on dehydrocostuslactone (isolated *Saussurea costus* roots)[2], finding phytotoxicity in bioassays against *L. perenne*, *Lolium rigidum*, *Echinochloa crus-galli* and *Amaranthus viridis* weeds. The phytotoxicity found for dehydrocostuslactone against these weeds, its structural features and the isolation yield highlighted the interest for obtaining and testing structural derivatives. A wide collection was obtained by key modifications following diverse synthetic strategies that included hydroxilations on different positions and functionalizations in the lactonic ring. The study of the derivatives in bioassays against different types of weeds allowed the discussion of structure-activity relationships and modes of actions, as well as the elucidation of key structural features, specially in relation to the degree of hydroxilation and position of such functional groups in the structures.

Hence, these results concerning the allelochemicals thapsigargin and dehydrocostuslactone provide evidence on their suitability as promising materials for the development of novel herbicides based on natural products, prompting further in-depth research on them. This multidisciplinary objective aims to achieve more efficient and sustainable control of weed pests that pose significant threats to agronomy.

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Using Silicone Tube Microextraction to Probe the Release of Harmala Alkaloids by Syrian Rue (*Peganum harmala*)

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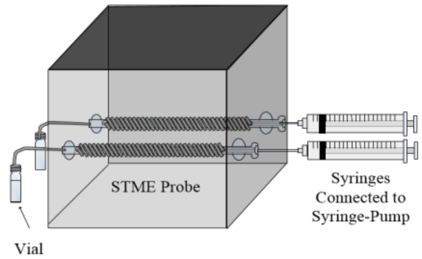
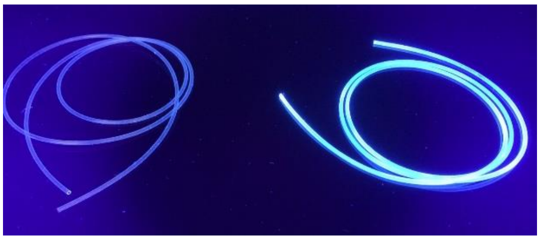
Syrian rue (*Peganum harmala* L.), a desert plant native to the Middle East and southern Asia, was introduced into the United States in the 1920's and has become invasive in southwestern deserts. Syrian rue produces six harmala alkaloids that have the potential to inhibit the growth of neighboring plants and negatively impact organisms living in soil near its roots. To better understand the toxic properties of these compounds toward other plants, the release of these alkaloids by Syrian rue roots was measured using silicone tube microextraction, a technique developed in our laboratory. Silicone sequesters and concentrates lipophilic organic compounds, such as the harmala alkaloids. This method also allows for repeated sampling of the soil without disturbance. The compounds are extracted from the silicone and the concentration of each compound is measured using high performance liquid chromatography (HPLC). Using fluorescence detection, harmine and harmaline can be detected down to 10 and 1 ng/mL, respectively. The spatial and temporal profiles of the dynamic release of harmala alkaloids in the rhizosphere were measured using various planting methods (pots of unique design) and variations of silicone probe designs. Harmine was most prevalent in soil, typically being found in ng amounts on silicone probes, while harmaline is more inhibitory in bioassays. Harmine concentrations in soil ranged up to 8 ppm.




Blank probe vs alkaloid-loaded probe

Effects of the allelochemical coumarin on soil microbial community

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Synthetic herbicides in agriculture can harm non-target organisms. Alternative compounds for weed control could be some secondary metabolites produced by plants. These include the coumarin (2H-chromen-2-one), produced by *Melilotus Neapolitan*, which has been reported as a natural herbicide against *Avena fatua*, a weed of the wheat (*Triticum durum*) crop[1]. Coumarin activity against weeds has been demonstrated in *in-vitro* conditions, but its effects on weeds and other organisms in natural conditions have been little investigated. A necessary step before field study is represented by microcosm experiments where natural conditions can be partially simulated by using undisturbed soil cores to verify the effect of coumarin also on non-target organisms, such as soil microorganisms. The soil microbial community plays a key role in ecosystem services such as regulation of climate (through C sequestration in humus), water and nutrient cycles. The aim of this study is to assess the effect of increasing amounts of coumarin (0, 100, 200, and 300 mg kg^{-1}) on microbial biomass, activity (soil potential respiration, N-mineralization, and nitrification) and bacterial and fungal genetic diversity. For each treatment, two experimental conditions were tested, respectively referred as soil (pots containing soil only) and soil plus plants (pots containing soil plus 5 oat and 5 wheat plants, deriving from seeds previously germinated on distilled water in Petri dishes). The results showed that coumarin had no adverse effects on total biomass, nitrogen mineralization, nitrification, bacterial diversity, and even stimulated total microbial activity. However, at higher doses (200-300 mg kg^{-1}), a reduction in fungal mycelium and alteration in the fungal community's genetic diversity was observed. This study points to the exclusion of an adverse effect of coumarin at the lower dose (100 mg kg^{-1}), therefore suggesting its application to the soil without risk to the microbial community.

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Evaluation of the herbicidal potential of some phytopathogenic fungi against *Amaranthus tricolor*

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Fungi are known as important sources of natural compounds that have been studied and applied for different purposes in distinct areas[1]. In this current study, thirteen fungi that produce compounds with herbicidal activities were isolated, identified, and extracted under the assumption that the mechanism of action occurs during seed exposure to the extract. The extracts from all the fungal strains considerably decreased the growth parameters of *Amaranthus tricolor* L. The EC010 strain extracts showed the greatest effect. Through ITS region gene sequencing methods, the isolated EC010 was identified as a genus of *Diaporthe*. The results showed a significant ($p < 0.05$) inhibitory effect of 91.25% on germination and a decrease in shoot and root length by 91.28% and 95.30%, respectively. The mycelium of *Diaporthe* sp. was extracted using sequential extraction techniques for the partial separation of the herbicidal fraction. According to the bioassay activities, the EtOAc fraction showed the highest inhibitory activity. The osmotic stress of the *A. tricolor* seeds was studied. Although the extract increased the accumulation of proline and soluble protein, the treated seeds showed lower imbibition. While the activity of α-amylase was dramatically decreased after treatment. Accordingly, the *Diaporthe* sp. may serve as a potential herbicidal compound resource.

Keywords: Allelochemicals, *Amaranthus tricolor*, *Diaporthe* sp.

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Potential utilization of soluble concentrates formulation of *Melia azedarach* leaf extract as a natural herbicide for controlling *Echinochloa crus-galli*

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Melia azedarach L. is interesting to produce as a natural herbicide. In literature, the methanolic extract from *M. azedarach* was found to have herbicidal activity against the weed *Rumex dentatus* L., but not to be harmful to the crop of wheat (*Triticum aestivum* L.)[1]. In our current work, *M. azedarach* dichloromethane crude extract was formulated into soluble concentrates as a natural herbicide for controlling *Echinochloa crus-galli* (L.) Beauv. seed. The inhibitory effect of *M. azedarach* soluble concentrates formulation on seed germination and seedling growth, seed imbibition, and α-amylase (EC 3.2.1.1) activity of *E. crus-galli* was evaluated. The soluble concentrates formulation was prepared using *M. azedarach* dichloromethane crude extract as an active ingredient mixed with a nonionic surfactant Tween 80 at a ratio of 30:70 (w/w). For pre-emergence herbicidal activity bioassays, the *M. azedarach* extract in soluble concentrates formulation was diluted at a concentration of 625, 1250, 2500, and 5000 ppm of an active ingredient. The results showed that increasing concentration of *M. azedarach* soluble concentrates decreased seed germination and seedling growth, seed imbibition, and α-amylase activity. *M. azedarach* soluble concentrates at the highest concentration (5000 ppm) can completely inhibit seed germination and seedling growth of the tested seeds. For seed imbibition and α-amylase activity, the tested seeds were soaked in *M. azedarach* soluble concentrates for 24, 36, and 48 h. Seed imbibition and α-amylase activity increased with the longer exposure time. As a result, soluble concentrates formulation containing *M. azedarach* crude extract have the potential to inhibit seed germination and seedling growth by limiting seed imbibition and α-amylase activity. Therefore, *M. azedarach* L. dichloromethane crude extract in soluble concentrate formulation might be encouraged as a natural herbicide for pre-emergence application to prevent the negative effects caused by synthetic herbicides.

Keywords: Herbicidal formulation, Crude extract, Pre-emergence herbicidal activity

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Evaluating Phytotoxic Potential of Valencene, Fenchone, Perillaldehyde, and Nootkatone in *Arabidopsis thaliana* (L.): Towards Sustainable Herbicide Development.

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Nowadays, weeds represent one of the primary causes of crop yield losses, and synthetic herbicides are the solution farmers rely upon. However, these products not only result in environmental damage and health risks but also contribute to the development of weed resistance. One potential solution employing phytotoxic compounds derived from plants for the development of sustainable herbicides. This study assessed the plant metabolites valencene, fenchone, perillaldehyde, and nootkatone, molecules known for their phytotoxic properties, using the model plant *Arabidopsis thaliana* (L.). Screening experiments were conducted on Petri dishes containing culture media with increasing concentrations of each molecule (0, 25, 50, 100, 200, 400, and 800 µM), which were then placed in a growth chamber. Based on their inhibitory activity, nootkatone exhibited the highest effectiveness, followed by perillaldehyde, valencene, and fenchone. The evaluated parameters during the morphophysiological analysis were the length of the primary root, the length and number of secondary roots, as well as the length, density, and number of root hairs. Valencene and fenchone showed minimal differences from the control, while nootkatone and perillaldehyde demonstrated the most promising results. All four molecules inhibited seedling growth at concentrations of 400 µM or higher. Nootkatone led to significant inhibition of primary root growth even at low concentrations, with inhibition rates increasing from 16% at 25 µM to approximately 61% at 50 µM. The same comparison between concentrations was conducted for perillaldehyde, where the inhibition intensity ranged from 26.5% at 100 µM to 53.3% at 200 µM. Further tests were conducted for nootkatone and perillaldehyde using different concentration levels (0, 25, 50, 75, 100, 125, 150, 175 µM) to refine the dose-response curves. From these curves, the IC_{50} values were calculated for both molecules, resulting in 45 µM for nootkatone and 190 µM for perillaldehyde, open the door for future studies.

Solanum curtilobum: a bitter potato with allelopathic potential

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This study explores the allelopathic potential of the methanolic extract of *Solanum curtilobum*, a bitter potato of the peruvian Andes characterized by its high content of glycoalkaloids[1], on the germination and growth of *Bidens pilosa*. Despite the well-documented allelopathic properties of various Solanum species[2], the specific impact of *Solanum curtilobum* on other plant species remains largely unexplored.

Aerial parts of *Solanum curtilobum* were collected from the highlands of Peru (S 12° 16’ 44.030064”, W 75° 4’ 21.94752”, 3939 m.a.s.l.) and its taxonomy was confirmed by the specialists of the Genebank of the International Potato Center. These plant parts were, dried, blended, and subsequently macerated with methanol in a ratio of 1:3 (w/v) for 14 days. The macerate was then filtered, and the methanol was evaporated using a rotary evaporator, yielding a concentrated extract. Germination tests were conducted following a completely random design using five concentrations of the extract diluted in distilled water (0, 1, 5, 20, and 50 ml L^{-1}) on 100 seeds for each treatment. Following, the seeds were kept in a growth chamber at 25°C in complete darkness for 14 days. The germination variables of all the treatments were compared with a Duncan test at a p value of 0.05.

Although there was no significant difference for the germination variables evaluated at the doses of 0, 1 and 5 ml L^{-1}, there was a significant decrease in the germination percentage at the concentration of 20 ml L^{-1}, and no seeds germinated at the concentration of 50 ml/L. Seedling length, mean germination time, uncertainty index, synchronization index and germination speed also decreased significantly at the doses of 20 and 50 ml L^{-1}. These findings highlight the potential of *Solanum curtilobum* as a natural herbicide, thereby opening new avenues for research into sustainable agricultural practices.

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Using *Hydrocotyle umbellata* extract as algicides for aquarium plant farm

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Algal blooms in aquarium plant ponds that cause serious effects on the growth of aquarium plants. This study investigated dominant algal species from water samples of an aquarium plant farm and determined the optimal dose range of *Hydrocotyle umbellata* extract for algicides. The two dominant species isolated from water samples at the aquarium plant farm were *Spirulina* sp. and *Chlorella* sp. The water and ethanolic extract of *H. umbellata* toxicity tests with the dominant algae were applied for the antialgal activity. This test is a 0–7 day algal growth inhibition assay. There were 5 levels of concentration at 20, 40, 60, 80 and 100 ppm. And the control treatments were water and ethanol. The inhibition ratio (IR) of *Spirulina* sp. Was 63.4-75.6 % and 87.8-93.4 % at 20-100 ppm of water and ethanolic extract, respectively, of *H. umbellata* in the 7-day assays. The inhibition ratio (IR) of *Chlorella* sp. was 64.7-77.1 % and 98.8-100.0 % at 20-100 ppm of water and ethanolic extract, respectively of *H. umbellata* in the 7-day assays. *H. umbellata* extracted by water and ethanol against *Spirulina* sp., where $EC_{50,7d}$ values were 118.38 and 92.14 ppm, respectively. Thus, *Chlorella* sp. was more sensitive to both extracts than *Spirulina* sp., showing $EC_{50,7d}$ values of 36.01 and 12.95 ppm for water and ethanolic extracts, respectively.

Keywords: Allelochemicals, *Hydrocotyle umbellata*, *Spirulina* sp., *Chlorella* sp.

The Allelopathically Effect of Grain Legume and Winter Rye Intercrops Biomass Extracts on Radish Germinations and Shoots Growth

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As a good strategy for competition between crops, allelopathy can clarify the compatibility of crop species and the appropriate sequence of different crops cultivation. Allelopathy is a fascinating phenomenon in which plants release biochemicals that affect the growth, survival and reproduction of neighbouring plants[1].

In the present allelopathy bioassay, we investigated the effects of the grain legumes pea and chickpea as well as winter rye on radish seed germination and root growth. Radish (*Raphanus sativus*) was selected as the recipient plant, while the grain legumes pea (*Pisum sativa* L.), chickpea (*Cicer arietinum*) and winter rye (*Secale cereale*), which were grown as pure and intercrops, were selected as donor plants. In 2023, aqueous extracts were prepared from undamaged shoot samples of the donor plants of the grain legumes before flowering. The extracts were prepared in duplicate from 250 g of fresh, ground shoot tissue in 1 l of distilled water at a constant 20 C and kept in the dark for 24 h, after which they were filtered through filler paper. The liquid is used as the starting aqueous extract. Each type of starting aqueous extract was diluted with distilled water to concentrations: 25,50, 75, 100 stored at 4 C until and during use[2].

The study aims to understand the effects of extract concentration on radish seed germination and root growth and how they influence plant communities in intercropping. The results show that the aqueous extracts of all donor plants grown as pure crop inhibited radish seed germination at both low and high concentrations. However, rye promoted root length at lower concentrations compared to the legume extracts. Extracts of grain legumes and winter rye intercrops (1:1) thus had a positive effect on root growth, while the pure extracts of pea and chickpea had an inhibitory effect, depending on the concentration.

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The First Report of Glyphosate Resistance in *Amaranthus viridis*, an Invasive Plant in Brazilian Citrus Orchards

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Glyphosate is the main tool for invasive plant management in Brazilian citrus orchards, *Conyza bonariensis* and *Digitaria insularis*, have been found with resistance to this herbicide.[1] Field prospections have allowed the identification of a possible new case of glyphosate resistance. In this work, the susceptibility levels to glyphosate on three *Amaranthus viridis* L. populations (R1, R2, and R-IAC), with suspected resistance, collected in citrus orchards from the São Paulo State, Brazil, as well as their accumulation rates of shikimic acid, were determined. The fresh weight of the susceptible population (S) was reduced by 50% with ~30 g ae ha^{-1} glyphosate [GR_{50}: the herbicide rate required for 50% reduction of fresh weight at grams of active ingredient per hectare (g a.i. ha^{-1}); ae: acid equivalent, concentration of the parent molecule], while the GR_{50} values of the resistant populations (R) were between 5.4 and 11.3 times higher than that for S population. The LD_{50} (herbicide dose to kill 50% of individuals of an invasive population) values of the S population were 150 g ae ha^{-1} glyphosate, while the LD_{50} of the R populations ranged from 600 to 920 g ae $há^{-1}$. Based on the reduction of fresh weight and the survival rate, the R1 population showed the highest level of glyphosate resistance, which had GR_{50} and LD_{50} values of 248 and 918 g ae ha^{-1} glyphosate, respectively. The S population accumulated 240 µg shikimic acid at 1000 µM glyphosate, while the R1, R2, and R-IAC populations accumulated only 16, 43, and 33 µg shikimic acid, respectively (between 5.6 to 15 times less than the S population). Enzyme activity assays suggested that at least one target site-type mechanism was involved in resistance. This result revealed the first report of glyphosate resistance in *A. viridis* reported in the world.

Keywords: Citrus, *Amaranthus viridis*, Glyphosate, invasive plant, herbicide

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Phytotoxic activity of a mellein-enriched fungal fitrate organic extract a *Biscogniauxia rosacearum, a pathogen of grapevine*

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The genus *Biscogniauxia* (family Graphostromataceae) includes more than one hundred species reported from a broad spectrum of woody hosts worldwide (e.g. oak, beech, vine, pine, almond)[1]. *Biscogniauxia* species are endophytes and opportunistic pathogens on old, stressed trees. The species, *B. capnodes*, *B. mediterranea* and, more recently, *B. rosacearum*, have been reported as pathogens of *Vitis vinifera* L.[2] *B. rosacearum* has been further identified as a pathogen of strawberry tree in North Africa.[3] In recent years, *Biscogniauxia* endophytic strains have received more attention, thanks to the identification and discovery of various secondary metabolites that exhibited antifungal, antimycobacterial, antiproliferative, antioxidant, antitumor, and antigerminative activities with promising application prospects. In particular, some pure specialized metabolites from *B. rosacearum* have been showed to be phytotoxic on *Quercus ilex* L., *Hedera helix* L., and *Vitis vinifera* L.[4]

In this context, our attention was focused on *B. rosacearum*, sampled in Iran. The freeze-dried culture filtrate, dissolved in MilliQ H_2O (pH 5.5), underwent liquid-liquid extraction using ethyl acetate (EtOAc). The residual culture filtrate was acidified to pH 2 with HCOOH and further extracted using EtOAc. UHPLC-QqTOF-MS/MS analyses highlighted that the main compounds, melleins, well know fungal phytotoxins,[5] were quantitatively different in the organic extracts investigated. This finding was in line with the different toxicity showed by the two organic extracts towards young tomato (*Solanum lycopersicum* L.) leaves as evidenced by the leaf puncture test, which used Ophiobolin A as positive control. The pH 2.0-obtained organic fraction showed necrotic symptoms after 24 h exposure time, while both the extracts were able to induce curling and chlorosis effects after 48 hours.

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On the phytotoxic metabolites produced by *Neoscytalidium hyalinum* the causal agent of grapevine dieback

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The genus *Neoscytalidium*, previously known as *Scytalidium*, encompasses dematiaceous molds within the phylum *Ascomycota*. These molds are widely distributed across tropical and subtropical regions globally[1]. Species within the *Neoscytalidium* genus are often identified as soil saprophytes and plant pathogens, and they can also act as airborne contaminants in both indoor and outdoor environments leading to it being defined as a mycosis agent in human and animals[2]. The genus includes over 10 species, with *N. dimidiatum*, *N. hyalinum*, *N. novaehollandiae*, and *N. orchidacearum* specifically recognized for their pathogenicity in plants[1], whose symptoms include branch wilt, dieback, canker, leaf blight, gummosis and tree death. Among these, recent studies have demonstrated that *N. hyalinum* can induce grapevine dieback[3], melon fruit[4] and cassava root rot[5]. However, limited information regarding the chemical identification and/or isolation of the secondary metabolites produced by this fungal are reported. The knowledge of the toxic fungal metabolites allows the complex process of fungal-plant interaction to be investigated, while could be also a starting point for the study of other biological activities for applications in agriculture and/or medicine. In this context, the culture filtrate of *N. hyalinum* was firstly fractionated by liquid-liquid extraction, obtaining an ethyl acetate extract, which was further chromatographed by RP-C8 eluting with different MeCN:MeOH:H_2O solutions at decreasing polarity. NMR spectroscopy and HR-ESIMS were applied to define the metabolic profile. Moreover, phytotoxicity of crude fungal culture filtrates extracts and chromatographic fraction was preliminarily assessed by leaf-puncture assay on a non-host plant (*Solanum lycopersicum* L.). Necrosis and curling symptoms were observed after 24 treatment hours.

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Can allelopathic potentialities of Mediterranean plant species reduce the spread of the invasive plant species *Arundo donax* in a climate change context?

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Invasive alien species are not only considered to be a major cause of biodiversity loss[1], they also generate economic costs, notably through reduced agricultural yields, restoration of invaded environments and management. *Arundo donax* L. (giant reed) is an invasive plant species that causes significant damages to the railway network in the Mediterranean region. Here, we aim to identify a management method of invasive plants, based on the allelopathic potentialities of two native Mediterranean plant species (*Cotinus coggygria* Scop. and *Cistus albidus* L.), in a climate change context. Indeed, in the Mediterranean region, climate model simulations predict an increase in the hydric stress already experienced by organisms[2]. Thus, the objectives of our study were to understand: i) how *A. donax* growth and soil microbial communities are affected by the leaf aqueous extracts of the two native plants in two different culture conditions (with and without organic amendment), and ii) what are the effects of hydric stress and how it interacts with plant chemical interactions. To this end, we set up a 12 weeks mesocosm experiment in a climatic chamber by growing *A. donax* seedlings under different culture conditions.

Under favorable water conditions, a decrease in root biomass was observed in the presence of allelochemical compounds, whereas under water stress conditions, this decrease in biomass was observed at the leaf level. In addition, it was highlighted a strong buffering effect played by the organic amendment on the allelopathic potentialities of the two source species. In contrast to the growth of *A. donax*, any effect of the leaf aqueous extracts on the soil microbial community was observed.

This study allowed to highlight clear allelopathic potentialities of *C. coggygria* and *C. albidus* for the control of *A. donax* whatever the water conditions considered.

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"Enhancing Pelargonium graveolens L. Growth Using Zn-Al and Mg-Al LDH Nanomaterials: A Biochemical Approach"

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The approaches of using nanoparticles (NPs) have been successfully applied to increase the growth and biological activity of aromatic and medicinal plants. In this context, Zn-Al LDH and Mg-Al LDH NPs have previously been used, however, there is a lack of research on their effects on the physiological and biochemical properties of the geranium plant. In the present study the synthesis of both LDH nanoparticles was carried out by applying the co-precipitation method, and their characteristics were assessed through SEM, FTIR, and XRD analyses. To study this gap an experiment was conducted as factorial based on a completely randomized design with a concentration of Zn-Al LDH and Mg-Al LDH NPs (10 ppm) using the spray method. Based on the results, both types of LDH NPs caused significant increases in Mg and Zn uptake. This consequentially improved plant growth as indicated by increased shoot length, number of branches and leaves as well as shoot fresh and dry weights. There was an enhancement in photosynthetic pigments and an increase in the total soluble sugars, indicating carbon availability for primary and secondary metabolites biosynthesis. Consistently, an increase in secondary metabolites such as essential oils, upon the application of both LDH NPs. Geranium plants treated with Mg-Al LDH NPs showed the highest levels of polyphenols (44.5%), flavonoids (33.5%), and total antioxidant capacity (31.7%). Analysis of essential oil revealed that the major components were monoterpenes, including geraniol (32.7%), and β-citronellol (29.18%). In addition, a significant increase in antioxidant enzyme activities (catalase and peroxidase) of Zn-Al LDH NPs treated plants and (catalase) of Mg-Al LDH NPs treated plants.It can be concluded that, for the first time, the foliar application of Zn-Al LDH and Mg-Al LDH NPs acted as an elicitor to enhance growth and bioactive metabolite accumulation in geranium plants, which consequentially improved the bioactivity.

Keywords: LDH NPs; *Pelargonium graveolens*; secondary metabolites; essential oils

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Allelopathic interaction between tomato plants and the plant growth promoting bacterium *Kocuria rhizophila*

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Members of plant microbiota play a pivotal role in plants growth and development through different mechanisms including allelopathy. In particular, plant growth promoting (PGP) bacteria can produce phytohormones, volatile organic compounds (VOCs) and antibiotics that increase nutrient availability, promote plant growth and mitigate biotic and abiotic stress resulting in higher crop yield. *Kocuria rhizophila*, a soil dwelling Gram positive bacterium commonly used in industry for antimicrobial testing and food preparation has been recently characterized for multiple PGP traits, such as indole-3-acetic acid (IAA) and bioactive molecules production, salt and drought tolerance [1]. Here, the molecular pathways by which *K. rhizophila* stimulated plant growth were investigated in tomato through a multi-omics approach. Transcriptomic analysis revealed an up-regulation of genes involved in amino acid, lipid and secondary metabolism, and in cell wall organization. In agreement, proteins involved in photosynthesis, cell division and plant growth were higher accumulated in PGPB-inoculated compared to control plants. Metabolomic analysis showed that amino acid and peptides, monosaccharides, and TCA classes of metabolites were mostly affected by *K. rhizophila* treatment. Interestingly, eight gene modules based on their correlation with differential accumulated proteins and metabolites were identified by a WGCNA approach. In particular, two modules showed the highest correlation with nine proteins, among which a nucleoside diphosphate kinase, and several metabolites, mainly belonging to amino acids and TCA. The results highlighted that sugars, amino acids, energy regulators, key factors for improving plant growth and yield, were strongly modulated by plant - *K. rhizophila* interaction.

Keywords: plant growth-promoting bacteria; multi-omics; soil actinomycete; *Solanum lycopersicum*; weighted gene co-expression network analysis

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UHPLC-HRMS/MS-based profiling and phytotoxicity of an organic extract from *Phaeoacremonium tuscanicum*

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Since the beginning of the 20th century, oak decline has increasingly emerged as an important ecological issue. It could be due the heavy damages caused by various biotic and abiotic factors, such as climate change, inadequate forest management practices, defoliating insects, bark and wood-boring beetles, bacteria, mycoplasma-like organisms, viruses, and fungal infections. The decline commonly affects *Quercus robur* L. and, to a lesser extent, *Q. petraea* (Matt.) Liebl., the dominant species in Europe[1], as well as other oaks such as *Q. brantii* Lindl., native to western Asia.

Several fungi associated with oak decline, particularly in the central part of the Zagros forests in western Iran, belong to *Cytosporaceae*, *Diatrypaceae*, *Biscogniauxia* families[2].

Recently, *Phaeoacremonium tuscanicum*, a member of the *Phaeoacremonium* genus (family *Tognoniaceae*), has been identified as a pathogenic fungal species affecting *Q. brantii* worldwide[3]. The first report of *P. tuscanicum* was from a 100-year-old vineyard displaying esca symptoms in Italy[4]. However, eleven species of *Phaeoacremonium* have been documented as causing human infections, with *P. tuscanicum* identified for the first time in a case of subcutaneous infection[5].

Due to the growing ecological interest in this fungal species, the aim of our study has been to analyse the chemical and phytotoxicity of secondary metabolites produced *P. tuscanicum* in *in vitro* cultures. The fungal culture filtrates were subjected to EtOAc:H_2O partitioning, and the resulting organic extract was analysed using an untargeted UHPLC-HRMS/MS approach. Low-molecular weight phenols were found as the most abundant compounds. When the phytotoxicity of the same organic extracts was evaluated by leaf-puncture assay, marked toxic effects were detected after 24 and 48 h exposure on young tomato leaves.

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NMR-Based Metabolomics And Bioassays To Study Extracts And Putative Phytotoxins From Mediterranean Plant Species

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Plant secondary metabolites are some of the most critical influencers of community structure and ecosystem processes. Mediterranean plants are known for their diverse bioactive secondary metabolites, and they play a crucial role in plant-plant interactions as plant growth regulators, which could be beneficial for development of eco-friendly herbicides. This study employed NMR-based metabolomics to investigate phytotoxic properties of extracts of five Mediterranean species: *Pistacia lentiscus*, *Bellis sylvestris*, *Phleum subulatum*, *Petrohrhagia saxifraga*, and *Melilotus neapolitana*. These extracts were applied to hydroponic cultures of receiving plants: *Triticum durum*, *Triticum ovatum*, and *Avena fatua* to assess their impact on plant growth.

Through morphological analyses and NMR metabolomics, *M.neapolitana* and *B.sylvestris* emerged as strong candidates for phytotoxic activity. The study revealed that these plants influence metabolic pathways, including those involving sugars, amino acids, and organic acids, which are vital for plant development. Key metabolites responsible for the observed phytotoxic effects were also identified.

This research demonstrates the effectiveness of NMR-based metabolomics in identifying and characterizing bioactive plant extracts. The insight gained highlights the potential of certain Mediterranean species to contribute to sustainable herbicide solutions by offering selective phytotoxic effects against specific plant targets.

Dereplication and Identification of phytotoxic metabolites from plant extracts by 1D and 2D NMR Spectroscopy

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NMR (Nuclear Magnetic Resonance) is a powerful analytical technique used extensively in various fields of science, including chemistry. It offers a unique advantage in identifying secondary metabolites in complex mixtures. Unlike traditional techniques such as gas chromatography-mass spectrometry (GC-MS), NMR[1-2] is expected to meet this need, being one of the most powerful and versatile techniques for studying the structural properties of molecules in solution. High-throughput identification and quantification of metabolites are achieved by this technique, allowing a large number of samples to be analyzed in a short period of time and observing and distinguishing a wide range of metabolites from different chemical classes within the crude extracts.

Nevertheless, in the case of complex mixtures, conventional NMR methods struggle to extract information clearly. Therefore, to discover and characterize potential allelochemicals from plants, novel NMR-based methodologies are urgently needed to reduce time and improve spectral resolution.

Here, we propose the screening of twelve Asteraceae species from the Mediterranean region by NMR-based metabolomics. Asteraceae species and subspecies, representing one of the most numerous plant families, are abundant sources of natural lead compounds used in agriculture, medicine, pharmaceuticals, and food. Multivariate data analysis of the results allowed the rapid selection of *Anthemis maritima* L., *Centaurea deusta* Ten. subsp. *deusta*, and *Xanthium italicum* Moretti as interesting extracts. Furthermore, the application of alternative NMR methods[3] has made it possible to characterize complex mixtures accurately and predict the nature of potential active compounds from 1D and 2D NMR analyses of the extract. All of them were sesquiterpene lactones, which are already known for their allelochemical potential.[4]

However, further work needs to be done to validate the biological activity of pure compounds and to understand the molecular mechanism of isolated compounds.

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Allelopathic Interactions between the Plant-based Indole Alkaloid Gramine and the Soil Microorganism Arthrobacter spec.

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Shaping of the soil microbiome by plant secondary metabolites covers an important domain in plant-microbe interactions. The addition of the indole metabolite gramine to soil revealed metabolite-depended shifts in the bacterial composition[1]. Bacterial strains were isolated from the treated soil to study the impact and degradation pathways of gramine. We found an *Arthrobacter spec*. strain capable to degrade gramine. First degradation products are indole-3-carboxaldehyde (I3A) and indole-3-carboxylic acid (I3C). Proteomic analysis and gene expression studies with the *Arthrobacter* strain revealed peroxidase(s) as enzymes involved in the first steps of gramine degradation. I3A and I3C are related to auxins and provide plant growth promoting properties. Therefore, we studied the functions of the degradation products for plant growth and physiology. Supplementation of I3A resulted in an increased fresh weight of *Arabidopsis thaliana* and, according to transcriptomics and qPCR studies, in an upregulation of auxin related genes. Taken together, our data highlight the importance of plant secondary metabolites not only in detrimental but also in dosage dependent plant growth promoting (PGP) interactions with bacterial strains in soil. As a conclusion, microbial species can produce catabolic intermediates from an exuded plant secondary metabolite that possess PGP properties. The PGP catabolites do not accumulate in higher, probably toxic concentration as they are further processed.

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Canavanine - a beneficial "toxin"

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Canavanine (CAN) is a non-proteinogenic amino acid synthesized in the photosynthetically active tissues of some Fabaceae plants. This analog of arginine is accumulated in seeds and serves as a readily utilizable nitrogen pool[1]. Seedlings are also a rich source of CAN, but its content decreases during ontogeny[2]. In some species such as alfalfa (*Medicago sativa*), CAN has been detected in root exudates[3]. It acts as a protective agent due to its high toxicity towards insects and functions as a phytotoxin for species that do not synthesize this non-proteinogenic amino acid[4].

The aim of the study was to investigate whether supplementation of alfalfa seedlings (a species producing CAN) with this non-proteinogenic amino acid alters nitrogen metabolism.

Significant inhibition of alfalfa root growth was observed after the application of 0.5 mM CAN, a concentration 10 times higher than that which completely inhibits tomato (*Solanum lycopersicum*) seedling root growth (a species not synthesizing CAN). In contrast, CAN at twice lower concentration (0.25 mM) stimulated the growth of alfalfa seedling roots. Single supplementation of 0.25 mM CAN did not increase the protein pool in alfalfa seedlings. However, stimulation of nitrogen assimilation metabolism from amino acids was observed. CAN increased arginase activity in alfalfa seedling roots after 24 hours of treatment. Urease expression and activity were also stimulated, particularly in the shoots, which was accompanied by an increased concentration of free ammonium ions.

In summary, CAN may serve as a valuable nitrogen source for young alfalfa seedlings (prior to establishing a symbiotic relationship), simultaneously inhibiting the growth of neighboring plants, potential weeds.

Key words: non-proteinogenic amino acid, legumes, nitrogen

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Cannabidiolic acid and allelopathy: an intriguing matter to pursue?

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Although available data about allelopathic activity of hemp (*Cannabis sativa* L.) are very limited, it is known for centuries as an effective weeds suppressing crop plant, which can serve to the control of pests. Previous findings are in line with the ability of a hemp aqueous extract to decrease the germination rate of monocots plants, and to seriously affect seedlings growth, roots elongation and gravitropism of dicot crop species, such as yellow lupine.[1] Cannabinoids and terpenes are hypothesized to be the main actors, so much so that cannabinoids were found to cause cell death in plant tissue culture, and terpenes were able to inhibit *in vitro* seedlings germination and growth.[2]

In order to get insights into allelopathic effects of hemp specialized metabolites, extractive and chromatographic techniques were employed to isolate and purify cannabidiolic acid (CBDA) from the hemp processing inflorescence waste, commonly called hemp pollen.[3]

CBDA, representing the main hemp phytocannabinoid, is a 22-carbon terpenophenol, whose content decreases moving from flowers and leaves to stems, fruits and roots. The chemical structure of the isolated compound was elucidated by means of spectroscopic (NMR 1D, ATR FT-IR, UV-Vis) and high resolution tandem spectrometric techniques, while leaf puncture assay was applied to ascertain its phytotoxicity. CBDA produced lesions on punctured detached green tomato young leaves, similarly to ophiobolin A, used a positive standard compound. This is the first report on CBDA phytotoxicity, which opens up to further investigations aimed at promoting its use in an innovative allelophatic scenario.

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Biodegradation of juglone by the walnut phytopathogen *Xanthomonas arboricola* pv. *juglandis*.

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Xanthomonas arboricola pv. *juglandis* is the causal agent of walnut blight, a disease affecting *Juglans regia* L. cultivations by causing severe economic losses worldwide [1]. Main symptoms include necrotic spotting on leaves and shoots, necrosis of pericarp, darkening of mesocarp and endocarp and premature fruit drop [2]. During spring, from dormant buds *X. arboricola* pv. *juglandis* colonizes the leaves, non-lignified shoots and fruits and, depending on climatic conditions (i.e., air humidity, temperature) and cultivar susceptibility, incites damages to the crop [3].

The content of phenolic compounds found in leaves, shoots and fruits of *J. regia* cultivars plays an important role in determining the extent of the infection during the vegetative season. In particular, it has been shown that the seasonal alteration in the juglone content exerts a strong influence on the disease development and severity. In fact, juglone, responsible for the notorious allelopathic effects of black walnut, together with other phenolic compounds, is strictly involved in the interaction with *X. arboricola* pv. *juglandis* during the walnut infection and represents one of the main compounds involved in the plant defence thanks antimicrobial activity towards human bacteria and fungi [4].

In this study the degradation of juglone in presence of *X. a.* pvs. *juglandis* and *pruni* was evaluated at three different times (24, 48 and 72 hours) by NMR respect to control, namely juglone-containing media without bacteria. The NMR analysis clearly showed the ability of *X. a* pv. *juglandis* NCPPB 1659 to degrade juglone, with a marked degradation of naphtoquinone derivative after 48 h. On the contrary, *X. arboricola* pv. *pruni* NCPPB 2588 was ineffective at all selected times. The seasonal variation of juglone collected during three months in six sampling dates was determined. The highest content of phenolic compounds was found in May and July. Agreeing to the data juglone is more concentrated in the leaves than in fruits.

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Sesquiterpene lactones produced by *Centaurea cineraria* L. subsp. *cineraria* as potential tools for the control of broomrape weeds

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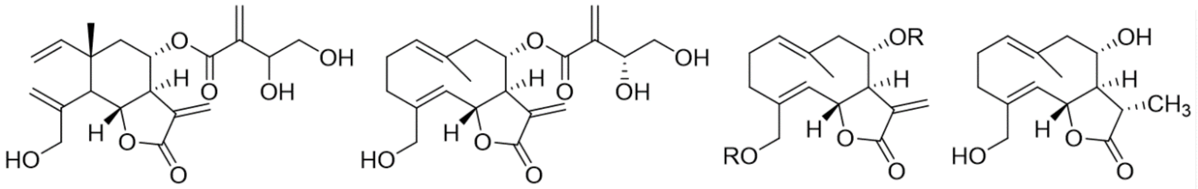
Broomrape weeds (Orobanchaceae), comprising various *Orobanche* and *Phelipanche* species, represent a great risk to important agricultural crops and few methods are available for their control.[1] In pursuit of sustainable strategies to manage these weeds, the plant *Centaurea cineraria* L. subsp. *cineraria* has been investigated as a potential source of inhibitors of broomrape radicle growth. Four sesquiterpene lactones have been isolated from its aerial parts and identified as isocnicin, cnicin, salonitenolide, and 11β,13-dihydrosalonitenolide (**1**-**4**, Fig. 1) using spectroscopic and optical methods.[2]



1 **2** **3** R=H **5** R=Ac **4**

Fig. 1. Chemical structures of compounds **1**-**5**.

Tested at 1.0–0.1 mM against the broomrape species *Orobanche minor*, *Orobanche crenata*, *Orobanche cumana* and *Phelipanche ramosa*, isocnicin (**1**), cnicin (**2**), and salonitenolide (**3**) demonstrated remarkable growth inhibitory activity at the highest concentrations. Some hemisynthetic derivatives have been also prepared and the acetylated derivative of salonitenolide (**5**, Fig. 1) showed the strongest activity among all compounds tested.

This communication will give an overview on the work carried out on the isolation and chemical and biological characterization of these sesquiterpene lactones and will illustrate some structure-activity relationship conclusions. The evaluation of their ecotoxicological profile will be also discussed.

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Soil Fungal Secondary Metabolites as a Novel Source of Allelochemicals

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Soil fungi play a crucial role in plant allelopathy by producing a variety of allelochemicals that impact plant growth, soil health and biodiversity. Allelochemicals including phenols, terpenes, and flavonoids produced by fungi impact soil structure, moisture holding capacity, and nutrient availability[1]. Soil fungi also influence the half life of allelochemicals by transformation and subsequent degradation[2].

Soil fungi produce significant quantities of lytic enzymes including glucanases and chitinases which degrade plant and other organic residues. The enzyme β-1,3-glucanase is known to degrade cell walls, thereby facilitating infections such as root and fusarial rots caused by *Pythium aphanidermatum* and *Fusarium oxysporum,* respectively[1]. Endophytic fungi also have been found to produce allelochemical metabolites such as N-(2-hydroxyphenyl) malonamic acid (HPMA) and N-(2-hydroxy-4-methoxyphenyl) malonamic acid (HMPMA) which have various effects on plant interactions and soil ecology[2]. Our own research on soil-borne entomopathogenic fungi have revealed the production of a diverse array of secondary metabolites including numerous toxins[3].

Understanding the ecological role of such metabolites in multitrophic interactions between plants and soil biota is essential for effective management of plant growth in agricultural and natural ecosystems, enhancing soil fertility, and effective soil reclamation[4]. Detailed study of soil fungal interactions also offers opportunities for improved management of pests and pathogens, and development of biocontrols, thereby facilitating sustainable agricultural practices[5].

Recently, we isolated and identified numerous soil-borne fungi from Australian agricultural sites generating a diverse library of Basidiomycota and Ascomycota associated with dung beetles. Following generation of pure cultures in liquid form, the analysis of broth and mycelial extracts via LC-MS QToF revealed structurally diverse secondary metabolites and a variety of bioactive compounds. We are currently evaluating the metabolic profiles of selected fungal isolates including mycotoxins from several *Penicillium, Aspergillus* and *Trichosporon* spp. isolates for insecticidal, fungicidal and bioherbicidal activity with a focus on novel modes of action.

Keywords: allelochemicals, crop protection, metabolic profiling, soil fungi, novel bioactives

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Isolation and structural elucidation of oleanane saponins from *Bellis sylvestris* Cyr. involved in plant-plant chemical interactions

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Saponins are triterpenoid or steroid glycosides produced by several plants and few other organisms. The interest towards these compounds is due to their potential applications in many fields, based on the wide range of biological activities they possess [1]. Furthermore, they very likely play a role in plant defence and in general in plant chemical ecology [1]. A few saponins have, for example, been reported for their phytotoxic activity [2,3]. The production of phytotoxic compounds by plants is linked to the phenomenon of allelopathy, according to which one plant species interferes (often negatively) with the growth and performance of neighbouring species, through the production and release of chemicals [4]. In a preliminary study aimed at studying chemical-mediated interactions among plants of Mediterranean area, the species Bellis sylvestris Cyr., belonging to the Asteraceae family, showed phytotoxic potential against the target specie Triticum ovatum (= Aegilops geniculata) [4]. A phytochemical study aimed at identifying the phytotoxic compounds from B. sylvestris was therefore carried out. Extracts were obtained from the leaf, flower head, and root components and fractionated through a combination of several chromatographic steps. The structure of the saponins was determined by the extensive use of 2D-NMR experiments, including COSY, TOCSY, NOESY, HSQC, HMBC, CIGAR-HMBC, H2BC, and HSQC-TOCSY, along with Q-TOF HRMS^2 analysis. The phytochemical analysis led to the isolation of new oleanane saponins, along with already known compounds. The phytotoxic activity of the isolated saponins was then assessed against T. ovatum. The metabolites were tested at three different concentrations (i.e., 1 mM, 1 µM and 1 nM) and their effects evaluated on the root and shoot elongation. Besides the inhibition of plant growth, an upwards root growth of T. ovatum was observed. It was therefore hypothesized that these compounds could interfere with the gravitropic response of the target plant. This was in good agreement with a previous study reporting that saponins isolated from Pisum sativum L. specifically interact with AUX1 protein in regulating the gravitropic response of Arabidopsis thaliana roots [5].

Keywords: saponins, NMR, allelopathy.

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Trichoderma parceramosum as a promising source of new nematicidal agents

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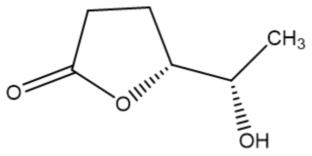
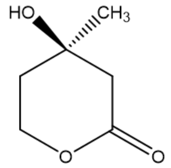
Trichoderma spp. are endophytic fungi broadly used in agriculture for the biocontrol of different pathogens affecting crops, such as fungi, nematodes and insects [1]. Metabolites biosynthesized by *Trichoderma* inhibit plant pathogens in the soil through their highly potent antimicrobial activity [2]. One major hurdle in agricultural crop production is the capability of parasitic nematodes, such as *Meloidogyne spp.*, to cause considerable damage and yield losses in vegetable crops. These nematodes invade plant root tissues and determine the formation of galls or "knots" which disrupt the plant's ability to absorb water and nutrients, thus resulting in stunted growth, yellowing, and wilting of the host [3]. The nematicidal activity of *Trichoderma* spp. against the plant-parasitic nematodes *Meloidogyne* spp. has been reported in guava, okra, mung bean [1]. In particular, *Meloidogyne incognita,* a globally distributed root-knot nematode, attacks more than 2,000 plant species including tomato, and Indian ginseng [1]. At the best of our knowledge, there are no reports about the use of *Trichoderma parceramosum* as a biological control agent against *M. incognita*.

The aim of this study is to investigate *T. parceramosum* crude extracts in order to isolate compounds potentially responsible of the nematicidal properties. The hydroalcoholic extract of a fungal culture in Solid State Fermentation (SSF) was subjected to liquid-liquid repartition, first with *n*-hexane and then with dichloromethane. This latter fraction showed strong activity against *M. incognita.* The purification of most active fractions, trough chromatographic techniques, led to the isolation of heterocyclic lactones. 1D-NMR experiments and exhaustive 2D-NMR investigations allowed to determine the structure of the pure compounds. The nematidicidal activity of pure compounds against *M. incognita* has been evaluated.





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(4Z)-Lachnophyllum lactone, a plant specialized metabolite as potential herbicide for parasitic weeds management: isolation, total synthesis, and biological studies

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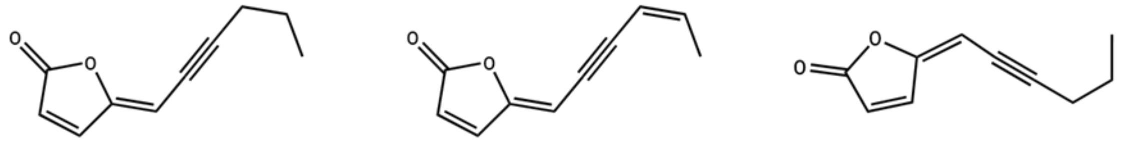
Broomrapes (*Orobanche* and *Phelipanche* spp.) and dodders (*Cuscuta* spp.), are holoparasitic weeds infesting a large number of important crops and causing severe yield losses. Plant allelochemicals has been proposed to develop bioinspired herbicide for parasitic weed management. *Conyza bonariensis,* is an invasive plant native to America that use allelopathy as part of a successful strategy to outcompete neighbouring plants. The main bioactive acetylenic furanones were isolated from the root organic extract and identified as the (4*Z*)-lachnophyllum lactone and (4Z,8*Z*)-matricaria lactone (Figure 1)[1,2].



(4*Z*)-Lachnophyllum lactone (4*Z*,8*Z*)-Matricaria lactone (4*E*)-Lachnophyllum lactone

Fig. 1. Bioactive acetylenic furanones.

Considering the low amount obtained from natural sources, a versatile strategy was developed for the total synthesis of (4*Z*)-lachnophyllum lactone that strongly inhibited the seedling growth of all parasitic weed species studied. The methodology allowed us to afford the metabolite on gram-scale and could be easily modified to obtain several structural analogues for SAR studies. The (4*E*)-lachnophyllum lactone (Figure 1) was also obtained by photo-isomerization to understand the role of the double bond geometry on the bioactivity. In this communication, the isolation of the natural acetylenic furanones, the total synthesis and their biological activity will be reported. Finally, first attempts to obtain suitable formulations for their application as bioherbicide will be discussed.

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The Importance of Plant Population Responses to Toxic Substances: Impacts on Size Hierarchies and Competitive Outcomes

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In an experiment to investigate the density-dependent responses of *Arabidopsis thaliana* to toxic levels of copper in the soil, striking effects were seen on the development of size hierarchies within pots. *Arabidopsis* plants were grown at densities of 1, 3, 5 and 7 plants per pot in soil treated with copper at concentrations of 0, 75, 125 and 175 micrograms per g. Plants were harvested two weeks after treatment application. Size hierarchies were impacted by both copper dose and plant density. For wild type plants, the coefficient of variation (CV) was 0.17 and 0.33 for control plants at densities of 3 and 7 plants per pot, respectively. For plants treated with 175 micrograms copper per g soil, the CV values at these densities were 0.75 and 1.36 respectively. In addition, for plants in the high-density pots, the average size of the largest plant per pot increased with the rate of applied copper. In controls, the average shoot dry weight of the largest plant was 15.5 mg, while for plants receiving 175 micrograms copper per g soil, the average size of the largest plant was 23.9 mg, and the largest individual plant was found in the 175 µg/g treatment. In this experiment, copper was applied through watering the pots, and was distributed heterogeneously in the soil. We hypothesize that plants with root systems that first penetrated less-toxic regions of soil grew larger under less stress and had a competitive advantage over smaller plants. In soils with a heterogenous distribution of contaminants, competition for non-toxic regions of soil may drive size hierarchies and thereby determine competitive outcomes. These findings may have relevance to studies of allelopathy, as heterogeneous distribution of naturally occurring allelochemicals is likely. Collection of data on individual as well as total plant growth may detect such effects.

Pre-emergence herbicidal effect and mechanism of action of *Callistemon lanceolatus* essential oil-based emulsifiable concentrate (EC) controlling *Amaranthus gracilis*

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In worldwide markets for agriculture, weeds are estimated to be the primary cause of potential yield loss compared to animals and diseases. Crop production is negatively impacted by weeds in terms of quality, economy, and environment[1]. *Callistemon lanceolatus* is an important medicinal plant that has several different chemical components, including fatty acids, triterpenoids, flavonoids, and phenolic substances [2]. Additionally, *C. lanceolatus* essential oil (EO) was documented as a natural herbicide that can inhibit seed germination and growth of *Echinochloa crus-gall* [3]. To the best of our knowledge, this study was to prepare an emulsifiable concentrate (EC) based on *C. lanceolatus* EO and determine its pre-emergence herbicidal impact controlling the broad-leaf weed *Amaranthus gracilis*, as well as the mechanism of action, which involves seed imbibition and α-amylase (EC 3.2.1.1) activity. The active ingredient in the EO-EC formulation is *C. lanceolatus* EO, which was mixed with dimethylformamide and a nonionic surfactant Tween 80 (hydrophile-lipophile balance 15) in a 50:40:10 ratio. The EO-EC formulation was diluted at a concentration of 25, 50, and 100 ppm of an active ingredient for the experiment. Our findings demonstrated that the EO-EC can completely prevent *A. gracilis* seed germination and seedling growth at a concentration of 100 ppm. Furthermore, seed imbibition and α-amylase activity increased with longer times (12 to 24 h), whereas decreased with higher EO-EC concentrations. As a result, the *C. lanceolatus* EO-EC formulation might be supported as a natural herbicide for environmentally friendly farming practices. Further, the compounds that caused the inhibition need to be investigated since they could serve as the foundation for the development of novel herbicides.

Keywords: Natural herbicide, pesticidal formulation, Allelopathy

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Development of robust methods to study allelopathy in soil and *in vitro*

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Today, our agriculture is facing many challenges, including the necessity to progressively reduce the use of herbicides for weed management. Innovative strategies could come from new knowledge on the natural mechanisms of plant-plant interactions, such as allelopathy, a process by which plants can modify the growth of neighbouring plants through the release of chemical compounds. Even though many allelochemicals have been identified, little is known about the genetic basis and molecular mechanisms involved in allelopathy[1].

Using *Arabidopsis thaliana* as plant model, we aim at identifying genes and metabolisms regulating allelopathic interactions by omics approaches. As a first step, the development of robust, reproducible and high-throughput methods to detect and quantify allelopathic interactions is crucial. One of the major challenges is to distinguish allelopathic effect from competition for nutrient and light. For this purpose, an original 'Plant Soil Feedback' (PSF) protocol has been established. The assay is based on growing a "donor" plant on a medium or in soil (conditioning phase), which is then removed and replaced by a "receiver" plant on the same medium/soil (feedback stage). Various characteristics of the receiver plant, such as growth, are then analysed and compared between different conditioning media, with the aim of highlighting the effect of any allelopathic compounds released into the medium by the donor plant. Assays performed on soil using the 'Phenoscope' phenotyping robot[2] (https://ijpb.versailles.inrae.fr/en/page/phenoscope) but also *in vitro* on Petri dishes or in liquid media will be presented.

Keywords: Arabidopsis thaliana, plant soil feedback, *in vitro* culture

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