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Allelopathy for sustainable ecosystems

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Plenary Session

Allelopathy: studies on the ecology of plant chemistry

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Plants release allelochemicals or bioactive metabolites into the environment that may suppress or exclude other plant species or populations. In the majority of studies, a binary approach is taken to establish the importance of allelopathy in community assembly. Ecosystem processes, however, mediate the production, release, and activities of allelochemicals. Quantitative approaches are thus requited to study the relative importance of allelopathy in comparison to other environmental drivers of plant exclusion or community assembly. In this presentation I will discuss the importance of ecological processes and evolution in selecting for allelopathic traits and determination of the relevance of allelopathy in community assembly. The role of plant metabolites in conferring competitive and invasive abilities to non-native invasive species will also be presented using examples from our research performed on non-native species such as *Prosopis juliflora, Ageratina adenophora, Eucalyptus globulus* and *Chromolaena odorata*.

Keywords: Invasion, non-native species, soil, microbes

[*]Speaker

Global Change and Terrestrial Ecosystem Experiments - Challenges from Climate Policy and Biodiversity Conservation

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Land ecosystems are affected by environmental change in numerous ways, rising annual mean temperature being only one indicative variable. Experimental facilities permit to address specific relationships between environmental conditions and the functioning of the ecosystem with increasing detail and precision, but does our capacity to predict the overall behaviour of the ecosystem actually improve? Providing useful knowledge for environmental policies requires a multi-dimensional approach that considers ecosystem and earth system theory, observations from experiments and natural conditions, numerical models, and a suitable scenario framework that can be used to explore alternative future conditions.

Keywords: global changes

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Message in the water column: Mediterranean sponges under scrutiny

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Sponges (Porifera) represent a high substrate cover in some marine ecosystems like Mediterranean coralligenous assemblages or the Caribbean Sea. As sessile filter feeders, sponge species have evolved an arsenal of chemical defence to thrive in this highly competitive environment with high concentrations of microbes and a constant struggle for space. While contact interactions have usually been involved in the communication with these key marine invertebrates, less attention has been paid to the distance communication despite outstanding filtration capacities. Metabolites released by sponges in the water column has usually been underestimated mainly because of technical issues related to a quick dilution factor and complex capture of the targeted molecules. After inspection of the state of the art in this field we will present our recent findings on the specialized metabolism of Mediterranean sponges. We will first describe the chemical diversity of the encrusting sponge *Crambe crambe* and its bioactive guanidine alkaloids named crambescins and crambescidins. We will then assess the presence of these compounds in the close environment of the sponge and extend this approach to other common Mediterranean sponges. Some preliminary results on the study of the distance interaction between marine cave-dwelling sponges and crustaceans will then be presented. These findings could pave the way for new key molecular data in marine ecology but also to produce important sponge metabolites.

Keywords: Porifera, marine communication

*Speaker

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Trophic interactions in the rhizosphere: applying chemical ecology to develop novel strategies for root pest control

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Root exudates can serve various functions to control abiotic and biotic processes. Particularly intriguing are root exudates that control mutualistic interactions with soil-dwelling organisms. These mutually beneficial plant-mediated signals are not only of fundamental ecological interest, but the root-produced signals may also be exploited for crop improvement and protection. This is illustrated by our work on root-feeding larvae of the beetle *Diabrotoca virgifera virgifera* (Western corn rootworm), which cause tremendous losses to maize growers in the USA and Europe. Entomopathogenic nematodes (EPN) are a possible solution to fight this pest. These tiny parasitic worms kill the rootworm larvae within days and we have found that they are attracted to E-(β)-caryophyllene, a sesquiterpene that is specifically emitted from maize roots after rootworm attack. American maize lines have lost the ability to emit this signal. Using genetic transformation we restored caryophyllene emission in one such line and in field trials this was shown to result in enhanced EPN attraction and better protection against rootworm damage. Using our knowledge of these rhizosphere interactions, we are currently developing new strategies for the application of entomopathogenic nematodes to control rootworms and other soil pests. These strategies involved: 1) the selection for highly effective nematode strains, 2) the application of nematodes in beads that can be planted with maize seeds, and 3) increasing the "shelf life" of nematodes with the use of a plant-derived compound that puts them in a state of quiescence.

Keywords: Nematode

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The ecology of chemical-defense polymorphisms in crop plants

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Over the course of their domestication, crop plants have frequently undergone reduction or loss of chemical defenses against herbivores and pathogens. However, in some crops, some defenses have persisted and they appear to play diverse, but often little-understood, roles in crop ecology. Crop species that are polymorphic for chemical defenses offer unique opportunities for exploring the roles of crop chemical defenses in agroecosystems. In these coupled human and natural systems, the level of defense has consequences not only for the plant but also for how farmers organize their activities. Understanding variation in chemical defense thus requires the analysis of costs and benefits of defense not only to the plant but also to the farmers. I will examine the biocultural ecology of chemical defense in crop plants characterized by such polymorphisms, focusing on a particularly dramatic example: manioc, also known as cassava (*Manihot esculenta*, Euphorbiaceae), in which there exist both non-toxic sweet and highly toxic bitter varieties, reflecting enormous variation in the cyanogen content of the plant's tuberous roots. I will show how variation in chemical defense is central to the coevolution of the plant and the cultural practices associated with it.

Keywords: Chemical defenses in crops

*Speaker

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Invited speakers

Identification of a ubiquitously-expressed vascular-specific UDP-glucosyltransferase involved in BOA detoxification in *arabidopsis*

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Plants have adapted to the presence of allelochemicals and other xenobiotic compounds within their environment through the use of detoxification schemes which mitigate the effects of these inhibitors. One such mechanism involves the formation of glucosyl conjugates via the action UDP-glucosyltransferases, and the resulting conjugates may subsequently be recognized by specific membrane-associated transporters during phase III detoxification. In *Arabidopsis thaliana*, prior studies have shown that detoxification of the allelochemical benzoxazolin-2(3H)-one (BOA) occurs predominantly through *O*-glucosylation of the intermediate BOA-6-OH, most likely requiring the sequential action of as-yet-unidentified cytochrome P450 and UDP-glucosyltransferase activities. Here we report on an *A. thaliana* UDP-glucosyltransferase mutant exhibiting hypersensitivity to exogenously provided BOA, which also exhibits a significantly reduced ability to generate BOA-6-*O*-glucoside *in vivo*. Histochemical studies employing UGT promoter::reporter gene fusions indicated pervasive and highly vascular-specific expression of this gene throughout both vegetative and reproductive development, with the exception of developing seeds where promoter activity was not detected. Collectively, our results strongly suggest an essential role for this gene in BOA detoxification.

Keywords: BOA, benzoxazolinones, glucosyltransferase

*Speaker

The Life Cycle of a Semiochemical

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Semiochemicals are the vocabulary of communication for the majority of organisms on our planet. Despite this we know very little about these chemical languages and their roles in ecology. While the concept of a fragrance that stimulates sexual behaviour is the perfumer's dream, for many animals it is a reality.
Our work has focussed on understanding the chemical ecology associated with strategies employed by Australian orchids of the genera *Chiloglottis, Caladenia* and *Drakaea* to lure wasps as specific pollinators by sex pheromone mimicry. Known as Pouyannian mimicry, the orchids emit a chemical, or chemicals, that mimic the volatiles released by female wasps in order to attract a mate. In efforts to copulate with the orchid the male wasp transfers pollen between flowers leading to pollination.[1]

This paper will discuss progress in our studies of these intriguing chemical systems by following the life cycle of a semiochemical from its birth to its ultimate demise.

Bohman B., Flematti G.R., Barrow R.A., Pichersky E., Peakall R. *Curr. Opin. Plant Biol.*, **2016**, *32*, 37-46.

Keywords: semiochemistry, pheromone, allomone, ecology, orchid, pollination

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Roles of Allelopathy in Subtropical Ecosystems, Case Studies in Taiwan

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Allelochemicals released from plants play very important roles in sustainable eocosystem, such as dominance, succession and climax of plant community, biodiversity and crop productivity. Since 1972 the author and his colleagues have conducted numerous studies in elucidating the mechanism of aforementioned subjects. For examples, that mechanism of formation of pure stand of *Rhododendron formosanum, Astonia scholaria, Acacia confusa, Miscanthus floridulus* and *M. transmorrisonesis*, etc. was due to the allelopathic interaction. The responsible compounds are phenolics, flavonoids, terpenoids, and alkaloids. In addition, we also investigated the cause of yield reduction of the second crop of rice plant in Taiwan, indicating that the allelochemicals released during the decomposition of rice residues in soil were through microbial interaction. The phenomenon was designated as autointoxication, in which six phytotoxic phenolics and short chain fatty acids were involved. The autotoxic phenomenon attracted many allelopathic scientists in many countries. Recently, the autointoxication mechanism of *Oryza sativa* was elucidated by an approach of molecular genomics, which turns out to be very interesting for future research. Furthermore, we even found that an allelochemical, (-)-epicatechin, released from the *Rhododendron* was biotransformed into a more phytotoxic active compound, protocatechuic acid, by soil microorganism and finally converted into glycerol as carbon source for soil microorganisms. This unique case will bring more scientists attention to further investigation on the role of microbial enrollment in allelopathic interactions. Our group also conducted experiments in laboratory, greenhouse, and field by applying the allelopathic compounds as naturally occurring herbicides to improve productivity and weed control in agricultural practice, which exhibits to be important for sustainable agriculture and will be beneficial to human wellbeing.

Keywords: Subtropical Ecosystems

*Speaker

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Use of Allelopathy as Natural Growth Stimulant

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Allelopathic crop extracts has the ability to enhance the productivity of various crops when used at low concentration. In a series of studies, we evaluated the potential role of sorghum, maize, rice, moringa and brassica extracts in improving the productivity of wheat, rice and maize under different levels of fertilizer inputs in Punjab, Pakistan. We recorded 15-35% increase in yield of wheat, rice and maize across growing seasons and locations. However, combined application of more than one extract was often more effective. The improvement in productivity of field crops was comparable to commercial growth regulators with very low cost involved. The growth promotion and yield enhancement from the application of allelopathic crop extracts was attributed to the presence of certain allelochemicals and growth promoting substances. In conclusion, application of Allelopathic crop extracts at low concentration extract may be opted to enhance the productivity and profitability of wheat, rice and maize.

Keywords: Growth promotion, Yield increase, Natural stimulant

*Speaker

Role of soil persistence and mineralization of sorgoleone on the allelopathic potential of *Sorghum bicolor*

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Sorgoleone is the major component of the hydrophobic root exudate of sorghum (*Sorghum bicolor*). Synthesis of this allelochemical occurs exclusively in root hairs and continues through-out the growth season. Sorgoleone production is optimum at 30°C but is suppressed when approximately 20 µg of exudate mg^{-1} root dry weight accumulates at the root hair tips. Pro-duction resumes after sorgoleone removal and there is some evidence that sorgoleone levels increase when other species grow in close proximity to sorghum. Great strides have been made in our understanding of sorgoleone biosynthesis, including the characterization of the genes and enzymes required for production, though more effort is still required to understand the mecha-nisms of exudation from root hairs to the soil. Once exuded, sorgoleone interferes with several molecular target sites, including photosynthetic electron transport, however, in planta activity requires translocation of the allelochemical to photosynthetic tissue. Low levels of translocation suggest that sorgoleone inhibits photosynthesis only in young seedlings within the rhizosphere of sorghum. Consequently, knowledge of the fate of sorgoleone in soil is essential to fully understand its mechanism of action. Sorgoleone is strongly sorbed in soil, which increases its persistence, yet experiments show that microorganism mineralization occurs over time. The methoxy group of sorgoleone is mineralized most rapidly, whereas mineralization of the remaining molecule (ring and lipophilic tail) is slower. Mineralization kinetics indicate that soil microorganisms previously exposed to sorghum are able to use sorgoleone as a source of energy.

Keywords: allelochemical, soil fate, root exudate, dynamics

*Speaker

An allelopathic interaction between barnyardgrass and momilactone deficient mutant of rice

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The allelopathic interaction between rice and barnyard grass was investigated. When barnyard grass and rice seedlings were grown together at non-limiting nutrient conditions, the allelopathic activity of rice towards barnyard grass was increased. However, momilactone-deficient rice mutants showed no increase in allolopathic activity. Thus, momilactone production may be involved in barnyardgrass-induced rice allelopathy rather than nutrient competition between both species. Barnyard grass root exudates also increased, together with the increased rice allelopathic activity, including momilactone B production and its secretion by rice plants. Momilactone B possesses strong growth inhibitory activity against barnyard grass and plays a particularly critical role in rice allelopathy. Therefore, barnyard grass-induced rice allelopathy may be due to the increased momilactone B production and secretion from rice. Rice may be aware of the presence of neighbouring barnyard grass by detection of the key compound in barnyard grass root exudates, and this sensorial function may trigger a signal cascade resulting in increasing rice allelopathy through increased production of momilactone B in rice and secretion of momilactone B into the rhizosphere. Barnyard grass-induced allelopathy of rice may provide a competitive advantage for rice through suppression of the growth of barnyard grass.

Keywords: Allelopathy, Barnyardgrass, Chemical interaction, Momilactone, Mutant

*Speaker

INVESTIGATION OF QUINONES PRODUCED BY LIVING ROOTS: THEIR POTENTIAL ROLES IN RHIZOSPHERE REGULATION AND ALLELOPATHY

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Naturally occurring quinones are well characterised with respect to their potent biological activities. Specifically, they possess antitumor, antiinflammatory, antiparasitic, antimicrobial, insecticidal, fungicidal and herbicidal activities. Quinones are known to inhibit electron transport involved in photosynthesis and mitochondrial respiration. Quinone-based fungicides are classified as "organic fungicides" and are known multisite inhibitors. This may be advantageous in the prevention of resistance development in fungal pathogens. Similarly, quinone-based natural herbicides were also described with multisite inhibitors. We have studied two unusual groups of root-produced quinones in *Sorghum* spp. (sorgoleones) and *Echium* spp. (shikonins) that may confer competitive advantage to plants producing them in high concentration. Localisation studies have shown these molecules to be produced by the living root hairs of both species, and in the case of *Echium* spp., also in root periderm. Although sorgoleones and shikonins have demonstrated moderate to good antifungal and antimicrobial activity on numerous organisms, they have also have demonstrated considerable phytotoxicity or interference ability in laboratory and field studies. Here, we present the results of recent localisation studies in both species using confocal and light microscopy for imaging, coupled to hyperspectral analysis. Metabolic profiling using mass spectrometry (UPLC-MS QToF) was utilised to study root chemistry and release of quinones into the rhizosphere. Both species released considerable quantities of a complex mixture of bioactive quinones into the soil rhizosphere over time. Whether differences in biological activity of quinones are associated with their lipophilicity, transport across membranes, or inherent ability of certain organisms to metabolise these molecules is currently not clear. The potential mode of action of these molecules in higher plants and plant defense, as well as the role of shikonins as anti-cancer therapeutics will also be discussed.

Keywords: naphthoquinones, sorgoleone, quinone, mode of action, root, root hair, shikonins, anti cancer therapeutics

*Speaker

Novel Pasture Legumes in Australia –The Case of Biserrula (*Biserrulla pelecinus* L.) and Metabolites Associated with Livestock Photosensitiization

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Biserrula and French serradella are annual legumes native to the southern Mediterranean. Serradella was widely cultivated throughout France and temperate Europe in the Middle Ages while biserrula was found more sporadically in mixed legume stands. These legumes were rediscovered in the 1990's through the efforts of Australian breeders and are well adapted to diverse Australian conditions. With ample rainfall, they can rapidly establish dense stands, are deep rooted and regenerate due to the production of hard seeds which germinate the next winter following false breaks (summer rainfall events). Both legumes are drought tolerant and adapted to acidic soils. Despite proving to be a valuable addition to the limited pasture toolbox, producers in NSW and WA have identified key factors limiting uptake. In the case of French serradella, intolerance to heavy weed infestation and high soil Mg resulted in reduced stands. Livestock have performed well on both forages as protein content ranges from 12 to 25%, and pastures offer considerable feed until early summer. Biserrula established rapidly and densely and was often weed suppressive. Despite the competitive growth habit, high protein content and low methane generation, the uptake of biserrula in southern Australia has been severely limited by the development of photosensitization in grazing livestock. The pathogenesis of biserrula photosensitization and associated metabolite(s) have been the focus of our recent experimentation. All commercial cultivars of biserrula caused primary photosensitization. MTT bioassays with cell suspension cultures have shown that shoot tissue extracts exhibited no photoactivity following forage senescence or drying. Bioactivity-guided metabolic profiling using UPLC/MS-QToF followed by separation of crude extracts using silica gel flash column chromatography and HPLC resulted in identification of multiple metabolites associated with photosensitization. The process involved in identification of bioactive phototoxic secondary metabolites will be further described. Strategies to limit photosensitivity to grazing livestock were also developed.

Keywords: hard, seeded annual legumes, biserrula, serradella, photosensitization, secondary metabolites, MTT bioassay, metabolomics

*Speaker

Allelopathy in crops

Use of *Sorghum bicolor* L. (Moench) residues in combination with lower rate of trifluralin herbicide for weeds control in cowpea (*Vigna unguiculata*) L. (Walp)

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A Field experiments were conducted to test the allelopathic potential of sorghum residues alone and in combination with half (1.2 L ha^{-1}) of recommended rate of trifluralin herbicide in controlling weeds in cowpea field. For preparation of sorghum residues, grains of sorghum cv. Enkath were grown in lines in plots of 4 × 3.5 m^2 keeping 10 cm between grains and 75 cm between lines. Plots of the same area were left without cultivation to be used in the next experiment as a control. At physiological maturity of sorghum crop, the grains were harvested and the plants were left on the plots to dry under sun for 2 weeks. After that, the sorghum residues were incorporated in to the plot soil at 5 T ha^{-1} and 10 T ha^{-1}. Plots without residues, plots with half dose of trifluralin herbicide, plots with label rate of herbicide and weed free plots (removing weeds weekly). Incorporation of sorghum residues at 5 T ha^{-1} reduced weed density by 6% of control at 75 days after sowing (DAS), while incorporation of sorghum residues at 10 T ha^{-1} reduced weed density by 43% of control at 75 DAS, and reduced dry weight biomass of weeds by 48 and 66% of control respectively. However, this reduction is further increased when half rate of herbicide was applied to plots amended with sorghum residues. Application of sorghum residues to the plots amended with half rate of trifluralin herbicide provided seed yield significantly higher than that achieved by sole application of label rate of herbicide.

Keywords: allelopathy, sorghum residues, lower rate herbicide, weed control, cowpea

*Speaker

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Allelopathic potential of cumin on seed germination and early growth of chick pea

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Allelophatic potential of cumin on seed germination and early growth of chick pea (*Cicer arietinum* L) was investigated in agronomy laboratory of Sabzevar branch, Islamic Azad university on 2014. An experiments was conducted as the factorial experiments based on completely randomized design with 4 replications including two cumin organ extracts (seed and aerial extract) at five concentrations (0, 25 50 75 and 100 %). The result showed that type of organ had not significant effect on the root length, shoot length. The mean germination time reached to 5, 10, 50 and 90% germination, Maximum germination and germination rate was affected by type of organ extract. Seed extract had more inhibitory effect on germination parameters. Increasing of concentration significantly reduced the Maximum germination, germination rate and root length (99.17, 94.54 and 99.84%, respectively) and increased the mean germination time reached to 5, 10, 50 and 90% germination (55.15, 57.71, 60.48 and 64.70%, respectively). Inhibitory effects of seed extract were higher than aerial extract with increasing of concentrations. 50% seed extract and 100% aerial extract had the most inhibitoriest effect. Overall, The results showed that allelopathic effects in seed extract was higher than cumin aerial part.

Keywords: allelopathy, cumin, chick pea, germination

*Speaker

The allelopathic effect of type and amount of cumin residues on growth and root morphology of chickpeas

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To determine the allelopathic effect of type and amount of cumin residues on growth and root morphology of chickpeas, an experiment was conducted in a factorial arrangement based on a randomized complete block design with three replications at Islamic Azad University, Sabzevar branch. Factors were residual type (seed, stem, and seed+stem) and residual amount (0, 250, 500 and 750 $g.m^{-2}$). The results showed that the increase in the amount of residue decreased stem length(18.96%), 7.40% in stem diameter, root length (41.25%), root dry weight (61.37%), shoot dry weight (51.25%), total root length (41.25%), root surface (31.74%), root volume (69.25%), and root specific weight (37.77%). Seed+stem residues had the greatest inhibitory effect in all traits. Overall, roots were more sensitive than stems to cumin residues.

Keywords: Allelopathy, chickpeas, Cumin residual, Root morphology

*Speaker

Chemical Ecology of fruit pulp – A case study with *Tamarindus indica*, L.

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Allelopathic effects through root, stem and leaves have been well documented but the effects mediated by fruit pulp has not been done so far. Majority of tree species contain inhibitors of germination in their fruit pulp. This makes sense in the ecological perspective, for, such inhibitors would not only prevent premature germination of seeds in the fruit on the tree but also of those dropped from the tree and lying below. It is thus our interest to study the chemicals in the fruit pulp and their ecological significance. *Tamarindus indica* L. is a native and widely distributed species of Asia which is of immense economic as well as traditional importance. The tamarind tree produces edible pod-like fruits. The fruit pulp is used as food preservative and as a traditional medicine in every household from the time unknown in India. Methanol Fraction, the major component of Tamarind fruit pulp revealed the presence of a strong acidulous compound in the fruit pulp. Identification and molecular elucidation of this strong sourish compound are in progress. The crude extract as well as the methanol fraction showed 100% inhibition of germination at a concentration of 500 ppm on rice seed bioassay. MIC50 value of shoot length of both methanol fraction and crude extract are 200 ppm and 250 ppm respectively whereas it is 225 ppm and 275 ppm in case of root length. At a concentration of 400ppm, the two extracts showed 90% inhibition on both shoot length and root length. Strong antifungal activity was also exhibited by the methanol fraction and crude extract against two fungal species namely *Aspergillus niger* and *Aspergillus tamarii*. The inhibitory and antifungal nature of the fruit pulp therefore may play a role by protecting the seeds until favourable condition are available for their germination.

Keywords: tamarind, allelopathy, antifungal

*Speaker

Allelopathy and allelopathic substance in Caesalpinia mimosoides Lamk.

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Caesalpinia mimosoides Lamk. (Fabaceae) is a medicinal plant distributed in Southeast Asia. The young shoots and leaves of *C. mimosoides* are mainly consumed as fresh vegetable in Thailand and also used for traditional treatments. Although its pharmacological values are well-known, but no study has reported on its allelopathic activity. Therefore, we evaluated the allelopathic potential of leaf and stem extracts of *C. mimosoides* on the growth of six test plants to find out allelopathic substances. The aqueous methanol extracts of *C. mimosoides* leaves and stems exhibited the inhibitory effects on the growth of cress, alfalfa, lettuce, foxtail fescue, timothy and barnyard grass. The growth of test plants decreased as the concentration of the extracts increased. These results suggest that leaf and stem extracts of *C. mimosoides* may possess allelopathic substances. However, the comparison of the $I50$ values between leaf and stem extracts indicates that the leaf extracts had more growth inhibitory effects on the tested plants than the stem extracts. The leaf extracts were subjected to partition with ethyl acetate, and the ethyl acetate fraction was then purified by column chromatographies. A growth inhibitory substance was isolated and identified by spectral analysis as methyl gallate. This is the first time of the isolation of methyl gallate from *C. mimosoides*. At the concentration of 10 mM of methyl gallate, the shoot of cress was completely inhibited and the root was inhibited by 4.7% of control growth. The $I50$ values of methyl gallate on the growth of cress shoots and roots were 2.4 and 2.9 mM, respectively. The growth inhibitory effects of methyl gallate suggest that it may act as an allelopathic substance of *C. mimosoides*.

Keywords: Allelopathic activity, Inhibitory effect, Caesalpinia mimosoides, Methyl gallate

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Increased Bacoside content in Brahmi (*Bacopa monnieri*) by the aqueous seed coat extract of *Tamarindusindica* – Scientific validation of indigenous traditional knowledge

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Bio-fertilizers are one of the best efficient, ecofriendly tools in our agricultural field as a replacement to our conventional chemical fertilizers. Use of aqueous tamarind seed coat extract (TSCE) as biofertilizers in rice and vegetable cultivation is an age-old practice in Indian tribal culture. But no scientific validation has been performed on this indigenous traditional knowledge. In the present research work, our main objective is to study the role of TSCE as biofertilizer, one of the most valuable plant Brahmi, *Bacopa monnieri* (L) Wettst. Bacopa is considered to be a "medhyarasayana", an herb that sharpens the mind and the intellect and also an important ingredient in many Ayurvedic herbal formulations designed to treat conditions such as memory loss, anxiety, poor cognition and loss of concentration. TSCE was prepared in each set by soaking 50 mgs of seeds in 250 ml of water for 5-7days. This constitute the standard or stock solution of 1:5 dilution from which further dilution 1:10 and 1:20 were made. Effects of aqueous TSCE at different concentration namely 1:5, 1:10 and 1:20 were tested on *Bacopa monnieri*. TSCE showed maximum stimulatory activity at 1:20 dilutions than in 1:10 dilutions whereas 1:5 dilution exhibited negative activity and after three weeks of application it turned to completely dry at this applied concentration. At 1:20 dilutions, length and breadth of the bacopa leaf increased 76.46% and 37%, whereas it is 23.53% and 12.5% respectively in 1:10 dilution. Biochemical analysis namely phenolics, flavonoids, tannins and antioxidant activities were also revealed to show increase value in 1:20 and 1:10 dilutions compared to that of control. Our experimental results indicated that TSCE at 1:20 and 1:10 may be used as a biofertilizers which function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity.

Keywords: Brahmi (*Bacopa monnieri*), Biofertilizers, Tamarind seed coat extract, Bacoside

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Qualitative and quantitative changes of allelochemical compounds in *Brassica napus* L. residues decomposing in the soil

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Complex studies of allelochemical compounds released by oilseed rape during vegetation and during decomposition of crop residues in the soil are necessary to understand and scientifically explain the allelopathic effect of oilseed rape on agroecosystem. The aim of this study was to analyse the composition of allelochemical compounds in winter oilseed rape and the different morphological plant parts residues (threshing remains, stubble and roots) after harvesting as well as to reveal the quantitative and qualitative changes resulting from the different residues' decomposition in the soil within different periods (3, 7, 14, 19 and 26 months) and estimate the influence on weed germination.

All winter oilseed rape residues after harvesting had inhibitory influence on the germination of *Galium aparine* L., after three months of this crop residues decomposition in the soil the highest inhibitory influence was on the germination of *Sinapis arvensis* L., after seven months – on the germination of *Sonchus arvensis* L. A phytochemical analysis revealed that the total content of phenolic compounds in investigated crop residues decomposing in the soil significantly decreased after three months. As decomposition of these residues continued, the content of total phenolic compounds increased and reached the highest value after 19 months, except for the threshing remains. Qualitative and quantitative analyses of glucosinolates revealed that the content of these allelochemical compounds was significantly higher in the rape stubble compared with the threshing remains, where aromatic glucosinolates prevailed. The highest content of volatile organic compounds was determined in winter rape residues after seven months, except in the threshing remains. The results of this investigation suggests that winter oilseed rape residues decomposing in soil are distinguished by their allelopathic properties and therefore have an impact on agroecosystems in the following two-year period.

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Keywords: Weed germination, Phenolic compounds, Glucosinolates, Volatile compounds, Winter oilseed rape

*Speaker

Impact of Crop Residue Mulches and Nitrogen Sources on Weeds and Productivity of Wheat

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Returning of allelopathic crop residues as mulches to soil may be one of the best management strategy to manage weeds and sustain the productivity on long term basis. A 2-year study was conducted to investigate the influence of residue mulches of different crops known for having allelopathic potential under different nitrogen sources on weeds and productivity of wheat. The experiment consisted of four mulches (plastic, wheat straw, rice residues, sorghum residues) each applied at 4 tons/ha, and three nitrogen (N) sources (Urea, calcium ammonium nitrate, ammonium sulphate) applied at the rate of 125 kg/ha. All the mulches significantly reduced the density and dry biomass of weeds. Maximum suppression in weeds density and dry biomass was observed with plastic mulch. Highest suppression in broad leaved weed density and dry biomass was observed with wheat mulch followed by sorghum and rice mulch over control. For narrow leaved weeds, maximum reduction of density and dry biomass was observed in wheat mulch over control followed by sorghum mulch, while, minimum suppression in density and dry biomass was observed with rice mulch over control. Total weeds density and dry biomass was also affected with the allelopathic crop residue mulches. Maximum reduction in total weed density and dry biomass was observed with wheat mulch over control followed by sorghum and rice mulch. Nitrogen sources also affected grain yield significantly. Maximum yield improvement was observed when calcium ammonium nitrate was applied and that was statistically similar with ammonium sulphate application. We observed a strong negative correlation of grain yield and water soluble phenolics with the density and dry biomass of weeds. In crux, the use of allelopathic crop residue mulches in combination with the mineral nitrogen fertilizer might be a pragmatic option to control weeds and improve the wheat productivity.

Keywords: Weed dynamics, wheat, crop residues mulch, allelopathy

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Allelopathy of Persian walnut (*Juglans regia* L) and its by-products

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Since antiquity, the walnut tree is known not only for the edible walnut and the highly prized timber but also for his deep roots in folklore and mythology; moreover, walnut provides amongst the earliest recorded suggestion of allelopathy since the tree was considered a source of substances harmful to other organisms by Roman scholars Varro, and Pliny the Elder, who wrote treatises on agriculture dealing with the methods of good crop husbandry. Here we show a brief re- examination of the historical background of the Persian walnut (*Juglans regia* L.) in ancient herbals, to approach to the more recent literature on the allelopathic effects of walnut, with a particular regard to the by-product of its processing, which are indiscriminately employed by farmers for crop irrigation, without preliminary risk assessment. The results of recent publications (cytogenotoxicity on crops development) have posed the necessity to consider them as a hazard to identify solutions for their management through technologies which minimize its environmental impact and ensure a sustainable use of resources. However, an efficient and economical treatment process for walnut husk washing waters, the purification of the wastewater and recovery of compounds with high added value, would be of strategic importance for the walnut industry. We report interesting preliminary results on the antibacterial activity of two main compounds isolated by walnut and its by-products, namely juglone and regiolone.

Keywords: walnut walnut husk, *Juglans regia*, juglone, regiolone

*Speaker

Phenotypic Evaluation of Weed-competitive Traits and Yield of Rice RILs from an Indica x Tropical Japonica Mapping Population

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Indica rice cultivars can suppress weedy grasses. To better understand the important traits and genes underlying weed suppression and crop productivity, a recombinant inbred line (RIL) F8 population was developed by crossing non-suppressive 'Katy' and high-yielding, allelopathic 'PI312777'. Three hundred RILs were evaluated in the field (2014-2016) for traits related to growth, tillering, leaf production, and yield. Emergence of several RILs was earlier than either parent. Tiller and leaf production by PI312777 exceeded that by Katy, and some RILs exceeded PI312777. RILs with diverse photosynthesis rates were identified. Yields of PI312777 were 30% greater than those of Katy, and few RILs yielded more than PI312777. In field studies using selected RILs (_˜10% of total) with extreme, contrasting phenotypes, several RILs exhibited as much or more suppression than parents. Midseason weed biomass was highly correlated with rice dry weight (r=-0.73) and tiller number (r=-0.67). In the greenhouse, the main culm node from which the first tiller arose (NT1) was 1.4 for both parents and as low as 0.5 for RILs. Low values for NT1 were associated with more panicles (r=-0.37) and tillers (r=-0.30), suggesting that NT1 might be useful in understanding interactions between yield, early vigor, and weed suppression. In the greenhouse, PI312777 produced twice the root mass compared with Katy. In agar, PI312777 produced more numerous, spreading roots compared with Katy, and initial root growth angles among RILs ranged from _˜5 to _˜40 degrees from vertical. Few RILs suppressed weeds better, or yielded more than PI312777. Genomic regions associated with key traits are being determined by combining phenotypic data (and allelopathic activity; in progress) with genotypic data generated by the 2nd generation Illumina 6K SNP chip. The resulting QTL information will provide a foundation for mapping, and a better understanding of the roles of genes in yield and weed suppression.

Keywords: allelopathic rice, root growth and architecture, tillering, photosynthesis, single nucleotide polymorphism (SNP) chip

*Speaker

Understand the important role of phenolic acids in rice allelopathy

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Phenolic acids are paying close attention by researchers in plant allelopathy, as well as rice allelopathy. However, there is a lot of discussion and controversy about phenolic acids as rice allelochemicals because of its concentration and effect, when a paper reported that no difference in the amounts of total phenolic acids released between allelopathic and non-allelopathic rice cultivars. As its low concentration released to hydroponic solution, for quite a while, phenolic acids seemed to be excluded from the list of rice allelochemicals. To clarify this confusion, 4-Aminoantipyrine(4-AAP) method, Folin-Ciocalteu(FC) method, and Solid-Phase Extraction/High Performance Liquid Chromatography(SPE-HPLC) were used to compare the levels of phenolic compounds in rice culture solutions. Only 0%-51.2% of recoveries of 8 phenolic acids showed 4-AAP method is inappropriate to evaluate phenolic acids, thus led to the unreal results. By FC method, the total contents of phenolic compounds were always significantly higher in the culture solution of allelopathic rice PI312777 than non-allelopathic rice Lemont at 3-7 leaf stages. The maximum was 710 μg/plant in PI312777 at the 6-leaf stage, significantly higher than 10 μg/plant/day reported earlier by 4-AAP method. By SPE-HPLC, the 7 phenolic acids was 1.29-2.15 folds in PI312777 than in Lemont at 3-7 leaf stages. The cinnamic acid which cannot be determined by 4-AAP method, was 6-times higher in PI312777 than in Lemont at 6-leaf stage. The ferulic acid was 48.54 ng/plant in PI312777 at 6-leaf stage and no detection in Lemont. Moreover, various stress conditions up-regulated the biosynthesis of phenolic compounds. The phenolic acids increased the number of Myxococcus xanthus which could significantly increased miRNA expression in barnyard grass. Joint effects of ferulic acid and M. xanthus led to strongest inhibition on barnyard grass. We suggested phenolic acids, are not the only one, but are essential and principal components in "allelochemical cocktail".

Keywords: rice (*Oryza sativa* L.), Allelopathy, phenolic acids, rice exudates, bioactivity

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Allelopathic Activity of Some Turkish Plant Species

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Turkey is one of significant and unique country in the world from the stand point of plant genetic resources and plant diversity. Two of the Vavilov's Centre of Origin (Near Eastern and Mediterranean Centres) extend into country, indicating that Turkey is the one of the centre of origin and/or centre of diversity of several crop plants and many plant species. The flora of Turkey is very rich in wild medicinal, aromatic and ornamental plants, also.The potential of plant diversity of Turkey was determined and recognized by the plant explorers who worked in Turkey and found the most richest diversity of some cultivated plants, their wild and weed relatives and other wild plant species, than elsewhere. Although Turkey's flora with its existing diversity present potential for allelopathy researches, there is a lack of studies that screen the large number of Turkish plant species by bioassasy for allelopathic activity. That is why, the main objective of current research is to screen allelopathic activity of some crops, herbs, spices medicinal and aromatic plants from Turkey and to determine strong allelopathic species for next studies. Regarding to this, dried samples of plant species supplied from herbalists in different cities in Turkey will be assayed by the Sandwich Method for allelopathic activity, using *Lactuca sativa* -lettuce- as the test plant. To evaluate allelopathic activity, standart deviation (SD) and SD of variance (SDV) of radicle growth inhibition will be calculated. This is the first comprehensive report on screening of large number of Turkish plant species by bioassasy for allelopathic activity. Further studies would be applied to determine allelochemicals which cause allelopathic activity in regarding plants. Prospective data could provide a pathway to identify new natural chemicals that would serve sustainable agriculture.

Keywords: Allelopathic activity, Turkey, Sandwich Method, bioassay

*Speaker

Mutual effect of different crops with different densities of horse purslane (Trianthema portulacastrum L.)

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In Pakistan the extensive use of herbicides not only created the herbicide tolerance in weeds but also pose environmental threats. Different alternative techniques can be effectively utilized for successful weed management. Crops have the potential to suppress the seasonal weeds. Keeping in view the possible suppressive potential of crops, in a research trial different summer crops (cotton, maize, sorghum, numgbean and pearl millet; 2 plants pot^{-1}) were sown in the pots in sole and with horse purslane (2, 4 and 6 plants pot^{-1}). Horse purslane itself was also sown in pots in sole for comparison. Data were collected using standard procedures and analyzed by using analysis of variance techniques. Different crops were observed having differential response against horse purslane. Highest inhibition in shoot length, fresh and dry weights of horse purslane was observed in millet and sorghum. Cotton had least suppressive effect horse purslane parameters. Horse purslane also has competitive ability with crops. Highest adverse effect was recorded against mungbean shoot length (20-39%), shoot fresh weight (22-47%) and shoot dry weight (19-47%). Sorghum and millet were least affected by horse purslane. Sorghum shoot length, shoot fresh and dry weights were suppressed by 6-19%, 3-17% and 10-18% respectively and millet shoot length, shoot fresh and dry weights were suppressed by 3-23%, 7-18% and 9-22% respectively. It is concluded that pearl millet and sorghum are the best crops for this weed suppression. These crops can be included in sequential cropping (crop rotation) and intercropping system for better horse purslane management.

Keywords: Mutual effect, summer crops, horse purslane, allelopathic interaction, weed suppression

*Speaker

Allelopathic potential and growth inhibitory substances in Rumex maritimus L.

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Rumex maritimus (Polygonaceae) is an annual herbaceous plant distributed in North American, European and Asian countries. Although the medicinal values of *R. maritimus* are widely known, but there has yet been no report that addresses its allelopathic activity. Therefore, we explored allelopathic potential of *R. maritimus* to search allelopathic substances. The aqueous methanol extracts of *R. maritimus* were applied on the growth of cress, lettuce, alfalfa, rapeseed, barnyard grass, Italian ryegrass, timothy and foxtail fescue at four different concentrations. The extracts had inhibitory effects on the shoot and root growth of test plants, and the inhibition was increased with increasing extract concentration. These results suggest that the aqueous methanol extracts of *R. maritimus* possess allelopathic properties and may contain allelopathic substances. The extracts were then adjusted to pH 7.0 with 1N NaOH, partitioned against an equal volume of ethyl acetate. The ethyl acetate fraction was subsequently purified by several chromatographic steps and three growth inhibitory substances were identified by spectral data as 2-methoxystypandrone, altechromone A and 5,7-dihydroxyphthalide. At the concentrations of 3, 10 and 300 μM, the 2-methoxystypandrone, altechromone A and 5,7-dihydroxyphthalide inhibited 70.1, 65.3, 66.3% and 67.5, 87.1, 70.0% of shoot and root growth of cress compare to the control, respectively. The concentrations required for 50% shoot and root growth inhibition of cress for 2-methoxystypandrone, altechromone A and 5,7-dihydroxyphthalide were ranged from 5-12, 662-1410, 1730-2482 μM, respectively. Therefore, it is suggested that the growth inhibitory activities of *R. maritimus* on the test plants may be caused due to the presence of 2-methoxystypandrone, altechromone A and 5,7-dihydroxyphthalide in the *R. maritimus* extracts. This is the first report of having allelopathic substances in *Rumex maritimus*.

Keywords: allelopathy, *Rumex maritimus*, aqueous methanol extract, plant growth inhibitor

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Effects of Maize Rotation on the Physicochemical and Biological Properties of Continuously Cultivated Soil and the Growth of American ginseng

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American ginseng (*Panax quinquefolius*) growth is often hampered by replant problem, and the longerm impacts of crop rotation on plant performance remain unclear. In this study, we investigated the alteration of soil nutrients, autotoxic phenolic acids, and fungal communities in American ginseng-cultivated soil after maize growth to establish an American ginseng-maize-American ginseng rotation system in Northern China. Soil nutrient levels were assessed in treatment soils using chemical analytical methods. Autotoxic phenolic acids were determined by HPLC. The fungal community was evaluated by T-RFLP analysis. The effects of rotation on replanted American ginseng growth were measured using a pot experiment. Soil nutrients of maize growth for three to five years (4G-3M, 4G-5M) on American ginseng-cultivated soil (4G) were grouped together with maize-cultivated soil (CM). Syringic acid, ferulic acid, salicylic acid, *p*-coumaric acid, and total phenolic acids contents in 4G soil were significantly decreased to CM level after one year of rotation (4G-1M)($P < 0.05$). Soils supporting maize growth for 5 years (4G-5M) showed a recovery in Simpson diversity and Pielou evenness. Root yield and lateral root numbers of American ginseng increased in 4G-1M soil while root disease severity was significantly lower after 3 year of rotation (4G-3M) ($P < 0.05$). Multi-linear regression showed that seedling survival rate, root yield, and root disease severity were related to scertain soil chemical and microbial properties while soil microbial properties were rimpacted by soil chemical properties. In summary, maize rotation improved the physicochemical and biological properties of American ginseng-cultivated soil, and these positive effects lasted for at least three years.

Keywords: *Panax quinquefolius*, replant problem, maize rotation, soil nutrient, phenolic acid, fungal communities

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Evaluation of the allelopathic potential of some crops against *Hypericum* spp.

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With the growing awareness of the problems associated with the excessive use of herbicides which include surface and ground water contamination and evolution of herbicide resistance in several weed species, interest in developing effective biocontrol methods as alternatives for weed control is increasing. Allelopathic crops may offer a promising approach to efficiently control weeds. Among weeds species associated to cereals in Tunisia, St. John's wort (*Hypericum* spp.), a perennial weed, is becoming a serious weed due to its toxicity to livestock and its ability to cause important losses in crop yields.
The present study is aimed to map St. John's wort infections and to evaluate the allelopathic potential of some crops on weed growth.

Field surveys carried out in the main cereal growing regions in the northern of Tunisia showed that Infestation varied among regions and are particularly related to the cultural practices used by farmers in each region.

The allelopathic potential of selected crops was evaluated by assessing the effect of water extracts on root growth of weed seedlings in laboratory bioassays. Tested crops included alfalfa, sorghum, sunflower, rapeseed, mustard, rye, and barley. Allelopathic crop species with the highest phototoxic activity on St. John's wort root development have been tested in field experiment and their effects on the weed dry matter were determined at crop harvesting.

Water extracts of tested crops resulted in significant reductions in weed root growth. The highest phytotoxic activity was observed using barley extract (up to 73%) followed by rape extract (70%). Similar results have been observed in field plots where the dry matter of St. John's wort was reduced by 85% and 37% in rape and barley crops respectively.
These results suggest that allelopathic crops grown in rotation may be a promising method for the management of this perennial weed in cereals crops.

Keywords: *Hypericum* spp. weed management, cereals, allelopathic crops.

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The allelopathic effect of winter oilseed rape residues treated with bio-preparations and organic fertiliser on *Sinapis arvensis*

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Slurry can be used as organic fertiliser in agro-technologies. Lately large number preparations of biological origin are recommended to be used for increasing crop productivity, resistance to environmental stresses and sustaining agroecosystem stability. But still little is known how these organic treatments are influencing weeds. The aim of this research was to evaluate the allelopathic potential of volatiles from winter oilseed rape different morphological parts residues (threshing remains, stubble and roots) after harvest treated with bio-preparations and slurry on wild mustard germination. Treatments of the weed germination bioassays: 1) without crop residues (Control); 2) crop residues without treatment (CropRes); 3) crop residues with bio-preparation (Bio1); 4) crop residues with slurry (S); 5) crop residues with slurry and bio-preparation (SBio2). In the composition of Bio1 three carrier materials included: dolomite, molasses and magnesium sulphate; in the Bio2: calcium carbonate and molasses. Experiments were carried out in climate chamber using Petri dishes with two compartments: in one compartment weed seeds were germinated, in second – placed winter oilseed rape residues in different quantities (10 and 30 mg cm^{-3}). Threshing remains and stubble of winter oilseed rape after harvesting at higher concentration stimulated the germination of *S. arvensis*. However, threshing remains treated with bio-preparations and slurry showed opposite results: the germination was suppressed. Stubble at higher concentration treated with SBio2 inhibited the germination of tested weed to compare with stubble without any treatments, but lower concentration showed opposite results: the germination in all treatments was stimulated. The roots of winter rape at lower concentration increased the germination of *S. arvensis*, but higher concentration the germination inhibited. Slurry and bio-preparations at higher concentration positively influenced germination of *S. arvensis* to compare with the roots without treatments. Acknowledgments: part of this research was funded by a grant (No. SIT-8/2015) from the Research Council of Lithuania.

Keywords: allelopathic effect, weed germination, winter oilseed rape residues, bio, preparations, slurry

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Allelopathic Activity of Iranian Native Medicinal and Aromatic Plants by Using Cotton Swab Method

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In recent years, awareness of the harmful effects of herbicides have persuaded researchers to seek for a suitable replacement of chemical herbicides. One of these alternatives is natural compounds. So using allelopathic compounds as a novel strategy reduces the chemical herbicides consumption. This study was aimed to identify strong allelopathic activity among medicinal and aromatic plants (MAPs). Through one of the modern bioassay methods "Cotton Swab Method" which is used for evaluating volatile compounds, this experiment was conducted as a randomized complete design with four replicates. The essential oils (EOs) were applied in two different concentration 2 μL and 5 μL per 25 mL glass vial on lettuce seed germination. In this context a total of 104 EOs of MAPs were evaluated. Germination parameters (germination inhibition percentage, seed vigor, seed germination index (SGI), T50, mean germination time, speed of germination and germination speed index) were evaluated. The results showed that 15 samples such as *Origanum majorana*, *Zataria multiflora, Thymus daenensis, Melissa officinalis, Pelargonium graveolnes,* and *Pimpinella anisum* had very strong inhibitory effect on lettuce seed germination (%80-100 inhibition). Based on T50 results, 12 samples such as *Rosmarinus officinalis*, *Artemisia scoparia*, and *Nepeta binaludensis* were highly effective on seed germination delay (More than 200%). So it can be concluded that there are very strong germination inhibitor EOs especially in Lamiaceae and Asteraceae families. According to the GC-MS analysis, components such as terpinen, thymol, carvacrol, citronellal, citronellol, geraniol and anethole were the main constituents of the strong inhibitor EOs. Moreover, camphor, p-cymene, β-pinene and 1,8-cineole were the main constituents in strong EOs which postpone seed germination. These results showed that cotton swab can be used as a rapid and suitable method for detecting allelopathic properties in volatile compounds. Furthermore, natural volatile ingredients of the mentioned MAP are good candidates as bio-herbicides.

Keywords: Medicinal and Aromatic Plants, Allelopathic Properties, Cotton Swab Method, Essential Oil, Organic Cultivation

*Speaker

Metabolic profiling for benzoxazinoids in weed-suppressive and early vigour wheat genotypes

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Replicated and randomised wheat (*Triticum aestivum* L.) cultivar trials were conducted in moderate to low rainfall zones at Wagga Wagga and Condobolin NSW, respectively in 2014 to 2016. At each experimental site, crop and/or weed growth were monitored at selected growth stages including tillering, vegetative, grain filling, harvest and after crop harvest. In addition, shoots, roots, rhizoplane and bulk rhizosphere soil samples were collected. All shoot and root samples were extracted in methanol using Buchi automated high pressure extractor, while soil samples were extracted using a rotary shaker. Extracts were profiled for unique secondary plant products acting as allelochemicals for weed suppression, specifically benzoxazinoids (BXs), using liquid chromatograph coupled to a triple quadrupole mass spectrometer (UPLC-MS QQQ). In addition, non-targeted metabolomics analysis was performed to evaluate relative abundance of diverse metabolites using a quadrupole time-of-flight mass spectrometry (UPLC-MS QToF) platform. Metabolic profiling of wheat shoots, roots, and soils resulted in detection of up to 14 BXs including BX glycosides and other metabolites of interest. Both qualitative and quantitative differences in BXs were observed and were cultivar-, growth stage- and location-dependent. Plant part and rhizosphere location (distance from root) also impacted BX concentration. The distribution of the secondary metabolites in wheat cultivar tissues suggest differential production of some key bioactive metabolites. Further metabolic profiling provided crucial information regarding crop metabolism, as well as the biosynthesis and release of metabolites associated with weed suppression in currently available commercial wheat cultivars, in contrast to weed suppressive rye (*Secale cereale* L.) and heritage wheat cultivars such as Federation, known for their potent ability to suppress weeds. This presentation will focus on the results of three years of field experimentation at two locations and predict which cultivars are best-suited for weed suppressive properties due to canopy architecture and allelopathic traits while maintaining high yield potential.

Keywords: Weed suppression, metabolomics, residue, competition, resource allocation.

*Speaker

Phytototoxicity study of *Bidens sulphurea* Sch. Bip. as a preliminary approach for weed control

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Farmers of the Franca region in Brazil observed that *Bidens sulphurea* was able to eliminate the *Panicum maximum* weed that infected the coffee plantations, without affecting the crop. To determine if the ability of inhibition observed can be due to the presence of phytotoxic compounds from leaves and roots, a biodirected isolation and spectroscopic characterization have been realized. The leaf dichloromethane and root acetone extracts were the most active, and the former appeared to be more phytotoxic to the target species, including four weeds. Twenty-six compounds were isolated from leaves and roots, and four of them have been described for the first time. The major compounds in the leaf extract are the sesquiterpene lactones that showed a marked inhibition. *A. viridis* and *P. maximum* were the most sensitive species among weeds tested. These phytotoxic lactones were also evaluated on *A. viridis* and *P. maximum* in hydroponic conditions, with *A. viridis* being the species most affected by lactones. This is the first time that sesquiterpene lactone phytotoxicity has been evaluated in hydroponic culture. This is a preliminary approach of the use of *B. sulphurea* for weed control in agriculture, both as cover crop or using its components as natural herbicide leads.

Keywords: *Bidens sulphurea*, costunolide, reynosin, santamarine, allelochemicals, hydroponic culture

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Allelopathic effects of some weeds as individually and in combination on seedlings growth and biochemical parameters of wheat

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Allelopathy is defined as harmful effects of a species on others directly or indirectly by releasing of biochemical compounds into the environment that called allelochemicals. The allelopathy is an important interaction among plant species in the natural and agricultural systems. Wheat (*Triticum aestivum*) is the most economical crop in the Iran. In the presented study, allelopathic effects of four frequently weed species in the agricultural fields of north-west of Iran including *Amaranthus retroflexus*, *Elymus repens*, *Convolvulus arvensis* and *Malva neglecta* on growth and some biochemical parameters of wheat was studied. Fresh materials of weeds were collected from fields and used for preparation of leachates. The experiments were conducted in petri dishes as individually and combination of leachates. Statistical analysis of experimental data showed that three weeds including *Amaranthus retroflexus*, *Convolvulus arvensis* and *Malva neglecta* had considerable inhibitory effects on wheat growth parameters such as shoot and root length, fresh and dry weight as leachates applied individually. In contrast, as leachates applied in combination (dual, triplex and quad) growth of wheat was improved compared with individually application. Total soluble protein content increased in all status at all treatments except *Elymus repens*. Generally, photosynthetic pigments content were reduced in all treatments. Also, activity of antioxidant enzymes were decreased by leachates of three mentioned weeds as applied individually, but increased at some treatments of the combination leachates. Malondialdehyde content was affected by the majority of treatments in all status. As a result, the three mentioned weeds had more allelopathic effects on wheat growth and physiological parameters and would manage these weeds in the wheat fields.

Keywords: Allelopathy, wheat, crop, weeds, leachate

*Speaker

Allelopathic Effects of Some Vegetable Crops on Selected Common weed Species in Jordan

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Allelopathic activities of some widely cultivated vegetable crops grown in Jordan namely; bean, cabbage, cauliflower, eggplant , pepper, potato, radish and tomato were investigated against six common weeds included *Amaranthus retroflexus*, *Chenopodium murale*, *Eruca sativa*, *Malva sylvestris*, *Portulaca oleracea* and *Solanum nigrum* under laboratory and glasshouse conditions. Shoot extracts, foliage leachates and volatile materials emanated from extracts of different crops significantly reduced seed germination and seedlings growth of different weed species in Petri-dishes. Effects of extracts was concentration-dependent and roots were more inhibited than shoots. The effects of dried shoot residues in potted soil was varied on different weeds. Certain residues were highly toxic and significantly reduced germination and growth of certain weeds others had stimulatory effects. Soil-incorporated dried residues of cabbage, cauliflower and bean were most toxic, while *A. retroflexus*, *C. murale* and *P. oleracea* were most sensitive to allelopathic effects of the tested crops. Decayed residues of tomato, cabbage, bean and eggplant in the soil were most toxic to the tested weed species. While soil-applied extracts significantly reduced germination and growth of certain weeds, foliage application of these extracts gave inconsistent results on weeds growth. Root exudates of many crops tested severely reduced dry weight of most weed species with radish, cauliflower and bean exudates were most toxic to *A. retroflexus*, *C. murale* and *S. nigrum*. Foliage leachates of Cauliflower and Pepper were highly toxic. Among weeds, *M. syvestris* was the most tolerant weed to allelopathic effects of the tested crop species.

Keywords: Vegetable crops, weeds, extracts, foliage leachates, volatiles, root exudates, crop residues

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Rice allelopathy could be increased by induction of Barnyard grass (*Echinochloa crus-galli*) root exudates

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The changes of rice allelopathy were investigated by induction of barnyard grass (*Echinochloa crus-galli* L., BYG) root exudates in a hydroponic system. Results showed that after induction by BYG root exudates, the inhibitory rates of allelopathic rice PI312777 leaves extracts were increased from 45.08% to 60.92% on BYG root length, from 41.77% to 62.25% on BYG plant height, and from 44.30% to 64.56% on BYG dry weight, respectively. Similarly, the inhibitory rates of nonallelopathic rice Lemont leaves extracts were increased from 12.74% to 27.11% on BYG root length, from 10.04% to 14.32% on BYG plant height, and from 10.97% to 26.16% on BYG dry weight, respectively. The inhibitory rates of two rice culture solutions had the same trends as their leaves extracts. The levels of phenolic compounds in PI312777 and Lemont culture solutions were 1.54 times and 1.39 times respectively, as high as the control solutions. HPLC analysis showed that the BYG root exudates contained *p*-hydroxybenzoic acid at 19.95 μg/plant, vanillic acid at 18.99 μg/plant, syringic acid at 47.95 μg/plant, cinnamic acid at 76.75 μg/plant, salicylic acid at 1.10 μg/plant, and ferulic acid at 92.51 μg/plant. After induction of the 6 phenolic acids mixtures at the concentration above, the inhibitory rates of PI312777 leaves extracts on BYG root length were 43.41%. These results indicated that in BYG root exudates, phenolic acids may be, but not the only compounds which contributing to the increase of rice allelopathy.

Keywords: Rice (*Oryza sativa*. L), allelopathy, Induction, Barnyard grass (*Echinochloa crus-galli*), Root exudates

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Contribution of allelopathy and competition to weed suppression by winter wheat, triticale and rye

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Above-ground competition and allelopathy are two of the most dominant mechanisms of plants to subdue their competitors in their closest surroundings. In an agricultural perspective, the suppression of weeds by the crop is of particular interest, as weeds represent the largest yield loss potential worldwide, if not controlled. In view of the current increase in the cases of herbicide resistance and no indication of new modes of action becoming available to the market in the next decade, the crop's inert ability to suppress weeds have become more important. This study aims to identify the contribution of competitive traits, such as early vigour, crop height and leaf area index and presence of phytotoxic compounds of the group of benzoxazinoids to weed suppression. Four cultivars of each of the winter cereals wheat, triticale and rye were grown in field experiments at two locations. Soil samples were taken in early spring 2016, extracted with a Dionex ASE350 Accelerated Solvent Extractor and analysed in the multiple reaction mode with an Applied Biosystems 4500 Q Trap LC-MS/MS for benzoxazinoid concentrations. Weed biomass of the natural weed population present in the plots was measured in the end of June 2016. Competitive traits were measured throughout the growing season. Partial least squares regression with weed biomass as response variable was used for modelling. Competitive traits, as well as benzoxazinoid concentrations contributed significantly to the models on winter wheat, winter triticale and winter rye data and explained 63, 69 and 58% of the variance in weed biomass in the first two components, respectively. Consequently, it can be concluded that competitive, as well as allelopathic traits, contributed significantly to weed suppressive outcome in winter cereals. This knowledge is of significant importance for future breeding programs aiming at increased weed suppressiveness of winter cereals.

Keywords: Partial Least Squares Regression, LC, MS/MS, Benzoxazinoids, Cereals, *Triticum aestivum*, Triticosecale, Secale cereale, Weed

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Determination of the allelochemicals in *Solanum lycopersicum* roots

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The work described herein shows the isolation and characterization of natural products present in the tomato roots (*Solanum lycopersicum*) with phytotoxic activity. Tomato was selected owing that almost all studies have focused on the fruit, but other parts have been less studied, ignoring in this way a possible source of new natural products with phytotoxic activity. As result of that work, stigmasterol, bergapten and α-tomatine have been isolated.

The major compounds, stigmasterol and α-tomatine have been evaluated on wheat coleoptiles, being α-tomatine the most active. In the bibliography has been reported that α-tomatine protect the leaves of tomato against microorganisms, fungus and pathogens. However, phytotoxic activity has not been reported yet.
α-tomatine was identified in root exudates using LC-MS/MS. The results obtained confirm that α-tomatine is exudated by roots to environment. To confirm the allelopathic roll, α-tomatine was tested on the model seed *Lactuca sativa* and on two weeds of tomato crops (*Lolium perenne* and *Echinochloa crus-galli*). The stimulant activity of the parasitic plant germination of α-tomatine was also evaluated on the parasitic plant of tomato, *Phelipanche ramose*, and also on *Orobanche crenata* and *Orobanche cumana*.

Keywords: Isolation, phytotoxicity, roots, α tomatine, parasitic plants, LC MS/MS

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Potential control of weeds and plant pathogens by *Cynara cardunculus* L. leaf extracts

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Allelochemicals from donor plants represent an eco-friendly strategy for weed and pest control in agriculture. *Cynara cardunculus* L. leaves present a high concentration of sesquiterpene lactones such as aguerin B, grosheimin, and cynaropicrin. However, the potential use of their extracts for weed and plant pathogen biological control is at the beginning of investigation. This study aimed to evaluate the allelopathic effects of leaf aqueous extracts (40 and 80% concentrations) of three *C. cardunculus* botanical varieties, globe artichoke [var. *scolymus* (L.) Fiori], cultivated cardoon (var. *altilis* DC.), and wild cardoon [var. *sylvestris* (Lamk) Fiori], on seed germination of six common weeds in Mediterranean agroecosystems (*Amaranthus retroflexus* L., *Portulaca oleracea* L., *Diplotaxis erucoides* (L.) DC., *Lavatera arborea* L., *Brassica campestris* L., and *Solanum nigrum* L.), compared with distilled water as a control. The autoallelopathic activity on wild cardoon also was considered. Different leaf extract typologies obtained by methanolic, ethanolic, and aqueous extraction, starting from fresh, dried, and lyophilized leaves were compared. Furthermore, the addition of acetic or citric acid was compared in order to reach the microbiological stabilization of the extract. Cultivated cardoon extracts exhibited *in vitro* antagonistic activity against pre- and postharvest phytopathogenic fungi and bacteria, with better inhibition efficacy of water and ethanolic extracts compared with methanolic extracts. On average, leaf aqueous extracts reduced seed germinationby 41% compared with the control. The best result was obtained with cultivated cardoon extract at 80%, which inhibited weed seed germination by about 64%. Methanolic, acidified extracts appeared to inhibit seed germination more than did the aqueous, non-acidified ones. These results hold promise for the development and production of bioherbicides, biofungicides, and biobactericides based on *C. cardunculus* allelochemicals as alternatives to chemical products.

Keywords: leaf extracts, weeds, globe artichoke, wild cardoon, cultivated cardoon, allelochemicals

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Phytotoxic activity of *Piper retrofractum* fruit extracts and its bioactive compounds

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Piper retrofractum is a medicinal plant. Bioactive compounds from its fruits have been recognized and used in food, cosmetics, and medicine. Insecticidal and antimicrobial compounds in this plant have also been reported, but there have been no relevant studies on their phytotoxic activity. Therefore, this study aimed at the evaluation of phytotoxic activity of an aqueous methanol extract of the fruits of *P. retrofractum* and the activity of the isolated compounds on seedling growth of test plants. The result showed that the fruit extracts were phytotoxic to cress, lettuce, alfalfa, barnyard grass, Italian ryegrass, and jungle rice seedlings. A significant reduction in growth of all test seedlings was observed for the extract at 1 mg dry weight equivalent extract/mL. The concentrations required for 50% growth inhibition ($I50$) of the extract on all test seedlings ranged from 0.3 to 14 mg dry weight equivalent extract/mL. The extract was then subjected to bioassay-guided fractionations, and seven compounds, 3-phenylpropanoic acid (**1**), (2*E*,4*E*)-methyl piperate (**2**), (2*E*,4*Z*)-methyl piperate (**3**), piperlonguminine (**4**), dihydropiperine (**5**), isochavicine (**6**), and piperine (**7**) were isolated. Those compounds inhibited seedling growth of cress and barnyard grass and the inhibition increased with increasing concentrations of the compounds. Compounds (**6**), (**7**), and (**4**) inhibited shoot and root growth of cress at concentrations greater than 30 μM, with $I50$ values ranging from 11 to 18, 23 to 35, and 25 to 10 μM, respectively. Compounds (**7**) and (**5**) inhibited shoot and root growth of barnyard grass at concentrations greater than 100 and 300 μM, with $I50$ values ranging from 238 to 37 and 487 to 131 μM, respectively. These results suggest that the compounds (**1-7**), especially (**4-7**), may be responsible for the phytotoxic activity of the fruit extract of *P. retrofractum*.

Keywords: phytotoxicity, natural herbicide, inhibitory compounds, weed management

*Speaker

Allelopathic potential of burdock leaves, roots and cultivated soil

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Burdock (*Arctium lappa* L.) is a biennial herb belonging to the Asteraceae family. Continuous cropping of burdock in the same field decreases the crop yield year by year (replant problem). Allelopathy has been reported to be one of the causes of replant problems in some other plant species. However, there is very limited information on allelopathy of burdock. The present study aimed to assess allelopathic potential of burdock leaves, roots, and cultivated soil. All extracts of burdock leaves, roots, and cultivated soil inhibited the growth of barnyard grass, foxtail fescue, ryegrass, cress, lettuce, and burdock. The level of inhibition increased with increases in extract concentrations. Those extracts inhibited the root growth of monocotyledonous plants (barnyard grass, foxtail fescue, and ryegrass) more than their shoot growth. The leaf extract of burdock showed higher growth inhibition than the root extract. The level of sensitivity of burdock to the extracts was intermediate among the six test plant species. After purification of the root extract by several chromatographic runs, a growth inhibitory substance, methyl caffeate, was isolated. Methyl caffeate significantly inhibited the growth of barnyard grass, cress, and burdock at concentrations $>$ 1 mM. The concentrations of methyl caffeate for 50% growth inhibition were 1.7–3.0, 1.1–1.4 and 2.6–4.2 mM for barnyard grass, cress, and burdock, respectively. The purification of growth inhibitory substances in the extracts of burdock leaves and cultivated soil is currently in progress. The present results suggest that the extracts of burdock leaves, roots, and cultivated soil had allelopathic potential. In order to more fully investigate this potential allelopathic involvement in the burdock replant problem, further identification of growth inhibitory substances in burdock leaves and cultivated soil will be necessary.

Keywords: burdock, autotoxic, allelopathy, growth inhibition, growth inhibitory substance

*Speaker

Sorghum has potential to suppress *Amaranthus hybridus* germination and seedling vigour

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Chemical and mechanical weed control strategies can be environmentally detrimental and therefore alternative weed management practices are continually being explored. Sorghum produces a potentially weed suppressing natural toxin called sorgoleone, which is the most abundant metabolite in this hydrophobic exudate. We conducted pot experiments to evaluate the potential allelopathic effects of sorghum root residues and root exudates on the germination and early growth of *Amaranthus hybridus* (L.), a problematic broad leaf weed of Zimbabwe, in the 2015/16 season at Henderson Research Station. A completely randomized design with four treatments (SC Smile, SC Sila, Macia and a control) replicated three times was used. For three consecutive times, sorghum was planted at a density of 10 seeds per pot using the same growing media. At 7 days old, stems of sorghum seedlings were excised using sterile surgical blades, leaving the root system undisturbed in the soil. After the third harvest of sorghum, seeds of *A. hybridus*, were then dribbled into the pots at a density of 3 seeds per pot. Germination and seedling vigour of *A. hybridus* were observed. In comparison with the control treatment, results showed that germination and early seedling development of *A. hybridus* grown in the media where sorghum had grown were greatly suppressed. There was a significant difference (P0.001) in the suppression of *A. hybridus* germination and seedling vigour across treatments. The control treatment had the highest germination (85%), while SC Sila had the lowest germination percentage (13.3%). The control treatment produced the most vigorous plants while SC Smile produced the weakest weed seedlings. Suppression of weed seed germination and weed seedling growth gives crops an early competitive advantage over weeds. There is potential in utilizing sorghum allelopathy for *A. hybridus* control.

Keywords: sorghum, weed control, allelopathy, *A. hybridus*, competitive advantage

*Speaker

Quantification of sorgoleone in 353 African sorghum accessions

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353 sorghum accessions comprising 148 landraces from South Africa, 97 landraces from Botswana, 66 landraces from Namibia, 15 landraces from Zambia, 11 wild sorghums and 7 sweet stem sorghums from Zimbabwe, and 9 released/breeding material grown in Zimbabwe, Mozambique, Malawi, Botswana, Tanzania and Zambia were screened for sorgoleone content by high performance liquid chromatography in 2016. Sorghum seeds were planted in petri dishes in three replicates in the Weed Science Laboratory (Department of Crop Science, University of Zimbabwe). Six days after germination, roots of seedlings were excised from the shoots and immersed in HPLC-grade methanol (1:20) w/v for 3 minutes to extract the sorgoleone. The crude extract was filtered and evaporated in a water bath with temperature set at 45∘C. The dried extract was dissolved in mobile phase (1mg/ml) and the solution filtered through a poly filter 0.45μm prior to HPLC analysis. Quantification was based on a calibration curve using purified sorgoleone as an external standard. Differences in sorgoleone concentration among accessions were observed. Very high levels of sorgoleone were detected in accessions that included a Zimbabwean wild sorghum IBS749 (355.24 μg/mg RFW); a Botswana landrace IS19450 (464.43 μg/mg RFW) and three South African landraces IS14002, IS14003 and IS9456 (381.20 μg/mg RFW, 472.69 μg/mg RFW, and 584.69 μg/mg RFW respectively). Sorgoleone was not detected in 11 of the 353 sorghum accessions tested. In the majority (316) of accessions, sorgoleone was in the range of 0.01 – 200 μg/mg RFW. In many of the accessions, a major compound that was eluted at about ≈0.3 minutes after injection, and 5 to 9 minor compounds were also detected. The results indicate that sorghum from some parts of Africa contains large quantities of sorgoleone and other major and minor compounds. There is potential for use of sorghum allelopathy for weed control in Africa.

Keywords: African sorghum, sorgoleone, high performance liquid chromatography, allelopathy, weed control

*Speaker

Allelopathic potential of different wheat cultivars on selected weed species under field and laboratory condition

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In order to compare the allelopathic potential of different wheat cultivars under severe competition with *Phalaris minor* Retz., an experiment was conducted as split plot in RCBD with three replications at experimental field of Agricultural Research Center of Golestan Province during growing season 2013-2014. The 12 wheat cultivars (Morvarid, Gonbad, N-87-20, N-91-8, N-91-9, N-91-10, N-91-14, N-91-17, Koohdasht, line 17, Karim and N-90-7) as main plots were planted in two subplots (weed-free and weed-infested with 50 *P.minor* individuals per square meter). Also, the allelopathic effects of different wheat cultivars on growth and germination of soybean crop, *Euphorbia maculate* L. and *Euphorbia heterophylla* L. weeds were separately compared in several laboratory experiments. All petri dish experiments were conducted as factorial in CRD with three replications. The treatments were wheat cultivars in twelve mentioned above levels as first factors and the concentration of wheat cultivars aqueous extracts in five levels (0, 25%, 50%, 75%, and 100) as second factors. Among different cultivars, N-90-7, N-91-8 and N-91-9 had the highest yield under weed free condition, but N-90-7 was more successful than the other cultivars under weed infestation condition too. The results of petri dish experiments showed that different levels of wheat extract had no significant effect on germination percentage of soybean. But *E. maculata* and *E. heterophylla* seed germination was affected by increasing extract dosages. Germination rate, length and dry weight of *E. maculata* and *E. heterophylla* seedlings were decreased as the extract concentration increased significantly. The results showed that some of wheat cultivars have allelopathic potential to reduce weed *E. maculata and E. heterophylla* growth. N-87-20 wheat cultivar was the most allelopathic potent plant among different wheat cultivars and it showed the highest inhibitory effect on *E. heterophylla* and *E. maculata* growth and germination.

Keywords: Interference, soybean, spotted spurge, *Triticum aestivum*, wild poinsettia

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A potential allelopathic substance in *Paspalum commersonii* Lam.

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Paspalum commersonii (Poaceae), a 15 to 20 cm tall perennial weed, is native to South Africa and widely distributed in India, Indonesia, Bangladesh and Myanmar. We explored the allelopathic potential of *P. commersonii* and determined the putative allelopathic substances in its, as no study has been found so far on allelopathy of *P. commersonii*. The aqueous methanol extracts of *P. commersonii* were tested against on the shoot and root growth of cress, lettuce, alfalfa, rapeseed, barnyard grass, Italian ryegrass, timothy and foxtail fescue at six concentrations. The aqueous methanol extracts showed growth inhibitory effects on the tested plants in a concentration dependent manner. The root growth of the test plants were suppressed more than the shoot growth. These results indicate that, the extracts of *P. commersonii* have allelopathic properties and thus may contain allelopathic substances. The extracts were partitioned with equal volume of ethyl acetate and the ethyl acetate fraction was subsequently purified by silica gel column, Sephadex LH-20, C18 cartridges and HPLC. An active substance was purified and characterized as loliolide by the specific rotation, HRESIMS and ^{1}H NMR spectrum. The seedling growth of cress and barnyard grass were inhibited by the loliolide at the concentration greater than 10 μM and 30 μM, respectively. The required concentrations for 50% growth inhibition of cress and barnyard grass were ranged 32.1 to 41.6 μM and 48.3 to 128.5 μM, respectively by the loliolide. Therefore, the allelopathic substance loliolide may be responsible for exhibiting the inhibitory activity in *P. commersonii*.

Keywords: Allelopathic activity, *Paspalum commersonii*, Allelopathic substance, Loliolide

*Speaker

Rice Allelopathy from Chemicals to Regulating Genes

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The phenomena of allelopathy were realized since ancient times in agricultural production. Allelochemicals from rice are secreted in the rhizosphere and suppress the growth of weeds. The process of allelopathy is regulated by many genes such as those signaling the synthesis and metabolism of allelochemicals by the plant. Identification of genes that regulate rice allelopathy is crucial to an understanding of the mechanisms of allelopathy in rice. In this study, the allelopathic cultivar PI312777 was selected for evaluation using gene chip technology and bio-informatics to analyze and select the relative genes in regulaion of rice allelopathy according to reported types of allelochemicals. Expression vectors were then constructed and transferred into rice by agrobacterium-mediated transformation for functional verification. The results showed that there were around 40 genes closely related to the stress conditions, and 10 genes related to synthesis and metabolism of phenolics. Bio-informatics analysis of gene chip data indicated that the pathways elicited under stress conditions were quite different for PI312777 and Lemont. Phenylpropanoid biosynthesis was the main pathway for PI312777, while carbon fixation was key for Lemont. C4H (Os05g0320700 or CYP73A38) was one of the key genes. The effects of transgenetic rice and PI312777 on the growth of barnyardgrass were similar, and 5 phenolic compounds were also detected by HPLC to identify the selected gene functions. The results proved that allelopathic potential could be improved by increasing gene expression, and phenolics were the most important chemicals in rice allelopathy. These results provided not only new evidence for understanding the molecular mechanism of regulating rice allelopathy, but also a clue for obtaining genes for improvement allelopathic potenial in modern rice molecular breeding.

Keywords: rice, allelopathy, Barnyardgrass, phenylpropanoid biosynthesis, C4H

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Allelopathic Response of Asphodelus tenuifolius Cav. on the Early Vegetative Growth of Zea mays L.

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Allelopathy is a mechanism in which allelo- chemicals produced by plants may inhibit or enhance the associated plant growth. Plants have a defense system to produce allelochemicals and localize them to the front line i.e. on the leaf surface, external stem and roots ,buds etc In the present investigation we were observed that the shoot and seed extracts of *Asphodelus tenuifolius* Cav. have shown the inhibitory effect on germination of the test crops, Zea mays . Further, different concentrations of *Asphodelus tenuifolius* Cav. shoot and seed extracts have caused inhibitory effect on germination and growth behavior of *Zea mays* L. seedlings significantly. Maximum germination and growth occurred in control, where as ,at lower concentrations significant growth in shoot length and root length observed. Similiarly the fresh weight and dry weight is also reduced in *Asphodelus tenuifolius* Cav. extracts. Therefore we conclude that *Asphodelus tenuifolius* shows significant allelopathic effect on seedling growth of *Zea mays.*

Keywords: Allelopathy, *Asphodelus tenuifolius*, plant water extract, maize growth

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Self-weeding canola - the lessons

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A project was undertaken to see whether canola allelopathy could deliver field outcomes. There have been 4 years of field data to this point and there have been important lessons each time. For the first two years highly consistent results were obtained and the allelopathy impacts were strongly identified. There followed two challenging years for weeds research the first being almost a non-weed year and then a very wet one with prolific weeds. The results continued to be consistent to the extent that annual ryegrass, the target weed, continued to be controlled by the same variety to the extent that it occurred. In the final year other grass weeds (Avena and Bromus spp) were present and in a commercial crop would have needed herbicide for control. However the pressure for ryegrass was removed. The other aspect worth commenting on was our publicity talking about self-weeding crops – this attracted a lot of media attention which would not have occurred if we had used allelopathy.

Keywords: self, weeding, canola, annual ryegrass

*Speaker

Impact of the use of *Trichoderma* spp. on greenhouse tomato crop and the bio control of late blight caused by *Phytophthora infestans* (Mont.) of Bary.

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As a part of the search for alternative methods that reduce the use of fertilizers and fungicides, the present work focused on the study of bio-stimulant and antifungal effects of two antagonist isolates collected from Algerian rhizosphere of tomato culture: *T. asperellum* and *T. atroviride*. The study was based on the evaluation of vegetative growth of tomato plants, yield of their fruit and the impact of their use on total polyphenol and foliar pigments contenents. Moreover, the biocontrol of downy mildew was assessed by *in vivo* antagonist activity on foliar discs of detached leaves of tomato against *Phytophthora infestans* (Mont.) de Bary. The results showed the biostimulant effect of these two isolates, particularly *T. atroviride*. The total polyphenols and leaf pigments contenents were significantly better on leaves of plants treated with *T. atroviride*. In addition, this study confirmed the antagonist activity of the two *Trichoderma* isolates against *Phytophthora infestans,* with the predominance of *T. atroviride*.

Keywords: *Trichoderma atroviride*, *Trichoderma asperellum*, Biostimulant and elicitor effects, *Lycopersicum esculentum* Mill., *Phytophthora infestans*.

*Speaker

Mediterranean ecosystems

Does Allelopathy escalate invasiveness of *Prosopis juliflora* (Sw.) DC in arid land environment?

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Prosopis juliflora is highly recognized for its invasive character and its detrimental effects on plant species due to the release of allelochemicals. From a preliminary investigation, we found that the number of seedlings of *P. juliflora* is 10-20 folds greater than other shrubby trees encountered in the area. In this research we aimed to assess the allelopathic effects of crude water extracts of *P. juliflora* on selected Qatari flora. Effects on germination of lettuce seeds indicated strong-dose-dependent allelopathic effects. The results on native Qatari flora showed that seed germination and/or radicle length of: *Acacia tortilis, P. cineraria, Sueda aegyptica, Halopeplis perfoliata, and P. juliflora* were affected differently due to different treatment levels. While 10% of significant reduction exerted on seed germination of *P. cineraria* only at higher concentrations (6 - 8 mg ml^{-1}), the seed radical length was significantly reduced starting at the lowest concentration (2 mg ml^{-1}) and with significant greater reduction at higher concentrations. The seed germination and seed radical length of *Sueda aegyptica* were significantly decreased ($\simeq$50%) at 4 mg ml^{-1} crude water extract of *P. juliflora.* Autotoxic effects of *P. juliflora* were also observed at higher concentrations of 6mgml-1and 8mgml-1. Seed germination of *C. imbricatum* was significantly reduced after treatment with the leaf-soil leachate of *P. juliflora.* The aqueous leaf extract of *P. juliflora* was also tested on seedlings of native Qatari plants. The seedlings growth of *Aeluropus lagopoides, C. imbricatum* and *Tetraena qatarensis* were all impacted upon treatment. The seedling growth and dry biomass of *C. imbricatum* were significantly declined at higher concentration of 8mgml-1. However, the seedling growth and dry biomass of *Tetraena qatarensis* were significantly reduced at all treatment concentration levels. *P. juliflora* is an invasive and has detrimental effects on associated native plants and establishing a proper management plan of this plant is imperative.

Keywords: *Prosopis juliflora*, Invasive species, arid land, Qatari flora, seed germination, seedlings.

*Speaker

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Allelopathic effect of *Thapsia garganica*

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In the present work, the phytotoxicity of *Thapsia garganica* was evaluated through the effect of its different organs aqueous extracts (10, 20, 30, 40, 50 g/L) and organic ones with petroleum ether, ethyl acetate and methanol at 1, 3 and 6 mg/mL, on lettuce germination and growth. The methanol extracts had the strongest inhibitory effect on germination, which was completely inhibited at all concentrations, followed by the aqueous leaves extract at 50 g/L and the germination rate did not exceed 5%. The inhibition of germination by leaves aqueous extracts could be attributed to membrane disintegration, shown by an electrolytes leakage increase, compared to the control, which reached 166.06 %. A germination delay was also recorded, with the exception of petroleum stem extracts. As regards lettuce growth, the measurement of roots and aerial parts length showed that the most significant toxicity was observed with the methanol and aqueous extracts. The ethyl acetate stems extract had a stimulating effect on roots length. The largest value was 83.43% of control at 1000 ppm. In front of allelopathic stress, the lettuce seeds have developed a defense strategy manifested by an accumulation of sugars, an increase of the dehydrogenase activity, and an improvement of seedlings hydration. The study of the effect of leaves methanolic extract on *Tribolium castaneum* and *Spodoptera littoralis* showed that this plant has an insecticide activity. The repellent and toxic activity (by forced ingestion and topical application) against *T. castaneum* was demonstrated. For *S. littoralis*, despite the low anti-feeding of caterpillars, a decrease in the growth rate and conversion of digested and ingested food to biomass, an increase in the approximate digestibility, and a delay of the larval development until mortality were recorded. The larval mortality was caused by exuviation difficulties and various degrees of morphogenesis abnormalities were observed in nymphs.

Keywords: *Thapsia garganica*, phytotoxicity, lettuce, insecticidal potential

*Speaker

Metabolomics study of allelochemicals from selected Mediterranean plant species

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Allelopathy plays a very important role in natural and agricultural ecosystems and it has been suggested to have a great impact on vegetation of Mediterranean area [1]. The understanding of this phenomenon has been partially constrained, among other things, by the methods available to study the secondary metabolites involved. A new method based on metabolomics has been recently developed [2, 3], and it is herewith applied to the study of allelochemicals from selected plant species of the Mediterranean region. Donor plant (*Arbutus unedo*, *Myrtus communis, Medicago minima* and *Daphne gnidium*) extracts were analysed by ^{1}H and 2D NMR in order to define their chemical composition. They were tested for their phytotoxicity on a receiving plant species (*Aegilops geniculata*). Morphological and metabolomics analyses were carried out on shoots and roots of *A. geniculata* plants treated with the extracts. Tests were carried out also with partially purified fractions and with the pure putative allelochemicals. The extracts of the four plant species showed a strong inhibitory activity on the receiving plant. NMR paired with multivariate data analysis of the receiving plant let to hypothesize the main metabolic pathways affected. Studies with pure compounds confirmed in some cases the putative allelochemicals, while in other cases it was possible to determine the occurrence of synergistic effects. Some of the compounds were taken up and, in some cases, modified by the receiving plant. Although phytotoxic activity is only one aspect of allelopathy, the identification of the active compounds lays the bases for in field studies, while the identification of the metabolic pathways affected by the allelochemicals offers new insights for the study of their mode of action. References

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Keywords: Allelochemicals, Mediterranean area, NMR based metabolomics, Aegilops geniculata

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An integrated and multiscaling approach to study the allellopatic role of *Carex distachya* Desf. in plant communities of Mediterranean coastal vegetation.

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Allelopathy has been showed to be one of the most important mechanisms regulating plant diversity and succession through several biological traits (e.g. germination, seedling growth, root symbionts and site quality). The modulation of these mechanisms can affect intra and inter-specific plant interactions and thus dynamical vegetation processes.
In Mediterranean-type ecosystems, climatic stress increases the production of allelochemicals amplifying the importance of allelopathic interactions under these ecological conditions.

However, the ecological relevance of allelopathy in Mediterranean plant communities is, still now, not well known because the effects of chemical interactions on germination and plant growth can be overlapped with those produced by competition and also because much of the information on this topic comes from laboratory experiments.

To better understand the role of allelopathy in the mechanisms regulating biodiversity maintenance and dynamical processes of Mediterranean plant community we undertook studieson steno-mediterranean *Carex distachya* Desf. (Cyperaceae), a perennial densely caespitose hemicriptophyte dominant in the intermediate successional stages of sand dune *macchia* vegetation.

Different approaches have been performed to investigated the potential allelopathic interference of *C. distachya* on the coexisting and weed species: i) chemical characterization and bioactivity of fresh and decomposing aqueous plant extracts; ii) bioactivity of pure metabolites; iii) analysis of pure metabolites/plant organ in relation to habitat; iv) analysis of pure metabolites in the soil with the characterization of their spatio-temporal dynamics and temperature effects; v) greenhouse experiment by monoculture of *C. distachya* on the selected plant species.
The obtained results evidenced that *C. distachya* metabolome is characterized by a wide spectrum of chemodiversity with different metabolic patterns in leaves and roots. The RP-HPLC analysis of soil community revealed that most of these compounds were released in the environment. Bioassay on the coexisting species and two common weeds highlighted a different behaviour with constantly toxic effects on these two latter.

Keywords: *Carex distachya* Desf., Secondary metabolites, Mediterranean vegetation, Coexisting species, Weeds

[*]Speaker

Insect plant interactions

PHENOLIC COMPOUNDS AS DUAL ACTORS ON PLANT-PLANT AND PLANT-INSECT INTERACTIONS

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Plant ecologists exploring the secrets of plant secondary metabolism often explain its tremendous diversity by the critical need for the plants to defend themselves against a wide range of herbivores. Considering this diversity as a result of herbivory pressure has to be questioned in relation with rising evidences of pleiotropic roles of many of the secondary compounds at different ecosystem levels. Polyphenols appeared to be especially good candidates to unravel the complexity of the roles of such metabolites as they have become the most studied compounds both in plant defense and as allelopathic agents. Is it a coincidence? an artifact link to technical constraint? or does it tells us something about the interactions between anti herbivore and allelopathic processes, with further implications on ecosystem functioning ?
Illustrations of this dual role of phenolic compounds both in allelopathic and response to herbivory contexts will be presented through experiments concerning a cespitous and conservative Poaceae dominating subalpine grasslands in the Alps : *Festuca paniculata*. First, a semi-natural and realistic design has allowed to identify growth inhibitory activity of this species through the release of soluble compounds (including polyphenols). Second, concentrations of Festuca leaf phenolics were not modified by insect (grasshopper) herbivory, unlike in other grass species. But these polyphenols disappeared more rapidly from grazed senescent leaves when compared to intact leaves, and their abundance was correlated to the slowdown of Festuca litter decomposition observed under herbivore pressure.
Considering these results together encourage to reinforce efforts towards better descriptions of the different classes of polyphenols (phenolic acids, flavonoids, etc..) which have different responses when plants are submitted to herbivory pressure and could have contrasted allelopathic potentials. But it also stresses the need of adapted experimental designs favoring the coexistence and co-expression of both herbivory response and allelopathic activity.

Keywords: Phenolic compounds, allelopathy, herbivory, litter decomposition, *Festuca paniculata*.

*Speaker

Effect of *Crematogaster* spp. visiting extrafloral nectaries of *Clerodendrum chinense* on insect herbivore

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Ants are the major protagonists in the Plant – insect interaction, they play a major role in the survival of plant by deterring herbivore and increasing Plant Growth which in turn increases the fitness of the plant. To get the better insights regarding the interaction the inflorescences of *Clerodendrum chinense* a perennial shrub, with extra floral nectaries on its sepals were monitored for one and half years, to test the hypothesis stating that "the presence of ants reduces insect herbivore and increases the number of flowers?" The experiment was performed by treating the inflorescences with antibarrier glue to restrict the ants' visit to the inflorescence keeping one set as control (untreated). The study revealed that the behaviour of ants, showed presence of herbivore and increase in no. of inflorescences. While the plants in which anting was continued, population also showed herbivore. However it showed less no. of flowers. To analyse the behaviour of ants the ecochemicals were extracted using ethanol which showed the presence of secondary metabolites like tannins, alkaloids, saponins, flavonoids and terpenoids. It may help to predict the community dynamics of this plant in forest ecosystem.

Keywords: Ants, extrafloral nectaries, herbivore, ecochemicals

*Speaker

Foliar Galls in *Trewia nudiflora*, L. – Morphological, Anatomical and Biochemical Changes Induced by Ant Infestation

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Trewia nudiflora Linn. (Euphorbiaceous) commonly known as false white teak is one of the important medicinal plants and also reported as a host of foliar gall caused by *Trioza fletcheri,* CRAWF. Gall-inducing insects have profound effects on their hosts. These insects live within plant tissues and induce tumor-like growths that provide the insects with food, shelter and protection from natural enemies. Pathogens inject some elicitors and lead to the synthesis of different type of enzymes and some secondary metabolites at high amount in the plant which plays an important role in gall resistance in plants. In order to understand the actual mechanism of gall formation by *Trioza fletcheri,* CRAWF., we have studied the morphological, anatomical and biochemical changes in *Trewia nudiflora*. Morphological study showed numerous simple pouch like galls are covering the upper surface of the leaf blades. Anatomical study revealed the presence of completely deformed cells compressed to form a hollow cavity. Each cavity measured approximately 0.5-2.0 cm in naked eye. Within the gall cavity we observed an ant species which may be *Brachymyrmex sp.* of the family Formicidae. Biochemical studies revealed positive correlation between **a**ctivity of antioxidant enzymes (namely catalase and peroxidase) and phenolics with different stages of gall formation whereas chlorophyll content, sugar content and Alpha amylase activity decreases. However, there is no report about ant infestation on gall in *Trewia nudiflora* leaves. Ants have a series of ecological relationships with gall-makers, as predators, mutualists or successori. The entering-exiting behaviour of the ants in respect to open galls could thus result simply from their own searching behaviour or, alternatively, from a situation of reward coming from the gall in the form of these exudates which conferring an additional defense against predation and/or parasitism of the galls through attraction of such predatory ants.

Keywords: *Trewia nudiflora*, L., foliar gall, antioxidant enzymes, phenolics, ant

*Speaker

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Rice allelopathy and anti-herbivore defense priming

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Rice (*Oryza sativa*) is one of the most important food crops. Momilactones are the most toxic allelochemicals released from rice plants. Our study showed that momilactones inhibited seed germination of *Arabidopsis thaliana* by enhancing abscisic acid (ABA) pathway leading to up-regulation of the transcription levels of key transcription factors and biosynthesis genes in ABA signaling pathway. The diterpenes inhibited the seedling root growth by affecting auxin distribution and transportation in the roots. Priming of plant defense refers to increased readiness of induced defense through the perception of indicative signal cues or the experience of previous enemy attack, which allows plant to induce more effective and rapid defense responses upon subsequent attack. We found anti-herbivore priming effects of silicon (Si) in rice. Si-pretreated rice plant showed enhanced resistance against *Cnaphalocrocis medinalis* (rice leaffolder, LF) and *Nilaparvata lugens* (brown planthopper, BPH). Upon LF attack, rice plants subjected to Si pre-treatment exhibited enhanced defense responses relative to untreated controls, including higher jasmonic acid (JA) accumulation levels, increased levels of transcripts encoding defense marker genes, and elevated activities of peroxidase, polyphenol oxidase and trypsin protease inhibitor. We compared the defense responses of rice ratoons generated from parent plants that had been either infested by LF caterpillars or treated with methyl jasmonate (MeJA) during vegetative growth, with ratoons generated from control parent plants. Ratoon plants generated from parents receiving prior LF infestation or MeJA treatment exhibited higher LF resistance, higher JA levels, as well as elevated levels of transcripts of defense-related genes associated with JA signaling. Appropriate utilization of plant allelopathy and defense priming is a promising approach to minimize application of herbicides and insecticides.

Keywords: Rice, allelopathy, momilactones, defense priming, silicon

*Speaker

Soil microbial plant/rhizosphere interactions

Spatial distribution patterns of root associated-microbiota mediated by *Camellia sinensis* (L.) leaves leachates in continuous monoculture system

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Soil sickness is a typical negative feedback which depends upon harmful interactions between plants and microbes resulting in autotoxicity, an imbalance of microbes and hence pathogenicity. Pyrosequencing approaches have provided recent insights into rhizosphere microbial variation in the chronosequence tea orchards. However, our knowledge of the connection between imbalance of plant-associated micro-biota with leaf lecheates in continuous monoculture is limited. The aim of this study was to show the impact of *Camellia sinensis* (L.) leaf lecheates on the distribution and assemblage of microbiota in the rhizosphere, rhizoplane and endosphere across a continuous monoculture system. High-throughput sequencing was used to determine the spatial structure of root associated-microbiota in fresh (2-years) tea plantation, old (30-years) tea plantation and adjacent uncultivated field. Moreover, High Performance Liquid Chromatography-Electro Spray Ionization-Mass Spectrometry was used to identify and quantify allelochemicals in leaf lecheates of *C. sinensis* (*L.*). Results indicated that the main phyla associated with root were Protobacteria, Acidobacteria, Cyanobacteria, Chloroflexi, Actinobacteria, Bacteroides, Nitrospirae, WD272, Gemmatimonadetes and Firmicutes showed distinct distribution patterns across chronosequenced tea plantation. Redundancy analysis showed that the Catechin, Epicatechin, Epigallocatechin, Epicatechin gallate and Epigallocatechin Gallate of leaf leachates were highly correlated with the majority of bacterial taxa. These results suggested that long-term tea cultivation resulted in the accumulation of autotoxin as the results of pruning and falling leaves. In addition, these autotoxins altered the composition and structure of the soil bacterial community in continuous monoculture soil directly or indirectly by changing the pH of rhizosphere soil. These results might be helpful to explore the reason why the quality and fertility were disturbed in long term monoculture tea field.

Keywords: Allelochemicals, Rhizosphere, Rhizoplane, Endosphere, Microbiome, High, throughput sequence, HPLC, ESI, MS, RDA

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Allelochemical-microbial interactions between ferulic acid and *Myxococcus xanthus* enhance the growth inhibition on barnyardgrass

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The populations of *Myxococcus*, a gram-negative bacteria, is accumulated in the rhizospheric soil of allelopathic rice PI312777 and significantly larger than that of non-allelopathic rice Lemont, which suggests that *Myxococcus* plays a potential role in the rhizospheric biological process of PI312777 in suppression of weeds. In this study, the *Myxococcus xanthus* separated from the rhizospheric soil of PI312777 was used to study the interaction with ferulic acid (FA). The results show that exogenous FA promotes the cell proliferation of *M. xanthus*. A dimethyl labeling quantitative proteomic technique was used to reveal the protein expression on *M. xanthus* induced by FA. A total of 76 proteins were found up-regulated whilst 56 proteins were down-regulated in the FA-induced *M. xanthus*. These up-regulated proteins were involved in the cell growth, motility, signal response and transduction, metabolic process, biosynthesis of secondary metabolites, and social predation. Some target proteins, including frzS, BolA/YrbA family protein, methyl accepting chemotaxis protein, ABC transporter, and ATP-binding protein were selected to detect their genes transcript level, which showed that transcript level of the five genes was also up-regulated in the 0.10 mM FA-induced *M. xanthus* compared to the FA absent control. Additionally, transcript level of five other gene members from frz multigene family including *frzA*, *frzB*, *frzCD*, *frzE*, and *frzG* were also significantly up-regulated. Jointed application of FA and *M. xanthus* led to strongest growth inhibition of barnyardgrass. The results indicated that FA induced the chemotaxis and cell proliferation of *M. xanthus*, whilst the interactions between FA and *M. xanthus* lead to enhance growth inhibition on barnyardgrass.

Keywords: Allelopathy, barnyardgrass, ferulic acid, interaction, Myxococcus xanthus

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Changes of autotoxic potential and microbial community structure in rhizosphere soil of continuously planted tea plant (*Camellia sinensis* L.)

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The effect of tea soils with 4-, 14-, 28-, and 32-planted years on the physiological indices and the quality indices of replanted tea leaves was examined. It was found that activities of superoxide dismutase, peroxidase, catalase decreased significantly with increase planted years, while the malondialdehyde levels increased significantly with increase of soil planted years. The levels of polyphenols, caffeine, and free amino acid in tea leaves were significant lower when the tea seedlings cultured in 14-, 28-, and 32-year soils than that in 0- and 4-year soils. In laboratory bioassay, the aqueous soil extracts inhibited lettuce (*Lactuca sativa* L.) growth significantly with increase planted years. The contents of three phenolic acids (protocatechuic acid, *p*-hydroxybenzoic acid, and cinnamic acid) showed significant higher in 14-, 28-, and 32-year soils than that in 0- and 4-year soils. By Biolog EcoPlate method, the carbon source utilization by soil microbe to fatty acid, carboxyl derivatives, and phenolic acids were significantly higher in tested soils, compared to the control. Principal component analysis (PCA) showed the 18 out of 31 carbon sources were significantly correlated with the PC1, PC2, and PC4. The 11 components were positively correlated including 3 of carbohydrates, 3 of carboxyl derivatives, 2 of amino acids, 2 of amides, and 1 of fatty acid, and 7 components were negatively correlated including 6 of carbohydrates and 1 of amino acid. Microbes utilizing carboxyl derivatives were significantly and positively correlated with the inhibitory rates of soil aqueous extracts on lettuce growth. These results indicate that the accumulation of acidic compounds and deficiency of some carbohydrate carbon sources were the main characteristic in the long planted soils. Microbial community led acidic compounds accumulation in tea soils might be a cause of soil toxicity in the long planted tea plantations.

Keywords: Wuyi rock tea, rhizosphere soil, continuously planted soils, soil toxicity, microbial community

*Speaker
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The roles of rhizosphere microbiomes mediated by root secretions in consecutive monoculture problems of *Pseudostellaria heterophylla*

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Many crops suffer from the replant problem in the modern cropping system, and the root-associated microbiome is important for the health of plants, especially in the consecutive monocropping system. In our study, we measured microbial population changes under a consecutive monoculture system of *Pseudostellaria heterophylla* and interaction between phenolic acids in root exudates and typical pathogen and beneficial bacteria. The results illustrated that successive cropping of *P. heterophylla* shifts the diversity and structure of microbial community in rhizosphere soil. The diversity of microbial community in rhizosphere soil of *P. heterophylla* was decreased with the increase of planting years while the structure of the microbial community deteriorated. Moreover, the numbers of typical pathogens increased and the amount of beneficial bacteria decreased with the increasing years of monoculture. These changes finally resulted in a microecology imbalance in P. heterophylla rhizosphere and caused more and more serious consecutive monoculture problems in the long run. Further study revealed that phenolic acids in the root secretion of P. heterophylla increased over time, which was closely related to changes in rhizospheric microorganisms. In conclusion, our research reveals that under consecutive monoculture system of *P. heterophylla*, allelopathic substances such as phenolic acids secreted by roots accumulate increasingly and they can mediate the changes of microbial community structure, which result in the changes of rhizosphere microbiomes of *P. heterophylla* with the phenomena of fewer beneficial microorganisms and more pathogenic microorganisms. Finally, a large number of pathogenic microorganisms cause severe disease. Adding the beneficial microbiomes into rhizosphere of *P. heterophylla* can alter the rhizosphere microbiome inducing the beneficial microbiome to become the dominant population which alleviates the replant disease. The result provides a new avenue for modulating the root microbiome to enhance crop production and sustainability, especially in the monoculture system.

Keywords: *Pseudostellaria heterophylla*, consecutive monoculture problems, replant disease, rhizosphere micro, ecology, root secretion, *Fusarium oxysporum*, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, Illumina Sequencing, DGGE, qPCR

*Speaker

Abutilon's Root Surface Detoxification of Benzoxazolinones: An Interspecies Cooperation

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The root of *Abutilon theophrasti* (Herbiseed, Twyford, UK) can be colonized by a facultative microbial community composed of several fungi and bacteria with *Actinomucor elegans* as a dominant species. The yeast *Papiliotrema baii*, a recently described novel fungal species and the bacterium *Pantoea ananatis* have key functions in the elimination and detoxification of hydroxylated benzoxazolinones by polymerization processes at the root surface. Several enzymes are involved in these root surface reactions, sincluding laccases and peroxidases. H2O2 is produced mainly by the yeast, providing a substrate for peroxidases. BOA-OH polymers precipitate at the root surfaces, forming black or brownish colouring of the epidermis, which has no obvious negative influence on the root growth. Roots with laccase produced a root surface covered by glycolipids produced by *Pantoea ananatis*. Depending on environmental conditions, including the availability of nitrate/nitrite in the rhizosphere, *Pantoea ananatis* transforms BOA-6-OH to a new, hitherto unknown nitro aromatic compound, which presents the first intermediate of a catabolic sequence. The entire micro-community is also able to degrade DIBOA and DIMBOA completely. The plant detoxification product BOA-6-O-glucoside and the BOA-OH derived bacterial compounds can all be found at the root surface or in the rhizosphere, but their presence and relative concentrations depend on environmental conditions. Shifts in activity of various biosynthetic pathways are likely and are thought to occur opportunistically. The results provide new insights in plant-microbe interspecies cooperation that assist plants in protecting against the presence of harmful allelochemicals. Those plant-microbe interactions, assumed to exist more commonly than previously thought, may help to explain the oft observed lack of sensitivity of certain plant species against allelochemicals. Such cooperations among plants and microbes may suggest a new field of research in allelopathic interactions in the rhizosphere.

Keywords: *Abutilon theophrasti*, benzoxazolinone, detoxification, *Pantoea ananatis*, *Papiliotrema baii*

*Speaker

Collapse of soil microbial biodiversity after rapeseed-glucosinolate exposure

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Land plants live in intricate communities with soil bacteria and fungi indispensable for plant survival and growth. Furthermore, plants are considered to be a major factor that affects communities of soil microbes. The plant-microbe interactions are largely governed by different allelochemicals. Here, we employed a combination of lipid-fingerprinting, enzyme activity assays and high-throughput DNA sequencing to uncover the dynamics of the microbial community structures in the soil after exposure to isothiocyanates, toxic glucosinolate catabolites with allelopathic properties produced by rapeseed and other Brassicaceae. We show that rapeseed-derived isothiocyanates drastically diminish diversity of bacteria and fungi. After isothiocyanate exposure, only few bacterial taxa of the Gammaproteobacteria (*Acinetobacter*, *Thermomonas*), Bacteriodetes (*Flavobacterium*) and Acidobacteria (*Geothrix*) proliferated while *Trichosporon* (Zygomycota) dominated the fungal soil community. The current findings provide evidence for the fundamental shift in soil microbial community structure after the introduction of rapeseed-derived isothiocyanates into the soil. Thus, plants of the Brassicaceae strongly modify the soil microbiota, leading to extreme losses within the microbial community structure and affecting the capacity to adapt to a changing environment. The community profiles revealed in this study are the basis for further investigations on specific microbial groups to understand their relevance to soil health. The present knowledge on microbial genetics, taxonomy and systematics and the largely unknown biochemical and physiological properties of many microbial organisms clearly limits the understanding of the true impact of glucosinolates on microbial diversity and community structure. Therefore, the relevance of these bacteria and fungi from soil can only be explained after conducting functional studies. Accordingly, future analyses will concentrate on the different strategies resulting in glucosinolate tolerance, by characterizing the microorganisms retrieved from glucosinolate treated soil during the present study.

Keywords: rapeseed, glucosinolates, soil microbial biodiversity, lipid, fingerprinting, high, throughput DNA sequencing

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Insights into the mechanism of proliferation on the special microbes mediated by root exudates in the *Radix pseudostellariae* rhizosphere under continuous monoculture regimes

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Radix pseudostellariae, belonging to the *Caryophyllaceaeis* family, is one of the most common and highly demanded Chinese medicines, which contains polysaccharides, ginseng saponins, flavonoids, cyclic peptides, amino acids and trace elements. However, consecutively monocultured *R. pseudostellariae* are prone to severe diseases, which may result in reduced biomass, especially of tuberous products. This phenomenon is known as replanting disease or soil sickness. More than 70 % of medicinal plants, especially tuberous root medicinal plants, are reported to be attacked by various replanting diseases. Therefore, replanting disease incidence has resulted in a tremendous hurdle to obtain maximum agricultural production of *R. pseudostellariae*. The root exudates of *R. pseudostellariae* cause deterioration of the microbial community in the rhizosphere, increasing host-specific pathogens at the expense of beneficial microorganisms. We found the effect of phenolic acids and organic acids were invoked as a driver of the changes seen in the rhizosphere soils. We studied the effects of artificially applied root exudates of *R. pseudostellariae* on *R. pseudostellariae* seedling growth, rhizosphere soil microbial communities, and soil physicochemical properties. The deep pyrosequencing and qRT-PCR analysis demonstrated that the treatment of phenolic acids significantly decreased the relative abundance of *Trichoderma*, *Penicillium*, *Pseudonocardiales*, *Xanthomonadales*, *Streptomycetales*. And the organic acids had significantly negative effects on the relative abundance of *Pseudonocardiales* and *Streptomycetales*, which significantly increased the abundance of *Fusarium, Xanthomonadales*, *Micrococcales* and *Gemmatimonadales*. The non-invasive micro-test technique (NMT) analysis indicated that the root exudates increased the H^+ efflux in the pathogenic fungi (*Fusarium oxysporum* and *Talaromyces helicus*) and decreased it in the beneficial fungi (*Trichoderma harzianum*), which creates an acid environment that inhibited proliferation of beneficial bacteria and facilitated build-up of specialized plant pathogens. This study explains the reasons why the root exudates are able to mediate a microflora shift and structure disorder in continuously monocultured *R. pseudostellariae* rhizosphere soil.

Keywords: *Radix pseudostellariae*, Replanting disease, root exudates, NMT, deep pyrosequencing

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Impact of global changes on allelopathy

Non vascular plant allelochemical interactions in terrestrial ecosystems under environmental changes: scientific challenges

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The study of structural and functional characteristics of plant secondary metabolites (PSMs) is a particularly valuable scientific research in relation to recent environmental changes, including those with changing climate or pollutants. The response of plants to ecological changes influences higher plant resource allocation and also PSMs. The accumulation of PSMs in vascular plant tissues is then considered as a common adaptive response of plant to adverse environmental conditions. However, although diverse and complex secondary metabolites produced in the plant kingdom are found in vascular plants, our knowledge of secondary metabolites in ancient terrestrial non-vascular plants is extremely limited. Among them, the Bryophytes or mosses represent an important branch of the plant kingdom with more than 16 000 species playing a fundamental ecological role in many terrestrial ecosystems.
Here we proposed to give a synthesis of chemical interactions of Bryophytes under environmental changes by focusing on some case study under controlled and *in situ* experiments. As example, living *Sphagnum* secondary metabolites involved in a peatland plant–soil feedback under environmental changes will be presented. Understanding how environmental changes interact to shape non-vascular plant secondary metabolisms remains an important challenge for chemical ecologists, especially where bryophytes are dominant community members.

Keywords: Bryophytes, Climate changes, Ericoid mycorrhizae, Plant Secondary Metabolites, Polyphenols, Phenoloxidases, Sphagnum

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Chemical interaction between mosses and mycorrhizal fungi of an Ericale species in peatland under climate warming

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Peatlands represent only 3% of terrestrial surface area but they stock one third of the world's soil carbon stock. *Sphagnum* genus have a fundamental role in peatlands, because it forms dense carpets that slowly decompose into peat, sequestering carbon. Belowground interactions between plants and microorganisms play a key role in ecosystem functioning and constitute a crucial challenge for peatland responses to climate warming.
Previous works clearly demonstrated that phenolics compounds from living Sphagnum are involved in microbial community structure, in the control of enzymatic activities and affect the germination of vascular plants. According to the key role of mycorrhizal fungi in the development of vascular plant, we investigated the interaction between *Sphagnum* phenolics and the fungal colonization of an Ericale species (*Andromeda polifolia).* A climate warming treatment (by around + 1∘C) were initiate since 2008 by Open-Top Chambers in a Jura mountain peatland (France) in two microhabitats (lawns and hummocks). We hypothesize 1) an allelopathic interaction of *Sphagnum fallax* phenolics on *A. polifolia* mycorrhizal colonization and that 2) a long experimental warming affects such interactions.
Results show that phenolic compounds was related to the microhabitat (0.6 mg g^{-1}DW in lawns and 1.3 mg g^{-1}DW in hummocks respectively). We observed a significant correlation of Sphagnum phenolic production between the capitulum and the senescent stems. Mycorrhizal colonization, including ericoid mycorrhiza and Dark Septate Endophyte (DSE) was also related to the microhabitat. In ambient plots, a positive correlation between DSE colonization and *Sphagnum* phenolics was observed whereas there was a negative correlation between mycor-rhizal colonization and *Sphagnum* phenolics in warming plots. These results strongly suggest that allelochemical interactions play a key role in peatland functioning and confirm that below-ground biological activities are crucial in ecological processes.

Keywords: Bryophytes, Sphagnum, peatland, phenolics, mycorrhizal fungi, Dark Septate Endophyte, climate change

*Speaker

Isoprene emissions under climate change, future impact on allelopathy

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Isoprene represents the major fraction of Biogenic Volatile Organic Compounds (BVOC) emitted by *Quercus pubescens*. This compound is highly useful to *Quercus pubescens* since it can maintain the stability of thylakoid membranes in plants. In the same way, isoprene could also be useful to non-emitter species which could benefit from isoprene advantages without production costs, especially when drought occurs. However, impacts of water deficit on isoprene emissions are not well understood yet. Indeed, isoprene emissions can increase, decrease or remain unchanged according to the stress severity and the studied species. There is also a lack of knowledge on isoprene modifications across recurrent drought that is, a water deficit period that occurs during few years.

In this study, we evaluated the impact of a recurrent water deficit on isoprene emissions of *Quercus pubescens*, a species that is well widespread in the Mediterranean region and the possible implications of isoprene on the ecosystem through allelopathic processes.

For this purpose, isoprene emissions were measured after 3-4 years of recurrent drought, with a PTR-ToF-MS on the experimental site O3HP in Southern France. This site is equipped with a rain exclusion device which allows to reduce by 30% the annual rain according to the most severe scenario of climate change.

We showed that isoprene emissions were reduced with recurrent drought which can be negative for the whole ecosystem. Indeed, it seems that isoprene emissions from *Q. pubescens* can enhance physiological performances of *Acer monspessulanum*, a non-emitter species, under moderate water deficit. Hence, if recurrent drought expected with climate change limits isoprene released into the atmosphere, non or low-emitter species could in turn be impacted but this hypothesis needs further research.

Keywords: isoprene emissions, recurrent drought, physiological performance, allelopathy

*Speaker

A comparison of novel weapons in European and Australian *Echium plantagineum* populations using metabolic profiling

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Native to the Iberian Peninsula, *Echium plantagineum* L. was introduced to Australia in 1800's and is a noxious invasive weed in Australian pastures. Its toxicity to grazing livestock causes > $250 M annual losses to the Australian industries. However, in its native range it is not particularly invasive or toxic. Plants from twelve geographically distinct European and Australian populations of *E. plantagineum* were collected and analyzed for accumulation of allelochemicals including antimicrobial and phytotoxic naphthoquinones (NQs) and shoot toxic pyrrolizidine alkaloids (PAs). NQs were extracted in ethanol from the root periderm, whereas foliage was extracted in methanol for analysis of PAs. Extracts were subjected to metabolic profiling using UHPLC-ESI-QTOF (Agilent, USA) in negative and positive mode. Data was processed using targeted and non-targeted analyses and compared to an in-house database of NQs and PAs using Mass Profiler Professional Software (Agilent, USA). Australian populations produced up to 6-fold higher concentrations of NQs and 3-fold reduced concentration of 14 PAs ($P< 0.05$) in comparison to plants collected from the Iberian Peninsula. *Echium plantagineum* plants established more densely across the Australian range, and field stands were also significantly less biodiverse as measured by plant species richness in contrast to stands in the native range. Plants in the native range retained their potent ability to defend against herbivory through the enhanced production of an abundance of PAs, while Australian plants defended through increased production of a diversity of phytotoxic and antimicrobial NQs in root periderm tissues. Our studies suggest that the invasion success of *E. plantagineum* in Australia, in contrast to establishment in the native range, may be related to post-introduction adaptive evolutionary changes in plant metabolism. Upregulation of key metabolites in Australia may be associated with climate-driven natural selection processes and plant's escape from natural enemies, further contributing to successful invasion following introduction.

Keywords: Plant invasion, naphthoquinones, pyrrolizidine alkaloids, UHPLC QTOF, biodiversity

*Speaker

Marine and freshwater interactions

Chemical interactions between host and epiphytes – example of benthic microalgae

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Chemical interactions including allelopathy are important processes in shaping communities in aquatic environment. However, the role and effects of allelopathic interactions in the structure and functioning of photosynthetic microbial biofilms is still poorly known.
In order to improve our understanding of the mechanisms associated with allelopathic interactions in biofilms, an in depth study of an interaction between the filamentous green alga *Uronema confervicolum* and benthic diatoms was realised. Benthic diatoms are competitors and potential epiphyte of the green alga. Bioassay-guided fractionation and comparative metabolomic profiling were used to identify allelopathic compounds produced by *U. confervicolum*. Growth, photosynthesis efficiency and adhesion inhibition assays were realised to measure the effect of allelopathic compounds produced by *U. confervicolum*. The responses of target cells were studied through scanning electron microscopy, transcriptomic and metabolomic analyses.

The results indicate that beside an allelopathic inhibition of diatom growth and photosynthesis by two polyunsaturated fatty acids, other allelopathic compounds inhibited specifically diatom ability to adhere to a substrate. The process of adhesion inhibition was further analysed in depth; our results revealed that this inhibition was associated with an inhibition of the formation of the extracellular polymeric substances matrix of the biofilm. Metabolomic profiling indicated an involvement of carnitine, in the response of diatoms to allelopathic compounds. Transcriptomic analyses suggested a stimulation of energy metabolism and photosynthesis while cell cycle and the production of adhesion related proteins were inhibited.
These results highlighted the importance of taking into account the multiplicity of allelopathic compounds produced by a single organism and acting together. The mode of action of these allelochemicals must be further studied. The compounds responsive for the inhibition of adhesion may be either allelopathic compounds inhibiting directly adhesion mechanisms or infochemical, *i.e.* a signal of the presence of inhibiting allelopathic compounds.

Keywords: biofilms, adhesion, diatoms, filamentous green algae

*Speaker

Unknown allelopathic compound from the chlorophyte Ankistrodesmus falcatus with inhibitory effect against the cyanobacteria Microcystis aeruginosa

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Phytoplankton succession consists in shifts in species dominance, belonging the antagonist dominant species to one or a few related taxonomic groups. Some examples could be chlorophytes/cyanobacteria, flagellates/cyanobacteria or flagellates/diatoms. These shifts occur during regime transitions (changes in mixing, light, temperature and nutrients). It has been hypothesized that, in some cases, these shifts are induced by allelochemicals working as signalling molecules, causing cascading effects that lead to abrupt collapse or slow replacement of phytoplankton populations. This mechanism might anticipate the onset of unfavourable environmental conditions.

In this work, we studied interspecific competition under nitrate limitation between the cyanobacteria *Microcystis aeruginosa* and the chlorophyte *Ankistrodesmus falcatus*. We aimed to determine the driving factors of competition.

Using nitrate as limiting resource in batch culture, we parameterized growth and uptake functions for each species. We found that the cyanobacterium was, by little, a better competitor than *Ankistrodesmus falcatus*. We also performed a bioassay to test for allelopathic effects. We detected an allelopathic effect of *A. falcatus* against *M. aeruginosa*, but only in the absence of nitrate limitation. This allelopathic effect was never reported before.

In order to test the relative importance of nitrate competition versus allelochemical properties in determining the outcome of competition, we ran long-term nitrate-limited continuous cultures, varying the initial relative abundances of each species (modifying then the potential effect of allelopathy).

We found that, when initial relative abundances favoured *Microcystis aeruginosa*, the outcome was the exclusion of *Ankistrodesmus falcatus*, as predicted by our estimates of growth and uptake parameters. When *A. falcatus* was initially at higher abundances, nutrient competition predicts that the dominance species shoeld be again *M. aeruginosa*, but, the observed allelopathic effect could overturn this result, as recent experimental works demonstrated. However, in this situations, *M. aeruginosa* was also the winner, suggesting that *A. falcatus* allelopathic effect was weak.

Keywords: Allelochemicals, *Ankistrodesmus*, *Microcystis*, interspecific competition

*Speaker

From resources to population dynamics: a two-species competitive system driven by allelopathy

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Recent works have shown the potential of allelopathy as a driver of biodiversity in two-species competitive phytoplankton systems. The interplay between allelopathy and resource competition generates contrasting outcomes: i) exclusion of the worst resource competitor and allelopathic species; ii) oscillatory coexistence and iii) exclusion of the best resource competitor and non-allelopathic species. The system undergoes a transition from states i) to iii) as the initial relative abundances of the species changes in favour to the allelopathic species. A mechanistic model of population dynamics accurately predicts the dynamics observed in the system both qualitatively and quantitatively. However, the allelopathy function in this model stablishes a negative exponential relationship between the abundance of the allelopathic species and the rate of allelochemical production. The biological mechanism behind this function is unknown, although it seems related with limiting resource (nitrate) dynamics.
The aim of this work was to study the causal relationship between the dynamics of the limiting resource (nitrate) in this system and the dynamics of allelochemical production.

We performed long-term interspecific competition experiments in continuous cultures using the allelopathic cyanobacteria *Phormidium* sp. and the chlorophyte *Ankistrodesmus falcatus*. We ran the cultures during 60-90 days until the outcome of competition was observed. We manip-ulated the initial abundances (in the inoculum) of each species in order to be able to obtain the three outcomes described above (from i to iii). On a daily basis, we sampled species abundances, light absorption, nitrate concentration (the stablished limiting resource) and allelochemical pro-duction, which was estimated indirectly through a bioassay.
We found that allelochemical production is strongly positively related with nitrate availability for the cells, and this relationship is the basis that can explain all the dynamic properties of this system.

Keywords: Allelopathy, phytoplankton, competitive exclusion, coexistence, resource competition

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Allelopathic mecanisms as a driver of the coexistence of mangroves species and cultivated species of the North Vietnam

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Mangroves are the only forests located at the sea-land interface in the tropical and subtropical regions of the world. Mangrove species, which develop in intertidal zones where wave energy is lower allowing thin sediments deposition suitable for mangroves settlement and development. These forests are key elements of tropical coastal ecosystems, providing numerous ecosystemic services such as raw material and food production, habitats, limitation of costal erosion and salt intrusion. Another service is the production of secondary metabolites by mangrove species and their potential use in medicine and/or agriculture. Indeed, contrary to the synthetic pesticides which are associated to negative environmental impacts (soil persistence, non-targeted toxicity, carcinogenic and mutagenic activities, etc.), use of natural pesticides and herbicides is generally considered safer for the environment and the human health, particularly due to their implicit biodegradability, their highly selective actions, etc. We explored the allelopathic potential of 8 mangrove species (*Avicennia marina, Kandelia obovata, Bruguiera gymnorrhiza, Sonneratia apetala, Sonneratia caseolaris, Aegiceras corniculatum, Lumnitzera racemosa* and *Rhizophora stylosa)* on the development of plants from an adjacent agrosystem (target species *Oryza sativa and Echinochloa crus-galli*). The results of our experiment reveal that the leaves aqueous extracts mangrove trees might affect in several ways the germination and the growth of the target species linked to their chemical signature. These fallouts seem especially important as Vietnam is currently facing serious challenges concerning both the quantity and the toxicity of the pesticides used in agriculture.

Keywords: Mangroves, chemical interactions, allelochemicals compounds, germination

*Speaker

Allelopathy of aquatic plants – Quoi de neuf ?

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Plants growing in the water column, so called submerged macrophytes, face multiple challenges, among them often light and carbon limitation, while nutrient uptake can be from sediment and/or water. This results in recurrent competition with other primary producers such as phytoplankton, periphyton or epiphytic algae. Also emergent or floating-leaved macrophytes may face competition with other higher plants or algae. The content and release of allelopathically active compounds can provide an advantage to producing plants, and may help to keep competitors within limits. Studies on allelopathy between submerged macrophytes and other primary producers focus mainly on extracts or leaching of plant material, or on water where the plants have been cultured in. So far, only few active compounds have been isolated from extracts, and we know even less about active compounds released to the water or sediment. This presentation will provide a short overview of studies published on the allelopathy of aquatic macrophytes, mainly focussing on the past 15 years, with a focus on which species and compounds have been identified as allelopathically active, which target organisms and test systems have been used, and which modes of action of the allelopathically active compounds were isolated. Open questions are how environmental conditions influence the production and release of active compounds, whether allelopathic interactions can be induced (or inhibited) depending on the prevalent interactions and environmental conditions, whether allelopathically active compounds have also other bioactive functions for the producing plant, or if exotic invasive species have a higher allelopathic potential than native plants. Indirect evidence for allelopathic interactions from mesocosm and field studies will be considered, with a quest to develop environmentally realistic studies taking into account natural settings.

Keywords: freshwater, submerged macrophytes, aquatic plants, phytoplankton, periphyton, epiphytes

*Speaker

Impact of Cyanobacterium *Microcystis* species on Native freshwater Phytoplankton Communities

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Invasion of non-native aquatic algae impact community assembly in freshwater ecosystem. We have a limited understanding of ecological drivers that mediate the invasion success of microscopic organisms. In addition to increased number of studies on invasive plants and animals in terrestrial and aquatic ecosystems, a few reports suggest the invasion of cyanobacterium *Microcystis* species to freshwater environments globally. Species of *Microcystis* are known to release toxic chemicals into environment, thus have potential to effect the composition of the native algal communities and ecosystem functioning. We quantified the invasion of *Microcystis* species to lakes and water reservoirs in subtropical zone and their impact on the native and the co-occurring species of *Monoraphidium* and *Scenedesmus*. We generated data on invasive traits such as high growth rate, better resource utilization efficiency and overall superior competitive abilities over the native species. Since global warming has been identified as a major driving force for the invasion of cyanobacteria, we visualize the future major impact of *Microsystis* invasion. We will discuss why algal invasion is important in context of freshwater communities, and how it could potentially damage our ecosystem services.

Keywords: Cyanobacterium *Microcystis*, phytoplancton

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Allelopathic interactions between *Ludwigia grandiflora* and phytoplankton are season-dependent

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Aquatic plants (macrophytes) are important components of freshwater ecosystems and serve numerous purposes that structure aquatic communities. Although macrophytes represent an essential component of stable aquatic communities, invasive macrophytes can negatively alter ecosystem properties. The success of invasive macrophytes may be related to allelopathic interactions that can have strong impacts on phytoplankton communities.
Ludwigia grandiflora is an invasive macrophyte in the north-west of France which competes with indigenous macrophytes (e.g. *Mentha aquatica*) and phytoplankton species. We tested over a yearly cycle of development, the allelopathic effects of *Ludwigia* on three target phytoplankton species as well as the allelopathic effects of both *Ludwigia* (i.e. the invasive species) and *Mentha* (i.e. the indigenous species) on the seed germination of *Ludwigia. Scenedesmus communis* and two *Microcystis aeruginosa* strains (one toxic producing microcystins and one non-toxic) were selected as phytoplankton species. Firstly, we examined the allelopathic effects of *Ludwigia* leaf extract on the photosynthetic activity of the phytoplankton species according to three distinct seasons (spring, summer, autumn). Secondly, we examined the allelopathic effect of both *Ludwigia* and *Mentha* leaf extracts on seed germination of *Ludwigia* according to the same seasons. We observed a strong seasonal effect of allelopathic interactions and a differential effect on the three phytoplankton species. The photosynthetic activities of *Scenedesmus* and the toxic *Microcystis* were stimulated at low dose extract whereas the photosynthetic activity of the non-toxic *Microcystis* was inhibited according to the increase of dose extract. The photosynthetic activities of the three phytoplankton species decreased at high dose extract in summer. The germination rate of *Ludwigia* was stimulated in presence of both *Ludwigia* and *Mentha* leaf extracts. The stimulation of germination rate was higher in summer compared to the two other seasons, concomitant to the strong negative effects observed on the phytoplankton species.

Keywords: allelopathy, aquatic ecosystems, biological invasion, *Ludwigia grandiflora*, macrophyte, phytoplankton

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The role of micro-algal toxin in allelopathy

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Marine coastal areas are facing increasing occurrence of toxic micro-algae blooms all over the world. The toxins produced by these photosynthetic organisms can cause severe afflictions not only to marine organisms but also to humans. In particular, the tropical benthic dinoflagellate Ostreopsis cf. ovata known to produce several analogs of the potent palytoxin has now spread over the Mediterranean Sea. So far, the ecological role and the mode of action of those toxins have been little studied, although some results point out their role as allelochemicals. Allelopathic interactions between *Ostreopsis* cf. ovata and co-occurring benthic micro-algae were investigated using bioassays along with co-cultures. The physiological state of the micro-algae was monitored measuring their photochemistry (active chlorophyll-a fluorescence) and their exo-metabolome (extra cellular content) was simultaneously investigated. A targeted metabolomic approach was first used to analyze the toxinic content followed by an untargeted analysis by UHPLC-HRMS to detect other metabolites eventually involved as allelochemicals.
The bioassays reveal a non-uniform toxicity of *Ostreopsis* exo-metabolome on its competitors and was particularly harmful against the benthic diatom Licmophora paradoxa. A co-culture without contact between the two species further showed no effects of this exo-metabolome on *L. paradoxa*, suggesting that the mode of action of the toxins is by contact rather than distant allelopathy.

Keywords: toxic dinoflagellate, marine, bioessay, allelopathy

*Speaker

Terrestrial invasion

Fast Invading Exotic Tree Paper Mulberry (*Broussonetia papyriferra*) in the Periurban Ecosystems: Does Allelopathy Play a Role?

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Paper mulberry (Moraceae; Botanical name *Broussonetia papyrifera*) is an exotic tree which is spreading very fast in various ecosystems especially the periurban areas surrounded by forest patches. It forms dense monospecific stands of shrubby nature and does not allow any other plant to grow underneath or in association. In order to find out the possible mechanism for the absence of other species in association with the tree, role of allelopathy was investigated. For this, the understorey soil and leaf litter of paper mulberry were collected and their allelopathic effect was determined on the commonly occurring understorey species– *Bidens pilosa*. The aqueous extracts of paper mulberry leaf litter severely reduced seed germination and growth of *B. pilosa*. The emergence and growth of test species were also reduced in the understorey soil and soil amended with the litter powder (0-40 g kg^{-1} soil) of paper mulberry, though no nutrient deficiency was detected in the soil. Even the incorporation of exogenous activated charcoal or nitrogen could not completely ameliorate the toxic effect of the litter. Further, the soils were found to contain allelochemicals which were identified as phenolic compounds using HPLC. The study concludes that the leaf litter of paper mulberry exhibits allelopathic effect on *B. pilosa* through water soluble phenolic allelochemicals that may have ecological implications.

Keywords: Invasion, Litter, Paper Mulberry, Understorey

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Sorghum halepense allelopathy: impact on soil and plant seedling growth

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Sorghum halepense is an aggressive invasive species in the arid and semi-arid regions throughout the world including India. We quantified the species richness in the *Sorghum halepense*-invaded communities and communities not yet invaded by the weed. Sorghum soil and no-sorghum soil were analysed for total phenolics, microbial activity, available nitrogen and organic carbon. Manipulative experiments were designed to understand the allelopathic potential of *S. halepense*. Soil was amended with root or shoot leachate of *S. halepense*, and its impact on plant growth and soil properties was studied. *Sorghum halepense* did not impact species richness in natural settings. *S. halepense*-invaded soil had higher levels of total phenolics and lower levels of available nitrogen. Root leachate had greater inhibitory effects on the root growth of two common species, *Brassica juncea* or *Bidens pilosa,* than did shoot leachate of the weed. Shoot leachate-amended soil had higher levels of total phenolics and available nitrogen than root leachate-amended soils. *Sorghum halepense* allelopathic potential is argued due to lower levels of available nitrogen. Higher inhibition in the root dry weight of assay species in root leachate-amended soil compared to shoot leachate-amended soil can be due to lower levels of nitrogen in root-amended soil. Relative allelopathic potential of root versus shoot leachates in bioassays for allelopathy should be examined to distinguish the role of chemicals versus nutrient availability.

Keywords: *Sorghum halepense*, Soil, Microbial activity, Nitrogen

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Allelopathy in the invasion by *Fallopia* spp.: from invasiveness to management

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Species of the genus *Fallopia* are among the major plant invaders in France and Europe. Our research team is investigating how allelopathy is involved in the invasion by these species. In the first hand, metabolomic studies detected flavanols (catechins), flavonols, anthraquinones, stilbenes (including piceid and resveratrol), and cinnamic acids in rhizome extracts of *Fallopia* spp. Some flavanols (B-type proanthocyanidins) have shown an inhibiting effect on denitrifying microorganisms, which could participate to the invasiveness of the species. In the other hand, allelopathic interactions could be used in biotic resistance against *Fallopia* spp. Greenhouse and lab studies showed the reduction of the growth of *F.* x *bohemica* when watered with an extract or a leachate from native species, compared to the control. Those results have potential applications in the management of the species in invaded sites.

Keywords: Japanese knotweed s.l., native plants, secondary metabolites, nitrogen

*Speaker

Direct and indirect allelopathic effects of the invasive weed *Solanum rostratum*

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The allelopathic effect of aqueous and ethanol extracts of different plant parts (i.e. root, stem, leaf and fruit) of the invasive weed *Solanum rostratum* were investigated. Leaf extracts exhibited the most potent inhibitory activity against various indicator species including *Amaranthus retroflexus* and *Poa annua*; the aqueous extract of leaves completely suppressed seed germination of test plants, while the ethanol extract of leaves inhibited root growth of test plants by 53_˜93%. The ethanol extract of leaves and stems of *S. rostratum* was further partitioned by petroleum ether, chloroform, ethyl acetate and butanol, which were subsequently subjected to phytotoxic bioassay against *A. retroflexus* and *P. annua* at 5 mg/ml. Chloroform and butanol extracts showed the strongest phytotoxic activity, inhibiting root growth of test species by 71_˜94%. Our results indicated that the major active allelochemicals were present in two fractions, the chloroform and butanol extracts of leaves and stems of *S. rostratum*. The indirect allelopathic effects of *S. rostratum* were also evaluated. We found that invasion of *S. rostratum* did not affect soil moisture, soil pH and electrical conductivity significantly; however, invasion of *S. rostratum* enhanced activities of several soil enzymes. Furthermore, pyrosequencing analysis revealed that invasion of *S. rostratum* triggered a significant shift in the composition and structure of soil fungi. We suggest that *S. rostratum* potentially releases bioactive compounds into the environment which can not only directly inhibit neighboring plant growth but also indirectly alter soil properties to facilitate its invasion success.

Keywords: allelopathy, invasive plant, Solanum rostratum, soil property

*Speaker

Evaluating toxin effects in stressed environments: Normalization supports comparisons between species and contaminants

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Current ecotoxicological laboratory techniques test toxin effects on plants in standardized laboratory conditions where other stress factors are virtually absent. It is challenging to estimate and compare how different toxins affect plants in natural environments where stress, such as resource competition is present. As multiple stressors may amplify or reduce toxin effects in the field, a method that reliably estimates changes in growth by toxin stress regardless of the level of non-toxin stress would be highly useful.

Here we utilize the density-dependence of plant response to toxins and develop a method that allows comparisons between species and toxins under competitive stress. We first normalize the biomasses at each toxin level so that mean biomass at a selected reference plant density equals to one at all toxin levels. Thereafter, we divide the real biomass at any higher plant density at a certain toxin level with the real biomass at the selected reference density at the same toxin level. This allows us to observe the relative change in plant biomass as plant density increases, i.e. in the presence of competitive stress. As an example, we normalize biomasses of lettuce and barley exposed to copper sulfate. We observe that lettuce growth is more severely inhibited than barley growth, and that the difference is hard to observe without normalization. We conclude that barley is more tolerant than lettuce under high competitive stress.

This approach may facilitate comparisons how toxins change plant growth under competitive stress, and whether the changes are similar in different plant species as competitive stress increases. Further studies are needed to investigate if toxin effects should be routinely tested at various plant densities and different species, and if upcoming ecotoxicological test standards should utilize the density-dependence of toxin effects while environmental threshold values are determined.

Keywords: modelling, competition, toxin, stress, comparisons between species

*Speaker

Adverse impacts of invasive *Ageratina adenophora* on Nepalese native trees

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Ageratina adenophora is considered one of the most problematic invasive species in Nepal. Tropical to subtropical Nepal forests are severely invaded by this species. Previous studies on *A. adenophora* are concentrated on crop plants and some weedy herbs but the studies on its effects on native trees have been very scarce. Moreover, previous tests have typically used crushed or ground leaves that may contain compounds that do not exist on soil surface. To avoid these drawbacks, we used different bioassays to investigate the modes of impacts of *A. adenophora* on native trees (*Schima wallichii* and *Alnus nepalensis*) in Nepal. Seedlings of these tree species were grown in *A. adenophora* invaded and uninvaded soils, soil with *A. adenophora* litter and without litter, and soil with extracts prepared by soaking intact *A. adenophora* leaves. Analysis of growth parameters showed that litter, extracts and invaded soil by *A. adenophora* were toxic to the native seedlings. Inhibition was shown after seedling exposure and the effect remained permanent. In conclusion, native tree species are vulnerable to *A. adenophora* invaded sites creating problems in the establishment and survival of native seedlings in Nepal forests.

Keywords: Crofton weed, invasion, native trees, growth, litter toxicity

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†Speaker

A tale of two invaders: Chemistry, ecology and genetics of invasive *Echium* spp. in southern Australia

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Echium plantagineum and *E. vulgare* are congeneric exotics that possess similar morphological and biological features and were introduced to Australia in a similar time frame. However, *E. plantagineum* is highly invasive in Australia, whereas *E. vulgare* is found only sporadically across southeastern Australia. Studies were conducted to evaluate the secondary chemistry, ecology and genetics of each species in an effort to understand their respective invasive success, or lack thereof. In a common garden experiment, *E. plantagineum* produced qualitatively and quantitatively higher levels of bioactive pyrrolizidine alkaloids (PAs) in its shoots when compared to *E. vulgare.* PAs have been well documented to be associated with reduced herbivory of insects and are highly hepatotoxic to grazing livestock. In contrast, the perennial *E. vulgare* produced somewhat higher levels of antimicrobial and potentially allelopathic naphthoquinones in its roots than did *E. plantagineum*, in a series of glass-house experiments. Similar trends were observed in field-collected plants. Potential ecological roles of these defensive metabolites in *Echium spp.* invasion will be discussed. The presence of *E. plantagineum* significantly reduced ($P < 0.01$) the number and density of other plant species co-habiting the same area in various field locations, while no significant impact of infestation was associated with *E. vulgare* co-habitation. *E. plantagineum* also exhibited a much smaller monoploid genome (1C = 0.37 pg) when compared to *E. vulgare* (1C = 0.43 pg), and these findings supported the large genome constraint hypothesis associated with reduced invasion success of plants exhibiting a larger genome size. In addition, intensively sampled Australian *E. plantagineum* populations suggested a much higher level of chloroplastidic genetic diversity ($h = 0.7661$ compared to $h = 0.3800$ in *E. vulgare*). We conclude that the upregulated production of defence compounds, significant impacts on endemic plant communities and elevated genetic diversity have all logically contributed to the successful invasion of *E. plantagineum* in Australia.

Keywords: *Echium plantagineum*, *Echium vulgare*, pyrrolizidine alkaloids, naphthoquinones

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New chemistry, biosynthetic pathways and modes of action

Net nitrate uptake, PM H^+-ATPase activity and related gene expressions in maize roots in response to coumarin

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Coumarin is the most simple plant secondary metabolite widely distributed in plant kingdom affecting root form and function, including anatomy, morphology and nutrient uptake. A physiological and molecular approach in maize roots exposed to different coumarin concentra-tions, with or without 0.2 μM nitrate (NO_3^-), was adopted to elucidate its mode of action. In particular, the time course of net NO_3^- uptake rate (NNUR), PM H^+-ATPase activity, proton pumping, and related gene expressions (ZmNPF6.3, ZmNRT2.1, ZmMHA3 and ZmMHA4) were evaluated. Coumarin alone did not affect NNUR, PM H^+-ATPase activity and transcript levels of ZmNRT2.1 and ZmMHA3. By contrast, coumarin alone increased ZmNPF6.3 and ZmMHA3 expression, as observed in response to abiotic stress. When coumarin and NO_3^- were concur-rently added to the nutrient solution, an increase in NNUR, PM H^+-ATPase activity together with ZmNRT2.1 and ZmMHA3 expression levels were observed, suggesting that coumarin af-fected the inducible component of high affinity transport system (iHATS) of NNUR and this effect was probably mediated by nitrate. Indeed, as the energy conditions were favourable in terms of PM H^+-ATPase, the active proton pumping became essential for nitrate uptake. Moreover, the results with vanadate, an inhibitor of PM H^+-ATPase, suggested that this enzyme could be a main target of coumarin. Surprisingly, coumarin did not affect the H^+-ATPase activity by direct contact with plasma membrane vesicle isolated from maize roots, indicating its possible role in the transcription processes as well.

Keywords: coumarin, nitrate uptake, H^+-ATPAse

*Speaker

Modification of reactive nitrogen species metabolism as a secondary mode of action of meta-Tyrosine in tomato roots

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A non-protein amino acid (NPAA) - *meta*-Tyrosine (*m*-Tyr) a structural analog of phenylalanine is a harmful compound produced by chewing fescue (*Festuca rubra* L. ssp. *commutata*) and donkey-tail spurge (*Euphorbia myrsinites* L.) roots. In animal cells *m*-Tyr is commonly considered as a marker of oxidative stress, due to its generation in the presence of reactive oxygen species. As a material we have used 3-4 days old tomato (*Solanum lycopersicum* L.) seedlings treated for 24-72 h with *m*-Tyr (50 or 250 μM), resulting in inhibition of root growth by 50 or 100%, without lethal effect. Fluorescence of DAF-FM and APF derivatives was determined to show reactive nitrogen species (RNS) level in roots of tomato plants. *m*-Tyr-induced restriction *of* root elongation growth was related to formation of nitrated proteins described as content of 3-nitro tyrosine. Supplementation of tomato seedlings with *m*-Tyr enhanced superoxide radicals generation in roots and stimulated protein nitration. It correlated well to increase of fluorescence of DAF-FM derivatives, and transiently stimulated fluorescence of APF derivatives corresponding respectively to NO and $ONOO^-$ formation. Modifications in RNS formation induced by *m*-Tyr were linked to metabolism of nitrosoglutathione (GSNO). Activity of nitrosoglutatione reductase (GSNOR), catalyzing degradation of GSNO was enhanced by long term supplementation of tomato seedlings with *m*-Tyr, similarly as protein abundance, while transcripts level were only slightly altered by tested NPAA. We conclude, that secondary mode of action of *m*-Tyr in roots of tomato plants involves perturbation in RNS formation, alteration in GSNO metabolism and modification of protein nitration level.

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Keywords: meta, tyrosine, nonproteinogenic amino acid, GSNOR, RNS, 3, nitrotyrosine

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Rosmarinic acid phytotoxicity in *Arabidopsis* roots is associated with mitochondrial dysfunction and metabolomic changes connected with the production of Reactive Oxygen Species.

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The phytotoxic potential of rosmarinic acid (RA), a caffeic acid ester largely found in aromatic species (Boraginaceae and Lamiaceae families), was evaluated *in-vitro* on *Arabidopsis thaliana* (L.) Heynh through a metabolomic (GC-MS) and microscopic approach (TEM and confocal microscopy).
In-vitro bioassays pointed out that rosmarinic acid was able to affect root growth and morphology and detailed microscope analysis evidenced a strong reduction of root meristem accompanied by an alteration of its organization and ultrastructure. In particular, a high vacuolization and alteration of mitochondria structure and function was observed. RA-treatment (175 μM) caused an increase of mitochondria number and a strong reduction of mitochondrial transmembrane potential ($\Delta\Psi$m). These data suggested an energy deficit, which was confirmed by the metabolomic data were a strong alteration of the TCA cycle and amino acids metabolism (alanine, aspartate, glutamate, ß-alanine, glycine, serine, threonine etc.) was highlighted. In addition, root meristem of treated seedlings where characterized by a high accumulation of H_2O_2 and O^{2-} accompanied by a reduction of catalase and superoxide dismutase activity, which suggest that seedlings exposed to RA are experiencing oxidative stress and are unable to cope with it. Finally, the trypan blue staining showed a high number of root death cells which perfectly correlate with the high vacuolization observed.

Based on these results, we proposed that the alteration of mitochondrial membrane potential accompanied by the inhibition of cell respiration, probably due the increase of reactive oxygen species, which consequently causes cell death, could be the mechanism by which rosmarinic acid induces its strong phytotoxicity on *Arabidopsis* seedlings.

Keywords: Rosmarinic acid, plant cell death, metabolomic, TCA cycle, mitochondria

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Farnesene affects *Arabidopsis* root meristem altering auxin transport

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Farnesene is a sesquiterpene with semiochemical activity commonly found in the essential oils of many plants and heavily involved in interspecies communication. This molecule, already known for its high phytotoxic potential and its effects on root morphology and anatomy, caused a loss in gravitropism, bold roots and a "left-handedness" phenotype. These clues suggested an alteration of auxin distribution, and for this reason, the aim of the present study was to evaluate its effects on: i) PINOID proteins (PIN) distribution, involved in polar auxin transport; ii) PIN genes expression iii) apical meristem anatomy of primary root (RAM), in *Arabidopsis thaliana* seedlings, treated with 250 μM farnesene, for 7 days. The following GFP constructs: *pSCR::SCR::GFP, pDR5::GFP, pPIN1::PIN1::GFP, pPIN2::PIN2::GFP, pPIN3::PIN3::GFP, pPIN4::PIN4::GFP* and *pPIN7::PIN7::GFP* were used to analyze auxin distribution. Farnesene caused a reduction in meristem zone size and an advancement in transition zone suggesting a premature exit of cells from the meristematic zone. Moreover, these effects were accompanied by a significant reduction in cell division. The auxin-responsive reporter *DR5::GFP* highlighted that auxin distribution was clearly impaired in farnesene-treated roots where auxin distribution appeared maximum in QC and columella initial cells, without extending to mature columella cells. This finding was further confirmed by the analysis on PIN transport proteins distribution, assessed on individual constructs, which showed an extreme alteration mainly dependent on the PIN7, involved in pattern specification during root development and auxin redistribution. Finally, farnesene treatment caused a down regulation of all the auxin transport genes studied. We propose that farnesene affected auxin transport and auxin distribution causing a severe alteration of root meristem, and consequently the left-handedness phenotype.

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Keywords: Farnesene, auxin transport, root meristem, PIN proteins

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Control of climbing plants by using allelochemicals affecting gravitropism

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Climbing plants, such as kudzu (*Pueraria lobata*), Japanese honeysuckle (*Lonicera japonica*), and burr cucumber (*Sicyos angulatus*) pose serious problems in many countries. They have negative impacts on productivity of crops in farmlands and could alter the landscape of natural habitats. Moreover, these climbing weeds also pose risks to mammalian safety and could potentially reduce the efficiency of power supply in urban communities as they invade electrical grids. In response to the request of electric power companies and government agencies in Japan, we design experimentation for the screening of allelochemicals for the effective management of climbing plants.
We have developed several bioassay systems to evaluate the effect of allelochemicals on gravitropism and climbing. Practical assays to prevent climbing of vine were also developed. We have evaluated the phytotoxicity of reported allelochemicals and new candidates including amino acids, polyphenols, flavonoids, alkaloids, and among others, and have found several candidates. *Cis*-cinnamic acid and its derivatives from *Spiraea thunbergi*, hinokitiol and its derivatives from Hiba (*Thujopsis dolabrata*), and chalcone derivatives from Ashitaba (*Angelica keiskei*), all native plants to Japan, were among those that impair gravitropic response.

Using transcriptome analysis, the modes of actions of the candidate allelochemicals were evaluated. *Cis*-cinnamic acid appears to influence the expression of auxin-responsive gene families in *Arabidopsis*. Auxins are putative mediators of gravitropism in plants. Our results suggest that exogenous application of these allelochemicals may effectively manage climbing plants.
Presently, we plan to develop derivatives by organic synthesis and conduct field evaluations for the development of practical management measures for climbing weedy plants.

Keywords: gravitropism, climbing plants, allelochemicals, cis, cinnamic acid, hinokitiol, calchone

*Speaker

A trial for making database for allelopathic activities by Specific Bioassays: Sandwich Method, Plant Box Method, Dish-Pack Method

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Tentative database for allelopathic activities by specific bioassays named sandwich method (for leaf leachates), plant box method (for root exudates), dish pack method (for volatile chemicals) was made from the data accumulated for about 20 years. Resulting from the sandwich method, about 6,000 species ware tested and the results obtained using 10 mg of leaves in 10 mL per 10 cm^2 dish followed normal distribution. *Sorghum* sp., *Oxalis* sp. and *Withania* sp. showed strong inhibitory activities. By the plant box method about, 2,000 species were assayed. *Mucuna pruriens* (velvet bean) and hairy vetch (*Vicia villosa*) showed strong activity by this method. The results by dish pack method have a little correlation with the data from sandwich method and plant box method. Based on this database, it was found that endangered plants, slow growing plants, unique plants with few relatives in a given plant family, are allelopathic. As a result of these bioassays, potentially dangerous invasive alien plants with high allelopathic activities were found to be: *Coccinia grandis, Rottboellia cochinchinensis, Phalaris brachystachys, Physalis angulata, Gypsophila paniculata, Trifolium incarnatum, Ipomopsis rubra, Silene armeria, Anisantha madritensis*. The reasons behind each allelopathic activity and the potential allelochemicals will be discussed.

Keywords: Invasive Alien Plants, Sandwich Method, Plant Box Method, Dish, pack Method, Biodiversityl

*Speaker

The coumarin scopoletin behaves as an auxin herbicide on *Arabidopsis* seedlings

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Due to the increasing side effects of synthetic herbicides (weed resistance, environmental toxicity, human diseases, etc.) many natural compounds with phytotoxic activities have been already identified and studied to be used as eco-friendly bioherbicides.
Scopoletin is a secondary metabolite belonging to the group of coumarins that can be found in different plant species like tobacco, sunflower, plum tree, *Avena sativa* and others. Although some studies have demonstrated its phytotoxic capacity, no in deep studies have been done to elucidate its mode of action on plant metabolism.

Analysis conducted by light and electron transmission microscopy revealed strong cell and tissue abnormalities at root tip level, such as cell wall malformations, multi-nucleated cells, abnormal nuclei and tissue disorganization. Besides, scopoletin induced reoriented microtubule assembly and ultimate cell death. Most of the effects observed after scopoletin treatment were similar to those observed after 2,4D, an auxin herbicide. Moreover, structural analyses of scopoletin revealed that it perfectly fits into the auxin-binding site of TIR1.
Our results show that scopoletin compromises root development of treated roots by inducing wrong microtubule assembling, mitochondrial membrane depolarization and ultimate cell death, which suggests a multi-mode of action on plant metabolism. Taken together, the results confirm the phytotoxic activity and plant growth regulator profile of scopoletin, whose mode of action on *Arabidopsis* metabolism is similar to auxinic herbicides.

Keywords: mode of action, auxin herbicide, coumarin, microtubule

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A Plant Growth Inhibitor N-trans-cinnamoyltyramine in Rice and Its Synthesis

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A bioassay-guided approach was used to identify allelopathic fractions from a Vietnamese rice cultivar (*Oryza sativa* L.cv. OM 5930) and was coupled with reverse-phase chromatography to isolate and identify as *N-trans*-cinnamoyltyramine (NTCT). An efficient and low cost total synthesis of NTCT was successfully achieved by one-step amidation from trans-cinnamic acid and tyramine. NTCT inhibited root and hypocotyl growth of cress, barnyardgrass and red sprangletop (*Leptochloa chinensis* (L.) Nees) at the concentrations greater than 0.24 μM. The means ED50 (the effective dose required for 50% plant growth inhibition) of *N-trans*-cinnamoyltyramineon cress and barnyardgrass hypocotyl and root elongation were 0.96 and 0.73 μM, respectively. The concentration of NTCT in the rice plants was 42 μg g^{-1} fresh weight. These findings suggest that NTCT may contribute to the growth inhibitory effect of rice plants, and may play an important role in rice allelopathy to control barnyardgrass and red sprangletop. The developed synthesis strategy of NTCT could permit production of this synthesized allelochemical at a commercial scale.

Keywords: *Oryza sativa*, barnyardgrass, N-transcinnamoyltyramine, synthesis, Allelopathic activity

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The mode of action and the genetic basis of resistance in the case of benzoxazinones

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Several grass species release precursors of benzoxazinones, a class of potent allelochemicals. Although the synthesis pathways and degradation processes of these compounds have been well characterized, the molecular mode of action by which they inhibit plant growth has remained elusive. Here, we present a combination of biochemical, molecular, and genomic analyses, by which we show that 2-amino-*3H*-phenoxazin-3-one (APO) and its methoxylated analog 2-amino-7-methoxy-*3H*-phenoxazin-3-one (AMPO) act by binding to the catalytic unit of histone deacetylases (HDACs), inhibiting these enzymes. APO and AMPO activity leads to genome-wide changes in histone acetylation, altering chromatin configuration and transcriptional profiles of the target plant, which ultimately results in a slow-down of growth. The high evolutionary conservation of the targeted pathway - APO and AMPO inhibit even human histone deacetylases - raises two questions: Is there specificity at the enzymatic and/or at the histone level? And, how do some plant species manage to be tolerant or even resistant to these allelochemicals? To answer the first question, we use biochemical and mass spectrometry analyses of HDACs and acetylated histones, as well as chromatin immunoprecipitation targeting specific acetylated histone residues. To understand the evolution of resistance, we employ a genomics approach, making use of the natural variation spectrum and the genomic resources of the model plant *Arabidopsis thaliana*, to map resistance-promoting alleles. Altogether, I will present the latest results of our attempt to comprehensively understand benzoxazinone-mediated allelopathy between plants.

Keywords: Allelopathy, benzoxazinones, DIBOA, DIMBOA, chromatin, histone

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C4H Coding Gene on Transcription Regulation of Rice Allelopathy

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Allelopathy to weed means that allelochemicals are released from crop into the environment to affect weed on growth or development. Allelopathy to weeds is an alternative way for reduce the usage of herbicide and protection eco-environment. Phenolic compounds were earliest found as one of bioactive chemicals from rice. Cinnamate-4-hydroxylase (C4H) is one of the key enzymes for phenolics synthesis. In this study, four C4H coding genes, CYP73A35, CYP73A39, CYP73A40, and CYP73A38, were cloned from allelopathic rice cultivar PI312777 (PI) and non-allelopathic cultivar Lemont (LE), and then were detected the expression level after the treatment of UV-B, SA. The results showed that the 4 genes of PI were up-regulated after treatment of UV-B, while three genes, CYP73A35, CYP73A39 and CYP73A38 were down-regulated in LE. After treatment of SA, the 4 genes of PI were down-regulated, while two genes of LE, CYP73A38 and CYP73A40, were up-regulated and the other two CYP73A35 and CYP73A39 were down-regulated. The results of Sequence alignment indicated that there was a little difference in the part of promoter and CDs sequences of the gene CYP73A40, and the gene encoding protein C4H with two amino acids mutation in carboxyl terminal. These results suggested that there was difference in expression regulated mechanism of C4H between allelopathic and non-allelopathic rice cultivar. This preliminary study provides a new evidence for revealing the regulation of C4H in rice allelopathy, and explains the reason of phenolic compounds difference in rice allelopathic potential.

Keywords: rice, allelopathy, Phenolic acid compounds, Cinnamate-4-hydroxylase

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Phytotoxic effects and mode of action of the alkaloid norharman on *Arabidopsis* seedlings

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The secondary metabolite norharman is an indol alkaloid that can be found in land plants of the families Graminaceae, Sapotaceae and Zygophyllaceae and in marine organisms such as the dinoflagellates *Nocticula miliaris* or in cyanobacteria of the Nostocaceae family. Although the algicidal, antibacterial and pharmacological activities of this secondary metabolite have been widely demonstrated, its herbicidal activity has been poorly investigated.
Therefore, in this work the phytotoxic activity of different concentrations of the compound (12.5, 25, 50, 75, 100, 200 and 400 μM) was analyzed on *A. thaliana* (L). Col-0 seedlings, selected as model species.

Seedlings showed a strong decrease in growth with an IC50 value (concentration that causes 50% inhibition) of 62 μM, and showed an altered morphology of the roots with a left-handed torsion and symptoms of necrosis. Electron microscopy showed important differences between treated and control seedlings, both at structural and cellular levels. Roots treated with IC50 norharman showed tissue disorganization, altered division planes with incomplete cell walls and multinucleated cells. The alteration of the microtubules was confirmed by immunofluorescence and increased cell death was detected by Trypan Blue staining. As well, norharman treatment caused the rupture and enlargement of many vacuoles at the cellular level.

The seedlings treated with the compound showed an altered morphology characterized by a decrease in the length of the main root, accompanied by an increase in the formation of lateral and adventitious roots and an increase in the production of root hairs. These alterations suggested the possibility of an alteration on the production or transport of auxins, which were measured by GC-MS.
Due to its strong phytotoxic potential, norharman appears as a promising molecule for studies of weed control.

Keywords: norharman, mode of action, phytotoxicity, cell death, microtubules

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Heterologous expression of the sorgoleone biosynthetic pathway and its effect on gene expression in *N. benthamiana*

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Sorgoleone, a hydrophobic compound exuded from root hair cells of *Sorghum* spp., likely accounts for much of the allelopathic activity associated with members of this genus. The enzymes involved in the biosynthesis of this compound have recently been identified and functionally characterized. To further examine the mechanism of action of sorgoleone, a multi-gene DNA construct was prepared for the expression of genes required for sorgoleone biosynthesis *in planta*. The DNA construct was deployed in *Nicotiana benthamiana* leaf tissues via *Agrobacterium*-mediated transient expression, and successfully directed the *in planta* accumulation of sorgoleone, as detected by GC-MS. The transient production of sorgoleone observed in infiltrated leaves resulted in the rapid formation of necrotic lesions, indicating that the compound possesses significant phytotoxicity to these tissues. To investigate the molecular mechanism(s) underlying the phytotoxicity of sorgoleone to the host plant *N. benthamiana*, RNA-Seq was employed to profile gene expression changes in the host plant during sorgoleone-induced necrotic lesion formation. The results from these experiments concerning whole transcriptome responses to sorgoleone production *in planta* will be presented.

Keywords: Sorgoleone, biosynthetic pathway, phytotoxicity, RNA, Sequencing

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Challenges in ecosystems functioning

Improvement of *Pisum sativum* salt stress tolerance by bio-priming their seeds using *Typha angustifolia* leaves aqueous extract

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This study aimed to evaluate the seed bio-priming effect on the salinity tolerance of pea (*Pisum sativum* L.) variety Lincoln. The aqueous extract of *Typha angustifolia* L. dried leaves at 40 g/L, was used for the pretreatment. Preliminary experiments were conducted to eliminate possible effect of aqueous extract pH and osmotic potential. Conductivity and pH of the aqueous extract used at 40 g/l were measured and solutions of PEG 4000 with same values of these two parameters were prepared and tested on pea. Results showed that PEG solutions had no affected neither germination index nor growth of the target plants. Indeed, all results were similar or improved relative to the control. The experiment was carried out in the presence of 0, 240, and 320 mM NaCl for germination and 0 and 120 mM for growth which was made hydroponically. Two seed lots were considered primed (P) and not primed (NP). Results revealed that salt stress adversely affected the germination, growth, membrane integrity, respiration, chlorophyll and carotenoid contents, mineral composition (K^+ and P) of pea. However, it increased Na^+, proline, total soluble sugars, and secondary metabolites (polyphenols, flavonoids, and alkaloids) accumulation. Seed priming reduced the negative impact of salt in all cases. Indeed, plants developed from primed seeds showed better response to salinity by the protection of membrane integrity, the maintenance of the highest values of osmotica (proline, total soluble sugars, K^+, and P) and by the amelioration of chlorophyll and carotenoid content. Hence, bio-priming of pea seeds seems to be a reliable procedure to increase the pea salinity tolerance and to win more biomass which can probably have an important impact on seed yield.

This work has been carried out thanks to the support of the A*MIDEX grant (n○ ANR-11-IDEX-0001-02) funded by the French Government " Investissements d'Avenir " program.

Keywords: *Pisum sativum* L., NaCl, Priming, Aqueous extracts, *Typha angustifolia* L.

*Speaker

Phytotoxic activity of flavonols isolated from *Annona coriacea*

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Annona coriacea is a Brazilian native species, which presents edible fruits and is very common in savanna areas of the country. Although phytotoxic activities were already described for the species, there are no data about the compounds responsible for this effect. The aim of this study was bioprospecting *A. coriacea* in relation to phytotoxic activity on the elongation of wheat etiolated coleoptiles fragments. Thus, the leaf ethanolic extract was subjected to chromatographic separation furnishing 9 groups (A-I). Group G (60% ethyl acetate: 40% methanol) had the highest phytotoxicity at 0.8 mg.ml-1, showing 66% of inhibition of coleoptiles elongation in relation to control. Therefore, this group was re-fractionated, enabling the isolation of 15 flavonols, most of them never identified in *A. coriacea*. Flavonols were quercetin (**1-5**), kaempferol (**6-9**), and isorhamnetin (**10-13**) derivatives. Compounds with complete identification were **2** (Quercetin-3-*O*-robinobioside), **3** (Rutin), **4** (Hiperin), **5** (Isoquercitrin), **6** (Biorobin), **9** (Nicotiflorin), **10** (Keioside), **11** (Narcissin), **12** (Cacticin) and **13** (Isorhamnetin-3-*O*-glycoside). Among the isolated compounds, those with higher phytotoxicity on coleoptiles elongation were **5** (IC50 2.20·10-4 M, r2 0.97) > **13** (IC50 2.84·10-4 M, r2 0.92) > **9** (IC50 4.80·10-4 M, r2 0.93) > **8** (IC50 1.16·10-2 M, r2 0.91). Although **5**, **13** and **8** showed satisfactory phytotoxicity in relation to other flavonoids described in literature(a), they did not exhibited significant effect on germination and initial growth of two standard-species (lettuce and tomato) and one weed (*Urochloa decumbens*, Poaceae). This behavior could be related to a higher activation of defense strategies in seedlings when compared to etiolated coleoptiles fragments. Different responses between these two systems have already been described for other compounds (a,b).

(CAPES/FAPESP)

a. Nebo et al. 2014. Phytochem Lett, 8: 226-232.
b. Lacret et al., 2010. J Chem Ecol, 36: 396-404.

Keywords: Allelopathy, Annonaceae, Kaempferol, Coleoptiles, Quercetin, Isorhamnetin

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Allelopathic interactions in grasslands: a systematic review

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Grassland ecosystems have been in the focus of many allelopathy studies. We conducted a systematic review including studies that evaluated the role of allelopathy in dynamic of grasslands. Several descriptive and methodological aspects were assessed in each article. We found that the studies have mostly investigated interactions involving herbaceous plants. Inhibitory effects of donor species on germination or growth of recipient species were mostly reported. We observed that in recent years, allelopathy research in grasslands has improved in some experimental design issues, but not in others. In relation to positive aspects, fewer studies used inadequate recipient species in recent years, and more studies used appropriate controls for field and greenhouse evaluations. Regarding negative issues, most of studies used artificial substrates, and did not perform chemical analysis. In recent years, more studies were only conducted in the laboratory, and field evaluations diminished. Therefore, some problems that led to critics about the relevance of allelopathy, and which have been extensively highlighted, are still present. Nevertheless, for all of the critical issues, there are also positive examples of studies that conduct experiments close to natural conditions and that observe interaction in the field. In spite of some progress, more caution should be taken with experimental design, in order to better understand the relevance of allelopathy in structuring grasslands, as well as other types of ecosystems.

Keywords: experimental design, plant community, progress and problems.

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Allelopathic Influences in Regeneration of High level Conifers in western Himalayas

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Silver fir (*Abies pindrow,* Spach) and spruce (*Picea smithiana*, Boiss), commonly known as high level conifers, are the important constituents of moist temperate forests of western Himalayas. Natural regeneration in these forests is a long standing problem in these forests. Allelopathy in many instances played a major role in the natural regeneration failure in spite of some limitations. So to investigate the regeneration problems in high level conifer forests, allelopathic research is undertaken to ascertain whether compounds released from litter, humus and under-storey species could negatively affect regeneration of *A. pindrow* and *P. smithiana* at two levels: (1) at the seed level by inhibiting seed germination in laboratory (2) at the plant level by decreasing seedling growth in nursery. The litter, humus and foliages of *Sarcococca saligna, Viburnum nervosum* and fern (under-storey plants) at 5, 10, 15, 20% concentrations were used to investigate their effects on seed germination, radicle & plumule growth in laboratory and seedling growth of silver fir and spruce. The various leachate concentrations inhibited the seed germination, radicle & plumule growth, seedling height and seedling basal diameter of both the species with some seedling mortality at higher concentrations. The allelochemicals present in the leachates of litter, humus and under-storey plants were also analyzed using HPLC technique. Allelopathic interactions of litter, humus and under-storey plants may explain the poor seedling establishment and growth of seedlings of *A. pindrow* and *P. smithiana* on the forest floor. The active substances/allelochemicals present in green foliage and decomposing litter are leached out by rainfall and other means and reach the soil underneath the canopy and hamper the regeneration process. Knowing such tree/under-storey interactions can ease the foresters in regenerating the forests by avoiding such situations in site preparations and eradication of ericaceous shrubs in assisted natural regeneration programmes.

Keywords: Regeneration, silver fir, spruce, germination, nursery growth, allelopathy, leachates.

*Speaker

Transfer of anti-herbivore defense signals among clonal plants of rice (*Oryza sativa* L.)

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Rice (*Oryza sativa* L.) plants can form a clonal plant network via clonal ramet and spacer. It has been demonstrated that clonal plant networks have physiological integration function. Chemicals and nutrients (such as photosynthetic assimilation product, mineral nutrients, water, etc.) can be transferred and shared among individuals in the same clonal plant network. Here we showed that anti-herbivore defense signals was shared among clonal plants of rice. When the main stem of wild type rice (donor) was fed by Cnaphalocrocis medinalis (rice leaffolder, LF) or treated with MeJA, weight gain of LF caterpillars on first order tiller (receptor) was significantly lower than that in the control group. The LF feeding on the main stem increased the activities of defense related enzymes including polyphenol oxidase (PPO), peroxidase (POD), superoxide dismutase (SOD), and lipoxygenase (LOX), and the content of trypsin proteinase inhibitor (TrypPIs) in the leaves of the main stem and primary tiller linked to the clonal network. Our results show that there exists defense signal transfer among clonal plants of rice, which may be important for protection of individuals in clonal plant networks.

Keywords: *Oryza sativa*, clonal plant network, defense signal transfer, plant communication, physiological integration

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Posters

Determination of allelopathic potentiality of *Rosmarinus officinalis*

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Management of weeds is important since weeds can cause significant crop loss. However, the excessive use of synthetic herbicides has led to the rise in herbicide-resistant/tolerant weeds (Heap, 2016) and this can be reduced by exploiting allelochemicals from plants. In this study, the contribution of some phenolic acids to rosemary allelopathic activity was evaluated. Leaves and stems of rosemary (30 g dry weight) were separately extracted twice with methanol (80% and cold methanol successively). The two filtrates were combined and the biological activity was evaluated on test plants. Soils incorporated with rosemary dried leaves were also tested on test plants in the greenhouse. The phenolic acid content (caffeic, ferulic, gallic, rosmarinic, and chlorogenic acids) of rosemary leaves were evaluated by HPLC to test their contribution to rosemary allelopathic activity. The specific activity and total activity of crude extract and individual phenolic acids were tested using *Lactuca sativa* as test plant. Seed emergence and dried matter of lettuce were inhibited by rosemary leaf debris incorporated into the soil at 1%. The growth inhibitory effects of the aqueous methanol extracts of the rosemary leaves were higher than that of the stems on all test plants. The inhibitory activity increased in a concentration-dose dependent manner and the roots were inhibited more than the shoot growth. At concentration 30 mg D.W. equivalent extract mL^{-1}, root and shoot growth of lettuce were completely inhibited. Ferulic acid had the highest specific activity and chlorogenic acid had the lowest. The specific activity of rosmarinic acid was medium level compared to other compounds but the total activity was high due to its high concentration in rosemary. Based on specific and total activity, as calculated by concentration and growth inhibitory effect, it could imply that rosmarinic acid, among other tested compounds, is the major allelochemicals in rosemary.

Keywords: allelopathic activity, *Rosmarinus officinalis*, specific activity, total activity, rosmarinic acid

*Speaker

Ethnobotanical survey as a benchmark for the screening for potential allelopathic species among medicinal plants in Ghana

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Recently, medicinal plants have been studied for the utilisation of isolated allelochemicals for sustainable weed control. However, there is no clear relationship between medicinal plants and corresponding potential allelopathy. The aim of this study was to document folk use of medicinal plants among traditional healers in Ghana and evaluates their allelopathic potentials to establish any possible relationship between medicinal plants and their allelopathic potential. Ethnobotanical survey was conducted in 2016 by using semi-structured questionnaire, field tour, and personal interviews. A total of 140 local informants in twenty communities in the Ejisu-Juaben Municipality, Ghana participated. Statistical tool (SPSS ver. 21.0) and quantitative ethnobotanical indices i.e. fidelity level (FL), relative frequency of citation (RFC), and use value (UV) were used to analyse the relevance of the ethnobotanical data. The sandwich (bioassay for leaf leachate allelopathy) and dish-pack methods (bioassay for volatile allelopathy) were used to screen allelopathic potential of the species mentioned. A total of 95 species with leaves (52%) being the most frequently used plant parts were recorded. *Cleistopholis patens* (UV=0.54; FL=90.7%; RFC=0.37), followed by *Ocimum gratissimum* (UV=0.37; FL=38.4%; RFC=0.35), and *Alstonia boonei* (UV=0.36; FL=70%; RFC=0.22) were rated as highly used medicinal species. *Alstonia boonei*, *Cleistopholis patens,* and *Ocimum gratissimum* were among the top inhibiting species in the sandwich bioassay (71.5, 67.6, and 62.3% respectively). The reported species showed inhibition on lettuce radicle in both sandwich (3.6-86.2%) and dish-pack methods (-6 – 84%). There was significant positive correlation (Spearman's rho) between the UV and RFC of medicinal plants and their corresponding allelopathic activity based on sandwich bioassay for leaf leachates (r=0.228, p=0.05 and r=0.203, p=0.05 respectively). It is evident from our report that highly used medicinal species with more therapeutic properties could have potential allelopathic properties. Thus, ethnobotanical survey can form the basis for primary screening for potential allelopathic plants.

Keywords: fidelity level, relative frequency of citation, use value, medicinal plants, allelopathy

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Highlighting the effects of coumarin on adult plants of *Arabidopsis thaliana* (L.) Heynh. by an integrated –omic approach

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In this study, the effects of the allelochemical coumarin through a metabolomic, proteomic and morpho-physiological approach in *Arabidopsis* adult plants (25 days old) were investigated. Metabolomic analysis evidenced an increment of amino acids and a high accumulation of soluble sugars, after 6 days of coumarin treatment. This effect was accompanied by a strong decrease on plant fresh and dry weights, as well as on total protein content. On the contrary, coumarin did not affect leaf number but caused a reduction in leaf area. An alteration of water status was confirmed by a reduction of relative water content and an increase in leaf osmotic potential. Moreover, coumarin impaired plant bio-membranes through an increase of lipid peroxidation and H2O2 content suggesting that coumarin treatment might induce oxidative stress. Coumarin reduced the effective quantum yield of the photosystem II, the energy dissipation in the form of heat, the maximum PSII efficiency, the coefficient of the photochemical quenching and the estimated electron transport rate, while it significantly stimulated the fluorescence emission and the coefficient of the non photochemical quenching. Finally, the proteomic characterization of coumarin-treated plants revealed a down-regulation of the ROS detoxifying proteins, responsible of oxidative damage and consequently of physiological cascade effects.

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Keywords: Coumarin, metabolomic, proteomic, chlorophyll a fluorescence, plant water status, oxidative stress

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Phytotoxicity of the monoterpenic phenol thymol on *Arabidopsis* adult plants: a physiological, molecular and metabolomic approach

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Thymol is a natural monoterpene phenol derivative of cymene, widely produced by several species belonging to the *Labiateae*. Although the phytotoxicity of thymol on seedling growth has been relatively explored, limited information are available on how this terpenoid affects physiology and metabolism of adult plants. For this reason, *Arabidipsis* adult plants were sub irrigated for 16 days with thymol (300 μM) highlighting the high phytotoxic potential of this molecule, which caused a developmental reduction, a high level of anthocyanins and necrotic areas on leaves blades. Moreover, plants showed a strong reduction in total protein content, fresh (FW) and dry (DW) weights as well as an increase in DW/FW ratio, H_2O_2 content, leaf osmotic potential, sugars and amino acids production, such as proline and galactinol, largely involved in osmoprotection under abiotic stress. The photosynthetic pigments content together with the parameters related to the photosynthetic efficiency were also negatively affected by thymol. Interestingly, treated plants showed an increase in Na^+ and NH_4^+ as well as a reduction in K^+, Mg^{2+} and Ca^{2+} content. At the same time, the expression of several genes involved in the short and long distance transport of these ions appeared to be affected by thymol. Taken together, these results suggest that thymol is an extremely phytotoxic molecule able to affect plant development, altering the nutrient absorption and photosynthesis.

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Keywords: thymol, plant metabolism, osmoprotectants, chlorophyll a fluorescence, mineral nutrient trasnsport

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Allelopathic effects of Cumin (*Cuminum cyminum*) Residues on root morphological characteristics of wheat and barley

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Allelopathic potential of Cumin (*Cuminum cyminum* L.) has been reported in many studies. These characteristics may negatively affect the growth of crop plants in rotation with cumin. After germination, the root is the first organ to be exposed to plant residues, and allelopathic properties of this material may affect root morphology. To determine the allelopathic effect of Cumin residue on growth and root morphology of wheat and barley, a factorial experiment was conducted in a randomized complete block design with three replications at Islamic Azad University, Sabzevar Branch in 2014. Factors were plant type (wheat and barley) and residual amount (0, 250, 500, and 750 g.m-2). The results showed that root length, root volume, average root diameter, and stem height were greater for wheat than barley, whereas the specific weight of roots was greater for barley than wheat. There was no significant difference in the total root length, root dry weight, and shoot dry weight of wheat and barley. The 750 g.m-2 residual amount resulted in decreases of 30.0% in stem length, 9.3% in stem diameter, 46.1% in root dry weight, 24.7% in shoot dry weight, 35.6% in total root length, 46.8% in root surface area, 24.6% in root volume, and 11.6% in root specific weight compared with the control. Seed+stem residual resulted in the greatests inhibition for all traits. Overall, the test results showed that the inhibitory effect of cumin residues on root growth was greater for barley than for wheat

Keywords: Allelopathy, Crop rotation, Cumin, Root morphology

*Speaker

Potencies of Sea Mango as Natural Repellent for *Rattus argentiventer* and Bio-herbicide for Weed Control

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Natural product utilization offers a safer method in pest control strategies to protect agricultural resources against pest. Sea mango (*Cerbera manghas* L.) was reported as a potential termiticide, insecticide and bactericide. These experiments were aimed to reveal other potencies of sea mango as natural repellent for *Rattus argentiventer* and bio-herbicide for weed control. The natural repellent potency of sea mango was determined by using T-maze (a simple arena for animal cognition experiments) and metabolic cage (a laboratory equipment with a spherical faeces-urine separator). The result showed that *R. argentiventer* tended to avoid sea mango fruits, which indicated that it took the food and beverage from the room without sea mango fruit as priority. The treatment of sea mango also caused metabolic disorder as indicated by the decrease of food and beverage intake by *R. argentiventer* and the significant decrease of its body weight, while the daily activities disorders were indicated by spending more time for locomotion and less time for foraging and resting.

The bio-herbicide potency of sea mango was determined by a series bioassay of seedlings growth on barnyard grass, foxtail fescue, Italian ryegrass, timothy, alfalfa, garden cress, lettuce and rapeseed. The result showed that seedling growth was varyable depending on the test plant species and extract concentrations. The inhibition of seedlings growth increased along with the increase of the sea mango leaf extract concentration. The I50 value showed that 8.50-32.30 and 4.26-34.67 mg dried weight equivalent extract mL^{-1} for shoots and roots respectively of sea mango leaf extract inhibited seedlings growth by 50%.
This study suggests that sea mango has potencies as natural repellent for *R. argentiventer* and bio-herbicide for weed control.

Keywords: *Cerbera manghas*, *R. argentiventer*, Weeds, repellent, bio, herbicide

*Speaker

Preliminary study of exploring the potential of *Cleome rutidosperma* - an invasive allelopathic species and its probable role as phytoremediator

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Cleome rutidosperma, L. (Capparidaceae) commonly known as Fringed Spider Flower or Purple Cleome is an invasive flowering plant, native to Tropical Africa. It is harvested from the wild for local use as a medicine and source of edible leaves. The allelopathic nature of invasive plants may be considered as an inherent silent feature. In order to establish their own existence, invasive plants use diverse mechanisms one of which is release of allelopathic compounds through the roots into the surrounding rhizosphere. *Cleome rutidosperma*, being a herbaceaous plant, mainly release the bioactive compounds required for ensuring its survival through their roots. Root exudates (RE) were collected after growing the plant in a root exudate trapping system. Allelopathic compounds were mainly recovered from the methanol and water fraction and bioassay guided fractionation revealed the methanol fraction to be the most potent bioactive fraction (IC50 400 ppm). Bioactive compounds of the methanol fraction have been isolated and purified by successive running through column chromatography and thin layer chromatography. Identification and molecular characterization of the compound are in progress. Isolation of microbial flora was done by plating 1ml of RE from both healthy and unhealthy plants. An intriguing fact was observed i.e., the fungal colony count in case of healthy roots was drastically greater than that of unhealthy roots. On the other hand, bacterial colony count did not differ much between healthy and unhealthy individuals. Interestingly, this species has a tendency to colonize in areas that are contaminated with sewage wastes. The mechanisms that help the plant to colonize the sewage contaminated sites are to be analyzed to reason the higher fungal colony counts in the root exudates. Such analysis may provide a new dimension to exploit this plant as a potential phytoremediator for decontamination of sewage waste land in a ecofriendly, cost-effective and sustainable way.

Keywords: Allelopathy, root exudate, phytoremediation

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Phthalimide-derived Strigolactone Mimics Complexation with Cyclodextrins

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Phthalimide-derived strigolactone mimics (PL) having a butenolide ring and lack-ing the typical enol ether bridge of strigolactone analogues have been reported recently as promis-ing compounds for use in the suicidal germination strategies for parasitic weed seed banks con-trol. These compounds were highly active, stimulating the germination of parasites with EC50 as low as 1.49 μM for *Phelipanche ramosa* and 38.3 μM for *Orobanche cumana*. Although low concentrations are useful to obtain the desired activity, their low water solubility would limit their future application in a soil water solution. In order to increase their water solubility and stability, we used α-, β- and γ-cyclodextrins to obtain complexes with selected phthalimide mimics PL01 (the simplest one), PL04 (including a nitro group in the aromatic ring) and PL07 (with two chlorines in the aromatic ring). The interaction of the PLs varied with structure, with each cyclodextrin and the coprecipitation compound with PL01 providing up to 190% improved water solubility. The theoretical complexes were modelled using geometry optimizations MM+ and PM3, including 250 water molecules, in which we observed higher stabilities of the complexes vs. the separated compounds. A wheat coleoptile bioassay and a parasitic seed germination bioassay on *Phelipanche ramosa* and *Orobanche cumana* were conducted to determine the effect on the complexation on the activity of these compounds. The results obtained from these experimentation showed that complexation with cyclodextrins is a potential alternative for development of strigolactone analogues acting as bioactive compounds for weed control.

Keywords: seed germination, phtalimide, strigolactone mimics, cyclodextrin

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Application of NMR-based metabolomics to identify allelochemicals in crude extracts of Mediterranean Fabaceae plants

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Allelopathy has been reported on some plants of Mediterranean vegetation and could contribute to structure this ecosystem. The allelopathic potential of fourteen Mediterranean plants belonging to the Fabaceae family has been assessed. The investigated plants of Mediterranean vegetation have been selected and studied by an NMR-based metabolomics approach.
Plants species (*Astragalus boeticus*, *Lathyrus cicera*, *Lathyrus clymenum*, *Medicago minima*, *Melilotus neapolitana*, *Ononis diffusa*, *Pisum sativum*, *Trifolium campestre*, *Trifolium cherleri*, *Trifolium scabrum*, *Trigonella esculenta*, *Vicia bhytinica*, *Vicia pseudocracca*, *Vicia sativa*) were collected in the Nature Reserve of Castel Volturno, a flat coastal area in the north of Naples (Southern Italy). The NMR metabolic profiling of the plant extracts furnished important information useful to identify the metabolite or metabolites responsible for the phytotoxicity.

All of the extracts were tested in Petri dishes against the *Cynodon dactylon* weed and, moreover, the phytotoxicity of the most interesting species have also been evaluated in hydroponic system against the target species *Triticum ovatum*. Using an NMR-based metabolomic approach, the effects of phytotoxins on this target plant was investigated evaluating the induced biochemical changes.
Most of the plant extracts employed in this study were found to have an activity, which could be correlated with the presence of flavonoids and hydroxycinnamate derivatives. These plant extracts affected the receiving plant in different ways, with different rates of growth inhibition at morphological level. The results of metabolomic analysis of treated plants suggested the induction of oxidative stress in all the receiving plants treated with active donor plant extracts, although differences were observed among the responses.

Keywords: Phytotoxicity, NMR, based metabolomics, Fabaceae, Mediterranean vegetation, Secondary metabolites

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Effect of stress on allelopathic activity of rice (*Oryza sativa* L.)

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Effects of nutrient, light, water, competition, and injury were studied on allelopathic activity of rice (*Oryza sativa*L.). Plants of the rice cultivar, Hawmjan, grown for two weeks under the effects of excess and limited amounts of nutrient, light, water, three levels of competition, and leaf injury were used in tests of allelopathic activity. The activity of rice water extracts was tested on lettuce (*Lactuca sativa* L.) germination, shoot and root length, and fresh and dry weight after three days. All five factors affected allelopathic activity of rice. Excess nutrients, limited light, and leaf injury increased allelopathic activity of rice by significantly reducing lettuce shoot and root length compared thors incorporate the several changes as noted in the revised version below. the control ($p < 0.001$ for all traits). However, excess and limited amounts of water reduced allelopathic activity of Hawmjan by significantly increasing lettuce shoot ($p < 0.001$) and root ($p= 0.006$) length compared with the control. Rice extracts significantly reduced lettuce shoot ($p< 0.001$) and root ($p = 0.005$) length compared with the water control, but the effect was not significantly different among the three levels. Lettuce germination percentage and dry weight were not affected by any treatment.

Keywords: rice, lettuce, stress, growth, allelopathic activity

[*]Speaker

Species dependant effects of forest trees leaves on *Pinus pinaster* germination and early growth

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Natural recruitement of *Pinus pinaster* is still uncertain in some areas of Atlantic coastal sand dune of South-West of France. Among the ecological factors likely to be involved, the allelopathic potential of co-occurring species such as *Arbutus*, *Quercus* spp. or even conspecific *Pinus* trees has been poorly investigated. Throughfall of dominant forest trees as well as litter leaching may release chemical compouds that could affect germination and early growth of *Pinus pinaster*.
In a controlled experiment, we tested the effects of two dilutions extracts from leaves or litter of the Ericaceae *Arbutus unedo*, the Fagaceae *Quercus ilex* and *Quercus robur*, and the Pinaceae *Pinus pinaster*. These species are the main tree species that can be found in Atlantic coastal sand dunes of south-west of France. The seeds germination and growth of seedlings of *Pinus pinaster* were surveyed.

Only one tree species alters the germination of *Pinus pinaster*: extracts from fresh leaves of *Arbutus unedo* showed a negative effect whatever the concentration of the extract. The extracts prepared from litter collected on the soil had no effects on *Pinus* germination. *Arbutus unedo* similarly decreased length and biomass of *Pinus* species.

Our result suggest that *Pinus pinaster* recruitement may be negatively affected by *Arbutus unedo* throughfall, which is very dense in the understorey of *Pinus* plantations to avoid erosion on littoral sand dunes. Other tree species such as *Quercus ilex*, *Q. robur* or *Pinus pinaster*, traditionnally used by forest manager, did not show any negative impact of *Pinus pinaster* germination.

Keywords: Allelopathy, atlantic coastal sand dunes, forest ecology, plant interactions

*Speaker

ALLELOPATHIC EFFECT OF TWO SPECIES: *Artemisia campestris* and *Opuntia ficus indica* ORIGINATING FROM TUNISIA

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Green chemistry has the main objective of reducing or eliminating the use or generation of hazardous substances and application of chemical products. Its applications are in favor of a sustainable agriculture, a clean environment and healthy food.
The aim of this study was the investigation, on one hand, of the chemical composition and allelopathic potential of *Artemisia campestris* essential oil against four species: *Daucus carota*, *Cicer arietinum*, *Phaseolus vulgaris* and *Triticum sativum* and, on the other hand, of the allelopathic ffect of *Opuntia ficus indica* against *Avena sativa*..

Three increasing concentrations of essential oil were tested: C1, C2 and C3. The seeds of weed species: *Daucus carota*, *Cicer arietinum*, *Phaseolus vulgaris* and *Triticum sativum* were cleaned and sorted to eliminate broken or damaged ones. Thereafter, they were dipped in water for 12 hours. Seed germination was monitored for seven days. In addition to this part, we measured stem and root length in the case of *Avena sativa* submitted to the effect of *Opuntia ficus indica* aquous extracts.

Artemisia campestris volatile oil was rich in monoterpenoids and a total of 27 components. Concerning the allelopathic potential of *Artemisa campestris* essential oil against the four forementioned species, we noted a significant reduction in germination for all the test species at the concentrations C2 and C3. However, an increase was notices at the concentration C1 which was the less important. Furthermore, there was a delay in germination for *Daucus carota* seeds and this delay was proportional to the essential oil concentration.

*Speaker

The aqueous extracts of *Opuntia ficus indica* pericarps exert an inhibitory action on the development of *Avena sativa* seedlings. This inhibition increases when parallel to the concentration of the extracts. The highest inhibition was noted at the highest concentration. Moreover, there was an inhibition of stem and root growth.

Keywords: Allelopathy/ Artemisia campestris/ Opuntia ficus indica/ Essential oil/ Aquous extract/ seedlings/ growth inhibition

Can the global change alter the allelopathic relationships between crops and weeds? The case of sorghum, rice and maize.

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Although there is little information about the changes in the secondary metabolism that will be produced by the rise of carbon dioxide concentration and temperatures associated to the global climate change, some experiments have shown important increases in several secondary metabolites. We have selected three crops, sorghum, rice and maize and we have compared allelopathic and non-allelopathic accessions, testing their production of allelochemicals and the *in vitro* effects on target plants, including *Arabidopsis thaliana* and weeds commonly associated to the crops. The changes in concentrations of sorgoleone, momilactones and hydroxamic acids in several carbon dioxide concentrations and temperatures are discussed. The differences in phytotoxicity are also taken into consideration.

Keywords: phytotoxicity, crop/weed interaction, climate change, momilactone, hydroxamic acids, sorgoleone, phenolic compounds

*Speaker

Physiological response of toxin producing *Microcystis aeruginosa* to *Tridax procunbens* L. extract

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Increase in urban, industrial and agricultural activities leads to the discharge of effluents and accelerates the process of eutrophication of water bodies. Consequently, the frequency of blooms of toxic cyanobacteria and the associated negative effects on animal and human health are increased. As an alternative to commonly used synthetic algaecides, chemical compounds of plant origin are employed to control microorganisms that are of public health importance. We evaluated the effect of *Tridax procumbes* L. ethanolic extract (50, 100, 250 and 500 mg L^{-1}) on the growth and antioxidant response of *Microcystis aeruginosa* (Kutzing) Kutzing. Throughout the experiment, there was an extract concentration dependent significant decrease in the cell density of the cyanobacterium. Furthermore, exposure to the extract increased internal hydrogen peroxide production, and consequently, upregulated peroxidase and superoxide dismutase activity. Glutathione S-transferase activity declined in the presence of the extract on day 6, while it increased on day 8 of the experiment. These results demonstrate that *T. procumbes* is a potential candidate for control of *M. aeruginosa* populations.

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Keywords: Algaecide, cyanobacteria, bioactive compounds, antioxidant response

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Phytotoxic activity of different naphthoquinone derivatives

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Naphthoquinones are known for their wide range of biological activities, including anti-inflammatory, phytotoxic, antioxidant and cytotoxic activities. Owing to the increasing demands of consumers in food quality and growing concerns about the impact caused by synthetic herbicides, the design of new agrochemicals with less environmental impact is needed. Natural products and allelopathy provide new alternatives to develop pesticides and these structures could be promising candidates for weed control.

The work described herein reports on the preparation of different compounds modifying the hydroxyl group position of three natural naphthoquinones (juglone, plumbagin and lawsone). A structure-activity relationship to evaluate the effect of bioavailability and the physicochemical properties to improve the activity was performed. The toxicity of these componds was first assessed against etiolated wheat coleoptiles. Compounds with better activity profiles were selected for further evaluation against Standard Target Species (STS) and four weeds, *Echinochloa crus-galli* L., *Lolium rigidum* Gaud., *Lolium perenne* L. and *Avena fatua* L.

Data showed a strong influence of lipophilic character. Acyl derivatives of juglone and alkyl derivatives of lawsone displayed strong inhibitory effects. Their potential structure and activity will be further described.

Keywords: naphthoquinones, bioassay, phytotoxicity, weed control

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Mediterranean litter allelopathic influence on soil microbial communities and oak seedlings

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In forest ecosystem, allelopathic effects of canopy trees or understory woody species may have important consequences on forest communities' composition and dynamics by influencing plant recruitment. These allelopathic interactions may be particularly important in Mediterranean forest ecosystems where woody plants produce high quantities of secondary metabolites. Secondary metabolites released by litter decomposition can be particularly influent in natural ecosystems due to the regularity and importance of litter inputs and the pronounced contact of seedlings with litter. Laboratory bioessays have suggested that litter extracts from a wide range of Mediterranean species inhibit germination or growth of other species seedlings. However, the expression of litter allelopathic potential can be modified by soil microbial communities: soil microbes can be negatively or positively affected by secondary metabolites, which influence allelopathic effects on plant seedlings. Thus, it is important to assess multi-level alleopathic interactions between litter, soil microbes and plant seedlings. Here, we tested the influence of leaf litter from 6 Mediterranean woody species on late-successional *Quercus ilex* and *Quercus pubescens* seedlings biomass, chemistry, mycorhizal associations and on underlying soil chemical and microbial properties. Chemical effects were separated from physical effects by using an artificial litter as control. Seedlings were grown for two years in pots with natural soil and different types of litter inputs. Litters had high quantities of terpenoids and phenolic coumpounds. Soil organic matter increased under the faster decomposing litter species. Microbial activity was stimulated and catabolic diversity influenced by litter type. However, no feedback on oak seedlings development was detected. We conclude that i) litter secondary metabolites can be used as C-sources by soil micro-organisms and ii) oak species are poorly sensitive to litter presence and chemical composition in their first years. However, soil modification induced by litter decomposition may lead to longer term litter effects on oak regeneration.

Keywords: litter decomposition, oak seedlings, mycorhizae, soil microbial communities

*Speaker

Citral and farnesene affect *Arabidopsis* seedlings through similar metabolic targets

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Citral and farnesene are two terpenoids previously found to show strong phytotoxic activity on Arabidopsis metabolism (Graña et al., 2013; Araniti et al., 2016). Assayed on seedlings of *Arabidopsis thaliana*, both affected several anatomical and physiological parameters, such as root morphology and anatomy, plant cell organization, plant hormonal balance, etc. Interestingly, the effects exerted by these two compounds were extremely similar suggesting similar metabolic targets. Therefore, metabolomic and physiological experiments were conducted in parallel in this study. In particular, seedlings of *A. thaliana* were grown for 14 days and then treated for 24 and 48 hours with 194 μM citral and 250 μM farnesene.
Imaging of chlorophyll *a* fluorescence was used to estimate effective PSII quantum yield (YII), quantum yield of regulated energy dissipation (YNPQ), quantum yield of non-regulated energy dissipation (YNO), maximal PSII quantum yield (Fv/Fm), and electron transport rate (ETR). Our results showed an early and similar damage on photosynthetic activity of both terpenoids, with decreased (YII) and increased (YNO); although farnesene showed slightly stronger effects than citral. Besides, damaged areas were mainly concentrated at the border of the older leaves, protecting the emergent central rosette, typical of early-senescence.

The metabolomic analysis concerning the pathways and networks affected by farnesene and citral was performed using the metaboanalyst module "MetPa", which pointed out that both molecules similarly affected the same pathways. In particular, the alanine aspartate and glutamate metabolism, the ß-alanine metabolism as well as the glycine serine and threonine metabolism were the most affected pathways for both molecules.

Taken together, these results confirm the hypothesis that citral and farnesene affect similar targets on *Arabidopsis*.

Graña, E. *et al.* (2013). *J. Chem. Ecol., 39*(2), 271-282.
Araniti, F. *Et al.* (2016). *PloS One*, *11*(8), e0160202.

Keywords: Metabolomics, terpenoids, mode of action, chlorophyll a fluorescence

*Speaker

Isolation and bioactivity of different *Annona cherimola* Mill. extracts

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Annonaceous acetogenins (ACGs) are waxy substances which are derivatives of C32 or C34 long chain fatty acids and are known for their wide range of biological activities such as cytotoxic, antitumoral, antiparasitic, pesticidal and immunosuppresive activities.
In order to respond to the growing demand for herbicides less toxic and harmful to the environment, allelopathy provides the possibility of using natural products as growth regulators and natural herbicides improving sustainable agriculture. ACGs could play a significant role in agro-ecosystems and affects the seed germination and growth, quality and quantity of crop products.

This work describes the bioassay-guided isolation of acetogenins from leaves of *Annona cherimola* Mill. from the south of Spain. The structures were elucidated by spectroscopic methods after isolation via column chromatographic separation and HPLC purification. The extracts were assessed by an etiolated wheat coleoptile bioassay as a first screening of activity. The fractions with better activity profiles were selected for their major purification.
Data showed a strong relationship between the activity profiles and the extracts which contain ACGs. Their potent activity and isolation will be further described.

Keywords: acetogenins, bioassay, allelopathy

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Phytotoxic activity of xylopine over seedlings of *Urochloa decumbens*

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Annona leaf extracts, rich in benzylisoquinoline alkaloids, was described as phytotoxic against standard species (lettuce, tomato, onion). Although xylopine, a common alkaloid in *Annona*, presents several biological activities such as antileishmania, sedative and analgesic, there are no studies related to allelopathic activity. The main objective of the present work was to verify the phytotoxic activity of alkaloid-enriched fractions and xylopine isolated from leaves of *Annona crassiflora* on wheat coleoptile elongation and on germination and initial growth of *Urochloa decumbens* (Poaceae), an important weed species in both agricultural and natural areas in Brazil. Leaves of *A. crassiflora* were extracted with acidified ethanol. This crude extract was subjected to standard chromatographic techniques to obtain alkaloid-enriched fractions. Semiprep-HPLC was used to isolation of xylopine, which was confirmed by UV spectrum and MS fragmentation pattern. Concentrations of the solutions of alkaloid fractions and xylopine varied from 0.8-0.2 $mg.mL^{-1}$ and 1000-10 μM, respectively. Alkaloid fractions showed 47% of elongation inhibition on wheat coleoptiles at 0.8 $mg.ml^{-1}$ and xylopine presented significant effect with IC50 of 263 μM. No significant effect of xylopine was observed in *U. decumbens* germination rate. By the other hand, the strongest effect occurred on inhibition of root growth, with IC50 of 1260 μM. The highest concentration (1000 μM) presented significant inhibition on shoot, with 42% of decrease of growth comparing to control. Root and shoot growth inhibitions have already been detected on bioassays using target species exposed to quinoline-alkaloids [1]. However, as long as we know, this is the first investigation of phytotoxic activity of this apor-phine alkaloid. Based on our results, xylopine could help in the control of this invasive plant, maybe causing less impact to the ecosystem compared with useful organophosphate pesticides.(CAPES-FAPESP)

[1] Nebo, L. *et al.*. *Phytochem. Lett.* **8,** 226–232 (2014).

Keywords: Allelopathy, Annonaceae, Alkaloid, Benzylisoquinoline, Aporphine

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†Speaker

The exogenous application of the brassinosteroid 24-epibrassinolide increases the antioxidant enzyme activities of the weed species *Bidens pilosa* L. under nonstressed conditions: comparison with the effects of other hormones and steroidal derivatives.

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The exogenous application of plant hormones has been intensively exploited to improve crop performance in the field. Some brassinosteroids have been demonstrated to increase crop tolerance for abiotic stresses, but their effects on weed species are little known. In this work, we evaluated the responses of the weed species *Bidens pilosa* L. to the exogenous application of 24-epibrassinolide (EBL), and a comparison was made with the effects of other steroidal derivatives brassicasterol, protodioscin, dioscin and diosgenin and also with the auxin indole-3-acetic acid (IAA) and with abscisic acid (ABA). The germination and the initial growth of *B. pilosa* L., as well as some parameters of antioxidant defense system and respiratory activity, were evaluated. EBL (0.1 to 5 μM) reduced the primary root lengths but not their fresh weights. ABA (1 to 10 μM) and protodioscin (50 to 250 μg ml^{-1}) inhibited all parameters of seedling growth. Root lengths and stem fresh weights were reduced by IAA (0.5 to 10 μM). Brassicasterol, dioscin and diosgenin produced milder effects. EBL, IAA or ABA did not alter the respiratory activity of primary roots, but protodioscin stimulated respiratory activity. EBL strongly activated the enzymes ascorbate peroxidase (APx), peroxidase (POD) and catalase (CAT). Protodioscin also stimulated these enzymes. IAA and ABA activated APx and POD but to a lesser extent. The strong stimulation of antioxidant enzymes by EBL occurred without changes in the malondialdehyde (MDA) content. In contrast, protodioscin induced a significant increase in the MDA content. Our results confirmed the role of EBL in strengthening the antioxidant enzyme defense system. Unlike other previous reports, however, this effect occurred in *B. pilosa*L. even in the absence of external stress. Thus, in the potential application of EBL for crop protection against abiotic stresses, a better performance of weed species is a problem to be considered.

Keywords: weed, brassinosteroid, antioxidant defense, respiration

*Speaker

Impact of *P. juliflora* an aggressive invader on soil microbial communities in its rhizosphere and its root extracts, on other crop Species

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Prosopis juliflora being one of the world's most invasive species, occupies a very broad range of habitats. Allelopathy may contribute to bare zone or inhibition zones below *P. juliflora* canopies because the invader produces a large amount of phenolic-rich litter throughout the year. We studied the microbial activities below canopies of *P. juliflora* and its native congener *P. cineraria*.. We observed significant differences in the fungal community in soil from canopies of two species. *Prosopis cineraria* had higher number of fungal colonies particularly of *Aspergillus niger* and other species of *Aspergillus*. This may explain the higher respiration rates in *P. cineraria* than open or *P. juliflora*. We also quantified mycorrhizal spores. The dominant mycorrhizae was *Glomus* species. We will discuss the impact of *Prosopis juliflora* invasion on soil microbial ecology and visualize its impact on soil processes. Here we link the effect of allelopathy on soil microbial communities to explain exotic plant invasion.

Keywords: *Prosopis juliflora*, invasion, mycorrhizae, soil microbial communities

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Screening for allelopathic activity of rice landraces in southern Thailand

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Allelopathic activity of 50 rice landraces in southern Thailand was tested from rice seedlings grown for 14 days. Rice growth parameters, i.e. shoot length, root length, root/shoot length ratio, shoot dry weight, root dry weight and root/shoot dry weight ratio were measured. Water extract from shoot of rice seedlings was applied to lettuce (*Lactuca sativa*L.) seeds to test for allelopathic activity. After three days, inhibition percentage of germination, shoot length, root length and dry weight of lettuce seedlings were measured. Results showed that rice cultivars Niaw Look Pueng and Niaw Daeng had the highest overall inhibition percentage of lettuce growth (93% for both cultivars), while cultivar Mae Mai had the lowest overall inhibition percentage (7%). Regression analyses between rice growth parameters and inhibition percentages of lettuce growth revealed statistically significant relationships mostly between rice root length, root/shoot length ratio, root dry weight and root/shoot dry weight ratio with inhibition percentages of lettuce growth ($p \leq 0.028$), indicating rice root length and root dry weight as important characters to predict its allelopathic activity.

Keywords: allelopathy, rice landraces, screen, growth, lettuce

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Deeping into the mode of action of the phenolic compound trans-cinnamaldehyde

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The phenolic compound trans-cinnamaldehyde (TC) can be found naturally in species of the Lauraceae family such as *Cinnamomun osmophloeum* or *C. zeylanicum*. This secondary metabolite is used as a flavoring in the food industry and has recognized fungicidal, cytotoxic and antimicrobial activity. The objective of this work is to approximate its mode of action in the model species *Arabidopsis thaliana* (L.) Col-0.
Trans-cinnamaldehyde IC50 and IC80 values (compound concentrations required to achieve 50% or 80% inhibition respectively) were established at 46 μM and 87 μM for germination of *Arabidopsis*, and at 367 μM and 671 μM, respectively, for root elongation. Analyzed treated roots after 7, 14 and 21 days of TC treatment showed a very characteristic 'octopus morphology', with a high number of root hairs and a lot of short roots with numerous primordia of lateral roots, suggesting alterations on the apical dominance. The ultrastructure of treated roots was analyzed by electron microscopy in seedlings treated with IC50 TC, and showed important differences between treated and control seedlings after 7 and 14 days, both at the structural and cellular levels. Treated roots showed tissue disorganization and altered division planes with incomplete cell walls. An increase in the number of mitochondria that could indicate mitochondrial function was also observed and confirmed by alterations in the mitochondrial potential measured by confocal microscopy with the fluorochrome JC-1.
The observed morphology suggests an alteration in the auxin balance due to an increase in its synthesis or to an alteration in its transport, which was confirmed by a bioassay with the anti-auxin PCIB. To verify if the alteration was due to an increase in the synthesis of the hormone, auxin quantification was realized by GC-MS.

Keywords: phenolic acid, phytotoxicity, auxin, mode of action

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Metabolomic fingerprinting of 8 mangroves species from North Vietnam

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Mangroves are tropical forest ecosystems occurring exclusively in intertidal zones. In such ecosystems, species are adapted to live in an extremely changing environment. Mangroves are present in more than 100 countries and cover around 152 000 km^2. Although their functioning is still misunderstood, these tropical forests are of special interest for the numerous ecosystem services they provide. For example, mangroves species are particularly used in traditional medicine and constitute thus a source of valuable molecules. Some of these chemical compounds are known to regulate the interactions between organisms of these ecosystems. They are considered as the ultimate expression of the metabolome, and they play key roles in allelopathic processes. The surrounding species, from bacteria to fungi and including invertebrates, are under constant pressure of this chemical production which will influence the quality of the environment. The aim of this study is to: (i) characterize the chemodiversity of eight trees species from the Northern Vietnamese mangroves: *Avicennia marina, Kandelia obovata, Bruguiera gymnorrhiza, Sonneratia apetala, Sonneratia caseolaris, Aegiceras corniculatum, Lumnitzera racemosa* and *Rhizophora stylosa ;* (ii) identify some chemotaxonomic biomarkers of these species.

For this purpose, leaf samples, harvested in the mangroves of the Nam-Dinh province, have been lyophilized and then crushed. A methanol extraction was carried out on the biological material and the resulting extracts were analysed by UHPLC-ESI-QToF in negative mode. After data extraction, filtering and statistical analyses, the results reveal, in agreement with phylogeny, a chemical proximity between species of the family Rhizophoraceae (*B. gymnorrhiza*, *K.* obovata, *R. stylosa*), on one hand, and between those of the genus *Sonneratia* (*S. apetala* and *S. caseolaris*), on the other hand.

MS/MS fragmentation and molecular networking will allow to go further in the annotation of these biomarkers while their isolation could be used to evaluate more precisely their ecological roles.

Keywords: mangroves, Vietnam, chemotaxonomy, metabolomics

*Speaker

Volatile constituent allelopathy of Saffron (*Crocus sativus*) and its mode of action

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Saffron is known for its continuous cropping problem which decreases its yield after 4-12 years. Studies have indicated the highly allelopathic nature of Saffron. However, studies using comprehensive chemical analysis of saffron allelochemicals have not been well documented. Identification of chemical(s) responsible for the allelopathic characteristic of Saffron and mode of action could potentially help understand this continuous cropping problem and even lead to finding newly discovered natural bioactive compounds. Previously established bioassay techniques including Sandwich method (SW), Dish pack method (DP), and plant box method (PB) were used for initial screening of plants with allelopathic potential. The inhibitory percentage on lettuce radicle growth by saffron in SW, DP, and PB bioassay were 100%, 60%, and 43% respectively. In HS-GC-MS analysis, safranal, isophorone, and D-limonene were identified as the main volatile constituents of Saffron. Safranal from Saffron stigma showed the strongest inhibitory activity on growth of lettuce seedling (EC50: 1.2 ppb). Moreover, microarray analysis of Arabidopsis in response to the volatile allelochemical was conducted to understand the mode of action of safranal. After 6-h treatment of the volatilized chemical, 579 genes were upregulated and 910 were downregulated by safranal. Upregulation of genes that belonged to a Gene Ontology (GO) category "response to heat" with highest level of significance was observed. Coexpression analysis of a heat shock factor (AtHSFA2) revealed that this gene was commonly regulated in many heat-responsive genes when Arabidopsis seedlings were exposed to safranal. Moreover after 72-h exposure, safranal triggered the generation of ROS species in lettuce seedlings. However the CAT and POX activity in lettuce seedling decreased significantly with increasing safranal concentration, resulting in dramatic damage in leaf.

Keywords: Allelopathy, Microarray analysis, Saffron constituent, safranal

*Speaker

Allelopathic Activity of Iranian Native Medicinal and Aromatic Plants by Using Cotton Swab Method

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In recent years, awareness of the harmful effects of herbicides have persuaded researchers to seek for a suitable replacement of chemical herbicides. One of these alternatives is natural compounds. So using allelopathic compounds as a novel strategy reduces the chemical herbicides consumption. This study was aimed to identify strong allelopathic activity among medicinal and aromatic plants (MAPs). Through one of the modern bioassay methods "Cotton Swab Method" which is used for evaluating volatile compounds, this experiment was conducted as a randomized complete design with four replicates. The essential oils (EOs) were applied in two different concentration 2 μl and 5 μl per 25 ml glass vial on lettuce seed germination. In this context a total of 104 EOs of MAPs were evaluated. Germination parameters (germination inhibition percentage, seed vigor, seed germination index (SGI), T50, mean germination time, speed of germination and germination speed index) were evaluated. The results showed that 15 samples such as *Origanum majorana*, *Zataria multiflora, Thymus daenensis, Melissa officinalis, Pelargonium graveolnes,* and *Pimpinella anisum* had very strong inhibitory effect on lettuce seed germination (%80-100 inhibition). Based on T50 results, 12 samples such as *Rosmarinus officinalis*, *Artemisia scoparia*, and *Nepeta binaludensis* were highly effective on seed germination delay (More than 200%). So it can be concluded that there are very strong germination inhibitor EOs especially in Lamiaceae and Asteraceae families. According to the GC-MS analysis, components such as terpinen, thymol, carvacrol, citronellal, citronellol, geraniol and anethole were the main constituents of the strong inhibitor EOs. Moreover, camphor, p-cymene, β-pinene and 1,8-cineole were the main constituents in strong EOs which postpone seed germination. These results showed that cotton swab can be used as a rapid and suitable method for detecting allelopathic properties in volatile compounds. Furthermore, natural volatile ingredients of the mentioned MAP are good candidates as bio-herbicides.

Keywords: Medicinal and Aromatic Plants, Allelopathic Properties, Cotton Swab Method, Essential Oil, Organic Cultivation

*Speaker

Insect antifeedant activities of sesquiterpene lactones from glandular trichomes on yacon leaves

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Yacon (*Smallanthus sonchifolius* (Poepp. & Endl.) H. Rob) is a perennial Asteraceae, and its enlarged roots are used as a foodstuff. Since this plant species exhibits high tolerance to pests, it is easy to cultivate using small amounts of pesticides. A large number of glandular trichomes are present on the leaves of this plant and have been suggested to function as a resistance factor. In order to collect the constituents of glandular trichomes, leaves were rinsed using dichloromethane (DCM) to obtain the rinsate, and plant residues were subsequently extracted by DCM to obtain the DCM extract. Biological evaluations revealed that insect antifeedant activity against the common cutworm (*Spodoptera litura* F.) was stronger for the rinsate than for the DCM extract. The main constituents of glandular trichomes were isolated by silica gel flash chromatography using a hexane and ethyl acetate solvent system, and were identified as the sesquiterpene lactone, uvedalin (5-Acetoxy-4-epoxyangeloyloxy-6-methylcarboxygermacrenolide) and its oxidative congener, enhydrin (5-Acetoxy-10-epoxy-4-epoxyangeloyloxy-6-methylcarboxy germacrenolide), known as melampolides based on spectroscopic data. These sesquiterpene lactones exhibited strong insect antifeedant activities, and, thus, may function as the chemical defense system in this plant toward phytophagous insects.

Keywords: Yacon, Asteraceae, Insect antifeedant, melampolide

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Field evaluation of Australian commercial wheat genotypes for competitive traits and weed suppression

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In 2014 to 2016, replicated field trials were performed to evaluate mechanisms of weed suppression in diverse Australian wheat genotypes in moderate to low rainfall zones at Wagga Wagga and Condobolin NSW, respectively. In 2014, a total of 11 winter wheat cultivars (*Triticum aestivum* L.) representing four major breeding family lines grown in Australia were evaluated; in 2015 and 2016, 13 cultivars were assessed including the heritage cultivar Federation. At each site, crop and weed growth were monitored at various phenological stages including early season, vegetative, grain-filling, harvest and post-harvest to the crop.

Significant differences between wheat cultivar and location were observed for crop biomass, early vigour, leaf area index (LAI), weed number, weed biomass, canopy architecture and yield in each year. Differences in weed suppression were largely impacted by crop architecture and phenology early in the growing season, particularly leaf shape and the ability to achieve early canopy closure. Cultivar competitive traits were also influenced by both genotype and environmental factors, as shown by clear differences in cultivar performance, yield and weed suppression. Cultivars Condo and Espada were superior performers in terms of weed suppression and yielding potential in both locations and all years.

Our results were replicated over multiple locations and years and clearly suggest that establishment of competitive wheat cultivars can result in effective suppression of weed growth (up to 90% or greater) in the absence of post-emergent herbicides. This suggests that weed suppression may be associated with cultivar competitive ability and/or allelopathy. In addition, the choice of wheat cultivars for desired yield and weed suppression impacts the subsequent ability of the crop to successfully interfere with weed growth and can prevent future weed propagules from entering the weed seedbank. Therefore, the choice of wheat cultivar can provide cost-effective and sustainable weed management and is useful tool.

Keywords: Weed suppression, canopy architecture, phenology, yield, weed seedbank.

*Speaker

Field evaluation of Australian canola genotypes for in-crop and post-harvest weed suppression

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In 2014-2016, replicated field trials were performed to evaluate mechanisms of weed suppression in Australian canola genotypes in moderate to low rainfall zones at Wagga Wagga and Condobolin NSW, respectively. In 2015-16, a split plot design with and without trifluralin (Tri) as the main plot and cultivar as the sub plot was employed for trials; 8 cultivars including hybrid and open-pollinated cultivars were assessed. At each site, crop and weed growth were monitored at various phenological stages including early season, vegetative, grain-filling, harvest and post-harvest.

Early vigour and ability to intercept light resulted in suppression of in-crop weed growth in canola trials, as well as post-harvest weed suppression associated with remaining crop residues. GT-50, Hyola 600RR and Hyola 50 were the most weed suppressive and consistently high yielding cultivars each year in both locations. CB Taurus and GT-50 provided greatest weed suppression when only residues remained in plots for 150 days post-harvest. Pre-emergence trifluralin treatment resulted in improved crop yields in contrast to untreated plots for most cultivars, but not all. In this case, these weed suppressive cultivars possessed rapid early crop growth and vigour and reduced light at the soil surface, potentially limiting weed growth in the absence of trifluralin.

Our results show that establishment of certain canola cultivars may effectively result in enhanced in-crop and post-harvest weed suppression, with or without the use of post-emergent herbicides during the growing season, especially when considering common spring and summer annual weeds which are problematic post-harvest. Therefore, canola cultivar choice may be an economical form of weed management due to competition by the crop and possibly other factors, such as production of allelochemicals by decomposing crop residues. Further investigation is underway to determine the allelopathic mechanisms in canola, particularly the identification of allelochemical(s) associated with post-harvest weed suppression in soils.

Keywords: Weed suppression, canopy architecture, phenology, crop residue, allelochemical.

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Growth inhibition of a climbing plant by wood chips

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Climbing plants are known to cause accidents on electricity power distribution via an event of short circuit. As a tool to control the growth of these nuisance plants, we focused on the use of wood chips that contain bioactive compounds inhibiting the plant growth. In this study, the leachates of wood chips were examined whether growth the climbing plant (*Pueraria lobata*) is inhibited. A series of assay showed that the periodical exposure of the shoot of *P. lobate* to the aqueous leachates of the wood chips (*Cryptomeria japonica* and *Quercus* sp.) resulted in the growth inhibition of this climbing plant, whereas the inhibitory effect of *Juglans mandshurica* and *Pinus densiflora* was not confirmed. The bioactive compounds were extracted using a solid extraction procedure, and the analytical results showed that the phenolic and fatty acids might contribute to the observed growth inhibition by the leachate of *Quercus* sp. wood chips.

Keywords: Climbing plant, Growth inhibition, *Quercus* sp., wood chip

*Speaker

Variation in chemical composition and allelopathic potential of mixoploid *Trigonella foenum graecum* L. with developmental stages

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Polyploidy plays an important role in plant evolution and constitutes an important mechanism of diversification and genetic variations creation. This study was conducted to evaluate the influence of developmental stages (vegetative, flowering and fruiting) of mixoploid fenugreek aerial parts on their chemical composition and allelopathic potential, assessed on lettuce germination and seedling growth. Aqueous and organic extracts significantly delayed germination, reduced its rate and affected seedling growth. Ethyl acetate and methanol extracts of aerial parts harvested at vegetative stage were the most toxic for lettuce germination and seedling growth, respectively. LC-MS/MS analysis of *T. foenum-graecum* aerial parts methanolic extract showed nine different flavonol glycosides (quercetin and kaempferol glucosides). Five novel components were identified, for the first time in fenugreek aerial parts, as Kaempferol 3-O-β-D- glucopyranoside, Kaempferol 7-O-glucoside, Kaempferol 3-O-α-L-rhamnosyl (1à2) β-D-xyloside, Kaempferol 7-O-β-D- glucopyranosyl (1-4) β-D- glucopyranoside and Kaempferol 3-O-β- glucosyl (1à2) (6'-O-acetyl)- β-D- galactoside, along with other known compounds of this species. Chemical composition of aerial parts differed with the developmental stage, indeed at the vegetative and fruiting stages, analysis revealed the presence of 9 compounds as compared to only 6 compounds at flowering stage. Thus, it is necessary to follow the qualitative changes of allelochemicals production at different developmental stages to identify the most productive one.

Keywords: Fenugreek, phytotoxicity, flavonol glycosides, mixoploidy, developmental stage

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Allelopathic effect from senescent leaves of *Eugenia dysenterica* (Mart.) DC.

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Eugenia dysenterica (Myrtaceae) is an arboreal species native to the Brazilian cerrado. Being deciduous, *E. dysenterica* sheds all of its leaves during the dry season, forming a dense cover of leaves underneath the mother-plant. Recognized for its medicinal properties, the chemical composition of the leaves is already known, but only on fresh green leaves, before the senescence. Based on this, the objectives of this study were to measure the amount of senescent leaves underneath the mother-plant; the phytotoxic effect of the senescent leaves extract; and the chemical substance possibly present in this tissue. Leaves were collected from three 1 m^2 areas on the ground underneath each of the five specimens chosen. The material was dried in an incubator for 72 h, at 50°C, and weighted. The phytotoxic effect of the shred senescent leaves was measured from a 10% (w/v) aqueous extract, extracted on ultrasound, and dilutions of 5%, 2.5% and 1.25% were made. Target species were lettuce (*Lactuca sativa*) and sesame (*Sesamum indicum*), 10 seeds each on 6 cm dishes, and 1.5 mL extract. The dishes were incubated for seven days at 25°C and 12 h photoperiod. The aqueous fraction was extracted with ethyl acetate to obtain the active compound. The organic fraction was chromatographed on silica gel and analysed by NMR and MS. The plant sheds approximately 406 g/m^2 of leaves, which produce aqueous extract at 1:0.242 g. The phytotoxic effect of the extract is significant, with 80% growth inhibition in the lowest concentration (1.25%). The major compound identified was (-)-catechin, extracted from leaves by 1:0.0056 g, known for its allelopathic properties usually found on root exudates, not reported yet on senescent leaves. We conclude that *E. dysenterica* sheds an expressive amount of leaves, which have phytotoxic properties, and that (-)-catechin is the responsible for this effect.

Keywords: Brazil, catechin, Cerrado, *Eugenia dysenterica*

*Speaker

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Chemometric analysis of UV-Vis spectrum – a glance into chemical diversity of plant's extracts

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In modern research of allelopathy metabolomics is by far the most accurate, if one wants to know the mechanism of plant interactions with other organisms. Although metabolomics provides a reflection of the chemistry of a living organism at a given point in time it is both labour and time consuming [1]. To weaken those obstacles, it is usually more convenient to apply targeted metabolic profiling, which gives a focused image of plant's response to e.g. applied allelochemicals. The question is: how can we learn what chemical compounds differ in response to a given stress?
In this study, we present a simple technique, based on exploratory factor analysis (EFA), which can help to get a clear picture of where to focus from a "global" picture to a specific place where all diversity resides. As a model, UV-Vis spectra of methanolic extracts from 136 plants were analysed, but this method could also be incorporated to IR, MS or NMR spectrum data in each case providing researcher with some new knowledge. In presented case the main factor was responsible for explanation of 70,17% of total variance and resembled spectrum of light harvesting complex. This variance was probably caused by differences in chloroplasts content as leaves were harvested from different parts of plants.

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Keywords: EFA, Factor analysis, spectroscopy, metabolomics

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Evaluation of effects of *Schinus lentiscifolius* March. essential oil on *Arabidopsis thaliana* (L.) Heynh. gene expression: preliminary results

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Induction and accumulation of reactive oxygen species (ROS) is a general response of plant defense mechanisms. ANP1 (*Arabidopsis* NPK1-like protein kinase 1) is induced by ROS and activates a specific class of stress-induced genes. Previous studies demonstrated that *Schinus lentiscifolius* essential oil (SLEO) interferes on germination and seedling growth of different target species, which reflects allelochemical action on cellular levels. Therefore, this work aimed to investigate the effect of SLEO on ANP1 expression by quantitative reverse transcription polymerase chain reaction (RT-qPCR). SLEO was obtained by hydrodistillation of leaves. *A. thaliana* Col WT seeds were surface sterilized, sown in Petri dishes containing 3% sucrose, 0.8% (w/v) agar and 0.1x MS minerals, and stratified during 48 h. After emergency of primary root, 5 μL of SLEO were applied on filter paper attached to the inner face of the Petri dish. Seedlings remained exposed to volatiles during 24 h. Roots were collected (3 repetitions of 25 mg material) and total RNA was prepared using Plant RNA Purification reagent and DNAse I treatment. RNA quantification was carried out using a NanoDrop. First-strand cDNA synthesis was performed with reverse transcriptase (M-MLV) using 1 μg of total RNA. Act and eEF1α were used as normalization controls. Comparisons between treatments were performed using ANOVA and a post hoc Tukey ($P \leq 0.05$). Results showed that ANP1 gene expression were not affected by SLEO, suggesting that phytotoxic effects observed seems to be explained in terms of cellular damage rather than by induction of stress-inducible genes. Volatiles interference on ANP1 pathway cannot be discarded. Often, studies involving phytotoxic activity are considered promising only when drastic effects are observed. However, when the aim is to understand the mode of action of allelochemicals, testing lower concentrations than those that cause harmful effects may represent an interesting alternative, in particular in ANP1 pathway.

Keywords: ANP1 gene, stress, inducible gene, reactive oxygen species

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Phytotoxic effects of *Schinus lentiscifolius* March. essential oil on *Arabidopsis thaliana* (L.) Heynh. germination and seedling growth

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Schinus lentiscifolius (Anacardiaceae) is a flowering woody shrub or small tree native to South America, recognized to present a pleasant scent related to essential oil (EO) production. EO show a wide spectrum of ecological interactions including phytotoxicity. Initial evaluation of phytotoxicity involves analysis of allelochemical interference on germination and initial growth of target species. Therefore, this work investigated the phytotoxic effects of *S. lentiscifolius* EO on *Arabidopsis thaliana* Columbia wild type germination and seedling growth. EO was obtained by hydrodistillation of *S. lentiscifolius* leaves. Thirty *A. thaliana* seeds were surface sterilized and sown in Petri dishes containing 3% sucrose, 0.8% (w/v) agar and 0.1x MS minerals. Seeds were stratified and EO (5, 10, 15, 20 and 25 μL) were pipetted on filter paper attached to the inner face of the Petri dish. Control was treatment without EO application. Parameters examined included germination rate (GR) and speed of accumulated germination (AS). In post-germination assays, EO was applied after emergence of the primary root. Ten seedlings remained exposed to the EO for 7 days. Photographs were taken to measure shoot length (SL) and root length (RL) using ImageJ 1.45s software. Comparisons between treatments and control were performed using ANOVA and post hoc SNK ($P \leq 0.05$). EO inhibited all measurements. Five μL treatment reduced in 30% the GR, while 20-25 μL reduced about 50% this parameter. AS was reduced by 55% and about 70% when 5 and 10-25 μL were applied, respectively. Treatments reduced in about 60% SL and by 47% (5 μL) and up to 60% (20 or 25 μL) the RL. Results demonstrate the phytotoxic activity of *S. lentiscifolius* EO on *A. thaliana* and can be used as diagnosis in the development of molecular approaches using *A. thaliana* as model plant in studies related to modes of action of EO.

Keywords: germination rate, root length, shoot length, speed of accumulated germination

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Phytotoxicity of Aqueous Extracts of Common Weeds and Medicinal Species to Wheat: A survey

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The aqueous extracts effects of 134 plant species of 39 botanical families including mostly common weeds and wild grown species with some selected medicinal plants in Jordan were investigated on wheat (*Triticum durum* L. cv. Stork) grown in Petri-dishes under laboratory conditions. All extracts reduced wheat germination and inhibited shoot (except *Smilax aspera* L.) and root growth (except *Launaea nudicaulis* (L.) Hook. f.) of wheat seedlings compared with the control. A wide array of variations between extracts of the tested species in their effects on wheat was found. Some extracts completely prevented wheat seed germination, others severely reduced germination, inhibited seedlings growth and imbalanced shoot/root growth ratio. Extracts of *Ranunculus asiaticus* L., *Sinapis arvensis* L., *Ecballium elaterium* (L.) A. Rich., *Crambe orientalis* L., *Salvia syriaca* L., *Rumex cyperius* Murb., *Calotropis procera* Ait. Fit and *Aristolochia maurorum* L. completely prevented wheat seed germination. In contrast, extract of *S. aspera* stimulated shoot and root growth of wheat seedlings and that of *L. nudicaulis* had no significant effect on shoot dry weight but stimulated root growth of seedlings compared with the control. In general, the effects of extracts were more pronounced on roots than on shoots and the severity of the harmful effects was plant species-dependent. Differences in the effects of different extracts on shoots and roots were evident and similar differences were obtained on their effects on wheat shoot/root ratio.

Keywords: Medicinal plants, Weeds, Wheat, Phytotoxicity, Aqueous extracts

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Silicone tube micro extraction: Repeated sampling of benzoxazinoids in the root zone of winter cereals

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Benzoxazinoids are secondary plant metabolites highly abundant in the *Poaceae*, including the grain crops wheat, triticale and rye. Benzoxazinoids have been shown to be phytotoxic to a range of weed species and suppress the growth of soil fungi, such as *Gaeumannomyces graminis*, the cause of the take-all disease in cereals. Hence, an improved knowledge on the prevalence and temporal distribution of benzoxazinoids in the root zone is of major interest. In this trial, four cultivars of winter rye, winter triticale and winter wheat were sown at the end of September and mid of October 2015 in the presence and absence of weed. Plants were grown outdoor in boxes (50x34x29) in a roofed area. Silicone tube micro extraction (STME) was used for repeated sampling of compounds in the root zone of the cereals during early growth in spring 2016. In each box, seven meters of tube were placed uniformly in the root zone and compounds were sampled with 100 % MeOH at a speed of 0.1 ml/min and analysed in the multiple reaction mode with an Applied Biosystems 4500 Q Trap LC-MS/MS. Samples contained mostly lipophilic or neutral compounds and it was possible to quantify the content of benzoxazinoids benzoxazolin-2-one (BOA), 2-hydroxy-1,4-benzoxazin-3-one (HBOA), 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one (HMBOA) and 6-methoxy-benzoxazolin-2-one (MBOA) and the phenozaxinones 2-amino-phenoxazin-3-one (APO) and 2-amino-7-methoxy-3H-phenoxazin-3-one (AMPO). Samples taken in April contained more of the microbial degradation products APO and AMPO and had generally higher benzoxazinoid concentrations, compared to samples taken in March. There was no difference between benzoxazinoid concentrations according to sowing date and presence or absence of weeds. In summary, STME is a promising method for repeated sampling of non-glycosylated benzoxazinoids in the root zone of winter cereals.

Keywords: Silicone Tube Microextraction, LC, MS/MS, *Triticum aestivum*, Triticosecale, Secale cereale, Cereals, Benzoxazinoids

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Antifungal and antiparasitic activities of essential oils and hydrosols of two medicinal plants *Pistacia lentiscus* and *Marrubium vulgaris* against the pests of the olive tree.

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Olea europea is one of the most characteristic trees in the Mediterranean region. *Bactrocera oleae* is considered one of the most dangerous pests among insect pests, also transmits fungus causing fruit rot

We studied the infestations of the pest *Bactrocera oleae* as well as pathogenic fungi.

In this work we are interested in the biological or rather natural control. The study of the infestation of the olive by *Bactrocera oleae* and the pathogenic flora associated with it on the one hand. The phytochemical study of essential oils and hydrosols of medicinal plants *Pistacia lentiscus* and *Marrubium vulgaris* on the other hand made it possible to obtain results to know the effectiveness of these species against the pests of the olive tree.

The antiparasitic and antifungal activities of the essential oils and hydrosols of the two plants *Pistacia lentiscus* and *Marrubium vulgaris* confirmed their efficacy and showed their powers of insecticidal and fungicidal toxicities on all the strains tested.

A 100% mortality of the adult insect at low doses of 0.8 μL and 5 μL of the essential oils of *Pistacia lentiscus* and *Marrubium vulgaris* respectively. And an important mushroom inhibitory activity with a concentration of 1 μL/L of essential oil of *P. lentiscus* and 0.5 μL of *Marrubum vulgaris* oil is inhibited with a 60% greater percentage of *Fusarium* and *Aspergilus niger*.

The lethal dose LD50 is very low, 0.34 μL for P. lentiscus and 3.47 μL for *M. vulgaris* confirming their toxicity against pests

The hydrosols of the plants studied showed an important fungicidal effect by in-vivo olive tests, which allows us to propose them as biopesticides, which is of great interest for agriculture, environment.

Keywords: *Olea europea*, *Bactrocera oleae, Pistacia lentiscus*, *Marrubium vulgaris*, mushrooms, essential oil, Hydrosol.

*Speaker

Allelopathic Effect of *Populus deltoides* on growth and yield of wheat crop under Agroforestry Ecosystems in Northern India

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The allelopathic effect of decomposed litter from trees inter-planted with crops is a key problem in the intercrop agroforestry business that could influence the economic benefits and sustainable development of ecoagriculture. Poplar is a prominent exotic species farmers like to plant along with crops. *Populus deltoides* is the most widely planted species in India. It is planted in plains of North-West India, i.e., Western Uttar Pradesh, Punjab and Haryana and to some extent in the outer plains of Uttaranchal and Himachal Pradesh. It has very high growth rate (M.A.I of 20 to 25 m^3/ha/yr). A lot of tree crop combinations are practised by the farmers in poplar based agroforestry system. Wheat during the winter season is most widely cultivated crop in the interspaces of poplar. However, variable performance of wheat varieties in association with various tree species has been reported by many workers. We review the evidence for allelopathy in poplar based agroforestry systems, and in several studies it has been observed that aqueous extracts of poplar leaves adversely affected the germination and growth of some wheat varieties at high extract concentration. Thus, such studies may help in identifying suitable wheat variety and amount of leaf litter to be retained in the field for higher productivity in poplar based agroforestry system. In another study, it has been reported that the aqueous extracts of partially decomposed poplar leaves retarded the germination and growth of wheat. Germination and growth of wheat were suppressed by extract of soil sampled at a distance of 0-3 m with 3 year old plantation, which further intensified and extended up to 6 m in fourth year. Thus, adverse effect of aqueous extracts of soil underneath poplar tree indicated the accumulate ion of allelochemicals in the soil and the effect was more pronounced with advancement of tree age.

Keywords: Allelochemicals, agroforestry system, extract concentration, *Populus deltoides*, wheat varieties

*Speaker

Can chemical cues drive trophic interactions in soil food web?

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Despite their important role in the litter decomposition process and nutrient cycling, soil arthropods and their trophic interactions in terrestrial ecosystems are still poorly understood. Some soil arthropods are detritivores (springtails) and participate directly to the litter decomposition process and others are predators (mites) and regulate the detritivores populations. To our knowledge, few studies attempted to decipher which sense (such as the sight or the smell) could be use by a springtail to detect a litter of good quality, and none of these studies attempted to decipher which sense could be use by a predator to detect a prey presence. In the present study, olfactory bioassays were conducted in order to test (i) the attraction behavior of two springtails (*Folsomia candida* and *Lepidocyrtus lanuginosus*) in response to the chemical cues from three contrasting litter types (*Cotinus coggygria*, *Pinus halepensis* and *Quercus pubescens*) and (ii) the attraction behavior of one predatory mite (*Stratiolaelaps scimitus*) in response to the chemical cues emitted by his prey (*Folsomia candida*). The detection of (i) the litter chemical cues by the two springtails or (ii) the *F. candida* chemical cues by *S. scimitus* was tested in a Y-shaped olfactometer made of a glass tube. *F. candida* ($P = 0.035$) and *L. lanuginosus* ($P = 0.015$) were only attracted by the chemical cues emitted from *P. halepensis* litter. *S. scimitus* tended to be attracted by *F. candida* chemical cues ($P = 0.052$). These first results suggest that springtails could detect and distinguish litter chemical cues and that predatory mites could potentially detect prey chemical cues. However, it seems necessary to improve these results by testing other individuals and by analyzing the chemical cues released by the litters and *F. candida*.

Keywords: Y tube olfactometer, mesofauna, soil food web, chemical cues, mediterranean litters

*Speaker

Detrimental allelopathic effects of glucosinolate breakdown products on Fabaceae

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Glucosinolates (GSLs) are a class of secondary metabolites with allelochemical properties found in members of the Brassicales. GSLs are relatively nontoxic, but after hydrolysis of the sugar moiety by myrosinase (thioglucosidase), the compounds are degraded. The breakdown products are highly bioactive, affecting germination, the growth of neighboring plants and can have antimicrobial effects. The GSLs and the myrosinases constitute a natural plant defense system against diverse pathogens due to their allelopathic properties. Potential negative long-term effects of the highly bioactive break-down products on beneficial soil microorganisms have not been studied sufficiently. However, this would be worthwhile with regard to a sustainable agricultural use of Brassicaceae-dominated producing areas for the subsequent cultivation of different crops, especially legumes, which can establish a symbiotic relationship with specific nitrogen-fixing bacteria. In order to address this issue we performed bioassays employing *Rhizobium leguminosarum* and *Mesorhizobium loti*, both of which are nitrogen-fixing bacteria belonging to the *Rhizobium* species. Analysis showed that both rhizobacteria are susceptible to glucosinolates. Likewise, the nodulation rate of the two legumes *Pisum sativum* and *Lotus japonicus* was significantly decreased when exposed to glucosinolates. Furthermore, the germination and growth of *L. japonicus* and *P. sativum* were drastically impaired after exposure to GSLs from rapeseeds as well as after exposure to Brassicaceae root exudates. The current findings provide evidence for the extensive negative effects of GSLs on the symbiosis of legumes with Rhizobia as well as on germination and growth of different legumes.

Keywords: Glucosinolates, *Rhizobium leguminosarum*, *Mesorhizobium loti*, nodulation

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Exposure to BOA-OH Isomers Elicits Zone Dependent Reactions of Maize Roots

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The response of maize exposed to benzoxazinoid derivatives is of special interest for estimating possible autotoxicity because these compounds are increasingly in the focus for agricultural uses. Except for BOA-6-OH, hydroxylated benzoxazolinones are commercially not available. Therefore, all isomers of hydroxylated benzoxazolinones were synthesized to compare their impacts on maize roots, e.g, and their ability to induce oxidative stress. We emphasized on reactions of the benzoxazinone releasing root hair zone, other root zones and on the maize endophyte *Fusarium verticillioides.* Analyses of root hair extracts and of washes of the root hair zone indicate that root hairs release the major portion of DIMBOAGlc/DIMBOA, either actively by exudation or during necrosis. Root hairs are sites of an early reaction which resembles a first phase oxidative burst indicated by a weak up to extremely strong catalase activity. An involvement of colonizing microorganisms is suggested. Root surface peroxidase assay expose youngest root zones, the tips and the bases of emerging roots as sites of high peroxidase activity. Within the maturation zone dark brown spots and rings appeared at areas where secondary roots emerged due to BOA-OH polymer production by peroxidase activity. The polymers may plug possible site of infections with pathogens. The intensity of spot development depends on the position of the OH group of the BOA-OH isomers. Maize endophyte *Fusarium verticillioides* did not degrade or modify any of the isomers, but amounts of fusaric acid released into the culture medium were reduced. The growth of the fungus was not inhibited.

Keywords: Zea mays, hydroxylated BOA isomers, root hair BX exudation, oxidative stress

*Speaker

The whole is greater than the sum of its parts: *Daphne gnidium* extracts and isolated pure compounds differently affect the receiving plant *Aegilops geniculata*

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Synergistic effects in allelopathy have been speculated and seldom demonstrated [1]. In the context of a large screening on phytotoxicity of plant species from Mediterranean area in the framework of allelopathic interaction studies, a donor plant species, *Daphne gnidium* (Thymeleaceae), showed peculiar effects on *Aegilops geniculata*. *Daphne gnidium* phytotox-icity on several plants has been reported [2]. Its extract, tested according to an established NMR based metabolomics method [3], showed strong inhibitory activity on the receiving plant. NMR analysis of the donor plant extract allowed to identify the putative allelochemicals as coumarin derivatives already reported from this species (daphnetin, daphnin and daphnetin-8-glucoside) [4]. Metabolomics analysis of roots and shoots of receiving plants after treatment with the extract showed the presence of daphnetin (the aglycone) in both plant organs. In order to prove their activity, pure compounds were tested at different concentrations (mM, μM, nM). The observed activity was significantly decreased even at the highest concentration tested, if compared to the extract. Furthermore, metabolomics analysis was crucial to point out a com-pletely different effect exerted by the extract and the pure compounds. When the plants were treated with pure compounds neither these nor their metabolites were detected in the shoots of the receiving plant, while a complex mixture of their derivatives was observed in the roots. This let to hypothesize that the receiving plant is able to metabolize the toxic compounds, but not in the presence of other components in the donor extract hindering this ability. Further studies are in progress in order to identify the responsible metabolites and clarify the mechanism.

Keywords: *Daphne gnidium*, *Aegilops geniculata*, NMR based Metabolomics, Mediterranean area, Synergistic effects

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*Speaker

Effects of Different Soil Amendments on Yield and Rhizosphere Microenvironment of *Panax notoginseng* in Continuous Cropping

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The yield and quality of *Panax notoginseng* suffers a significant decline under consecutive monoculture. In this study, effective microbes (EM) and *Trichoderma* (TRI) were repectively used to amend the rhizosphere soil. The deep pyrosequencing and denaturing gradient gel electrophoresis (DGGE) was applied to characterize the composition and structure of soil microbial community under monoculture and different amendments. The results showed compared with the 1st-cropping planted (NP), 2nd-cropping monoculture (SP) of *Panax notoginseng* led to no harvest and resulted in a significant increase in pathogen but a decline in beneficial microbes. The EM and TRI amendments could promote the rate of emergence, rate of reservation, and yield by regulating rhizosphere microbial community. The results indicated the fungal structure of rizosphere soil in EM and TRI treatment was close to that in new planting. The bacterial structure of rizosphere soil in TRI treatment was close to that in new planting. The bacterial structure of rizosphere soil in EM treatment was close to that in second planting. So the EM and TRI amendments could improve the rhizosphere microenvironment. However, the yield of EM and TRI treatments was respectively 15.41% and 29.90% significantly lower than of NP. Therefore, we need further study the ecological process of rhizosphere microbial catastrophe mediated by the monoculture of *Panax notoginseng* plants and create its regulation strategy.

Keywords: *Panax notoginseng*, Consecutive monoculture problem, Soil amendment, Rhizosphere microenvironment, Rhizosphere microbe

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A field assessment of the allelopathic potential of *Eucalyptus saligna* leaf litter on grassland species

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In South Brazil, large areas of grasslands have been replaced by eucalypt plantations. In laboratory assays, *Eucalyptus saligna* SM. leaf litter has shown phytotoxicity. Vegetation is scarce under *E. saligna* plantations, which may be related to allelochemicals released from its leaves. We investigated effects of *E. saligna* leaf litter on establishment of grassland species and if these effects are related to allelopathy. In an *E. saligna* plantation, we removed vegetation and litter and established groups of plots (0.8 m^2), with four repetitions per group. The groups consisted in addition of eucalypt leaf litter at full quantity (eucalypt leaves) - mean quantity found in plantations; leaf litter at half quantity (half eucalypt); absence of litter (control); and artificial leaves at full quantity (artificial leaves). After one year, we characterized vegetation in the plots. We assessed effects in species richness, diversity, plant cover, biomass and height. Plant establishment was slow, and plant cover reached maximum of 50% per plot. Plant richness was higher in the control than in all other groups. Shannon's diversity index, vegetation height, total plant cover, as well as graminoid, forb and bryophyte cover were lower in eucalypt leaves and artificial leaves plots than in control. For most of parameters, half eucalypt leaves did not differ from the other groups. Our results show that *E. saligna* leaf litter, at least partly, causes suppression of grassland vegetation. However, artificial leaves cause similar effects, which are not observed when litter quantity is reduced. Therefore, allelopathy does not seem to play a relevant role in plant inhibition.

Keywords: Brazilian Pampa, eucalypt plantation, plant community.

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Impact of environmental stress on production of bioactive metabolites in the invasive Australian weed *Echium plantagineum*

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Echium plantagineum L., a highly invasive weed in Australia, possesses a unique combination of chemical and physical defenses against pathogens, herbivores and potentially other plants. It often establishes in dense monocultures and was reported to upregulate production of secondary metabolites in response to exposure to certain plant stressors. Allelochemicals of particular interest in this species include toxic pyrrolizidine alkaloids (PAs) and antimicrobial and phytotoxic naphthoquinones (NQs), but limited information has been reported on the regulation of these metabolites. Therefore, the impact of simulated drought, herbivory, temperature and plant competition on the production of NQs and PAs was examined in controlled environment. Following stress treatment, plant parts were sequentially harvested and extracted in methanol/ethanol and PAs and NQs were profiled using UHPLC-ESI-QTOF (Agilent, USA). Data was analyzed using Mass Hunter software (Agilent, USA). NQs production in roots was rapidly elicited in response to drought, herbivory and high temperature, whereas PAs production was impacted mainly by water withholding. Deoxyshikonin and shikonin, key precursors in the NQ biosynthetic pathway, significantly increased in abundance (up to 3 fold levels) within 3 days following water withholding. However, prolonged drought resulted in reduced abundance of PAs and NQs. Exposure to elevated temperatures over a 10 week period resulted in significant upregulation of the shikonin biosynthetic pathway. NQ upregulation was also observed under intraspecific plant competition. In contrast, simulated herbivory rapidly resulted in elicitation of selected NQs and PAs, within 6 hours post-treatment. Differential regulation of PAs and NQs in response to specific plant stressors suggests that biosynthesis of these metabolites was either readily up- or down-regulated depending on the plant stressor and length of exposure to stress. As NQ concentrations increased in roots, PA levels typically decreased in shoots with exposure to stress, suggesting a carbon trade-off mechanism regulating secondary product metabolism above- and belowground.

Keywords: Naphthoquinones, pyrrolizidine alkaloids, shikonins, Paterson's curse, weed

[*]Speaker

Canavanine - a nonproteinogenic amino acid impacts growth of tomato roots by modification of ROS/RNS metabolism

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Canavanine (CAN), structural analog of arginine (Arg) is a non-protein amino acid of high toxicity produced by legumes. CAN is mostly present in seeds e.g. in *Canavalia ensiformis* (L.) DC., but also in leaves of *Sutherlandia frutescens* (L.)R.Br. It is abundant in seeds and sprouts of alfalfa (*Medicago sativa* L.). Harmful effect of CAN in other organisms, mostly herbivores, but also in plants is connected with its primary mode of action - incorporation into proteins in the place of Arg leading to formation of aberrant proteins. As an antimetabolite of Arg CAN may be considered as a useful tool for modulation of the endogenous level of RNS in the cells. The aim of our work was to investigate indirect action of CAN in plants. As a material we have used young seedlings of tomato (*Solanum lycopersicum* L.) exposed to 50 μM CAN for 3 days. CAN totally inhibited elongation growth of tomato roots. It was accompanied with alterations in root diameter and amount of root primordia, typical symptoms of stress induced morphogenic response. Changes in root growth resulting from tomato seedlings' supplementation with CAN correlated with induction of oxidative stress manifested as over-production of ROS, lipid peroxidation, and enhanced activity of catalase, dismutase, peroxidase and NADPH oxidases. Moreover, high level of protein carbonyl groups was observed suggesting oxidative damage of the tissue. To build up a comprehensive overview of ROS action in CAN treated roots we visualized also protein ubiquitination. Increased root diameter matched well to elevated concentration of auxins (IAA) and decline in NO production in root apex.
We may conclude that secondary mode of action of CAN in plants is based on disturbances in ROS/RNS production and metabolism.

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Keywords: canavanine, oxidative stress, protein carbonylation, root growth

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Weed infestation in peppermint or cereal cultivations depending on the type of soil and selected chemical and biological soil properties

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Laboratory experiments show, that water extracts of peppermint (*Mentha x piperita*) as well as the essential oil display allelopathic potential , including antibacterial properties. Mint (*M. suaveolens* and *Mentha x verticillata*) plants also act as a green manure for weed suppression in maize. The above results suggest, that cultivation of peppermint may affect the soil biota through allelopathic interferences, and thus we hypothesize, that it may influence the number and composition of weeds.
This study aimed at comparison of the weediness of peppermint or cereal fields, if the fore-crop was cereal or peppermint respectively, depending on the selected biological and chemical soil properties.

Field analyses were carried out in Michalów (southern Poland) during summer 2012 and 2015, at 45 farmers' fields, where organic peppermint has been grown for the last 50 years. The analyses were carried out in the fields located on two types of soil: 1) brown soil, 2) rendzinas, and in two types of cultivations with different fore-crops: peppermint-cereal, cereal-peppermint and peppermint-peppermint. On each field, weed surveys were taken with the assessment of weed cover according to Braun-Blanquet scale. Also, on each field soil samples were taken. The soil was transferred to the lab, to assess: soil dehydrogenase, total content of water-soluble phenolic compounds and the content of mesophilic bacteria, fungi and actinomycetes. The results were tested statistically using multivariate analysis DCA by CANOCO.
The multivariate analysis showed, that weed infestation of cereals was similar to this of peppermint on rendzinas, and there was no clear correlation with any of the studied soil characteristics. On the contrary, weed infestation of peppermint and cereals was different on brown soil, and that was mostly correlated with soil pH and the activity of soil dehydrogenase as well as the number of soil bacteria.

Keywords: dehydrogenase, mesophilic bacteria, actinomycetes, soil fungi, detrended correspondence analysis

*Speaker

Allelopathic seed meals as an option for weed control

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Species from *Brassicaceae* botanical family as well as *Fagopyrum esculentum* are a rich source of allelopathically active substances, which can be used for weed control, i.e. as seed meals. This experiment aimed at studying the allelopathic potential of meals from seeds of mustard, oilseed rape and buckwheat and from pods and shoots of wild radish on growth of corn and weeds.

Two series of pot experiment were set up in 2014. Each meal was separately mixed with soil in the amount of 2% and 4% (w/w) and placed in pots. Control pots did not contain meals. On the next day seeds of redroot pigweed, barnyard grass and corn were sown into pots. Germination and growth of plants in pots was recorded. The relative chlorophyll content in leaves was measured. After 2 months, the plants were cut and their length was recorded. Their shoot and root biomass was weighed.

In 2015, a field experiment was set up on sandy soil. Seed meals were mixed in Spring with the upper layer of soil, at a rate of 9.4 t ha^{-1} and 18.8 t ha^{-1} for buckwheat and 10.9 t ha-1 and 21.8 t ha^{-1} for mustard. Control plots were not treated with meals or herbicide sprayed. On the same day corn was sown. Weeds were recorded during the corn growth and at harvest. Both, in the pot experiment as well as the field experiment the meals reduced weed biomass in a dose-response manner. Mustard meal reduced growth and biomass accumulation of weeds and corn the most. Mustard meal reduced also the relative chlorophyll content in the leaves of both, corn and weeds. In a field experiment buckwheat meal did not influence negatively the growth of corn. Summing up, meals of allelopathic plants display a good potential for weed control.

Keywords: mustard, buckweat, corn, weed growth

*Speaker

Rye (*Secale cereale* L.) cover cropping as an useful tool for a sustainable weeds control strategy

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Cover cropping eases weed control maintaining an high agroecosystems biodiversity and a low environment impact. The inclusion of cover crops within conventional rotations designs more sustainable agroecosystems, which provide many additional ecosystem services such as improvement of soil and water quality, increase of nutrient cycling, pest control, and cash crop productivity. Rye (*Secale cereale* L.) is one of the best cool-season cover crops, widely used for its soil and climate adaptability, high biomass production, early termination, and exceptional weed suppression potential. Rye mulch has both physical and allelochemical mechanisms to suppress weeds. Exploitation of allelopathy can contribute to reduce herbicides use, crop production costs and environmental risks.

The weeding potential of 8 rye varieties was investigated in a field trial, both during rye growing and after mulch preparation at 2 termination stages: (T1) earing, and (T2) ten days later. Rye biomass and allelochemicals (Benzoxazinoids and Flavonoids) content varied with termination stage. At T2, biomass (on d.m. basis) was on average 7.4 Mg ha-1 (+73% compared with T1) with differences among varieties ($P \leq 0.001$). Mean Benzoxazinoids concentration was 219 μg g^{-1} d.m. at T2 stage (+9% compared with T1) with differences among varieties ($P \leq 0.01$).

Flavonoids content slightly decreased from 23% (T1) to 21% (T2). Weeds suppression, correlated to biomass production and allelochemicals content, increased from 19% (T1) to 31% (T2) with differences among varieties ($P \leq 0.001$), at both the two termination stages.

In a greenhouse pot trial, the same rye mulches significantly reduced the germination of redroot pigweed ($P \leq 0.01$), purslane ($P \leq 0.001$) and lambsquarter ($P \leq 0.001$) seedlings, but not velvetleaf. This fact suggests the presence of detoxification mechanisms in velvetleaf, promoted by an associate microbial community. As a consequence, the inhibition of these microorganisms could be the crucial way to pursue a sustainable control strategy versus resistant weeds.

Keywords: cover cropping, rye, allelopathy, weeds control, sustainable agroecosystems, sustainable agriculture

*Speaker

Antimicrobial activity of essential oils of the genus *Thymus numidicus* and *Thymus algeriensis* against the bacterial strains responsible for nosocomial infections

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The human organism has a complex defense system that allows it to encounter or harbor pathogens (viruses, bacteria, fungi etc.) without allowing them to invade its tissues (Hill et *al*, 1964). However, under certain conditions The latter may be responsible for more or less serious diseases (Heart *et al* 2006, Persidis et al 1999). The management of these diseases differs depending on the nature of the infectious agent involved: viruses, fungi, parasites or bacteria. Nosocomial infections are acquired in a care facility, and appear after 48 hours of admission, the severity of these infections lies in the therapeutic difficulties they pose. This is because they are resistant to commonly used antibiotics. The use of natural resources, in general, and medicinal plants in particular, becomes one of the most important and interesting avenues for exploring new and more effective antibacterial products (Wright *et al* 2007).

Our work consists in studying the antimicrobial activity of the essential oils of *Thymus numidicus* and *Thymus algeriensis* with respect to some bacterial strains responsible for nosocomial infections, in particular: *Klebsiella oxytoca, Acinitobacter baumanii, Satphylococus ATCC 25923 and Pseudomonas ATCC 27853.*

The results obtained are very satisfactory, the bacterial strains tested have proved to be very sensitive with our essential oils, with inhibition diameters of 26 to 28 mm for the *Kelbsiella.o* strain, 32 to 35 mm for *Acinitobacter.b* And 38 to 54 mm for *Satphylococus ATCC 25923.* This activity results in the chemical composition of the essential oils which is rich phenolics, the latter have a broad spectrum of antimicrobial activity resulting in irreversible lesions on bacterial membranes (Trombetta *et al* 2002, Setrani *et al* 2008).

Keywords: *T. numidicus*, *T. algeriensis*, huiles essentielles, GCMS, nosocomial infections

*Speaker

Phytotoxicity of flavones and chlorogenic acids from *Moquiniastrum floribundum* (Asteraceae)

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Asteraceae is one the largest families of Eudicots comprising several species used for economic and medicinal purposes due the production of various bioactive metabolites[1]. Among the 1700 genera of the family, *Moquiniastrum* is restricted to South America and comprises 21 species, 12 of them endemic of Brazil1. The aim of this work is to report the phytochemical study of polar phases from *Moquiniastrum floribundum* (Cabrera) G. Sancho and the phyto-toxic activities of their isolated compounds. Aerial parts (421.7 g) were extracted with hexane and subsequently with methanol. Methanol extract was partitioned successively with hexane, dichloromethane, ethyl acetate and butanol. The partition phases were subjected to silica gel and preparative HPLC, yielding the compounds **1-8**. The structures were elucidated by ^{1}H and ^{13}C NMR, gHMBC and comparison with literature data. Thus, the compounds were identi-fied as two flavones: genkwanin **(1)** and hispidulin **(2)**, and six chlorogenic acid derivatives: 4,5-**(3)**, 3,5- **(4)** and 3,4- **(5)** dicaffeoylquinic acids, 3,5-dicaffeoylquinic acid methyl ester **(6)**, 3,4,5-tricaffeoylquinic acid methyl ester **(7)**, and 5-caffeoylquinic acid **(8)**. Isolated compounds were assayed on elongation of etiolated wheat coleoptile at concentrations from 10-0.1 mM. The higher concentration of compounds **2** and **6** inhibited, respectively, 50% and 60% of coleoptile elongation. The lowest IC50 value was observed for compound **6** (0.29 mM) followed by compound **2** (10.4 mM). Comparing the two flavones, the presence of an additional methoxyl group in **2** could be responsible to the higher activity observed. For chlorogenic acid derivatives, the methyl ester of quinic acid and the presence of free hydroxyl group in C4-position contributed to the highest phytotoxicity of compound **6**.

[1] Sancho, G.; Funk, V.A.; Roque, N. *Phytotaxa* **2013**, *147*, 26.

Keywords: Allelopathy, caffeoylquinic acid derivatives, coleoptile, flavonoids.

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Are red seaweeds compounds biostimulators of lettuce?

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Marine algae have been used for a long time as biostimulants in agriculture with very promising results. In the present work, extracts from two agar-producing red seaweeds (*Gracilaria caudata* J. Agardh and *Gracilaria domingensis* (Kutzing) Sonder ex Dickie) were assayed to evaluate their effect on germination rate and lettuce initial growth. The bio-guided isolation indicated that aqueous extracts and nonpolar extracts, obtained with hexane or dichloromethane, were biologically active. Palmitic acid, the major compound of the nonpolar extracts, showed significant stimulant activity, increasing 83% of lettuce root length in relation to negative control at 0.5 mg.mL-1. Furthermore, stimulant effect of aqueous extract might probably be related with the presence of agaran, a typical sulfated polysaccharide produced by red seaweeds, which is almost the exclusive constituent of this extract. The polysaccharide obtained from *G. domingensis* promoted 60% of increase in lettuce root length against 40% by *G. caudata* (both at 1 mg.mL^{-1} of extract). The difference between two polysaccharide is probably related with the different content of sulfate groups (two-fold higher for polysaccharide from *G. dominguen-sis*). These last results indicated that modifications in agaran structure could lead to different response intensities depending on the level and the type of polysaccharide substitution.

Keywords: Biostimulator, *Lactuca sativa*, Palmitic acid, red alga, Sulfated polysaccharides

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Role of terpene emission in chemical plant-plant interactions under water deficit between Mediterranean forest species

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Volatile terpenes are well known to be involved in the response of plants to abiotic stresses as well as biotic interactions. In forest ecosystems, terpene emitters can cohabit, in the same place, with non-emitter species. The influence of terpene emissions of a species on a non-emitter species are not really known. On the one hand, non-emitters could benefit from terpene emissions of their neighbors as these BVOCs could allow them to improve their physiological performances under abiotic stress. On the other hand, terpenes could have negative allelopathic effect on their development and growth.
Mediterranean forest ecosystems host strong terpene emitters such as *Quercus pubescens* (isoprene emitter), and *Cotinus coggygria* (monoterpene emitter) but also non-emitter species (e.g. *Acer monspessulanum*). In order to analyze plant-plant interactions mediated by volatile organic compounds, we performed two laboratory experiments to evaluate the effect of isoprene and monoterpenes from *Q. pubescens* and *C. coggygria* respectively on *A. monspessulanum* under three water stress treatments. The effect of terpene emissions was assessed in terms of gaz exchange (net photosynthesis, stomatal conductance, water use efficiency) phenology and proportion of reactive oxygen species (ROS) with a focus on hydrogen peroxyde (H_2O_2).
Our results show that monoterpenes from *C. coggygria* tended to delay foliar development in *A. monspessulanum.* Also, fumigation of *A. monspessulanum* with isoprene emissions allowed a slight increase of both, net photosynthesis during moderate water stress and water use efficiency in *A. monspessulanum* during severe water stress starting at 30∘C. Finally, isoprene-fumigated trees showed a smaller proportion of H_2O_2 compared to trees that did not receive isoprene. These results indicate that *Acer monspessulanum* is likely to benefit from isoprene emissions from neighboring *Q. pubescens* - the highest isoprene emitter in the Mediterranean Basin -which favours its photosynthetic performances during abiotics stresses.

Keywords: Isoprene, monoterpenes, ecophysiological traits, phenology, *Acer monspessulanum*, oxidative stress

*Speaker

Variability in regeneration of the European Beech (*Fagus sylvatica*) : Interactions between allelopathy and climatic conditions

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The influence of temperature and water availability on early stages of tree recruitment was investigated on germination, mortality and Fagus sylvatica growth. This tree forest species is sensitive to climate changes, as shown by several niche-based models. Integrating a community ecology scale in our investigations, we hypothesised that chemical interactions of several dominant forest species may interact with the climatic variable susceptible to affect Fagus Sylvatica recruitment.
In a controlled experiment, leaf extracts of evergreen (*Hedera helix*, *Ruscus aculeatus*, *Pinus pinaster*) and deciduous species (*Fagus sylvatica*, *Quercus robur*) were tested on beech seeds. Most of these forest species were chosen because they were dominant in cover in vegetation surveys performed at the warm margin of the beech distribution range in France. *Pinus pinaster* was absent from these data, but was locally dominant on the border of some beech forests remaining in south-western lowlands of "Landes de Gascogne" forest (France).

Three factors, "Temperature", "Watering" and "Allelopathy", were studied at several stages of development of beech seeds and seedlings. The allelopathic effect influenced mainly seed germination. It was in interaction with temperature for the seedlings growth. Contrasted effects were observed depending on the donor species. *Hedera helix* had a negative impact on germination and was significantly different from *Quercus robur* effect that tends to be positive. *Pinus pinaster* leaf extract had a negative impact on growth in warm condition.

Our results suggest a limitation of beech recruitment in warm margin of distribution area, and a modulation of recruitment success according to plant neighbourhood type, with especially a negative impact of the evergreen species *Hedera helix* and *Pinus pinaster*.
We concluded that the inclusion of allelopathic effect from plant communities surrounding our focal species may be necessary to understand the dynamic of recruitment of tree species in a context of climatic changes.

Keywords: below, ground vegetation, regeneration, allelopathy, temperature, drought

*Speaker

Comparison of Australian and African Sorghum root exudates: A case of long-term evolutionary adaptation?

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Sorghum progenitors were separated into three distinct regions in Australia, Africa and Asia approximately 96 million years ago (Ma) as a result of continental separation and drift. Evolutionary adaptation and speciation resulted in 25 recognized modern *Sorghum* spp., including 17 Australian and 5 African endemic species that are today concentrated in the humid coastal area of Australia and arid inland Africa. From an evolutionary perspective, African *Sorghum* spp. are more successful in terms of distribution when compared to Australian *Sorghum* spp. African *Sorghum* spp. include two well-studied examples: the fully sequenced *S. bicolor* and the noxious weed *S. halepense*. Both species produce large quantities of the potent allelochemical sorgoleone exuded specifically from their root hairs. Compared to African *Sorghum* spp., the majority of Australian *Sorghum* spp. are distributed in small isolated niches around the northern coastal area and their root architecture and root exudation patterns have not been studied. Seeds of Australian and African sorghum were provided by the Australian Grains Genebank and were germinated at 28 ∘C (24 h dark) for 7 days. Upon harvest, root tissues were weighed, observed for exudation microscopically and extracted by swirling in methanol for 15 minutes followed by evaporation under N_2 gas at 40°C. Metabolic profiling of root exudates was performed using HPLC QToF on a C18 Agilent poroshell column (ACN: H_2O gradient). Sorgoleone production among species was compared and contrasted; results suggest that certain Australian genotypes produce unique metabolic profiles with little to no sorgoleone. Differential sorgoleone production may be the result of long-term evolutionary adaptation to diverse climates after continental drift 96 Ma. Study of sorgoleone biosynthesis and gene expression in Australian and African *Sorghum* spp. will potentially assist in grain sorghum improvement, as well as our understanding of plant defensive strategies with respect to long-term evolutionary adaption processes.

Keywords: Australian native sorghum, sorgoleone, HPLC, evolutionary adaptation

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Phytotoxic activity of two medicinal plants *Cassia absus* and *Nigella sativa* seeds and aerial parts

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For several years, scientists focused their attention on secondary plant products to develop bio-herbicides as an alternative strategy for weed control. The present study was conducted to assess the phytotoxic potential and the phytochemical composition of two medicinal plants *Cassia absus* and *Nigella sativa*. Seeds and dried aerial parts were extracted successively with petroleum ether, chloroform and methanol. Growth bioassays were carried out using lettuce (*Lactuca sativa* L.) as the test species. Three different concentrations were used to evaluate the phytotoxic activity (1, 3 and 6 mg/ml). Total phenolics, flavonoids, and proanthocyanidins contents of organic extracts of *Cassia absus* and *Nigella sativa* were estimated by colorimetric methods. Our results showed that *Cassia absus* organic extracts caused significant reduction of root growth of *Lactuca sativa*. Seed extract was more effective than aerial part extract. The methanolic seed extract exerted a strong phytotoxic effect on seedling growth, followed by petroleum ether extract of the aerial part. The phytochemical investigation showed that among the organic extracts, methanol extracts of seeds and aerial parts contained the highest amounts of total phenolics and proanthocyanidins. Regarding *Nigella sativa,* the phytotoxicity study revealed that seeds and aerial parts negatively affected the growth of lettuce plants. The degree of inhibition was largely dependent on the developmental stage at which material was collected and the nature of extracting solvent. The methanolic extract of aerial parts harvested at the vegetative stage was the most active to seedling growth of lettuce. The phytochemical investigation showed that among the organic extracts, methanolic extract from aerial parts harvested at fruiting stage contained the highest amounts of phenolic and flavonoid contents.

Keywords: *Cassia absus*, Tunisian *Nigella sativa*, Phytotoxicity, developmental stages, phytochemicals.

*Speaker

The first phytochemical and biological study of *Lobularia maritima*

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Lobularia Maritima, known as sweet alyssum, is a perennial plant species specific to the Mediterranean region and used in traditional medicine to treat abdominal pains, cough and cold. Few works were achieved on this plan Studies on *L. maritima* are mainly focused on cultivation and *in-vitro* rapid propagation. Phytochemical composition and biological activities of this species were not yet conducted. In this work, we investigated polyphenols, flavonoids and tanins content of *L. maritima*'s flowers, roots, stems and leaves using GC-MS analysis. Moreover, plant extracts were tested for their antioxidant activity using DPPH scavenging assay. Antimicrobial and antifungal activities were investigated throughout RDA assay. Enzymatic inhibition assay was performed on collagenase and ACE activities. *L. maritima* tested fractions showed positive results for total phenolic content where roots contain the highest amount of polyphenol. Leaves contain the highest amount of flavonoids and flowers as well as stems contain the highest amount of tannin. The tested fractions exhibited important antioxidant activity measured by DPPH free radical-scavenging ability in comparison to BHA activity. Antimicrobial and anti-fungal screening of the different fractions against gram positive and gram negative bacterial strains shows promoting results in almost all the plant fractions. Collagenase inhibition assay of steams extract exhibited the highest enzyme inhibition rate in comparison to the other extracts. Mass spectrometry coupled to gas chromatography– analysis allowed the identification of the most important compounds endowed with antimicrobial activity.

Keywords: *Lobularia Maritima*, traditional medicine, polyphenols, flavonoids, tanins, GC-MS, antioxidant, antimicrobial, antifungal, collagenase inhibition, ACE inhibition

*Speaker

Does climate change affect plant-plant interactions in Mediterranean Oak forest?

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Plant chemical interactions can have a strong impact on biodiversity and dynamics of forest ecosystems, particularly in Mediterranean forests where plants produce a wide variety of secondary metabolites. These allelopathic interactions may have different effects depending on climatic conditions. In this study, we performed a mesocosm experimentat to test the effect of litter and aqueous extract of green leaves of four woody species (*Quercus pubescens, Acer monspessulanum, Cotinus coggygria* and *Pinus halepensis*) among two water conditions (stressed and unstressed) on the growth of downy oak seedlings and some biotic parameters of soil. Germinated acorns of downy oak were planted in pots, covered by fiber glass cloth (control) or by leaf litter of the studied source species and watered once a month with aqueous extracts of green leaves of the source species. The downy oak stimulates the growth of its own seedlings especially in stress conditions. On the other hand, in unstressed conditions, *C. coggygria* showed great allelopathic potential by decreasing the total biomass, root biomass and the number and biomass of leaves of the seedlings. The effect of this source species could be related to a strong decomposition rate of its litter linked to a large abundance of organisms responsible for fragmenting and releasing of more allochemicals into the soil. *P. halepensis* also reduces root biomass in unstressed conditions. This shows that (**i**) the downy oak has self - stimulation property, (**ii**) climate change could alter plant - plant interactions and soil biodiversity (**iii**) understory species could interfere with the regeneration of dominant species, and (**iv**) the positive effect of oak could balance the negative effect of *C. coggygria* on oak seedlings to ensure the continuity of this ecosystem.

Keywords: Mediterranean forest, Downy Oak, Climate change, Plant plant interaction, Allelopathy, leaf litter, leaf leachates

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Trade-off between allelopathic activity and growth of rice under nutrient stress.

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Plant stress results in the redistribution of internal resources between growth and allelopathic activity. In this study, we examined the effect of nutrient stress in rice seedlings cultivar Hawmjan and Sansuay, which have high and medium allelopathic activity, respectively. Both rice cultivars were grown for 14 days in three concentrations of Hoagland nutrient solution (1N, 0.1N and 0.01N). Rice shoot length, plant dry weight, leaf greenness (SPAD value) and maximum quantum yield (Fv/Fm) were measured. Allelopathic activity using whole rice plant water extract was tested on lettuce seeds for three days. Inhibitory activity of germination, shoot length, root length, plant length and dry weight of lettuce seedlings were measured. Results showed that as nutrient concentrations decreased from 1N to 0.01N, rice growth decreased while its allelopathic activity increased for both cultivars. Regression analyses between rice growth and allelopathic activity parameters showed seven statistically significant negative associations between rice plant dry weight, leaf greenness and maximum quantum yield with inhibition percentage of germination, root length and plant length of lettuce seedlings in both cultivars (0.004

Keywords: allelopathic activity, stress, rice, growth, relationship

*Speaker

Companion plants in pastures – friend or foe?

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In Australia pastures for livestock are either long term 'permanent' pastures or short-term pastures in a rotation phase with crop production. In both cases the important component is a legume which commonly is grown with a perennial grass for the permanent pasture or with another annual legume or grass with respect to the short-term pasture. The companion species are chosen according to common practice which matches the species to the environment. At no stage are varieties chosen for their compatibility with companion species based on knowledge of their mutual chemical tolerances. We have looked at the compatibility of alfalfa-based pastures in respect of possible companion species. These include annual legumes and annual ryegrass. We found that there is considerable variation in alfalfa variety responses and in the associated species to alfalfa root exudates. The presence of annual ryegrass interfered with alfalfa nodulation in some cases. Our work suggests that the selection of companion species and varieties may be more complex than is practised if high production levels are to be achieved.

Keywords: pastures, legumes, nodulation

*Speaker

Response of weeds to litter leachate of *Mucuna bracteata* and persistence of its phytotoxic effects in soil

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Mucuna bracteata as a cover crop is most impressive as it forms a thick pure cover. The allelopathic effect of *M. bracteata* on weeds was investigated in the laboratory and greenhouse. The litter leachates of *M. bracteata* did not reduce seed germination (%) of *Asystasia gangetica* or *Pennisetum polystachion*. However, *M. bracteata* litter leachate delayed seed germination of *A. gangetica* and reduced radical length of *P. polystachion*. In pot culture, increased concentrations of *M. bracteata* extracts incorporated with soil did not affect root or shoot length, dry weight, or chlorophyll content of seedlings of either weed species. A decomposition study of *M. bracteata* in soil for five weeks showed that the phenolic compounds in *M. bracteata* litter did not remain stable in soil. These phenolic compounds decreased abruptly at 1 week after incubation, and then remained more or less steady until the end of the incubation period. The results of this experiment showed that the allelopathic effect of *M. bracteata* on seed germination and seedling growth depended on the target weed species, and residues of *M. bracteata* in mixture with soil did not affect the seedling growth of either weed species. The allelopathic activity did not persist for long periods of time in soil.

Keywords: *Mucuna bracteata*, weeds, germination, phenolic compounds.

*Speaker

Evaluation of different cover crop/ tillage systems on weed control for organic tomato production

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Field experiment was conducted at the experimental station of Iranian Plant Production Institute in Karaj, to investigate the effect of cover crops mixtures and reduced tillage on weed control and yield of tomato. The experiment was conducted in a randomized complete - block design with four replications with seven treatments consisting of a 3×2 (tillage × cover crop) factorial design and a control (hand weeding). Cover crop mixtures were legumes mixtures (hairy vetch/bell bean/ common vetch) or those legumes with grasses (cereal rye/triticale). Tillage treatments included an incorporation of the cover crop at planting, no tillage and a single strip-till. Cover crops were sown in autumn 2013 and in May 2014 (inflorescence emergence of the cover crop) three different tillage was performed. In July, total weed biomass was about 3 times higher in cover crops/ strip-till than cover crops/ no till or incorporation. Tomato fruit yields were not different among the cover crops/ tillage treatments. However, yield in control treatment reduced and had significant different with cover crops/ tillage treatments. These results demonstrate the important of appropriate selection and termination of cover crops for their successful adoption in organic conservation tillage system. Also, results have shown that a combination of cover crop and tillage can have a positive impact on the yield of tomato compared to conventional method.

Keywords: weed biomass, tomato yield, no tillage, strip tillage.

*Speaker

Phytotoxicity of Inula viscosa and effect of its leaves aqueous extracts on lettuce germination

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The phytotoxic activity of aqueous (10, 20, 30 and 40 g/L) and organic extracts (petroleum ether, ethyl acetate and methanol, at 1, 3, 6 mg/ml) of *Inula viscosa* (leaves, stems. and flowers) was tested on germination and seedling growth of *Lactuca sativa*. The effects of aqueous leaf extracts of *I. viscosa* on severlal biochemical processes in lettuce were evaluated. Germination was not affected significantly by any aqueous extract. However, a slight delay in germination was observed (GI = 78.9%) at the leaf extract concentration of 40 g/L. Extracts from leaves and flowers were the most toxic, especially to root growth, inducing an average inhibition of 77.6% at 40 g/L. Shoot growth was stimulated at low concentrations. In organic extracts, the methanol fraction was the most toxic to germination and seedling growth. Our results showed that lettuce seeds were able to germinate in the presence of *I. viscose* leaf extracts. They were able to overcome the allelopathic stress and maintain their germination by accumulating sugars, metabolites essential for energy-intensive process, and also by maintaining the integrity of their cell membrane, thus preventing the loss of electrolytes and lipid peroxidation. However, the observed delay in germination, could be attributed to a slight decrease in respiration noted under exposure to leaf extracts.

Keywords: *Inula viscosa*, phytotoxic activity, lettuce, biochemical process

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Evaluation of allelopathic potential of previous crops against chrysanthemum (*Glebionis coronaria* L.) resistant to chemical herbicides in cereal

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Continuous use of synthetic chemicals has a very negative impact on the quality of agricultural products, on the environment and on the overall human health. Moreover, heavy reliance on herbicides resulted in the development of resistant weeds. Recently, the edible chrysanthemum (*Glebionis coronaria* L.) has become difficult to control with chemical herbicides. The failure to control this weed is increasingly reported by farmers in cereal crops including wheat in North of Tunisia. Earlier in our study, we showed through experimental field assays that edible chrysanthemum has developed resistance to inhibitors of ALS.
As an alternative to herbicides, the allelopathic potential of many crops could be an environmentally friendly alternative for chrysanthemum management. For that, Water extracts of "oat", "barley", "rye", "mustard" and "sunflower" were analyzed for their inhibitory activity on chrysanthemum. Laboratory essays showed a significant effect of all crops on the weed seedlings growth. The highest inhibitory activity was observed by barely water extract and the least one by sunflower extract. Reduction percentages are 53, 05% and 14,94% respectively. A more complete evaluation *in pot* of these crop potential on chrysanthemum are still going on.

Keywords: Allelopathic potential, *Glebionis coronaria*, resistance

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