GHENT UNIVERSITY

FACULTY OF BIOSCIENCE ENGINEERING

ABSTRACTS

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CONTENT

SESSIONS

POSTERS

PLENARY Session

Plenary
09:30

CHALLENGES AND OPPORTUNITIES FOR AUTOMATION OF WEED CONTROL IN SPECIALTY CROPS

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Herbicides provide the basis for economical weed control in major crops, and are significant markets for the agricultural chemical industry (ACI). Worldwide herbicide sales in 2016 were $21 billion USD, and of this total, 63% of sales were in cereals, maize, rice and soy. However, given the business model of the ACI, it is difficult for them to support development of new herbicides for specialty crops such as vegetables. In contrast to the heavily regulated ACI, development of automated weed removal tools by small companies may lead to improved weed control programs for specialty crops.
Intra-row weeds compete with vegetable crops and because herbicides used in vegetables do not adequately control weeds, hand weeding is required. However, fewer laborers are available to hand weed and labor costs are increasing. Our project integrates novel engineering and automation technologies to develop cost-effective weed control systems for vegetable crops. This system will reduce the need for both hand labor and high-rate herbicides while increasing productivity and sustainability of organic and conventional vegetable production.
Weed/crop differentiation is a challenge for the process of weed removal automation. Differentiation, would allow for precise weed detection and removal while leaving the crop untouched. We differentiate weeds from the crop by placing a distinctive marker on the crop that is detectable by a camera. We use three marking methods:

1. Physical markers that do not contact the crop plant;
2. Topical markers on the plant;
3. Root absorbed markers that translocate from the roots to the foliage. Lettuce and tomato are the crops of focus for the project.

Physical markers include biodegradable plastic straws placed close to the crop. The advantage to the straws is that they do not contact the crop and could be used in organic fields. The disadvantage is that the straws stunt the crop when placed in seedling trays with the germinating crop, and growers do not like plastic cutter in the fields. Field evaluations of this technique found that it reduces hand-weeding times by nearly 50%.
Topical markers are dilute fluorescent paint applied to the crop before or during transplanting. Bright fluorescent markers are simple for cameras to detect. Additional advantages are that the paint does not contact harvestable portions of the crop. Disadvantage of topical markers are that the markers are hidden by crop growth and can be washed off by rain or irrigation.
Root absorbed markers have been difficult to implement. An ideal marker is applied as a seed treatment and absorbed by the crop root from where it is translocated to the foliage where it can be rapidly detected. The product also must be food safe if it is to be used on crops like lettuce and tomato. Products tested thus far do not translocate readily and are hard to detect unless high doses are applied.
Automated weeders are being adopted commercially in California specialty crops. However, these machines use pattern recognition and do not truly differentiate between crops and weeds. The process of developing commercial-scale weeders that can truly differentiate between crops and weeds will likely continue into the future.

Plenary
10:00

BIOLOGICAL CONTROL OF INVASIVE WEEDS AND ESCAPED ORNAMENTALS IN EUROPE

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Europe has a long history of collecting, acclimatising, and growing exotic plant species from all over the world and the vast majority of these species are welcome and benign assets to garden owners across the region. Unfortunately, a small minority jump the fence and become established and harmful invaders in their new environments. One factor that is always true for such invaders is that they have lost the natural enemies that keep them in check in their native range during their translocation. Classical biological control aims at reintroducing those specialist natural enemies that pose no threat to the receiving environment for the permanent suppression of the target weed.
Despite over a century of use the world over, Europe has been very slow to adopt this approach despite the extensive use of arthropod predators and parasitoids for controlling crop pests. This paper examines the history of weed biocontrol in Europe and highlights the current and emerging successes which are actually dominated by accidental introductions. The current weed biocontrol research activities are reviewed with special attention paid to aquatic and riparian weeds including *Fallopia japonica*, *Impatiens glandulifera, Hydrocotyle ranunculoides* and *Crassula helmsii*, as well as *Acacia longifolia*, which represent the only species for which intentional introductions have been made. The current challenges facing weed biocontrol are reviewed and the potential future targets discussed considering various drivers including the recent European Invasive Species Regulation highlighted.

Plenary
15:00

HERBICIDE HORMESIS: IMPORTANCE AND IMPLICATIONS FOR CROP PRODUCTION AND WEED CONTROL

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Hormesis is the stimulatory effect of a subtoxic level of a toxin or a stress that is observed with almost all toxicants and on almost all organisms. The phenomenon is common with most herbicide classes and modes of action on most plant species. Hormesis is also common in insects exposed to low doses of insecticides and plant pathogens exposed to low fungicide doses. Although hormesis is a common, relatively simple concept, it is yet poorly understood, and little is known of the mode of action of any herbicide-mediated hormesis. The mechanism in some cases of herbicide hormesis appears to be related to the herbicide target-site, whereas in other cases hormesis may be by overcompensation to moderate stress induced by the herbicide or a response to disturbed homeostasis. The magnitude of and the dose required for hormesis is influenced by many biological and environmental factors, such as plant growth stage and physiological status, environmental factors, endpoint measured, and time after exposure. Since the exact interactions of these influencing factors with hormesis are barely known, hormetic effects are difficult to predict with the currently available knowledge. This is why the effect is generally difficult to quantitatively repeat, even under laboratory conditions. Moreover, this unpredictability makes hormesis too risky as a production practice for yield or crop quality improvement in general crop production for most crop/herbicide combinations. Therefore, subtoxic levels of herbicides are only scarcely used to stimulate certain desired crop responses (e.g., sucrose accumulation in sugarcane). Nevertheless, unpredictable herbicide hormesis may almost certainly occur in crops. The economic impact of this is unknown. Little consideration is also given to what the environmental, ecological, and evolutionary implications of herbicide hormesis could be. We know that weeds and non-target plants are commonly exposed to a range of herbicide doses, including those that will cause hormesis. New information indicates that some weeds that evolved herbicide resistance may have hormetic responses to realistic herbicide application rates. Furthermore, it is indicated that the variable hormesis parameters for subpopulations of the same weed species within a field results in selection for and against certain subpopulations by low herbicide doses. Both phenomena may impact the evolution of weed resistance to herbicides. Little is however known about such effects under field conditions. The implications of herbicide hormesis are clearly many. A more complete understanding of herbicide hormesis is needed to exploit its potential benefits and to minimize its potential harmful effects in crop production.

Session
HERBOLOGY

Herbology
11:00

STUDY OF TUNISIAN PLANT EXTRACTS AS BIOHERBICIDE

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Weeds constantly compete with crops for water and nutrient resources reducing yield, quality and consequently causing huge economic losses (Araniti *et al*., 2015) that can rise to 34% in major crops (Jabran *et al*., 2015). The current trend is to find a biological product to minimize the perceived impacts from synthetic herbicides in agricultural production (Sbai *et al*., 2016). In this context, the herbicidal activity of ten different crude extracts, obtained from aerial parts of Tunisian spontaneous plants, was determined on post-emergence at 7.5, 20 and 34 gL^{-1} against *Trifolium incarnatum*, *Silybum marianum* and *Phalaris minor*. Aerial plant materials were grounded and macerated with methanol for 24h. Methanol was then eliminated using a rotavapor. The yield of plant extracts varied between 5.29% and 29.71% following the species. Extracts 6, 8, and 3 exhibited the best activity in terms of visual effect by spraying on weeds. Moreover, a formulation was carried out to improve their efficiency. The results showed that formulated E6 has completely punctured *Trifolium incarnatum* and has inhibited growth of *Phalaris minor* and *Silybum marianum*. A fractionation of E6 was then carried out. Five fractions were obtained and tested on *Trifolium incarnatum*. Among these fractions, F2 formulated at 20 gL^{-1} showed a very similar effect to a commercial bioherbicide. It caused the total death of *Trifolium incarnatum* 9 days after spraying. Based on bioassay-guided fractionation, five compounds were identified which can be employed in developing new types of bioherbicides for controlling weeds on crops. In addition, the strong weed suppressive ability of formulated F2 therefore offers interesting possibilities as an effective natural environment-friendly approach for weed management.

Key words: weeds, Plant extract, bioherbicide, Formulation

Herbology
11:20

IMPACT OF *BRASSICA JUNCEA* BIOFUMIGATION ON VIABILITY OF PROPAGULES OF PERNICIOUS WEED SPECIES

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Biofumigation may be a promising tool for depletion of persistent weed seed/bud banks. This technique is based on the incorporation of glucosinolate-rich fresh chopped plant biomass into the soil, which releases isothiocyanates with herbicidal properties. To gain acceptance by farmers and foster its implementation, the biofumigation process should be optimised. This study aims at (1) determining sensitivity of economically important and hard to control weed species to *Brassica juncea* biofumigation; (2) elucidating pedo-hydrological factors affecting efficacy of *B. juncea* biofumigation. To determine interspecific differences in susceptibility to biofumigation, seeds and vegetative propagules of 15 weed species were buried at three soil depths (5, 10, 20 cm) and subjected to three dosages of fresh, finely chopped *B. juncea* tissue (0, 70, 200 ton ha^{-1} uniformly incorporated to 10 cm depth), in presence or absence of a plastic soil cover. To assess the impact of pedo-hydrological factors, seeds of 10 species were buried in 20 L pots with equal amount of *B. juncea* biomass but different soil organic matter content, soil moisture content and soil temperature. Propagules were exhumed 14 days after burial and were tested for viability by a germination test followed by a tetrazoliumtest.
In general, efficacy of biofumigation increased with decreasing seed mass and burial depth and increasing dosage of *B. juncea* tissue. Biofumigation was highly effective (mortality>85%) against small-seeded species, moderately effective (mortality >50%) against annual grasses and poorly effective (mortality 0-20%) against hard-seeded and large-seeded species. Mortality of vegetative propagules was high (>90%) for *Sonchus arvensis*, *Equisetum arvense* and *Calystegia sepium*, medium (±60%) for *Elytrigia repens* and *Persicaria amphibia*, and low (±0%) for *Cyperus esculentus*. Viability reducing capacity of *B. juncea* biofumigation was most pronounced in moist warm soils and in the presence of a plastic soil cover. Impact of soil organic matter content was less clear-cut.

Key words: Perennials, weed seed bank depletion, vegetative propagules, integrated weed management

Herbology
11:40

AMARANTHUS PALMERI, NEW THREAT FOR TURKISH AGRICULTURE

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Amaranthus palmeri S. Wats. (Palmer amaranth) is native to the south-western United States and it's one of the most troublesome weeds in crop production systems in the United States. Human activities and some other factors are responsible for increasing distribution of *A. palmeri*, including the spread of seeds by equipment, transportation, animals, wind, and water. *Amaranthus palmeri* is not a native species of Turkish flora, however, it has been introduced 4-5 years ago and is able to settle under different environmental conditions. It was first reported in citrus orchards in the Cukurova region in a restricted area. From here it started to spread through annual crops such as maize and cotton. It is thought that *A. palmeri* has been brought to Turkey by contaminated animals which are imported from South America and USA. *Amaranthus palmeri* distribution and frequency in the fields were assessed by throwing 0.5 sq. m^2 squares randomly in 10 different locations in each field. Spatial distribution of *A. palmeri* in maize, cotton and citrus fields was characterized by fitting negative binomial distributions to frequency distributions of weed counts in each field. The density of *A. palmeri* in this study showed that spread of this weed species was very high in maize field. However, a seed sample of different biotype was collected in maize and citrus fields to examine the differences in germination behaviour. The seeds collected from 9 different fields were sterilized by soaking them in a 0.25% sodium hypochlorite solution for 2 min. After this procedure, seeds were rinsed again with deionized water. Fifty undamaged and intact seeds were selected from each population and placed in 9 cm diameter Petri dishes on two layers of filter paper that were moistened with 5 mL of distilled water. Petri dishes were sealed with parafilm to avoid contamination and drying. Subsequently, they were incubated in a growth chamber at 15, 20, 25, and 30 °C with alternating light/dark (12/12 h). Freshly harvested *A. palmeri* biotypes had different levels of primary dormancy and germination percentages when stored either at dry conditions at 24 °C. These findings show us that the origin of biotypes may have been different. A recent observation by us shows that *A. palmeri* is spreading very rapidly and will be one of the troublesome and economically important weed species within 10 years.

Key words: invasive weed, weed management, germination, new weed species

Herbology
12:00

WEED CONTROL IN POME FRUIT ORCHARDS: RECENT EVOLUTIONS AND FUTURE PERSPECTIVES

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Low-stemmed trees used in high-density orchards are grafted on a less vigorous rootstock with a smaller root system, making them more susceptible to competition with weeds for water and nutrients. Consequently, weed control in high-density orchards is more important than in high stem orchards to avoid a negative impact on tree development and fruit production. From the 1960s onwards, high stem commercial orchards in Belgium were replaced by low-stemmed, high density orchards and the use of herbicides allowed the maintenance of a weed free strip. Starting in the 1990s, integrated fruit production became mandatory and pest control including the use of herbicides became more and more restricted. Atrazine, simazine, diuron and other herbicides were withdrawn from authorization. This resulted in an increased interest in alternatives for weed control such as mechanical techniques. The latter, however, only remained operational in organic fruit growing due to several disadvantages compared to chemical weed control. Nowadays, fruit growers largely rely on the use of glyphosate. However, together with other key herbicides like Basta®, glyphosate is on the verge of being withdrawn from the market and very few effective alternatives are available. This strengthens the need for thoughtful and reliable herbicide schemes that control weeds within the limits of authorization. We recently studied different strategies for weed control based on herbicides of both chemical and biological origin or based on mechanical or thermal techniques. None of the post-emergence herbicides that were tested as replacements for Amitrol®, Basta® and glyphosate (diquat, MCPA…) showed the same efficacy and versatility as glyphosate. Future chemical weed control will rely on herbicides with a smaller weed spectrum and most likely a lower efficacy, necessitating combinations of products. Furthermore, weeds with a developed root system are more difficult to kill using the currently available products, which hence need to be applied sufficiently early. For biological herbicides such as pelargonic (nonanoic) acid, the latter disadvantages of the current chemical herbicides are even more pronounced. Moreover, no biological pre-emergence herbicides are available. Thermal weed control strategies can be based on hot air or hot water. For the moment, the use of hot air in fruit orchards, however, was economically not very promising. For hot water, the technical and economic efficiency are still largely unknown. Mechanical weed control is feasible using different systems such as hoeing blades, rotary cutters, a non-motorized plough disc or a cord weed mower (speed green®). So far, it seems best to combine different mechanical techniques to achieve effective weed control. The speed green technique is very versatile and very useful for controlling weeds around the trunks. In the future, especially when glyphosate becomes unavailable, we expect mechanical weed control to become more competitive with its chemical counterpart.

Herbology
15:45

PRE- AND POST-EMERGENCE HERBICIDE SCREENING FOR WEED CONTROL IN QUINOA (*CHENOPODIUM QUINOA*): FIELD TRIAL

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Both farmers and consumers are interested in alternative crops that can give a higher economic and nutritional value. The multidisciplinary research project at University College of Ghent ALTERGRAIN studies a selection of alternative and pseudo cereals through a whole chain approach which is deployed in the field and processing phase, the nutritional aspects before and after processing into derived foods and the economic potential of these crops for the Flanders's region.
Due to our moderate climate with regular rainfall weed control is an important issue especially controlling the weed *Chenopodium album* in the crop quinoa (*Chenopodium quinoa*). Following a 2-year herbicide screening in pots, a field trial was set up at the Experimental farm in Bottelare (University College of Ghent – Ghent University) to determine the selectivity of several combinations with selected pre- and post-emergence herbicides. The pre- and post-emergence tolerance of the quinoa crop was evaluated to respectively 25 combinations of active ingredients with 4 replications. Plants were evaluated for emergence density, phytotoxic effects on plant growth, weed determination and raw yield measurement. Field net plot size was 7,5 m² and all plots were machine-sown and combine-harvested.
The results give interesting information regarding herbicide tolerance for the crop studied on the field. Based on these results an application for authorization of herbicides can be submitted for the crop quinoa.

Key words: weed control, quinoa, herbicide, phytotoxicity, field trial

Herbology
16:05

PYRAFLUFEN-ETHYL: A NEW TOOL FOR WEED CONTROL IN POTATOES

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Weed control in potatoes in Belgium is mainly based on a pre-emergence application with residual herbicides. However, in some cases weeds are still able to germinate due to a reduced activity of these residual herbicides. Pyraflufen-ethyl can be used as a contact herbicide around emergence to control small germinated weeds. A pot trial was done to check the efficacy and rainfastness of pyraflufen-ethyl on difficult weeds in potato (*Datura stramonium* (DATST), *Atriplex patula* (ATXPA), *Matricaria chamomilla* (MATCH)). Weeds were sown in pots and treated in the 2-3 leaf stage for DATST and 4-6 leaf stage for ATXPA and MATCH. A part of the pots was irrigated with $5Lm^{-2}$ of water, 30 minutes after application in order to check rainfastness. For all weeds, mortality and biomass reduction was measured. The results show that pyraflufen-ethyl is an interesting solution as contact herbicide in potato for the mentioned weeds. In terms of rainfastness, the results show that pyraflufen-ethyl is quickly taken up by the weeds. We can conclude that pyraflufen-ethyl can be used as an extra tool for better weed control in potatoes,

Key words: Pyraflufen-ethyl, potato, *Atriplex patula, Datura stramonium, Matricaria chamomilla*.

Herbology
16:25

SELECTIVITY OF SEVERAL HERBICIDES TO SOYBEAN (*GLYCINE MAX*)

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Because of the lack of protein production, Europe still must import million tons of genetically modified soybean grown in North and South America. Growing soybean is not easy because the available varieties are not well adapted to European climatic conditions. Indeed, soybean is sown in mid-May to be harvested in October, but moisture can seriously jeopardize the production quality. For some years, the availability of more precocious varieties has raised the interest in growing soybean.
Therefore, a field trial was set up during the growing season 2016 to study the selectivity of several herbicides to soybean. Four pre-emergence herbicide treatments were compared to the registered mix (1138 g/ha^{-1} pendimethalin + 90 g/ha^{-1} clomazone): aclonifen (1800 g/ha^{-1}), S metolachlor (960 g/ha^{-1}), metribuzin (280 g/ha^{-1}) and flufenacet + metribuzin (480+350 g/ha^{-1}). Four post-emergence treatments were also tested: bentazon + imazamox (480+22.4 g/ha^{-1}), thifensulfuron (11.25 g/ha^{-1}), propaquizafop (200 g/ha^{-1}) and diquat (600 g/ha^{-1}). These post-emergence treatments were applied as a second spraying (on plots already sprayed with the registered mix in pre-emergence. As a selectivity trial, all tested products were sprayed at single and double rates. Crop safety symptoms were assessed, and harvest was performed.
All treatments showed some crop safety symptoms. Sprayed in pre-emergence, the registered mix and aclonifen were more phytotoxic than other products. Crop safety symptoms assessed in post-emergence treatments seemed mostly due to the pre-emergence registered mix. Recorded yields presented few significant differences.

Key words: soybean, herbicides, selectivity, post-emergence, pre-emergence

Herbology
16:45

MAJOR WEEDS IN BARLEY FIELDS OF DIYARBAKIR

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This study was carried out to quantify frequencies and densities of primary weeds in barley fields in Diyarbakır in 2015. For this purpose, field observations were made in 216 locations. 89 weed species belonging to 18 families, divided into 16 monocotyledons and 73 dicotyledons were detected. The families of Asteraceae, Poaceae, and Brassicaceae were the most dominant in the barley fields. *Avena fatua* L., *Sinapis arvensis* L., *Galium aparine* L., *Turgenia latifolia* (L.) Hoffm. and *Papaver rhoeas* L. were identified as the most common species, their ratios were 65, 57, 52, 52 and 51% respectively. *Avena fatua* was found in the highest density (8.73 plants m^2), followed by *Sinapis arvensis*, *Galium aparine*, *Anthemis arvensis* L., *Papaver rhoeas* (6.15, 5.04, 3.16 and 2.63 plants m^2 respectively). The results showed that effective weed control was not achieved. The density of weeds in monoculture barley production fields is a big question. It has been seen that alternative control tactics are urgently required.

Key words: Barley, weeds, survey, frequency, density

Special session
BIOCONTROL OF PLANT DISEASES

Special session
Biocontrol of plant diseases
11:00

REFIT OF REG. 1107/2009 - NOT ANOTHER MISFIT: CALL FOR DEVELOPMENT OF FAST TRACK AUTHORISATION FOLLOWING ADAPTED DATA REQUIREMENTS FOR BIOCONTROL AGENTS

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e-nema GmbH

Since the introduction of EU Dir. 91/414, biocontrol SMEs today organized within the International Biocontrol Manufacturers Association (IBMA), are calling for a fair and adapted regulation of their products. The regulatory framework, procedures and methods of assessment are inappropriate and restrict biocontrol agents (BCAs) from entering the market. Rules have been designed for single mode of action chemical PPPs and are not suitable for multi-mode of action BCAs. Very little has changed, even with the introduction of EC Reg. 1107/2009. Despite this situation the biocontrol business has been growing by more than 15% p.a. mainly due to an increase in sales of those products, which are not covered under 1107. If adapted rules would have been followed, the business would have grown much faster and many more microbial BCAs would be available to the ag sector. Due to non-functioning regulation an increasing number of products are flooding the market under various guises (e.g. fertilisers, soil amendments) other than as PPPs. IBMA members respect the regulations in place and are penalised comparatively to unscrupulous competitors selling products controlling pests but not subject to assessment. Authorisation of all PPPs is required for each plant. This was introduced with 1107 and is reasonable considering that different crops can differentially metabolize chemical PPPs with consequences on the MRLs. BCAs usually do not fall under residue requirement (Dir. 396/2005). So why not provide general authorisation for all crop plants? Only efficacy data are necessary, and use can easily be extended. It would ease and accelerate market access. At the same time a general authorisation for minor use can be granted because the major requirements are data on MRLs, not necessary for BCAs. Re-evaluation is another point. Reasonable for PPPs when new findings document safety concerns. But why for BCAs after more than 10 years of safe use? Why even extend the re-evaluation to a second RMS and double fees, when the workload cannot be met by one MS since introduction of 1107? Why charge fees? BCAs are safer, and less data are necessary. Today BCAs subsidise evaluation of chemical PPPs. Why kill animals for tox studies with microbial BCAs when medical science has identified all human pathogens? Checking data banks would be much more adequate. We should introduce more scientific expert knowledge and Qualified Presumption of Safety instead of rules developed for chemical PPPs. The REFIT procedure should open up for development of a fast-track system, incorporating time unlimited authorisation with provision for data call-in if triggered by adverse scientific evidence. A new approach for regulation of biological inputs into agriculture must include biostimulants and natural fertilisers. SMEs need fast track systems with supportive guidance. For the sake of agriculture biodiversity and future innovative agriculture approaches we need to avoid another MISFIT.

Key words: IBMA, innovative authorisation, proposal for new system

Special session
Biocontrol of plant diseases
11:20

ELICITATION OF PLANT DEFENSE BY COS-OGA AGAINST TOMATO EARLY BLIGHT

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The induction of plant immune response by using natural or synthetic elicitors is a promising strategy for the control of plant disease, compatible with sustainable agriculture. This study investigates the protective effects and the modes of action of FytoSave® and Fytosol, two COS-OGA based products, against the necrotrophic pathogen *Alternaria solani*. Bioassays were conducted under controlled conditions on tomato 'Moneymaker' and two mutants deleted either on salicylic acid (NahG mutant) or jasmonic acid (Def1 mutant) pathways. The elicitation of tomato with COS-OGA significantly increased protection against *A. solani* with the strongest protection when FytoSol is used. Significant protection against tomato early blight occurred after elicitation of the plants using FytoSol and benzothiadiazole (BTH) on the two mutants NahG and Def1. Using FytoSave®, a significant protection was observed only on the Def1 mutant but not on NaHg mutant. The quantification of the expression of plant defense genes by RT-qPCR reveals that PR-1, GLU and P69b were up-regulated after treatments with the elicitors COS-OGA and BTH. Furthermore, plants treated with elicitors and inoculated showed a higher expression of defense genes compared to uninoculated plants. Increased activities of β-1.3-glucanase and polyphenol oxidase enzymes were observed on COS-OGA treated plants elicited tomato leaves and only on tomato treated with FytoSol for peroxydase Moreover, these enzymes activities were significantly higher after 48 hpi in elicited and *A. solani* challenged tomato plants. Our results show that FytoSave® and BTH activate a defense response of tomato against *A. solani* through the SA pathway as it was observed for other biotrophic and hemibiotrophic pathogens. A higher protective effect occurred using FytoSol, combined with a significant augmentation of endogenous SA in tomato treated leaves, but the molecular pathway induced by this elicitor probably included other pathways.

Key words: Elicitation, tomato, COS-OGA, *A. solani*

Special session
Biocontrol of plant diseases
11:40

ECOLOGICAL RELATIONS INSIDE PLANT MICROBIOTA CAN IMPROVE THE EFFICACY OF BIOLOGICAL CONTROL AGENTS: THE CASE OF *PICHIA ANOMALA* STRAIN K ON APPLE AGAINST *BOTRYTIS CINEREA*

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Pichia anomala strain K is a Biological Control Agents (BCA) against apple two postharvest pathogens (*Penicillium* spp. and *Botrytis cinerea*). Progress have been made during the past two decades to understand its modes of action through various studies. To further improve and stabilize its efficacy, Massart *et al*. (2015) suggested to focus on benefice interactions inside host microbiota to flavor BCAs. Indeed, once applied, BCAs will face a complex microbiota where ecological interactions such as competition, parasitism, mutualism, and commensalism occur. In this study, we investigated the potential of apple microbiota to improve the efficacy of strain K against *B. cinerea*. Sampling were carried out in Belgium, by harvesting fruits of fifteen apple varieties grown in four disease management practices [no phytosanitary treatment (conservation orchard), light-organic, organic, and conventional]. Epiphytic microbiota was collected by 15 min sonication and by washing apples with a phosphate buffer (pH=7.4). Apple washing solutions were stored at -80 °C to generate a microbiota bank made up with eighteen different microbiota. The quantity of microorganisms (bacteria and fungi/yeast) in each microbiota type was first estimated by plating. Afterward, biological assays on wounded apple fruits have been carried out by co-inoculating each apple microbiota with the strain K, then with *B. cinerea* 24h later. The results show that apple skin harbours around $2x10^4 \pm 1,5x10^4$ microorganisms/cm^2. The biological assays revealed that the apple microbiota can either raise till 100%, drop or have no effect on the efficacy of strain K. Thanks to next generation sequencing that makes the microbiota profiling possible, studies are ongoing to identify ecological strains/species or groups of taxa which are benefice to the strain K efficacy.

Key words: Apple, Microbiota, Strain K

Special session
Biocontrol of plant diseases
12:00

EFFECT OF CYCLIC LIPOPEPTIDES PRODUCED BY *PSEUDOMONAS* SPP. ON *MAGNAPORTHE ORYZAE* ON RICE

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Rice is a staple crop consumed by about 3.5 billion people worldwide. However, rice yield is limited by several biotic and abiotic factors. Among the biotic factors, the rice blast disease caused by the fungus *Magnaporthe oryzae* is responsible for approximately 30% global rice yield loss. Fluorescent *Pseudomonas bacteria* produce secondary metabolites including cyclic lipopeptides (CLP), which are potent in the biocontrol of phytopathogenic fungi. In this study, twelve novel and previously characterized CLPs obtained during a previous study were used to investigate their capacity to control the rice blast disease. Crude extracts containing CLPs were added to *M. oryzae* spores before inoculation of rice plants to assess their potential to suppress rice blast infection. Additionally, we investigated the capacity of certain CLPs to elicit defence responses in rice cell suspension cultures and to suppress M. oryzae spore germination or appressorium formation under in vitro conditions. Results showed that certain CLPs could significantly reduce rice blast infection caused by *M. oryzae* spores. Furthermore, some CLPs triggered defense-related responses in rice cell cultures, either or not in a dose-dependent manner. More so, some CLPs blocked appressoria formation by germinating spores of *M. oryzae*. In conclusion, CLPs produced by *Pseudomonas* spp. show potential for the control of rice blast disease, which is at least in part due to their ability to block appressoria formation.

Key words: Rice, *Magnaporthe oryzae, Pseudomonas*, cyclic lipopeptides

Special session
Biocontrol of plant diseases
12:20

IDENTIFICATION OF FUNGAL VOLATILE ORGANIC COMPOUNDS EMITTED DURING QUORUM SENSING AS POTENTIAL BIOCONTROL AGENTS

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Quorum sensing enabling cells to chemically measure the density of their surrounding population and regulate it is a well-known phenomenon in bacteria. Recently, it has become apparent that fungi, like bacteria, can also use quorum regulation to affect population-level behaviours such as biofilm formation and pathogenesis.

This context, we have investigated whether such a phenomenon could occur for two pathogenic fungi infecting barley: *Fusarium culmorum* and *Cochliobolus sativus*.

Both pathogens were cultivated in stressful conditions (high density, poor medium) and the volatile organic compounds (VOCs) emitted were identified using solid phase micro extraction (SPME). In parallel, we have monitored the effect of the blends of VOCs emitted on the growth of each pathogen cultivated in normal conditions. We have observed that VOCs emitted by both fungi cultivated in high densities, significantly decreased the growth of their own species cultivated in normal conditions, suggesting that auto inhibition was occurring.

Having identified the VOCs composing the blend emitted by the pathogens cultivated in high densities, we have tested the effect of eight pure compounds individually at several concentrations. Among these, we have identified four compounds, emitted by *F. culmorum* cultured in high densities that significantly decreased the growth of *F. culmorum*; while two compounds emitted by *C. sativus* cultivated in high densities were significantly reducing the growth of *C. sativus*.

These results are promising and open perspectives for the use of fungal VOCs as an alternative to conventional pesticides.

Key words: barley, volatile organic compounds, quorum sensing, auto-inhibition

Session
PHYTOPATHOLOGY II

Phytopathology II
15:45

HEALTHY GERMPLASMS FOR SUSTAINABLE AGRICULTURE, THE CASE OF AN IMPORTANT CLONAL CROP: BANANA

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Banana plants (*Musa* sp.) bear the most important tropical fruit in the world, in terms of production and consumption, and it is a staple food for half a billion people across the planet. The most important viral diseases infecting *Musa* sp. are BBTV, BSV, BBrMV, CMV and BanMMV. Therefore, a virus free planting material is crucial to ensure a proper food security and to avoid significant economic losses. To answer this need, an ongoing multiyear collaboration has started between the Bioversity's International Musa Germplasm Transit Centre (ITC - KULeuven, Belgium) and the recognized Bioversity's Germplasm Health Unit (GHU - University of Liège, Belgium). ITC is hosting the largest and most widely used collection of Musa germplasm with more than 1,500 accessions. In the framework of this project, 265 accessions from the ITC collection and infected by at least one virus have been selected by both partners, for indexing and sanitation processes. Viruses were mainly BSV and BanMMV, and less commonly but to a more critical extent BBTV and BBrMV. The virus sanitation protocols developed by the GHU are currently being applied to these accessions. These protocols include meristem culture, thermotherapy, and chemotherapy to eradicate the virus from the plant. After sanitation, the plants are grown in the greenhouse before virus full indexing. To date, at least one plant/accession tested negative has been grown for 55% of the accessions (success rate for the sanitation). For 12% of the accessions, no virus-free material has been identified. For these accessions, and for the 33% of other accessions, the sanitation process is still ongoing. Among the sanitized accessions, 63% of the accessions that were subjected to thermotherapy were sanitized, whereas the sanitation occurred in 35% of the accessions receiving the chemotherapy.

Key words: Banana, Virus, Sanitation, Germplasm Health Unit (GHU), International Musa Germplasm Transit Centre (ITC)

Phytopathology II
16:05

ELUCIDATING THE RICE SHEATH ROT COMPLEX IN MALI AND NIGERIA – A CONTRASTING TALE OF TWO DIFFERENT WORLDS

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During an extensive survey of rice fields across West Africa, sheath rot symptoms were notably observed in Mali and Nigeria. The objectives of this study are (i) to identify the pathogens associated with sheath rot symptoms on rice, (ii) to ascertain the roles of the different pathogens associated with sheath rot of rice in Africa and (iii) investigate whether or not (and to what degree) the potentially ecological differences between the regions would affect variation among pathotypes. Pathogens and other microorganisms associated with the sheath rot complex appear to vary in different regions in Africa and might need to be studied separately in order to fully elucidate the complex. Results obtained so far in Mali recorded 50% abundance of *Bacillus*, 30% *Fusarium* spp. and 15% *Sarocladium oryzae* on both sheaths and seeds. However, samples collected from the two locations in Nigeria (North and West) recorded 60% *S. oryzae*, 20% *Fusarium incarnatum*, 5% *Curvularia* sp, 15% *Pantoea* sp. in Chanchaga village near Badeggi, while 65% *S. oryzae*, 20% *Fusarium incarnatum* and *andiyazi,* 5% *Curvularia* sp., 5% *Bacillus amyloliquefaciens*, 5% *Pseudomonas oryzihabitans*, 5% *Burkholderia gladioli* and *Rhizobium* were isolated in Ibadan. *S. oryzae* isolated and used in this study exhibits a high degree of diversity. Analysis of molecular variance revealed genetic differences among the *S. oryzae* from the two countries. Isolates of *S. oryzae* from Mali and Nigeria could be delineated into three genetic groups. Pathogenicity study with *S. oryzae* isolates on rice variety (FARO 44) showed that the most pathogenic isolates emerged from Ibadan-Nigeria.

This study of sheath rot complex elucidates the global biogeography of the associated pathogens and may also reveal whether the causal agents are native or alien to West Africa. This is important because determining whether distributions are natural or human-induced has implications for decisions regarding quarantine related issues.

Future work will focus on the Nigeria scenario in order to elucidate the *Sarocladium-Fusarium* interaction and the interplay of both in the incidence of the rice sheath rot complex

Phytopathology II
16:25

ROLE OF THE TOXINS CERULENIN AND HELVOLIC ACID IN THE RICE-*SAROCLADIUM ORYZAE* INTERACTION

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Sheath rot is a very aggressive, emerging disease of rice causing significant yield losses every year. During the last decades, sheath rot has spread worldwide to all rice-producing areas. The most important causal agent of sheath rot is the poorly studied fungal pathogen *Sarocladium oryzae*. *S. oryzae* produces at least two toxins, helvolic acid and cerulenin. Up to now, these toxins were believed to be the main pathogenicity factors since they are both phytotoxic. Besides that, helvolic acid has antibacterial activity, while cerulenin inhibits melanin and polyketide biosynthesis in other fungi. As a clear correlation with disease severity is still missing, this research aims to thoroughly study the role of cerulenin and helvolic acid in rice - *S. oryzae* interaction. Rice plants were infiltrated with crude extract from a liquid culture of *S. oryzae* containing different concentrations of toxins. According to literature, exogenous application of both toxins can mimic sheath rot symptoms, but these observations could not be confirmed. Using the standard grain inoculum technique, rice plants were inoculated with *S. oryzae* isolates with different levels of virulence. The toxin production of the different isolates was measured in vitro and in planta using HPLC-ESI-MS/HRMS and was correlated with disease severity. Cerulenin or helvolic acid levels were not linearly correlated with pathogenicity. It was observed, however, that the toxin levels of the most aggressive isolates were all within a certain concentration range. Based on these data, we can conclude that both toxins are produced in planta and interact with the rice plant, but they are not the main pathogenicity factors of *S. oryzae*. Ongoing research with non-toxin producers will further elucidate the role of helvolic acid and cerulenin in the interaction of *S. oryzae* with the rice plant.

Key words: *Sarocladium oryzae*, helvolic acid, cerulenin, sheath rot, *Oryza sativa*

Phytopathology II
16:45

ASSESSING THE PATHOGENIC ABILITY OF *RALSTONIA PSEUDOSOLANACEARUM* (*RALSTONIA SOLANACEARUM* PHYLOTYPE I) FROM ORNAMENTAL *ROSA* SPP. PLANTS

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In 2015, upon persistent manifestation of symptoms in *Rosa* spp. plants an outbreak of bacterial wilt caused by *Ralstonia pseudosolanacearum* (*Ralstonia solanacearum* phylotype I) was diagnosed in the Netherlands. In all cases this included *Rosa* spp. plants cultivated in greenhouses. The symptoms included stunting, wilting of young shoots and flower stalks, yellowing and early abscission of leaves, and dieback with black necrosis of pruned branches. This was worldwide the first documented report of *R. pseudosolanacearum* infecting *Rosa* spp.
Isolates of *R. pseudosolanacearum* found in symptomatic roses were assessed for their pathogenic ability in two rose cultivars (cv. “Armando” and cv. “Red Naomi”) and in four solanaceous crops: tomato (*Solanum lycopersicum* cv. “Money Maker”), tobacco (*Nicotiana tabacum* cv. “White Burley”), eggplant (*Solanum melongena* cv. “Black Beauty”) and sweet pepper (*Capsicum annum* cv. “Yolo Wonder”). Two modes of inoculation were included: 1. stem inoculation after wounding and 2. soil drenching. Stem inoculation after wounding was applied to all plant species and soil drenching was applied to plants of tomato and rose only.
Significant differences were observed in susceptibility between the two rose cultivars as well as between the two modes of inoculation performed. The cultivar “Armando” was significantly more susceptible than cultivar “Red Naomi,” exhibiting higher disease severity and incidence.
Similarly, stem inoculation after wounding was found to be significantly more effective than soil drenching, resulting in higher disease severity. Additionally, a temperature dependency in susceptibility was observed for both cultivars irrespective of the mode of inoculation, however, this was significantly more pronounced upon soil drenching. The solanaceous crops all showed to be susceptible to the R. pseudosolanacearum isolates originated from the *Rosa* spp. plants. Furthermore, our results clearly demonstrated that latent infections in a rose cultivar such as cv. “Red Naomi” do occur even at temperatures as low as 20°C. This latency poses high risks for the entire floricultural industry as latently infected *Rosa* spp. plants are propagated and distributed over various continents, including areas where climatic conditions are optimal for the pathogen. Stem inoculation of a variety of *R. pseudosolanacearum* (other than those isolated from rose) and *R. solanacearum* isolates into the two rose cultivars showed that both cultivars were able to harbour symptomless infections of all isolates inoculated.

Key words: *Ralstonia pseudosolanacearum*, *Rosa* spp., disease severity, disease incidence, temperature dependency

Session
PHYTOPATHOLOGY I

Phytopathology I
11:00

BIOSENS, DEVELOPMENT OF SPR SENSORS APPLIED TO EARLY DETECTION AND REAL-TIME MONITORING OF PATHOGENS AND BIOCONTROL AGENTS IN AGRICULTURE

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New environmental and public health requirements encourage the agricultural community to reduce the quantity of chemical pesticides. The SMARTBIOCONTROL research portfolio (Interreg program) proposes to develop alternative phytosanitary products: biopesticides produced by biocontrol agents. However, the efficiency of these biopesticides may vary in function of several environmental factors. It is therefore essential to characterize them in real-time in the field.

Within the portfolio, BIOSENS is a multidisciplinary project gathers the cross-border expertise of several partners in order to develop a new generation of biochips allowing the real-time detection, quantification and in situ monitoring of pathogens, biocontrol agents, and/or their biocidal molecules. The biochip is based on the physical phenomenon of surface plasmonic resonance in association with molecular methods of characterization (nucleic acids and/or protein markers).

In this project, the laboratory of Biotechnology of CARAH is in charge of the molecular design by grafting specific nucleic acids on the biochip in order to detect, quantify and follow the evolution of pathogens and biocontrol agents. Here, are presented preliminary results obtained during the first year of the project. These first results concern the validation of the nucleic acids grafting design by using classical molecular methods (PCR and real-time PCR) in controlled laboratory conditions. At first, and in agreement with all the partners of the portfolio, we focused our research on the target *Zymoseptoria tritici*, the causal agent of one of the European Union's most devastating disease of wheat.

Key words: Biochip, real-time detection, molecular probe, surface plasmonic resonance, septoria blotch

Phytopathology I
11:20

EVALUATION OF THE IMPLICATION OF *FUSARIUM GRAMINEARUM* AIRBORNE INOCULUM IN FUSARIUM HEAD BLIGHT OF WHEAT IN THE FIELD

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Fusarium head blight (FHB) is an important fungal disease of wheat ears, causing yield losses as well as mycotoxins accumulation in the grains. Among the complex of fungi involved in the disease, *F. graminearum*, which produces the harmful mycotoxin deoxynivalenol (DON), is the species most frequently associated with FHB in Europe and throughout the world. A preventive fungicide treatment applied at flowering stage is not always necessary because the intensity of this disease is highly variable from one year to another. Forecasting models based on meteorological conditions, have therefore been developed to predict the extent of the disease. However, the availability of the primary inoculum during the infection period (i.e. flowering) is not considered in such models and the addition of this parameter could potentially enhance their accuracy. In order to study the relationship between *F. graminearum* airborne inoculum and FHB, a network of Burkard volumetric air samplers was set up in five wheat fields distributed in Belgium from 2011 to 2013 and daily amounts of *F. graminearum* inoculum were quantified using a newly developed qPCR assay. A window-pane analysis was used to determine the most relevant time windows around wheat flowering in which the measured inoculum quantity or frequency of detection would be associated with FHB parameters (i.e. FHB severity, frequency of grains infected by *F. graminearum* and DON). Strong and significant correlations were found for multiple window lengths and starting times for almost all the combinations of variables. Inoculum quantities as well as frequencies of days with inoculum detection were both highly correlated with the frequency of *F. graminearum* infection and DON. Most importantly, the frequency of inoculum detection during time periods finishing before the beginning of wheat flowering was significantly associated with DON concentrations at harvest. These results highlight the relevance of inoculum monitoring for the development of accurate disease forecasting tools.

Key words: Fusarium head blight, *Gibberella zeae*, deoxynivalenol, disease forecasting, spore trap.

Phytopathology I
11:40

EVOLUTION OF *ZYMOSEPTORIA TRITICI* RESISTANCE TO 14-ALPHA DEMETHYLASE INHIBITORS IN NORTHERN FRANCE: AN UPDATE

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Zymoseptoria tritici, responsible for *Septoria tritici* blotch (STB), is today the most common and devastating pathogen on wheat crops, particularly in north western Europe. A total of 100 strains isolated in 2016 were characterized for their resistance level to DMI (inhibitors of 14-α-demethylase) fungicides using phenotyping (toward epoxiconazole, metconazole, tebuconazole and prochloraz) and molecular (sequencing of cyp51 gene) assays. Based on the molecular polymorphism of the cyp51 sequence, 27 haplotypes of *Z. tritici* were detected. Among the identified nucleotide and indel changes (19 in total), 13 have already been identified in the literature and 6 of them are new. The IC_{50} levels for the fungicides tested are much higher than the IPO323 reference sensitive strain. Comparative analyses revealed a weak correlation between the molecular and the phenotype of the strains studied, indicating the involvement of other processes in the mechanisms of resistance to DMI in *Z. tritici*.
On the other hand, the highlight of high cross-resistance against certain active ingredients suggests the presence of multidrug resistant strains (MDR) in the assessed population. Additional studies confirmed the occurrence of such strains, as revealed by the detection of the 519-bp insert in the promoter of the MgMSF1 efflux transporter, responsible for the MDR phenotype. In addition, modulization (docking and dynamics) analyses were performed to determine the impact of the alterations detected on fungal resistance at the CYP51 protein level. This study provides an updated overview of the resistance to DMI in *Z. tritici* population of northern France which contribute to a better management highly resistant strains to these fungicides.

Key words: *Zymoseptoria tritici* 14-alpha-demethylase resistance update

Phytopathology I
12:00

APPROPRIATE FUNGICIDE DOSES FOR THE CONTROL OF PHOMA STEM CANKER, CAUSED BY *LEPTOSPHAERIA MACULANS*, IN WINTER OILSEED RAPE

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Phoma stem canker (*Leptosphaeria maculans*) is one of the most damaging and widespread diseases of winter oilseed rape in the UK. Control is predominately through the application of fungicides and the use of more resistant varieties.

Objective: The objective of this paper is to present a preliminary review of fungicide performance, considering both dose and product, against Phoma leaf spot and stem canker over a 10 year period from 2007-2017.

Methods: Fungicides have been evaluated across 20 trials over ten years, funded by AHDB, using a two spray programme based on rates of 50 and 100% of maximum permitted dose (2010-14) and 25, 50, 75 and 100% of maximum permitted dose (2007-2009, 2014-17). The priority for inclusion for testing is given to products not currently approved to allow independent data to be available when they come to market. Fungicide groups tested either as mixtures or solo products include azole (prothioconazole, tebuconazole, metconazole), QoI (picoxystobin) and SDHI (penthiopyrad, boscalid) fungicides. In order to evaluate fungicides in high pressure situations, trials were located in areas with known history of phoma stem canker and on susceptible varieties with good resistance to light leaf spot (*Pyrenopeziza brassicae*). Trials plots were evaluated for efficacy against Phoma leaf spot following the autumn applications and phoma stem canker assessed pre-harvest (presented as a canker index 0-100). Trials were combine harvested and yield data (adjusted to 91% dry matter) was collected to establish the effect of spray programmes on yield. Dose response curves were produced to interpret the effects of fungicide application on disease and yield.

Results: The benefits of controlling phoma leaf spot with fungicides varied depending on the year. In most cases, canker indices of less than 30 did not result in substantial yield losses. Data from dose response curves suggest that a two-spray programme, applying 50% of the recommended label dose, in a typical season, was sufficient to control the disease. Average yield responses in most years ranged from 0.3–0.81 t/ha, as a result from either stem canker reduction or physiological plant growth regulation effects.

Conclusions: Good control of phoma leaf spot and stem canker can be obtained with two-spray programmes, applied in relation to thresholds observed in the field, on susceptible varieties. Applying more than half the recommended label dose of fungicide rarely resulted in additional yield or additional disease control benefits. It is likely that IPM strategies could result in greater use of resistant varieties and reduced fungicide doses.

Key words: *Leptosphaeria maculans*, fungicide performance, dose response curves, efficacy

Phytopathology I
12:20

TRACKING VECTORS OF BACTERIA AND PHYTOPLASMA THREATENING OUR CARROT PRODUCTION

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Several harmful phytoplasmas and bacteria such as Aster Yellows phytoplasmas (AY phytoplasma) or '*Candidatus Liberibacter solanacearum*' have an important impact on our carrot production. These obligate phloem pathogens are also threatening other Apiaceae, such as celery, and even our potato industry. These pathogens are mainly transmitted by phloem and xylem feeding insects belonging to the *Auchenorryncha* or *Sternorrhyncha*, commonly referred to as leaf-, plant- and treehoppers, as well as psyllids. However, for many of them, transmission of the diseases has not yet been investigated. Knowledge on vectors involved, their phytosanitary status in specific areas, their host range, alternative hosts, and influence of abiotic factors on the vector occurrence and disease transmission capacity is fragmented or lacking. A comprehensive scientific insight is indispensable in order to improve risk evaluation and define effective regulation and phytosanitary management strategies adapted to local conditions for this type of vectored plant diseases. One of the selected crops for which a 2-year intensive vector survey was organised, was carrot. Two of these fields appeared to be infected with both AY phytoplasma and '*Ca. L. solanacearum'*. A number of widely used trapping methods were compared, proving sticky traps to be the most effective. The known vector *Macrosteles sexnotatus*, as well as the experimental phytoplasma vector *Empoasca decipiens* (most abundant in summer), *Zyginidia scutellaris* (most abundant in autumn) and other species such as *Javesella pellucida* and *E. atropuncata* were found and population dynamics were assessed. The number of psyllids that were collected from the carrot fields was rather limited. The (potential) vectors were tested for the presence of the phytoplasmas and '*Ca. L. solanacearum*' and an overview of positive testing insects will be presented and further investigated through transmission trials.

Key words: *Auchenorrhyncha, Sternorrhyncha*, psyllids, aster yellows phytoplasma, '*Candidatus Liberibacter solanacearum*

Phytopathology I
15:45

GLIMPSES ON THE GLOBAL MOLECULAR EPIDEMIOLOGY AND ETIOLOGY OF LITTLE CHERRY DISEASE USING NEXT GENERATION SEQUENCING

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Over the past decade, cherry production in Europe and Asia has been severely affected by Little cherry disease symptoms. Little cherry disease (LChD) is a worldwide important pathogen of commercial sweet (*Prunus avium* L.) and sour cherry (*Prunus cerasus* L.) and occasionally also stone fruit trees (*Prunus* sp.). During 2014 and 2017, intensive surveys have been conducted to monitor the incidence and spread of LChV-1 and -2 in symptomatic and non-symptomatic host trees, eventually leading to re-evaluate the status of LChV infections in Belgium. Diseased plants display mild leaf symptoms such as premature reddening or bronzing, as well as the development of small fruits, uneven ripening and an insipid taste were observed in many orchards. A total of 306 trees together with international LChV-infected samples were sampled and tested by RT-PCR for the 2 viruses. Both viruses were widely detected in individual or mixed infections, with a slightly higher incidence for LChV-2 in samples from sweet and sour cherries. Additionally, both viruses were also found in ornamental *Prunus* spp. in private gardens and in lane trees. The disease was found to be prevalent in many cherry production areas in nearly all places where cherries are grown. In an attempt to characterize both virus population genetic diversity, the LChV-1 or -2 specific PCR products spanning both open reading frames from the partial RNA-dependent RNA polymerase (RdRp) and Coat Protein (CP) genes, were sequenced and used as markers in a phylogenetic study. Along with published homologous genomic data from other isolates, the genetic diversity of Belgian Little cherry virus (LChV-1 and 2) isolates originating from different hosts and geographic locations was assessed. Phylogenetic analysis revealed well-defined phylogroups with low genetic variability for the Belgian LChV-1 and LChV-2 isolates yet suggesting a long-term establishment for both viruses in Belgium. Several of these positive samples, one from LChV-1 and one from LChV-2, were selected for further complementary analysis by high throughput sequencing of siRNAs. De novo assembly was performed using different pipelines, including the automated VirusDetect pipeline. Analysis confirmed the presence of LChV-1 and LChV-2 in the samples yet revealed the presence of other Prunus viruses including Prune dwarf virus and Cherry virus A. Near full-length LChV1 and LChV-2 genomes were recovered by contig extension and by mapping the Illumina reads on reference genomes. The use of high throughput sequencing as untargeted tool for diagnostics and epidemiological studies offers new opportunities unravelling the complex etiology of the little cherry disease.

ARTIFICIALLY INDUCED SPORE RELEASE TO PREDICT ACTUAL APPLE SCAB PRESSURE

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During the potential infection moments of apple scab (*Venturia inaequalis*), the Research Station for Fruit Cultivation (pcfruit npo) sends out warnings to refine existing spraying schedules. Until now, these warnings are based on climate conditions and the number of ascospores released above severely infected leaves. These selected leaves are placed at ground level nearby the research station and spores are sampled on a daily basis using a Burkard spore trap that is placed above those leaves. In this way, warnings for targeted treatments are based on a worst-case scenario and similar for all growers regardless of the severity of their apple scab problem. To obtain more site-specific warnings, a technique was developed to determine the local scab inoculum pressure (*V. inaequalis* ascospores) in the orchard. This was performed by a forced maturation and release of the ascospores from a representative number of collected overwintered leaves from the orchards. The released ascospores were captured with the aid of rotorod spore traps and molecularly quantified with a qPCR method so that the potential scab inoculum pressure is known ahead of the primary scab season. The natural spore releases from the same batches of leaves was determined with the same type of spore traps during the course of the primary scab season. The rods with spores were gathered after each rain event and subjected to the same qPCR method to determine the number of naturally-released spores. A positive correlation was observed between the potential scab inoculum and the total actual spore releases during the season. This indicates that the artificially induced spore release can predict the scab disease pressure during the season and that this 'forced release' method can be used to determine the potential scab inoculum. Based on these results, growers can adjust their management strategy during (the beginning of) the season.

Key words: apple scab, potential scab inoculum, natural spore releases, management strategy

Phytopathology I
16:25

THE SEASONAL PATTERN OF *RHIZOCTONIA SOLANI* CAUSING BASAL ROT IN BELGIAN LETTUCE

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Butterhead lettuce (*Lactuca sativa*) is an important crop for the fresh vegetable market in Belgium and is mainly produced in glasshouses with up to five harvests per year. Basal rot is a common disease in lettuce and can be caused by four different pathogens: *Rhizoctonia solani, Sclerotinia* spp., *Botrytis cinerea* and *Pythium* spp. An increased concern about environmental and public health stimulates research on growing vegetables in a sustainable way. Lettuce growers use a standard scheme of fungicides to control basal rot. However, some pathogens may not be active in certain seasons, making their control superfluous. It has been reported that basal rot caused by *R. solani* was mostly observed in summer. Field trials in three different glasshouses were set up to test this assumption. Lettuce was grown continuously without any disease control and the pathogen causing basal rot was determined every time symptoms appeared. Our results showed that *R. solani* was able to cause basal rot throughout the year. The anastomosis groups that were identified for twenty-six isolates showed seasonal fluctuations, with cold adapted anastomosis groups dominating at low temperatures. This was shown by culturing the isolates on potato dextrose agar over a range of temperatures (7-33 °C). As a result, a reduced use of fungicides against *R. solani* during winter cannot be a general advice.

Key words: lettuce, basal rot, *Rhizoctonia solani*, temperature, anastomosis groups

Phytopathology I
16:45

ROLE OF *COLLETOTRICHUM* AND *VERTICILLIUM* IN WILTING OF PEPPER PLANTS

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In the organic cultivation of sweet pepper in the Netherlands, pepper plants are wilting, leading to yield losses. Previously, *Verticillium dahliae* was isolated and identified as the causal agent of wilting in sweet pepper. More recently, *Colletotrichum coccodes* was also frequently isolated from affected plants. *Verticillium dahliae*, a soil-borne pathogen, causes vascular diseases on many crops worldwide. *C. coccodes* has mainly been described as the causal agent of fruit anthracnose and to a lesser extent as a root rot pathogen of solanaceous crops. Both pathogens have been reported to cause disease in pepper plants, but the interaction of *C. coccodes* and *V. dahliae* in pepper plants has not been studied. In this study, the role of *C. coccodes* in Verticillium wilt of pepper plants was further investigated. In the greenhouse, several pepper plants were collected showing either no symptoms, moderate wilting, or severe wilting. To isolate the pathogen(s), segments of root and stem tissue were plated on PDA and MSEA, a selective medium for Verticillium. *V. dahliae* could only be isolated from plants showing moderate or severe wilting, while *C. coccodes* was isolated from the roots of both wilted and non-wilted plants. Remarkably, all plants had a damaged root system with root necrosis, with a loose cortex that could easily be removed. Root damage was more severe on plants that also showed wilting. Therefore, the role of *C. coccodes* in symptom development by *V. dahliae* in pepper plants was investigated. Plant experiments were carried out with single or combined inoculations of *C. coccodes* and *V. dahliae*. Pepper plants were root-dipped in a spore suspension of the pathogen(s) at different concentrations. Symptoms were observed on the roots 42 days after inoculation with the highest *C. coccodes* concentration (10^5 spores/mL). The roots were slightly discoloured, but no wilting or stunted growth was observed. *V. dahliae* alone caused severe stunting of the plants, already 21 days after inoculation. *C. coccodes* could decrease disease incidence and severity caused by *V. dahliae*, when the latter pathogen was present at low concentrations, but had no effect on disease development or made symptoms more severe when *V. dahliae* was present at high concentrations. In conclusion, *C. coccodes* is a weak root pathogen of pepper plants that can have antagonistic, neutral, or synergistic effects on symptom development by *V. dahliae*, depending on the *V. dahliae* concentration.

Key words: *Colletotrichum, Verticillium*, root rot, wilting, pepper

Phytopathology I
17:05

BIOACTIVITY AND UNEXPECTED INTERHALOGEN PRODUCTION BY THE LACTOPEROXIDASE SYSTEM

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The potential of ions produced in water by the lactoperoxidase system against plant pests has shown promising results [1]. We tested the bioactivity of ions produced by the lactoperoxidase oxidation of I- and SCN- in several buffers or in tap water and characterized the ions produced [2]. In vitro biological activity was tested against *Penicillium expansum*, the causal agent of mold in fruits, and the major cause of patulin contamination of fruit juices and compotes. Reaction product was tested with 13C-labelled SCN- in a large range of buffer and substrate ratio using 13C RMN and ESI-FT-ICR. We found that in very precise conditions which are specific ionic strength, precise ratio of substrates and pH of the solution, the mixing solution turned to yellow and a product is formed, I2SCN-, giving an intense signal at 49 ppm in 13C RMN. The formation of the 49 ppm-signal was unambiguously favoured by an acidic medium, but less favoured or inhibited by neutral or basic media. The use of citrate buffer pH 6.2 or phosphate buffer pH 7.4 counteracted this lowering of the signal when the buffer ionic strength was increased. But in basic media, even the use of a highly concentrated buffer failed to induce the formation of the product. The iodide concentration had to be higher than that of thiocyanate to allow I2 and in turn I2SCN- to form. The use of acidic media or highly concentrated neutral buffers optimized oxidoreduction of iodide and thiocyanate by lactoperoxidase and yielded more concentrated ion solutions. As a result, the quantity of solution to be prepared and to be sprayed in pre- or postharvest applications could be reduced.

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[2] F. Bafort, C. Damblon, N. Smargiasso, E; De Pauw, J.-P. Perraudin, M.H. Jijakli, Reaction product variability and biological activity of the lactoperoxidase system depending on medium ionic strength and pH, and on substrate relative concentration, Chem. Biodiversity (2017). doi: 10.1002/cbdv.201700497.

Key words: Lactoperoxidase, biological activity, *Penicillium expansum*, iodine-thiocyanate complex, biocontrol

Session
AGRICULTURAL ENTOMOLOGY AND ACAROLOGY I

THE SHOTGUN APPROACH OF COCOA PEST MANAGEMENT IN WEST-AFRICA

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Two thirds of global cocoa (*Theobroma cacao* L.) supplies are produced in West-Africa (mostly in Ghana and Côte d'Ivoire) by resource-poor, small-scale farmers. Average West-African cocoa yield is 480 kg of dry beans ha^{-1} $year^{-1}$, whereas on research stations, cocoa yield can reach 2,500 kg ha^{-1} $year^{-1}$. The latter yield gap is mainly caused by soil and landscape deterioration and inadequate farm management, which have led to pest and disease problems that are hard to control. In West-Africa, the main cocoa disease is Phytophthora Pod Rot (PPR) whereas the mirids *Distantiella theobromae* and *Sahlberghella singularis* (Hemiptera: Miridae) that suck the sap from cocoa pods and young shoots, are the most devastating pests. In West-African cocoa cultivation, chemical phytosanitary practices (applied by more than 75%; in some areas 100% of farmers) almost exclusively target mirids. Farmers who use insecticides, apply commercial products containing a systemic neonicotinoid insecticide, usually in combination with a contact pyrethroid insecticide two times per year (July-August and January-February). The pyrethroids would thereby kill the mirid adults as well as the nymphal instars, whereas the systemic neonicotinoids would ensure that mirids that hatch after insecticide applications are also killed. However, in Côte d'Ivoire, we found evidence that despite this generally applied practice, mirid infestation is highly variable between different cocoa growing areas. A 3 x 2 x 2 factorial experiment in which the application of 3 different insecticide products (acetamiprid + bifenthrin, imidacloprid and thiamethoxam + deltamethrin) in combination with two different doses (recommended dose and 80 % of normal dose) and with two application periods (early July 2017 and late August 2017) revealed no significant differences in the share of affected cherelles or mature cocoa pods. We recommend that West-African cocoa farmers apply a straightforward mirid monitoring system to guide insecticide applications, rather than rely on general application practices that are often recommended by pesticide companies. This would not only reduce production costs and consequently increase cocoa profitability but would also reduce health risks of cocoa farmers and environmental risks such as water pollution and pollinator (mostly *Forcipomyia* spp., Diptera: Ceratopogonidae) population reduction.

Key words: mirids, *Theobroma cacao*, Côte d'Ivoire, monitoring

Agricultural Entomology and Acarology I
11:20

UN-ANTICIPATED SOCIETAL BENEFITS OF A (GLOBE-SPANNING) BIOLOGICAL CONTROL CAMPAIGN AGAINST THE CASSAVA MEALYBUG

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Insects are the most speciose class of animals on Earth and provide vital ecosystem services to humanity. One such service, biological control, is a central component of sustainable intensification schemes for agriculture and can constitute a near tailor-made solution for the management of endemic and invasive pests alike. In this talk, we illustrate how biological control can deliver an environmentally-friendly and durable suppression of the globally-invasive mealybug *Phenacoccus manihoti* (Homoptera: Pseudococcidae). *P. manihoti* arrived in Southeast Asia in 2008, where it caused an 18% drop in aggregate crop yield of cassava, a yearly loss of >8 million-ton fresh root in Thailand alone, and 2- to 3-fold surges in commodity prices. To mitigate *P. manihoti* outbreaks, systemic insecticides were promoted and the host-specific parasitoid *Anagyrus lopezi* (Hymenoptera: Encyrtidae) was introduced and released in-country. Through observational studies, region-wide population surveys and manipulative assays, we illustrate how *A. lopezi* attains consistently high levels of parasitism at a continent-wide scale and effectively suppresses *P. manihoti* below non-damaging levels. Next, we employ network methods to quantify and visualize shifts in the global trade network of cassava-derived commodities (including starch) and reveal how *P. manihoti* biological control reduces price volatility in global futures markets. Lastly, we illuminate how successful biological control helps slow the expansion of the agricultural frontier in settings such as rural Cambodia, Lao PDR, or Vietnam, and even eases pressures on tropical forested areas. Our research illustrates how ecologically-based pest management helps restore resilience of tropical agro-ecosystems and provides long-lasting solutions for (invasive) pest problems. We use this highly-successful case to further underline the immense potential of ecologically-based tactics to safeguard agricultural production across the globe, and to deliver multiple (often un-anticipated) societal benefits at large spatial scales.

Key words: Biological control, tele coupling, environmental protection, invasive species, ecological intensification

CONTROL OF TOMATO LEAF CURL NEW DELHI VIRUS AS A MAJOR CHALLENGE IN HORTICULTURE

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Tomato leaf curl New Delhi virus (ToLCDNV) is a begomovirus, persistently transmitted by the whitefly *Bemisia tabaci*, and which causes severe losses in solanaceous and cucurbitaceous horticulture crops in Asia. Since 2013, a new strain of this virus, which we named ToLCNDV-ES, has entered, and spread in Spain where it caused considerable damage in zucchini that is produced both in the greenhouse and in the open air. Melon and cucumber were also affected, although to a lesser extent. Subsequently, crops in the neighbouring countries of Italy, Morocco and Tunisia also were invaded by this virus. The damages produced and the high efficiency of virus transmission by the whitefly in zucchini represent a challenge for the horticultural sector in the West-Mediterranean and forces us to look for new ways to fight against this virus. At present, the best options for control include integrated vector management. Zucchini growers currently improve the exclusion of whitefly from greenhouses, protect seedlings with agrotextiles, and maximize the monitoring of insect-vector entry. In Spain, the area where integrated and biological control programs are applied has increased massively in protected solanaceous crops, which has led to a significant reduction in the presence of whiteflies and in the spread of virus diseases. On the other hand, the situation in cucurbitaceous crops is different: it is challenging to convince the farmer to invest in tools and methods based on biological control organisms in the case of these short-cycle crops, where natural enemies have little time to install. Therefore, semi-field trials were set up to optimize the installation of phytoseiid mite *Amblyseius swirskii* in zucchini plantlets. We found that the correct early installation of the predator-mite reduced the infestation of *B. tabaci*. Despite of the high virulence of ToLCNDV, the secondary spread of the virus was very well controlled. Although this procedure did not affect the primary virus infection, the use of pre-installation of natural enemies in seedlings, could improve the control of ToLCNDV in zucchini.

Key words: virus, whitefly, biological control, courgette

Agricultural Entomology and Acarology I
12:00

SEASONAL OCCURRENCE AND LIFE CYCLE OF THE ALLIUM LEAF MINER (*PHYTOMYZA GYMNOSTOMA*) IN BELGIUM

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The allium leaf miner, *Phytomyza gymno*stoma (Diptera: Agromyzidae), is a damaging species to Alliaceae plants including leek, chives, onion, garlic, and shallot. *P. gymnostoma* has spread quickly throughout Europe and North America. Adult flies emerge in spring from overwintered pupae and create feeding punctures in leaves and feed on the sap. However, economic losses are caused by the larvae which mine leaves, stems and bulbs and lower the marketability of the crop. Plants affected by *P. gymnostoma* larvae tend to rot due to secondary infections by fungi and bacteria in damaged tissues. Although *P. gymnostoma* has caused considerable damage to leek production in Flanders (Belgium), there is lack of information on its distribution, life cycle and seasonal phenology under Belgian climate conditions. Therefore, this research aims to 1) investigate the occurrence and distribution of *P. gymnostoma* in Belgian leek fields, and 2) study the life cycle and the emergence of developmental stages in the field, and at different constant temperatures in the growth chamber to develop a degree-day model.

Monitoring was performed weekly during the period 2016-2017 using yellow sticky traps and yellow bowls containing soapy water and a preservative. *P. gymnostoma* adults were reared on leek or chives plants and maintained at 15 °C, 68% relative humidity and a photoperiod of 16h:8h (L:D). To study the life cycle, leek plants were placed in nylon cages and exposed to 2-day-old adults for 3 days. Then, plants were incubated at five constant temperatures (10, 15, 20, 25 and 30 °C) and the emergence and duration of different developmental stages were determined. *P. gymnostoma* was detected in 16 organic and conventional leek fields in Flanders. The pest developed two generations in leek fields, the first in early June and the second in October. The duration of egg and larval development of *P. gymnostoma* decreased with increasing temperature. The pupal development was delayed at 10 and 25 °C. The time required for completing the life cycle was 123 days at 15 °C. Except for 30 °C, at which no pupa turned to adult, *P. gymnostoma* completed its life cycle at all tested temperatures. The highest reproduction rate was observed at 15 °C. Based on the thermal requirements of *P. gymnostoma* and the annual temperature of Belgium, maximum two generations can be completed per year. The degree-day model obtained from this study will help to develop an integrated management strategy against the allium leaf miner.

Key words: Agromyzidae, leek, degree-day model, phenology, temperature

IMPROVED CONTROL OF SPOTTED WING DROSOPHILA WITH FEEDING ENHANCERS

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The objective of this paper was to identify the most efficient feeding enhancer in the lab and confirm its ability to improve chemical control of spotted wing drosophila (SWD) in the field. Tested feeding enhancers were white sugar 0.2% (WS), brown sugar 0.2% (BS), Attracker® 0.2% (A), Combi-Protec® 0.2% (CP), Blossom Protect® 0.1% (BP) and a mixture of bakers' yeast 0.125% (*Saccharomyces cerevisiae*) and brown sugar 0.125% (BYBS). A wetting agent, Trend® 0.1% (T), was also included.
The methods used in the lab were a capillary feeding assay, a petri dish assay (where the attraction of SWD to drops of feeding enhancer solutions was evaluated) and a fruit bioassay (where 10 SWD were exposed to fruit (untreated and dipped in cyantraniliprole 0.05% with or without the feeding enhancers) in condiment cups and their mortality was scored after 16, 24, 48 and 72 hours). The field trial was performed in a sweet cherry orchard. It consisted of a random block design trial with four replicates of five trees that were sprayed weekly with water and spinosad 0.015% solo and combined with WS, CP and BYBS. The applications were made with a Stihl SR450 gasoline powered backpack sprayer at 1000 litres of water per ha leaf wall area. On 10, 12 and 19 July 2017 the number of larvae per 50 cherries was assessed by immersing the fruit in a 10% NaCl solution for 24 hours and sieving the larvae out prior to counting with a binocular.
The results of the capillary feeding and petri dish assay revealed a shift in preference: at the beginning of these trials sugar-based feeding enhancers are preferred by SWD, but as the tests progress this preference shifts to protein-based products. It is hypothesized that the SWD first ensure their survival by consuming a lot of sugar. Once this need is fulfilled the focus shifts to anabolism through proteins. Both assays were however unable to identify the best feeding enhancer. In the fruit bioassay only BYBS significantly increased the mortality caused by cyantraniliprole after 16 hours. BS and CP also increased the mortality after 16 hours, but this was not significant. After 24, 48 and 72 hours no feeding enhancer could improve the mortality in a significant manner. The wetting agent T significantly decreased the mortality at 16 hours and 24 hours. This drop-in mortality was so pronounced that there was no significant difference anymore with the untreated control. In the field trial only, the mixture of bakers' yeast 0.125% and brown sugar 0.125% was able to improve the effect of spinosad on all three sampling dates but this was not significant. Statistical analysis was made with a Friedmann non-parametric and a Student-Newman-Keuls test (non-transformed variables, α=0, 05).
In conclusion, the mixture of bakers' yeast 0.125% and brown sugar 0.125% was the best feeding enhancer, both in lab (significant) as in field (not significant). The wetting agent Trend 0.1% was revealed as a feeding 'disturber' in the lab (significant).

Key words: Spotted wing drosophila (SWD), feeding enhancer, insecticide, yeast

Agricultural Entomology and Acarology I
15:45

EFFICACY SPECTRUM OF A NEW INSECTICIDE BASED ON *CLITORIA TERNATEA* EXTRACT

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Clitoria ternatea (Fabaceae), or Butterfly Pea, is a plant native to tropical equatorial Asia but currently cultivated in other warm regions. The species has been extensively studied for its pharmacological activities. Besides the medicinal uses, in agriculture it is considered an excellent forage legume, with very good regrowth and yield, and cover crop. Recent studies also indicate that *C. ternatea* possess insecticidal effects, mainly related to its content in cyclotides and flavonoids.
In this study we investigate on the efficacy spectrum of a newly produced biological insecticide based on a *C. ternatea* extract, named Sero-X. Sero-X is produced in Australia, where it is currently authorized on cotton and Macadamia nut. Efficacy spectrum in the Australian registration includes thrips, whitefly, different caterpillars and green mirids. The registered active ingredient in Sero-X is the *C. ternatea* extract as such, which is composed by mix of compounds. In addition to that, Sero-X has very low toxicity to mammals.
Considering its complex nature, we anticipated that the product could effectively control pests with different biology and behavior. We followed a two-steps approach by starting the efficacy testing in laboratory conditions and repeating it on crop in commercial farms in groups of pests with different feeding behavior. Sero-X was tested on the following targets, in laboratory conditions: Western flower thrips (*Frankliniella occidentalis*, Thysanoptera: Thripidae), Diamondback Moth (*Plutella xylostella*, Lepidoptera: Plutellidae) as a model for chewing caterpillars, Antispila oinophylla (Lepidoptera: Heliozelidae) representing the leaf miners, the Two-Spotted Wings Drosophila (*Drosophila suzukii*, Diptera: Drosophilidae) and the Cotton Aphid (*Aphis gossypii*, Hemiptera: Aphididae). All tests were repeated in commercial conditions on the same species except for thrips, the chewing and leaf miner Lepidoptera; in these cases, the field trials have been run with the Onion Thrips (*Thrips tabaci*, Thysanoptera: Thripidae), Codling Moth (*Cydia pomonella*, Lepidoptera: Tortricidae) and *Tuta absoluta* (Lepidoptera: Gelechiidae), respectively.
Additionally, we tested the product in field conditions on the pear psylla (*Cacopsylla pyri*, Hemiptera: Psyllidae).
We observed a generally positive performance of the product, that controlled efficiently all tested targets. On aphids the level of control in field conditions was lower than in the laboratory.
We can overall conclude that Sero-X might be a promising broad-spectrum insecticide with a favorable toxicological profile.

Key words: biocontrol, plant extract, *Clitoria ternatea*, biological insecticide

REGULATORY MECHANISMS OF TOMATO *GLANDULAR TRICHOMES* AGAINST THE APHID *MACROSIPHUM EUPHORBIAE*

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Alternative pest management to pesticide use requires a solid knowledge on the plant-pest-natural enemy relationship. In tomato (*Solanum lycopersicum*), one of the most important crops worldwide, the presence of type IV glandular trichomes and acylsucrose production is associated with high levels of resistance to diverse arthropod species. Some species of aphids as *Macrosiphum euphorbiae* are of particular importance as they not only produce severe plant damage but are also vectors of viruses. Nonetheless, it remains undescribed whether this resistance trait protects tomato plants from aphids. The aim of this study is to unravel the mechanisms driving this resistance trait from a molecular and an ecological perspective. Firstly, we analyzed the performance of *M. euphorbiae* on two near-isogenic lines (ABL 10-4 and 'Moneymaker') that vary in the presence of type-IV trichome density and acylsugar production. Because of the significant reduction on aphid's growth found on ABL 10-4 line, we studied the role played by the acylsucroses. Since phytohormones can modulate the density of defense-related glandular trichomes, transcriptomic analyses were also conducted to monitor changes in JA and SA signalling pathways. We noticed that previous herbivory, both by sap-sucking and leaf-chewing insects, had an antagonist effect on aphids´ performance attributed to the display of type-IV trichomes. In addition, a series of experiments were also performed to assess aphids' performance on tomato lines impaired in the JA/SA signalling pathway.

Key words: Tomato, glandular trichomes, defence, aphid, *Macrosiphum euphorbiae*

Agricultural Entomology and Acarology I
16:25

CROSS-RESISTANCE SCREEN OF A NOVEL COMPLEX II INHIBITING ACARICIDE ON THE TWO SPOTTED SPIDER MITE *TETRANYCHUS URTICAE*

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Pyflubumide is a novel carboxanilide acaricide that inhibits mitochondrial complex II of the spider mite *Tetranychus urticae*. Pyflubumide toxicity was examined on adult females of a number of laboratory and field-collected *T. urticae* strains. All strains displayed similar high levels of susceptibility (LC_{50} = 2-7 mg L^{-1}), with the exception of the JPR strain that was less susceptible and exhibited an LC_{50} value of 49 mg L^{-1}. Toxicity of the novel acaricide was further tested on eggs and adult males of JPR and of the susceptible WASATCH strain. The decrease in toxicity was also observed when comparing males and was even more pronounced when comparing eggs, with LC_{50} values reaching 300 mg L^{-1} in JPR. Sequencing complex II subunits did not show any polymorphism related to the target-site insensitivity. To further explore the possible underlying metabolic resistance mechanisms, synergism/antagonism experiments were conducted using the WASATCH and JPR strains. Our results suggested that cytochrome P450 monooxygenases are involved in the decrease of pyflubumide toxicity in JPR. Genome-wide gene expression confirmed the overexpression of three P450s in JPR compare to its ancestral strain JPO. Functional expression and in vitro metabolism assays supported the role of P450s in the metabolism of pyflubumide. Together, we conclude that the low level of cross-resistance to pyflubumide in JPR is likely due to P450 monooxygenase activity.

Key words: Cross-resistance; pyflubumide; P450; synergists; transcriptome analysis

LEVELS OF SALICYLIC ACID CORRELATE WITH BROAD MITE (*POLYPHAGOTARSONEMUS LATUS*) SUSCEPTIBILITY IN POT AZALEA

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The broad mite, *Polyphagotarsonemus latus*, is an important pest in many ornamental crops. Pot azalea (*Rhododendron simsii*) is one of the main Belgian ornamental crops. In pot azalea, broad mites cause malformations of shoot tips, leaves and flowers. Due to restrictions in availability of broad spectrum pesticides, problems with this pest have been increasing in open air and greenhouse cultivated pot azalea.

During the 2017 growing season the occurrence of mite communities was observed at 14 of the 33 pot azalea nurseries visited in Belgium. In total 245 samples were collected; several mite species were found, with *Polyphagotarsonemus latus* being the most prevalent.

In a separate test, mite infestation was followed on three cultivars of pot azalea in the period February to September 2017. These cultivars were chosen based on known differences in broad mite susceptibility: 'Elien' (resistant), 'Mevrouw Gerard Kint' (moderate tolerant) and 'Nordlicht' (susceptible). In 14-day intervals, the natural occurrence of mites was monitored, and leaf samples were taken for LC-MS/MS analysis of stress related hormones: salicylic acid, salicylic acid glucoside, jasmonic acid and abscisic acid.

The expected differences in broad mite susceptibility for the different cultivars were confirmed and could be related to differences in levels of stress hormones. In literature jasmonic acid (JA) is the plant stress hormone mostly related to the presence of pests. In our experiments we found that mainly the salicylic acid (SA) content was influenced by the presence of broad mites in the susceptible azalea cultivars. Further research to study the role and interaction of JA/SA pathways in defense reactions is therefore needed.

Agricultural Entomology and Acarology I
17:05

GROWTH IMPACT OF DOUBLE-STRAND RNA ON THE HONEYBEE GUT PARASITE *CRITHIDIA MELLIFICAE*

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Domesticated bees used for pollination purposes play an important role in agriculture. Those pollinators are frequently threatened by pathogens, a potential driver of worldwide wild bee decline. Reduced levels of pathogens in domesticated bees is therefore not only a clear benefit for the bee species in question but could have broader benefits on the whole population of bees. Reduction of bee pathogens with dsRNA have been successfully reported, but no data exist on the effective use against trypanosomatids. The objective of this study was to assess the response of a honeybee parasite *Crithidia mellificae* exposed to dsRNA. With the purpose of employing RNAi technology as a therapeutic tool, we targeted kinetoplastid membrane protein 11 (KMP-11) that is an essential protein responsible for cell division in trypanosomatids. The effect of KMP-11 dsRNA was tested on in vivo cultures of *C. mellificae*, where it was administered via electroporation and soaking. Two parasite densities (lower and higher) in logarithmic phase were exposed to 20 µg/ml of dsRNA in both experiments. Parasites were grown in incubator at 27 °C and the cell density was checked daily for three days. The *C. mellificae* growth was inhibited through transfection and by feeding dsRNA homologous to specific KMP-11 compared to the control. Although not lethal, targeting this gene resulted in a reduction of *C. mellificae* growth under higher density and optimum living condition showing the potential to minimize this parasite in bees.

Key words: DsRNA, RNAi, pathogen spill over

Session
AGRICULTURAL ENTOMOLOGY AND ACAROLOGY II

CHEMICAL ECOLOGY ON A PROMISING ROUTE INTO ITS SECOND CENTURY AND BEYOND

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Chemical Ecology had a long incubation period reaching back to the turn from the 19th to the 20th Century. J.H. Fabre (1823-1915) then gained evidence of sex attraction of Saturniid silk moth males towards a single calling virgin female in a wire cage. Unexplainable then on mechanistic terms, this easily reproducible observation had to wait until the advent of modern analytical chemical methods for qualitative and quantitative verification at the sub microgram level. Fabre in his wildest dreams could not have anticipated the impact of his discoveries onto modern pest management beginning half a century later and projecting far into the future. Chemical communication, although age old and well established in global biology, suddenly challenged scientists' phantasies, and made pest managers aware of new opportunities in the wake of R. Carson's influential book "Silent spring" whose ecological warning signs are now more up to date than ever before.

A long list of accomplishments followed the Fabre era during the first half of the 20th century. The main push of innovation in plant protection and pest management, however, no doubt occurred because of the sensational chemical structure elucidation of the *Bombyx mori* sex pheromone accomplished early in 1959 by Butenandt and his assistants at the MPI of biochemistry in Munich, Germany. Entomologists and chemical ecologists thereafter entered the field by the dozens. They soon found the basic principles of olfactory communication being realized in many arthropod species where the new strategy of mating disruption immediately found applications for the purposes of species specific pest management.

In this contribution, major accomplishments and growing points in chemical ecology will be highlighted. Examples will be drawn from 1. analytical chemistry which can penetrate down to levels of sub picograms; from 2. ecology with its insights into populations and assemblies of various guilds guided by pheromones, and from 3. automation and data handling capabilities for coping with the flood of generated data.

A short but generally optimistic outlook into the possible future(s) of chemical ecology will be attempted. Today we are still in the process of assembling the vocabulary of the chemical language thus deciphered while also trying to understand the meaning of the behavior coded by these chemical signals. Ultimately, "in silico" methods could be sought shortcutting the lengthy process needed for extracting, purifying, isolating, and identifying signal compounds by classical and extremely time consuming and thus expensive methods.

Agricultural Entomology and Acarology II
16:05

ELECTROPHYSIOLOGICAL RESPONSES AND FIELD ATTRACTION OF CAROB MOTH TO POMEGRANATE AND CONSPECIFIC FEMALES

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The carob moth, *Ectomyelois ceratoniae* (Zeller), is a devastating pest in high-value crops around the world. An efficient sex pheromone attractant is still missing for the management of this pest, because the major pheromone component is unstable. Host plant volatiles attract herbivore insects and have shown to have good potential to be exploited as alternatives or supplements to sex pheromones. To explore this possibility in carob moth, we assessed the attraction of moths to the volatiles of different fruit stages of pomegranate alone and in combination with virgin females using sticky delta traps in pomegranate orchards of Iran. Traps baited with mature pomegranates, whether cracked or uncracked, infested or uninfested, caught significantly larger numbers of male and both mated and virgin female carob moths than unbaited traps. Traps baited with cracked fruit caught more mated females than traps baited with uncracked fruit, and the headspace extract of cracked pomegranate only attracted mated females. Pomegranate flowers and unripe pomegranate did not attract moths. Males were attracted similarly to traps baited with cracked-infested pomegranate as to traps baited with virgin females alone. Interestingly, the combination of cracked pomegranate and virgin female enhanced the attraction of virgin females. Using coupled gas chromatography-electroantennography and gas chromatography-mass spectrometry, β-caryophyllene was identified from the headspace extract of pomegranate as a compound that consistently elicited the strongest antennal responses from female carob moths. Significant variation in the amount of this compound was found between different fruit stages tested, and moth attraction was negatively correlated with the amount of β-caryophyllene in fruits. Together, our results show that volatiles from cracked pomegranates alone or in combination with female sex pheromone have great potential to be exploited as lures to trap both male and female carob moths or to pull them in a push-pull strategy, while β-caryophyllene can be used in a host finding disruption program against this pest.

Key words: Attractants; electroantennography; fruit baits; plant volatile–pheromone interaction

WIREWORMS UNDER THE SWORD OF DAMOCLES: DIFFERENT VOCS ATTRACTION AND REPULSION OF FOUR DIFFERENT MAIZE VARIETIES

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Volatiles Organic Compound (VOCs) are one of the many features of defense used by plants in their eternal fight against pests. Their main role is to attract the natural enemies of the herbivores. But on another hand, the same herbivores can use them to locate plants while foraging. To fill a gap of knowledge in a complex web of interactions, we focused on wireworms (Coleoptera: Elateridae). Wireworms whose larvae feed on roots are one of the most spread pests of valuable crops such as maize and potatoes, causing important economic damage. Little is known about the root compounds that are playing a role in the attraction of the larvae. To know more about these compounds, we compared four different maize varieties (*Zea mays mays*) that are known to have different levels of attraction, from weak to strong, for wireworms in fields. We tested the attraction of larvae in laboratory conditions in dual-choice olfactometer assays where they were offered all possible combinations of the four maize varieties. Contemporary, we collected the VOCs of each variety during 24h using a push-and-pull system. The collected samples were then analyzed by gas chromatography coupled with a mass spectrometer (GC-MS) to identify their molecular profiles. The choice of the larvae was dependent on the offered combination and some varieties were preferred to others. Differences were also observed in terms of quantitative and qualitative emissions of volatile profiles between the maize varieties. Our aim is to develop traps based on VOCs from maize roots to open a new frontier in wireworm's management.

Key words: Direct defense, Integrated pest management, Maize roots, Volatiles organic compounds, Wireworms.

Agricultural Entomology and Acarology II
16:45

IMPACT OF CARBON DIOXIDE CONCENTRATION ON THE PLANT-APHID-NATURAL ENEMIES RELATIONSHIP

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While the impact of climate change on plant or insect communities has been receiving increasing attention during the last decade, plant-insect interactions under a changing environment remain to be studied. These interactions are of importance as regard to the economically dimension of some crop plants and the associated species of insect pests. Insect-plant interactions are mediated by plant secondary metabolites, including volatile organic compounds (VOCs). Modifications in greenhouse gases concentrations, as predicted for 2100, could alter these chemically mediated interactions.
Here, we raise the hypothesis that a raise of carbon dioxide concentration (beyond 700 ppm) affects the volatile emission of Broad bean plants, with a cascade impact on insect pests and its natural enemies.
For this purpose, an experiment was made to determine the impact of CO_2 concentration on aphid choice for their host plant. Choice tests were made on winged aphids between two plants grown under two CO_2 concentrations. Differences were found in the choice made by the aphids. We decided then to generate odour samplings on *Vicia faba* plants reared under the same CO_2 concentrations, hypothesizing that differences in plant semiochemicals may induce preferences for aphids. No differences were found when identifying the volatile organic compounds.
Because atmospheric carbon dioxide (CO_2) concentration directly impacts plants physiology, we raise the hypothesis that elevated CO_2 concentrations impact the quantity of honeydew produced by aphids, as well as the diversity and quantity of honeydew VOCs, with cascade effects on the foraging behavior of aphid natural enemies. Using solid-phase microextraction, we quantified the VOCs emitted by honeydew from pea aphids (Acyrthosiphon pisum Harris) reared either under 450 ± 50 ppm of CO_2 (aCO_2) or 800 ± 50 ppm of CO_2 (eCO_2). While the total amount of honeydew (honeydew release by 190 ± 50 individu's in both conditions) is not impacted by the CO_2 concentration, we found qualitative and quantitative differences in the semi chemistry of aphid honeydew between CO_2 conditions. Three VOCs were not found in the honeydew of eCO_2 aphids: 3-methyl-2-buten-1-ol, 2-methyl-1-butanol and isobutanol. However, no difference was observed in the searching and oviposition behavior of hoverfly (*Episyrphus balteatus* De Geer) females exposed to infested plants reared under both CO_2 conditions, in a dual choice bioassay.

Key words: climate change, *Vicia faba*, VOCs, *A. fabae*, honeydew, *Episyrphus balteatus*

Session
NEMATOLOGY

Nematology
11:00

IDENTIFICATION AND PHYLOGENY OF THE ENTOMOPARASITIC NEMATODES OF THE GENUS HETERORHABDITIS: MULTIGENE APPROACH

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Presently, the genus *Heterorhabditis* contains 16 valid species. In the study we used samples from eleven species: *H. amazonensis, H. bacteriophora, H. baujardi, H. becherriana, H. downesi, H. floridensis, H. georgiana, H. indica, H. megidis, H. noenieputensis* and *H. zealandica* to amplify and sequence five gene fragments: the D2-D3 expansion segments of 28S rRNA, ITS rRNA, COI, thin filament (F-actin) associated protein (unc-87) and calmodulin (cmd^{-1}) genes. Sequences of the D2-D3 expansion fragments of 28S rRNA, ITS rRNA and COI genes differentiated all species from each other. Sequence and phylogenetic analysis of these genes using Bayesian inference, maximum likelihood and statistical parsimony confirmed a division of the genus into three clades (groups): 'Indica', 'Bacteriophora' and 'Megidis'. The analysis of more than 500 ITS rRNA and 100 D2-D3 of 28S rRNA gene sequences downloaded from the GenBank and identified as *Heterorhabditis* revealed several cases of species misidentifications and presence of reading mistakes in some studied sequences. Molecular analysis did not reveal nucleotide differences in the ITS rRNA gene sequences of *H. somsookae* Maneesakorn *et al*., 2015 with those of *H. baujardi, H. sonorensis* Stock *et al*., 2009 with *H. taysearae, H. pakistanense* Shahina *et al*., 2017 with *H. indica*. Application of molecular data, some phylogenetic methods for differentiation of sibling species and intra- and interspecific variations for some genes are also discussed.

Key words: *Heterorhabditis*, gene, molecular phylogeny

Nematology
11:20

COMPARISON OF *STEINERNEMA SIAMKAYAI* AND *STEINERNEMA CARPOCAPSAE* AGAINST *SPODOPTERA LITURA* (COMMON CUTWORM) IN MARIGOLDS

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Entomopathogenic nematode (EPN) species and insect host developmental stage are one of the main factors for increasing EPN efficacy. Therefore, the objective of this experiment was to compare the efficacy between *Steinernema siamkayai* and *Steinernema carpocapsae* on median lethal dose (LD_{50}) of the first, third and fifth instars in larval stage and pupal stage of 1-2 days, 3-5 days and over 5 days of common cutworm, *Spodoptera litura*, under laboratory and field conditions. The LD_{50} value showed that *S. siamkayai* was more effective than *S. carpocapsae* on the first instars at 15.82 and 55.79 IJ/host, respectively. On the third instar there was similar efficacy but on the fifth instar *S. carpocapsae* was more effective than *S. siamkayai* at 54.77 and 104.08 IJ/host, respectively. All the results on common cutworm pupal stage showed that *S. carpocapsae* was more effective than *S. siamkayai* on age of pupae 1-2 days, 3-5 days and over 5 days at 461.24 and 621.10, 586.45 and 665.27, 607.89 and 840.15 IJ/host, respectively. Results from the field experiment reviewed that percentage of damaged marigold flowers by common cutworm was low after treated with *S. carpocapsae* (2.55%) and *S. siamkayai* (3.02%) whereas farmer's practice and control gave high percentage of damaged flower at 4.21 and 4.15%, respectively. Results from this experiment can be used for farmers as alternative control agents against common cutworm in marigolds.

Key words: *Spodoptera litura, Steinernema carpocapsae, Steinernema siamkayai*, marigolds

Nematology
11:40

EFFICIACY OF *TRICHODERMA VIRIDE* IN THE CONTROL OF ROOT LESION NEMATODES (*PRATYLENCHUS THORNEI* AND *P. NEGLECTUS*) AND DAGGER NEMATODES (*XIPHINEMA INDEX* AND *X. PACHTAICUM*) IN TURKEY

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Plant parasitic nematodes are considered the most important pests infecting different crop plants under field, greenhouse, and plastic tunnel conditions. It is known that chemical control, one of the most used control methods against plant parasitic nematodes worldwide poses a potential risk in terms of environmental pollution and human health. Therefore, as an alternative control method, the present study was carried out to determine the comparative efficacy of *Trichoderma* species (will know as biological control agents against several plant pathogens without environmental hazardous effects) against root lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and two *Xphinema* spp. Biological control of plant parasitic nematodes is a distinct possibility for future and it can be successfully exploited in modern agriculture. The main aim of this study was discovery of new biocontrol agents and the demonstration of their value in reducing disease incidence and severity have opened new promising avenues for practical applications in agriculture and for promoting environmental safety. The efficacy studies were conducted in vitro and under greenhouse conditions. Fungal *Trichoderma viride* was isolated from grass and wooden plant materials taken from several plantations in İstanbul and inoculated on Potato Dextrose Agar (PDA) medium for growth. The purified fungi culture was prepared by several dilutions in PDA medium. The plates were incubated at 24 °C for 14 days. The produced conidia were collected from the culture surfaces by flooding with sterile distilled water and scraping the colony surface with a sterile scrapper. Females of *X. index* and *X. pachtaicum* reared on fig tree and *Pratylenchus* individuals reared on carrot disc were used in studies. Near 100 individuals of each species were added to 14 cm Petri-dishes containing spores of fungi at different doses (10^4, 10^6, 10^8). These petri dishes were incubated at 24 °C for 14 days for attachment of fungi to nematode. This study was repeated in greenhouse conditions. In this study spore cultures at the same dose were mixed with soil and chickpea plant were transferred to these soils. After planting of seedlings nematode inoculum of 100 individuals were inoculated separately to each pot. The study was carried out with four replicates and two controls. In petri dishes all doses of *Trichoderma viride* was highly significant and gave a significant reduction ($p > 0.01$) in vitro and decreased the population density of root lesion and *Xiphinema* spp nematodes the fungi inhibited the nematode activity and movements in vitro during one week of exposure and was greatly significant on root lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) larvae than adult form. The same results were obtained in pot cultures. The effect of fungi is significantly rise parallel to increase of application dose and the higher effect was counted in 10^8 inoculum applications.

Key words: Biocontrol, *Xiphinema* spp., *Pratylenchus* spp., *Trichoderma viride*

Nematology
12:00

HOW TO BECOME ATTACHED TO A NEMATODE: NEW DEVELOPMENTS IN PASTEURIA ENDOSPORE ADHESION TO JUVENILE CUTICLE

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Pasteuria penetrans is a Gram-positive endospore forming bacteria that has potential to be developed into a biological control agent of plant-parasitic nematodes. If this bacterium is to be deployed to control nematodes in the field an understanding of the biochemistry and the underpinning molecular biology by which it adheres to one nematode but not another is key. A Velcro-like attachment process has been hypothesised and currently we have identified and started to characterise several collagen-like sequences from Pasteuria that are glycosylated and present on the endospore surface. We believe these fibrous structures that produce a hair-like nap on the surface of the endospore are important in the initial endospore attachment process to the nematode cuticle. Regarding the nematode cuticle to which the endospores bind we have identified two genes, that code for a FAR-protein and a mucin-like protein, that when knocked down affect endospore attachment.

Nematology
12:20

SOIL RESET: AN INNOVATIVE METHOD OF ANAEROBIC SOIL DISINFESTATION TO CONTROL SOIL-BORNE PATHOGENS AND NEMATODES

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Chemical control measures to control harmful soil-borne plant pathogens are at stake. Innovative solutions to combat these harmful organisms are therefore essential.
Anaerobic soil disinfection (ASD) can be realized by inundating soil or by working in fresh grass and covering it with VIF (virtually impermeable foil). This is the classical way (Blok *et al*., 2000). The (almost) oxygen-free situation and degradation products formed under these conditions, such as gasses and fatty acids, are deadly for various fungi, nematodes, bacteria, and weeds. Thatchtec BV and WUR PAGV developed since 2005 an improved version of ASD named Soil Reset (SR), (Runia, 2012)). Hereby the grass is replaced by the means of Herbie®, an easily degradable product with a constant and known quality.
In a field trial on sandy soil, conducted in 2016, the efficacy of SR treatments, with different doses of Herbie®87 (1Crude Protein, 2CP and 4CP) and ASD-classic (grass) in combination with a treatment duration of 3 or 6 weeks, to control Columbian root knot nematode (*Meloidogyne chitwoodi*), Potato Cyst Nematode (*Globodera pallida*) and the soil fungus *Verticillium dahliae*.
In this trial, the decline of potato cyst nematodes in ASD classic and BR with a dosage of 1CP, both with a treatment duration of 3 weeks, was more than 99%. This was comparable to SR treatments with higher doses (>1CP) and longer treatment duration (6 weeks).
M. chitwoodi was effectively controlled in all treatment combinations (> 99% killing). Except for the SR-treatment-1CP and ASD-classical both with the short treatment duration of 3 weeks, where the infection did not decline below detection level. To determine the effect of the treatments on a following crop which is sensitive to *M. chitwoodi*, potatoes (cv Hansa) were grown in this test field in 2017. The quality of the potato tubers reflected the final population levels of *M. chitwoodi* as measured in autumn 2016.
The soil fungus *V. dahliae* was very well controlled by the BR treatments of 2RE and 4RE and ASD-classic, all with a treatment duration of 6 weeks. The contamination decreased below detection level. The effects of BR treatments with lower doses and/or a shorter treatment period have not been studied in this experiment.
Both ASD-classic and Soil Reset are promising non-chemical control measures.
Further optimization is needed to make it economically feasible within arable crops. For cash crops like asparagus and strawberry these methods are introduced in practice with success.

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Runia W.T., Molendijk L.P.G., Ludeking D.J.W. & C.H. Schomaker (2012). Improvement of anaerobic soil disinfestation. Comm. Appl. Biol. Sci, Ghent University, 77(4):753-762.

Key words: non-chemical control, nematodes, soil-borne fungi, anaerobia

Nematology
15:45

ANALYSIS OF PARTIAL RESISTANCE TO FOLIAR HERBIVORES AND *MELOIDOGYNE INCOGNITA* IN WILD AND MEDITERRANEAN TOMATO ACCESSIONS

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A commonly invoked hypothesis explaining the high susceptibility of many crops to pests and diseases is that crops in the process of domestication have lost defensive traits that result in a decreased resistance to pests and diseases. At the same time, ecological theory, based on the molecular cross-talk regulating defensive responses of plants towards insect pests and diseases, predicts the occurrence of resistance trade-offs i.e. the resistance to a certain pest goes at the cost of the resistance to a different pest. To test these two hypotheses, we conducted a combination of field and greenhouse experiments using 24 genotypes of tomato and a selection economic relevant pest in subtropical and Mediterranean areas. We used wild tomato species and traditional Mediterranean cultivars from different geographic origins; all of them representing different stages in the primary and secondary domestication of tomato. Moreover, we included in the study accessions carrying the Mi-1 resistance gene to address not only the potential resistance of these accessions to root-knot nematodes but also to foliar insect pests. We compared the performance of three different pests, the aphid *Macrosiphum euphorbiae,* the cotton leafworm, *Spodoptera littoralis*, and the root-knot nematode *Meloidogyne incognita.* The results of the experiments show strong differences in the performance of pests according to tomato provenance. The detected partial resistances of tomato accessions to foliar pests and nematodes do not seem to be correlated with the degree of domestication. The implications of these findings to understand plant-pests interactions during the domestication process are further discussed.

Nematology
16:05

RESEARCHES ON GROWING WINTER BRASSICA SPECIES TO REDUCE THE DAMAGE OF ROOT KNOT NEMATODES IN TOMATO FIELDS

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The main aim of the study was to evaluate alternatives to the control of root knot nematodes by using biofumigation of Brassica group plants which were commonly cultivated in Samsun provinces in Turkey. Six genotypes (Arugula (*Eruca sativus*), whitehead radish (*Raphanus sativus*), red head radish, (*R. sativus*), turnip *B. rapa* triocularis 426422, cabbage *Brassica oleracea* L. var *capitata* subvar, alba S-38, cauliflower (*Brassica oleraceae* L. convar Botrytis subvar Botrytis), and susceptible winter crop spinach as a control for the experiments conducted in open fields that was heavily infested with *Meloidogyne arenaria*, conducted in Bafra (Doğanca) and Atakum villages. Winter Brassica varieties were grown till flowering stages, then whole plants were mixed to soil and covered with plastic screen for 1 month. At that time, gall indexes, female/plant, and total nematode/plant were recorded using 10 plant samples for each plot. Soil populations of root knot nematodes for each plot were counted taking samples for monthly and tomatoes were planted after taking the screen out. Soil populations of root knot nematodes were less then all plots in *R. sativus* and *E. sativus* have grown before. This situation was exactly the opposite in the development criteria of tomato plants grown after the plants were mixed into soil. Growth criteria for tomatoes grown after red head radish, white head radish, arugula, and cauliflower were remarkably high. Gall index of tomatoes after planting *R. sativus* were 2,25, 2,75 for *E. sativus*, cauliflower and *B. rapa triocularis* while 3,75 for spinach. The yield of tomato also affected positively when Arugula and *R. sativus* have grown before and yield was the less in the plots spinach has grown before. Perhaps the most important target for the farmers was yield and it could be advised to grow radish and Arugula in root knot infested fields to get high yield comparing the other winter plants.

This study was supported by Scientific and Technological Research Council of Turkey, TUBITAK-TOVAG, 109 O 544

Key words: Field experiment, *Brassica* spp., Root knot nematodes, tomato yield

Nematology
16:25

DISTRIBUTION OF THE ROOT-KNOT NEMATODE, *MELOIDOGYNE* SPP. IN VEGETABLE CROPS IN THE SOUTH REGION OF MOROCCO

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Root-knot nematodes (*Meloidogyne* spp.; RKN) are the most common nematodes attacking vegetable crops and causing serious damage worldwide. Therefore, we aimed to review the status and impacts of this nematode on vegetable crops in the Saiss region of Morocco. The earliest report of nematodes attacking vegetable crops in Morocco was in 1982, by Janati, who recorded the presence of four species of *Meloidogyne*, *M. javanica, M. incognita, M. arenaria* and *M. hapla*. Since then and up to now several surveys of nematodes associated with vegetable crops were conducted and revealed the presence of four species. However, these surveys were limited and primitive since not all vegetable crops production areas were sampled to detect RKN. An intensive survey of RKN was conducted on vegetable crops growing regions of Sous-Massa and showed that the root-knot nematodes (RKN) were detected in seventy-five out of 87 localities sampled (= 86%). Four species were detected, viz. *M. javanica*, *M. incognita*, *M. arenaria* and *M. hapla*. Both *M. javanica, M. incognita* were the most dominant species. The highest density of J2 of *Meloidogyne* spp. was recorded in Khmit Ait Amira with 349 J2 (*Meloidogyne* spp.) (100 g soil)$^{-1}$. In view of the estimates of nematode densities obtained in this survey, once can assume that this genus damage vegetable crops in many cases. Field studies on the population dynamics and the damage function are necessary to estimate the economic impact of this nematode on vegetable crops in the Souss region. The status of RKN on vegetable crops in the Souss region is advanced and much work is needed on the distribution, damage threshold and on the management issues.

Key words: Root-knot nematodes, Vegetables crops, Survey, Souss- Massa,

Nematology
16:45

PLANT PARASITIC NEMATODES ASSOCIATED WITH OLIVE TREES AND PATHOGENICITY OF *XIPHINEMA INDEX* AND *MELOIDOGYNE JAVANICA* IN TEKIRDAĞ TURKEY

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Having favorable soil and climate conditions Tekirdağ Province is the biggest Olive producer in North-western Turkey. Olive trees serve as hosts to many plant-parasitic nematodes which cause moderate to high depending on population density, host susceptibility and environmental conditions. The important plant parasitic nematodes in wild (*Olea europaea* L. spp. *sylvestris*) or cropped olive (*Olea europaea* L. ssp. europaea) were identified and damaging level of two important nematode species were evaluated in Tekirdağ Province. For nematode prevalence determination a study was carried out in 53 olive orchards between 2015-2017. Soil samples were collected from the upper rhizosphere under the canopy of each tree in olive orchards within a distant. Nematodes were extracted from 200 g soil samples by combination of Cobb's decanting and sieving method (Flegg, 1967) and sucrose flotation (Jenkins, 1964) method. For the species identification nematode suspensions from each sample were fixed in TAF solution, transferred to glycerine, and mounted on slides by wax-ring method (Hooper, 1990). Species identification was conducted by morphological and morphometric observations under microscope. The damaging potential of *Xiphinema index* and *Meloidogyne javanica* were evaluated in olive under controlled condition. About 500 females of *X. index* reared on fig tree were inoculated to olive growing pots while *M. javanica* individuals were reared on tomato and inoculum level per olive plant was 1000 juveniles. After four months growth at 22 °C plants were removed, and nematode damage was estimated by plant growth parameters observations, root observations, root galling, dry and fresh root weight measurements. Severity of root galling in plants inoculated with *M. javanica* was assessed on a 0 to 6 scale according to the percentage of galled roots. Reproduction factor (Rf) was determined by final nematode counts. 17 genera of plant parasitic nematodes were identified in soil samples. The most important plant-parasitic nematodes detected were spiral nematodes *Helicotylenchus* spp. (95%), *Criconema* spp. (14 %), *Pratylenchus* spp. (19 %). And *Xiphinema* spp. (38%). The predominant nematode species in the surveyed area was *Xiphinema pachtaicum*, *Helicotylenchus digonicus*, *Helicotylenchus dihysteria*, *Merlinius brevidens*, *Mesocriconema xenoplax*, *Pratylenchus neglectus* and *Pratylenchus thornei*. Vertical distribution of these nematode genera varies at soil depths. *Aphelenchus, Aphelenchoides, Filenchus, Helicotylenchus* individuals was present at 0-20 soil depth while *Xiphinema* spp. mostly found at deeper than 40 cm. Pathogenicity tests with *M. javanica* shows severe root damage compared to *X. index*. Large galls were observed. On the other hand, an increase in populations of both species were observed as well.

Key words: Plant Parasitic Nematodes, Olive, *Meloidogyne javanica*, *Xiphinema index*, Turkey

Nematology
17:05

SEASONAL FLUCTUATIONS, POPULATION DYNAMICS, VERTICAL AND HORIZONTAL DISTRIBUTIONS OF *XIPHINEMA INDEX* AND *XIPHINEMA PACHTAICUM* IN VINEYARDS INFECTED BY GRAPEVINE FANLEAF VIRUS IN TEKIRDAG, TURKEY

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Many soil-borne pathogens and pests as economically one of the most important. Within the Longidoridae family *Xiphinema* Genus members is considered as serious with species that has ability to transmit several virus diseases to host plants. Predominant grapevine disease Grapevine Fanleaf Virus (GFLV) is semi-persistently vectored by the ectoparasitic nematodes *Xiphinema index*. Within *Xiphinema* spp. *X. pachtaicum* (77%) and *X. index* (23%) were found highly distributed in Tekirdağ. In our recent nematode study two vineyards in Tekirdağ were found highly infected with both species. Besides after DAS-ELİSA (Double Antibody Sandwich ELİSA) tests with antisera of BIOREBA, Grapevine Fanleaf Virus (GFLV) was found widespread in both vineyards.

This study was carried out to determine the distribution and population dynamics of *X. index* and *X. pachtaicum* and explain virus dispersal relationship. On this purpose soil samples were collected regularly from these vineyards at 0-20, 20-40, 40-60, 60-80 cm. and in row at 25, 50, 75 cm. distance from trunk. Nematodes were extracted from 200 gr soil samples by combination of Cobb's decanting and sieving method (Flegg, 1967) and sucrose flotation (Jenkins, 1964) method. Population counts were carried out under microscope. The populations of *X. index* on sampled vineyards depended on climate and soil conditions by contrast with *X. pachtaicum* which is present in adult and juvenile form through all year all kind of soils. *X. index* population was higher in vineyard with more sandy soil. Nematode population rise after regular rainfalls in autumn and spring. The lowest *X. index* count was observed between 0-20 cm as the population of *X. pachtaicum* was more than 20 individuals per 200 g soil. There was a significant decrease in nematode population with the increase of the distance from trunk base. Total numbers of all encountered nematodes were significantly differed ($P > 0.05$) from distance to another. Besides after serological test of grapevines Grapevine Fanleaf Virus was detected in anterior and posterior vines as well. Based on these data, due to higher incidence of GFLV, *X. index* is considered as it may be a responsible for virus spread. Furthermore, soil sampling at 40-60 cm. soil depth and moist soil conditions is essential for the determination of presence of virus vector nematodes prior to new vineyard establishment. In accordance with results *X. index* is considered to complete one life cycle in one year.

Key words: *Xiphinema index, Xiphinema pachtaicum*, Grapevine Fanleaf Virus, Distribution, Vineyard, Turkey

Session
FORMULATION AND APPLICATION TECHNOLOGY, PESTICIDE RESIDUES, TOXICOLOGY AND ECOTOXICOLOGY

Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
11:00

TRANSPARENCY IN ACADEMIC AND REGULATORY SCIENCE RELATED TO PLANT PROTECTION PRODUCTS

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Phytophar, Brussels, Belgium

The EU regulatory system in plant protection products is very sophisticated and complex. The whole process and scientific background leading to so called (eco)toxicologic “endpoints” in the approval system is well documented on the internet but in general poorly known and appreciated outside the branch. On the other hand, academic research is a more readily understood process as most academic degree holders were exposed to this in their student live or in their professional career.
Both regulatory science and relevant peer reviewed academic science are included in the EU evaluation procedure under Regulation 1107/2009 for placing plant protection products on the market. Regulatory studies are however often perceived and framed as non-transparent as study results and raw data are not always to full extend available for the public and public scrutiny. This task is assigned to several independent dedicated regulatory bodies and specialised experts.
A comparison is made between the two systems with respect to transparency, process reliability and critical control points.
It can be concluded that both are valuable contributors to the regulatory system. The general use of non-standardised testing in academic research offers the advantage of developing new insights being a driver in developing new solutions and in updating methodology and procedures to improve the validated testing in the regulatory framework. Academic research has its own constraints: it inherently produces publication bias, in the short term it has a high risk on being non-reproducible and academic significance does not always imply relevance in practice. Regulatory research uses robust validated methodology that makes international comparison and interpretation of data easier in the two-year evaluation process. The whole data process is systematically inspected, controlled, and supervised by third parties. These standards ensure that all data submitted is of high quality, can be reproduced and provides reliable testing data. The costly high-level quality standards generally exclude most academic labs to meet these standards.
Both sources will increasingly need to address not only purely academic and regulatory aspects, but also societal concerns to sustain and grow trust in their scientific output.

Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
11:20

HIGH-RESOLUTION WATER MONITORING PROGRAM GIVES FURTHER INSIGHTS ON SOURCES OF RESIDUES FROM HERBICIDES IN SURFACE WATER

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[3] Human Safety, Bayer AG, Monheim, Germany
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The EU Water Framework Directive has set environmental quality standards for pesticides, which are the benchmarks for the ongoing EU wide water monitoring and the evaluation of water quality. In addition, national water standards exist that may deviate or include different compounds.
The explicit understanding of the exposure routes to a specific water body is crucial in taking the appropriate mitigating measures to avoid exceedances of these standards in surface water.
In the agricultural catchment area of the Grote Kemmelbeek in the West of Flanders in Belgium, Bayer set up a high-resolution water monitoring program by taking one or more mixed water samples daily in the period 2010-2013 at two different measuring points. During sampling, high intensive flow data from the river was registered by ISCO automated samplers. Concurrently, a survey was carried out among the involved farmers within the catchment area. Agronomic data from the applied herbicides, application rate and dates were collected for each field along with soil data, tile drainage situation in the field and weather data.
Samples in the first year were taken anonymously as a reference year. During the following three years farmers were asked to implement mitigating measures such as the use of low drift nozzles, vegetative filter strips, micro-dam technology in potatoes and conservation tillage. The objective of this research was to show an intrinsically better environmental situation in surface water after an awareness campaign at farmer's level and the establishment of these agronomic Best Management Practices (BMPs).
The water samples were analysed by LC-MS/MS for residues from some of the applied herbicides. The analytical results displayed a high correlation with the precipitation data. Intensive rainfall results in high concentration values. The highest concentrations were specifically measured during the application period. Surface water exposure occurred quickly, within a few hours, and usually disappeared significantly slower through dilution, dispersion, and degradation. The impact of the weather conditions and the quantity of applied herbicides as well as their specific properties show an important role in the contamination level of surface water. Next to diffuse sources, point source entries were also found as a significant exposure route rendering a consequent reduction of these contributions necessary.
Of the diffuse sources run-off was often the predominant exposure pathway into surface water. Applying agronomic Best Management Practices focussing on run-off mitigation (conservation tillage, micro-dams, vegetative filter strips, cover crops) would therefore be most effective in combination with an intensive stewardship program at farmer's level to help reducing pesticide concentrations to meet the standards of the EU Water Framework Directive.

Key words: surface water contamination, monitoring, herbicides, residues.

Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
11:40

IDENTIFICATION OF HERBICIDE SOURCE AREAS AND EXPOSURE PATHWAYS IN A WATERSHED BASED ON LANDSCAPE MODELLING AND HIGH-RESOLUTION MONITORING DATA

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The occurrence of herbicides in surface waters of intensively cultivated catchments can originate from a variety of sources. These include transport via runoff and erosion during storm events, subsurface transport through lateral flow and subsurface tile drainage, groundwater return flow, and from spray drift during applications. The Soil and Water Assessment Tool (SWAT) is widely used in the United States and the EU for catchment scale hydrologic and water quality modelling of non-point source chemicals in the environment. The SWAT model was applied to a 992-ha agricultural catchment in the Flanders region of Belgium to help in better understanding the sources of the herbicide detections observed in daily sampling over 3.5 years at two locations along the catchment's primary stream. The SWAT model was modified to include transport of herbicides through tile drains as an additional process to lateral subsurface transport already simulated. The simulation of herbicide transport to the stream via spray drift was accounted for through a rigorous spatially explicit approach that accounted for field proximity and orientation relative to the stream and the prevailing wind speed and direction during pesticide application. This level of analysis was enabled by detailed documentation of herbicide applications at the field level throughout the catchment and the high temporal resolution in-stream monitoring. The SWAT model was calibrated to observed flow and chemical monitoring data, then used to characterize the relative contributions of herbicides via surface processes, subsurface processes, and spray drift. In addition, very vulnerable fields with significant contributions to surface water exposure were identified. A comparison between observed and simulated exposure profiles was made to single out those events that could not be attributed to any of the diffuse exposure pathways by the model. Potential causes for these observations will be discussed (point sources: misuse, non-compliance with best management practices; modelling approach).

Key words: monitoring, surface water, SWAT, pesticides, modelling

Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
12:00

PROTECTING DRINKING WATER QUALITY IN AGRICULTURAL CATCHMENTS USING A MULTI-ACTOR APPROACH

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High-quality, safe, and sufficient drinking water is essential for life. In Europe agriculture is the biggest source of pesticides and nitrate pollution in fresh waters. Although there has been extensive research on the effectiveness of measures mitigating the impact of pesticides on water bodies, the effective uptake and realization of management practices and mitigation measures is lagging. This presentation focuses on how to set up catchment studies and engage actors to select the most effective mitigation strategy and enable uptake of measures to improve water quality at the catchment scale. Results are presented from the Cicindria case, a small agricultural catchment where we developed a targeted approach to mitigate pesticide runoff. First a map was derived with priority zones for applying mitigation measures. This was used to communicate to local farmers with focus on those farmers with potentially a significant impact on the pesticide load to the river to encourage them to participate in a voluntary erosion control program. Preliminary results show an additional 11 grass buffer strips have been installed at this point in the catchment all in priority zones. Also, the H_20_20 project Water Protect is presented where seven case studies are set-up involving multiple actors (farmers associations, local authorities, water producers, private water companies, consumer organizations) in implementing good practices to ensure safe drinking water supply. The seven cases cover different pedo-climatic conditions, different types of farming systems, different legal frameworks, larger and smaller water collection areas across the EU. In all these cases innovative instruments including spatially explicit GIS analyses, cost-efficiency analysis for mitigation, cost-benefit analysis for society, innovative water governance structures and predictive models accounting for temporal and spatial scaling issues will be proposed that enable local actors to monitor, to finance and to effectively implement management practices and measures for the protection of water sources.

Key words: water quality, stewardship, pesticide pollution, participatory approach

Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
12:20

CEDROZ®, NEW TERPENE NEMATICIDE AGAINST ROOT KNOT NEMATODE ON SOLANACEOUS AND CUCURBITS

E. MEDICO, E. VAN HENDE, K. EL OUADI & M.M. VILLAGRA
Eastman European Technology Center
Technologiepark 21, BE-9052 Zwijnaarde, Belgium

CEDROZ® is an innovative suspension of capsules (CS) based on Thymol and geraniol better known as terpenes. These have a nematicidal action identified, developed, and tested by Eden Research plc and Eastman Chemical B.V. Thymol and geraniol are phenols and alcohols respectively with the characteristic of being volatile, lipophilic, and insoluble in water. Thanks to the innovative formulation of capsules that promotes suppressibility without the use of solvents, terpenes can be dissolved in solution and applied to the soil with a slow and gradual release. The mode of action of Thymol and Geraniol is explained by their synergistic interaction on the lipid substances contained in the cell membranes of nematodes and their eggs. In experimental tests on solanaceous plants and cucurbitaceae Cedroz has shown to have an efficacy comparable to that of the existing post-transplant control solutions. Thymol and geraniol have a favorable toxicological, ecotoxicological and environmental profile. The commercial product Cedroz, will not have pre-harvest interval and will be exempt from MRL

Key words: formulation technology, nematicides, terpenes

Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
15:45

CHARACTERIZATION OF GREENHOUSE SPRAY

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Pesticide application techniques are studied to achieve a better efficiency, using the right dosage, and applying to the targeted areas with the least possible loss. Achieving greater efficiency makes it possible to reduce pesticide losses and to avoid additional costs for the farmer.
The purpose of this work is to characterize the distribution of sprays through a greenhouse canopy. To do this, we tested six different mixtures based on an insecticide and two adjuvants compared to water. For each formulation, we used hydro sensitive paper at three different levels of the plant. After spraying, we collected the papers to count the number and size of impacts. This operation enabled us to characterize the distribution of greenhouse sprays.

Key words: agricultural spray, greenhouse, droplet size distribution, canopy.

Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
16:05

EVALUATION OF 69 PESTICIDE RESIDUES IN MAJOR AGRICULTURAL PRODUCTS FROM THE WESTERN HIGHLANDS ZONE OF CAMEROON USING THE QUECHERS METHOD EXTRACTION AND LC-MS/MS & GC-ECD ANALYSIS

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To increase the crop yield and to protect harvested products during storage, Cameroonian farmers and traders apply different pesticides which residues may remain on/in the food product. It is critical to know the occurrence of pesticides residues in foods, for assessing the health risk and preserving consumer health. But, little is known on pesticide residue levels in major food commodities harvested in Cameroon, especially from the western highlands zone, the food basket of the country. Hence the present study evaluated pesticide residues in 72 samples of 13 agricultural products collected in the region, using the QuEChERS method extraction and analysed using LC-MS/MS and GC-ECD. The results present a validated multi-residue method for screening 69 pesticides in agricultural products from Cameroon. They also depict the amounts and frequencies of the most prevalent chemical class, the commodities in which residues were most frequently detected, the samples exceeding the maximum residue limits (MRLs), and the sample areas showing the most contaminated products. Our data present scientific evidence that investigation into continuous monitoring and good regulation of pesticide residues in Cameroon are needed to efficiently control the human health risks.

Key words: Food safety, Pesticide residues, QuEChERS method, Cameroon.

Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
16:25

RISK ASSESSMENT OF BELGIAN FLORISTS TO PESTICIDE RESIDUES

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Pesticides are known to be excessively applied to control insects and diseases during cropping of flowers. As a result, florists who handle daily many flowers can be exposed to the pesticide residues. Therefore, a study was conducted among Belgian volunteer florists to assess their risk of exposure to pesticide residues: sampling of flowers, residue analysis, transfer from flowers to hands and absorption through the skin after contact. 90 bouquets (roses, gerberas, and chrysanthemums) were collected in Belgium and analysed. Florists were requested to wear during professional activities two pairs of cotton gloves two consecutive half days to assess the potential transfer to hands and the dermal exposure. Finally, during the three most important periods for the sale of flowers in Belgium (Valentine's Day, Mother's Day, and Toussaint), 84 urine samples from florists and control groups (24-hour urine) were collected to assess their total exposure by measuring the concentrations of pesticides (parent compounds and metabolites). Many active substances were detected: 107 on bouquets, 111 on the gloves and 76 in urines of florists. A clear majority of pesticide residues measured on cut flowers and on cotton gloves were found in urine samples. A clear relation exists between dermal exposure and excretion of pesticide residues in florist urines. Exposure could be particularly critical for clofentezine with a maximum systemic exposure value four times higher than the threshold (393% AOEL). Moreover, clofentezine was detected in urine of florists during the three periods of samples. In conclusion, the study leads to conclude that Belgian florists are exposed daily to pesticide residues, with a potential effect on their health. Therefore, there is an urgent need to raise the awareness about pesticides residues among florists who should adopt better personal hygiene rules and among authorities who could set safety standards such as Maximum Residue Limits for residues on cut flowers.

Key words: pesticide residues, dermal exposure, biological monitoring, risk assessment, florists

Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
16:45

EXPOSURE ASSESSMENT OF OPERATORS IN NORTHERN BENIN

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Centrifuge canes and backpack sprayers are the two most popular sprayers used in the cotton area of the Northern Benin. Exposure of operators greatly varies with their skill and experience. Previous surveys showed that local practices are far from the recommended rules of use issued by the fabricants and the local extension officers. A study was carried out to compare the risks of contamination for each type of sprayer. The "patch method" was used to determine with a dye (tartrazine) which parts of the operators' bodies are the most exposed to the mixture. Ten spraying trials with the centrifuge cane sprayer and 10 trials with the backpack sprayer were performed by different producers in cotton fields at 1 m and 1.5 m height. The deposits on patches were measured thanks to the absorbance value determined after their extraction in water with a colorimeter (MN Nanocolor 500 D) and a calibration curve. The method allows to compare the contamination pattern for each sprayer and results show that the thighs are the most exposed parts of the body whatever the device. It has been also showed that the height increases the contamination. In conclusion, even if the centrifuge cane is usually preferred to the backpack sprayer (heavier and more expensive) the potential dermal exposure is significantly higher with this kind of device unless farmers are well trained.

Key words: risk assessment, dermal exposure, cotton, Benin

Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
17:05

PROBLEMS OF ORGANOCHLORINE PESTICIDES IN KAZAKHSTAN (OF THE FORMER STOREHOUSE PESTICIDES, MILITARY AND MINING SITES)

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Pesticides are a wide range of chemical compounds of the different classes that are not found in nature. They are consciously introduced for pest control in agriculture. Unlike other pollutants, the real danger of pesticides is not fully understood.

Persistent organochlorine pesticides are one of most dangerous environmental pollutants prohibited by the Stockholm Convention because of their inherent toxicity. Despite the ban since 1970, they are still found in soil and water, and accumulate in tissues. The widespread use of organochloride pesticides created serious environmental problems in Kazakhstan, Ukraine, and many other countries of the world.

The object of this research was soil from 90 former pesticides storehouses (Almaty region), 1 military site (village Maili, Balkhash district) and 1 mining site (Tekeli city). Concentrations of metals were determined using 7500 ICP-MS (Agilent, USA) instrument. Gas chromatography with mass spectrometric detection (Agilent 7890/5975c, USA) was used for determination of concentrations of pesticides in the soil

It was established that the soil of the studied sites is contaminated with interacting metabolites of DDT (4,4'-DDE, 4,4'-DDT, 4,4'-DDD) and heavy metals. Concentrations of DDT metabolites exceeded the MAC 10 times in soils from 26 territories of the former pesticides storehouses, and the soil from military and mining sites –2-4 times. MAC of DDT metabolites in soil is 100 mg kg^{-1}. It should be noted that the main dominant pollutants in the soil from the territory of the former storehouse were DDT metabolites, in the soil from the territory of military and mining sites – heavy metals of the 1st class of danger (Pb, Zn, As) and 2nd class of danger (Co, Ni, Cu, Cr). Based on these data the electronic database about stocks of obsolete pesticides in the Almaty region, concentrations of organochlorine pesticides and heavy metals in soil was developed. Structure of the database has been developed based on Enterprise Architect software using c # in Visual Studio 2013.

For the management of contaminated soils, the technology of phytoremediation using biomass fuel crops such as *Miscanthus* is being developed. *Miscanthus* can be grown at the contaminated and marginal lands for up to 30 years and produce valuable biomass. The production process does not practically demand fertilizers and pesticides, and, in addition, does not deplete the land. *Miscanthus* growing on contaminated soil allows restoring contaminated sites and simultaneously obtaining biomass for production of solid biofuels

This work was supported by the projects of the Ministry of Education and Science of the Republic of Kazakhstan and NATO (G4687).

Key words: organochloride pesticides, soil, the former storehouses pesticides, military and mining sites

POSTERS

Posters
HERBOLOGY

Posters
Herbology
H01

EFFICACY OF REDUCED RIMSULFURON DOSES IN COMBINATION WITH SUNFLOWER WATER EXTRACTS ON THE WEEDS

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Rimsulfuron is a sulfonylurea herbicide used to control broad and narrow leaved weeds in corn and tomato. The corn and tomato production in Turkey are part of a wheat-corn and wheat-tomato crop rotation system, respectively. Corn has been opted by the farmer as a first or second crop in some regions in which irrigation opportunities exist while tomato has been cultivated as a main crop. Rimsulfuron can be safely used in these crops, but it may degrade slower than expected due to drought conditions. Therefore, reduction of the herbicide used for weed control is important to prevent this type of risk. Using promising allelopathic plant extracts like sunflower with reduced rate of herbicide is a convenient solution to reduce herbicide rate without compromising the control of problematic weed species such as redroot pigweed and volunteer wheat. In this study, efficacy of reduced rates of rimsulfuron in combination with sunflower water extract on redroot pigweed and volunteer wheat were determined. The experiment was carried out in a greenhouse with a 16h photoperiod at 22±1/18±1 °C and completed 30 days after treatment. Sunflower water extracts at 1:10 and 1:20 concentrations combined with 50% of the recommended dose of rimsulfuron significantly reduced redroot pigweed and volunteer wheat growth and chlorophyll compared to the control while sunflower water extracts without rimsulfuron did not suppress seedling growth of weeds. Recommended rate of the herbicide effectively controlled the weeds, but half the recommended dose fairly controlled the volunteer wheat.

Key words: Rimsulfuron, sunflower water extract, weed, allelopathy

Posters
Herbology
H02

CONTROL OF *OROBANCHE CERNUA* ON TOMATO PLANT WITH BENZOTHIADIAZOLE AND ITS ENZYMATIC CHANGES

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Tomato (*Solanum lycopersicum* L.) is one of the most important plant hosts for the parasitic flowering plant broomrape (*Orobanche cernua* Loefl.) in the Middle-East, especially in Iran. Broomrape is the dominant species of Jiroft region in South-East Iran. In this study, plant defense responses of tomato plants treated with different concentrations (0, 10, 100 and 500 mgL^{-1}) of Benzothiadiazole (BTH) were measured to assess the effect against broomrape. After evaluation of the growth parameters of seven tomato plant varieties and root phenols, two varieties (namely Early Urbana and Super Chef respectively the most tolerant and sensitive cultivar) were selected for following experiments. In the second experiment, roots of two varieties of tomato as mention above, were soaked before transplanting into pots with soil infected by the pathogen, and then irrigated with different concentrations of BTH fifteen days later. According to the assessment of the growth parameters of host and pathogen, there was a significant effect of all the concentrations of benzothiadiazole on controlling the *O. cernua*. To evaluate the activity of Phenylalanine Ammonia Lyase (PAL) and Peroxidase (POX) enzymes, roots of the two mentioned varieties of tomato were soaked for an hour into different concentrations of BTH. The plants were then transplanted into pots with infected soil. Tomato seedlings were irrigated a second time 15 days later. After that, at 24, 48, 72 and 96 hours, the enzymatic changes in the plants were evaluated. The results showed that there was a significant effect of the concentrations of BTH and pathogen on the activity of PAL and POX enzymes that are involved in plant defense mechanisms. The highest enzymatic activity was obtained in the Early Urbana at a concentration of 500 mgL^{-1} in the presence of *O. cernua*. Based on the results, the use of BTH as a plant defense activator is a new, simple strategy and compatible with the environment that can be used to control broomrape damages with a promising counter.

Key words: Biological control, Broomrape, BTH, Defense Mechanisms

Posters
Herbology
H03

MORPHOLOGICAL AND GENETIC DIVERSITY OF ACETOLACTATE SYNTHASE-INHIBITING, HERBICIDE-RESISTANT *SCIRPUS MUCRONATUS* BIOTYPES

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The purpose of the study was to determine the genetic and morphological diversity of 50 biotypes of *Scirpus mucronatus* L. that are resistant to ALS-inhibitor herbicides (azimsulfuron, bensulfuron-methyl, ethoxysulfuron and halosulfuron-methyl) using the ISSR (Inter Simple Sequence Repeat) marker system. To determine the morphological diversity, five seeds from each population were grown in pots in a greenhouse, following a randomized block design. The parameter values subjected to a hierarchical clustering analysis showed significant variation among the populations. From the total of 13 characteristics examined, three PC compounds were obtained, representing 80.07% of the total variation. The genetic variation among populations was determined using 40 oligonucleotide primers. The genetic associations between varieties were evaluated by calculating the Jaccard similarity coefficient for pairwise comparisons based on the proportion of shared bands produced by the primers. Populations were classified into two main groups. The morphological and molecular analyses revealed differences in terms of several quantitative characteristics among the populations examined. Remarkable similarities were found among different *S. mucronatus* populations grown in different regions in terms of their morphological characteristics, the genetic diversity was found to be higher. High genetic diversity and low differentiation between populations, strongly suggest the potential for spread of resistance and the need for management that limits seed and pollen dispersal in *S. mucronatus*.

Key words: Bog bulrush, morphologic parameters, diversity, ISSR, rice

Posters
Herbology
H04

POSSIBILITIES OF HERBICIDES FOR WEED CONTROL IN PARSNIP

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The possibilities of chemical weed control in parsnip are fairly limited in Belgium. In addition, the active ingredient linuron has recently disappeared. In 2017 a screening of new active substances in parsnip was carried out. Fluorochloridone and metamitron were tested before emergence. Dimethenamid, aclonifen and flumioxazinIin were tested in post-emergence. These herbicides all appeared to offer possibilities for the weed control in parsnip. Mostly some phytotoxic was seen shortly after application, but this was limited to a few weeks after the treatment.

Key words: weed control, herbicides, parsnip

Posters
Special session
BIOCONTROL OF PLANT DISEASES

Posters
Special session: Biocontrol of plant diseases
S01

AUREOBASIDIUM PULLULANS STRAIN ACH1-1 BIOCONTROL OF POSTHARVEST DISEASES OF APPLES: 15 CRUCIAL YEARS OF RESEARCH BEFORE STARTING COMMERCIAL DEVELOPMENT

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Losses in harvested fruits are thoroughly associated to decaying fungi. Biological control using microbial agents including yeasts, has been reported among several alternatives to the use of synthetic chemical fungicides for managing postharvest fruit decay. *Aureobasidium pullulans* strain Ach1-1 was isolated as potential yeast antagonist form the surface of Golden Delicious apple fruit. The biocontrol agent exhibits high efficacy towards controlling blue and grey mold infection disease caused respectively by the two fungi *P. expansum* and *Botrytis cinerea*.

Competition for nutrients as mode of action, was evident for strain Ach1-1 against *P. expansum*. The results demonstrated that exogenous amino acids, applied at high concentrations on apple wounds as a mixture of specific amino acid groups or as individuals, significantly decreased strain Ach1-1 efficacy towards *P. expansum*.

Furthermore, molecular markers and a semi-selective medium method were developed, in order to monitor the population dynamic of *A. pullulans* strain Ach1-1 on fruit surface. The random amplified polymorphic DNA (RAPD) technique was applied to 15 strains of *A. pullulans*, including the strain Ach1-1. Among the five specific RAPD fragments amplified for strain Ach1-1, a 528 bp fragment was selected and used to design sequence-characterized amplified region (SCAR) primers. The results showed that these specific SCAR primers can clearly identify strain Ach1-1 among different strains of A. pullulans and other yeast strains commonly present on apple fruit surface. Furthermore, a semi-selective medium based PDA and supplemented with euparen, sumico, hygromycin B, streptomycin sulphate, cycloheximide, specific for strain Ach1-1 was developed.

These outcomes in fundamental and applied research, relevant for *Aureobasidium pullulans* strain Ach1-1 lead to a collaboration establishment with Elephant vert group towards the commercial development of this strain as potential biocontrol agent to control apple diseases in postharvest conditions.

Key words: Biocontrol, Efficacy, mode of action, Monitoring, commercial development

Posters
Special session: Biocontrol of plant diseases
S02

INTEGRATION OF *BACILLUS SUBTILIS* STRAINS AND PLANT DEFENSE ACTIVATORS FOR FIELD FIRE BLIGHT CONTROL

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Bacillus subtilis strains GB03, QST713, Y1336, plant defense activators [acibenzolar-S-methyl (ASM), fosetyl aluminium (F-Al), potassium phosphites (PH) and prohexadione-Ca (ProCa)] were evaluated individually and in combination for efficacy in controlling fire blight in Morocco using a split-split-plot design. The treatments were applied on the trees at timings based on their modes of action. Results showed that, when used alone, *B. subtilis* QST713, B. subtilis GB03 and *B. subtilis* Y1336 reduced blossom infection under field conditions by 63.8, 64.2, and 53%, respectively. For plant defense activators this reduction was 61.5, 56.6, 50 and 49% for ASM, ProCa, F-Al and PH, respectively.
On shoots, it ranged from 40 to 80% for biocontrol agents, but for plant defense activators it varied from 46 to 96.5%. Two individual applications of ProCa were the most effective treatment for reducing shoot blight incidence. The combination of plant defense activators and *Bacillus subtilis* strains allowed the highest protection rate against blossom and shoot blight ranging from 76 to 98.2%. The greatest protection was insured by *B. subtilis* QST713, combined with ASM or ProCa

Key words: fire blight, *Bacillus subtilis* and plant defense activators

Posters
Special session: Biocontrol of plant diseases
S03

INDUCED RESISTANCE IN CUCUMBER BY SOME MEDICINAL PLANT EXTRACTS AGAINST *PSEUDOPERONOSPORA CUBENSIS*

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Using of pesticide is a regular method for control of Downey mildew of cucumber. Natural compounds with induced resistance properties are promising for management of this disease. In this study, the effects of aqueous extracts of milkweed and colocynth were investigated in inducing resistance in cucumber. The effective concentration of each extracts was sprayed on one side of plants in greenhouse condition and the effect of extracts on induced resistance was evaluated on detached leaves from another side of plant. The leaves were inoculated with pathogen 24 hours after application of plant extracts. The results of experiment showed that aqueous extract of colocynth and milkweed (200 mg/ml) with 84, and 74% reducing the disease index respectively has significant differences with control. The evaluation of changes in Peroxidase, Glucanase and Phenylalanine ammonia lyase activities in cucumber at different times point after inoculation with pathogen showed that aqueous extract of colocynth and milkweed increased the activity of these enzymes and induced resistance in plants.

Key words: Downy mildew, Defense Enzyme, Cucumber, Induced resistance

Posters
Special session: Biocontrol of plant diseases
S04

COMPATABILITY ASSESSMENT OF BIOSURFACTANTS FORMULATED AS TANKMIX ADJUVANT WITH BIOCONTROL SOLUTIONS

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Mannosyl Erythritol Lipids (MELs) are biosurfactants which are manufactured by fermentation with yeast which convert a triglyceride substrate to functional glycolipids. These amphipathic molecules have a unique structure with potential in different facets of the industry. Oleon has developed different tank-mix adjuvants containing these MELs for the crop protection industry. MELs are of added value in this latter market due to their excellent surface and interfacial tension lowering actions and a very low critical micelle concentration. These characteristics allow the biomolecules to be used as spreaders/wetter's.
As the use of biocontrol solutions in the market increases, the compatibility of these solutions with (bio)surfactants is of great interest. It is understood that preferentially these biocontrol solutions are combined with biosurfactants to maintain the overall environmental friendliness of the formulation. To evaluate the compatibility or potential synergism of the developed MEL formulations with commercially available biocontrol solutions, a series of in vitro and in planta test were performed. Two microorganisms known to have a good performance in biocontrol were evaluated in the presence or absence of the formulated adjuvants at different dose-rate to identify any growth inhibition or potential synergism. The evaluations were carried out under the form of bioassays with plant disease (*Botrytis cinerea*) on tomatoes leaves. The biological efficacy was assessed by comparing results of plant growth after infestation without treatment to that after application of different formulated biocontrol solutions.
The test did not show any adverse effects on plant growth of MELs alone, nor in combination with the tested commercial biocontrol solutions, meaning that MELs are compatible with existing biocontrol solutions. Furthermore, some tests indicated the possibility for a synergistic effect where the presence of MEL boosts the performance of the biocontrol solutions, although no conclusive results were obtained here. A more elaborated test set up is needed to further explore these results.

Key words: biosurfactants, tank-mix adjuvants, biocontrol

Posters
Special session: Biocontrol of plant diseases
S05

AQUAPONIC WATER: A NOVEL SOURCE TO ISOLATE BCAS AGAINST *PYTHIUM APHANIDERMATUM*?

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Aquaponic systems, define as recirculating soilless systems combining aquaculture and hydroponic, are at the core of innovative researches. However, plant pest and more especially plant pathogens management is still unclear. In fact, in this kind of one loop device, where the nutrient plant solution returns to the fish part, chemical pesticides and disinfecting agents are not allowed due to the presence of fishes. Furthermore, they might be toxic for beneficial bacteria present in the system, such as nitrifying bacteria. Among the large possibility of diseases occurring in soilless systems, oomycetes pseudo-fungi, responsible of root rot diseases like *Pythium aphanidermatum* (Edson) Fitzp, are problematics due to their capacity to produce a mobile form, making the dispersion of the disease easier. Two recent articles open the hypothesis of a natural protective action of aquaponic water or fish effluents against plant pathogens during in vitro trials (Gravel *et al*., 2015; Sirakov *et al*., 2016). This phenomenon could be linked to the presence of antagonistic microorganisms or inhibitory compounds in fish water. Assumptions that don't seem aberrant considering suppressive action already observed in hydroponic systems (Postma *et al*., 2008). To confirm these observations, in vitro experiments with aquaponic water have been carried out and completed for the first time with in vivo trials to assess its capacity to procure a plant protection effect towards *P. aphanidermatum*. In vitro results show a very highly significant decrease of mycelium production when 25% of aquaponic water is added to a V8 $CaCO_3$ broth. But no difference was made between the control (standard V8 $CaCO_3$ broth) and the broth containing 25% of 0.2 µm filtrated aquaponic water. Based on this test, inhibitory action of aquaponic water seems to be linked to a microbial action. Nevertheless, an indirect action on pathogens by the way of plant stimulation by water compounds cannot be totally excluded. During in vivo tests, aquaponic lettuces inoculated with *P. aphanidermatum* have significantly less disease symptoms and a better root yields compared with inoculated lettuces grown with hydroponic water. These results highlight that aquaponic water can contribute to find a novel source of BCAs. To complete the study, water samples of both experimentations were conserved for 16S rDNA Illumina sequencing, to identify bacteria potentially antagonist against *P. aphanidermatum*.

Key words: aquaponics, suppressive, biocontrol, lettuce, *Pythium aphanidermatum*

Posters
Special session: Biocontrol of plant diseases
S06

BIOCONTROL OF COCOA POD BORER AND VASCULAR STREAK DIEBACK IN SOUTHEAST ASIA

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Vascular streak dieback (VSD), caused by *Ceratobasidium theobromae* (P.H.B. Talbot & Keane) and the cocoa pod borer (CPB), *Conopomorpha cramerella* (Snellen) (Lepidoptera: Gracillariidae) are respectively the most important disease and pest in South-East Asian cocoa (*Theobroma cacao* L.) cultivation. Decades of chemical treatments could not control CPB or VSD. Chemical VSD control is hampered because the fungus infests the trees through the xylem tissue. We have therefore looked at more integrated ways to control both VSD and CPB.

We set up two VSD biocontrol experiments on a cocoa plantation in peninsular Malaysia, studying the effect on VSD development of (i) *Trichoderma harzianum*, superficially applied to leaves of mature cacao plants, and (ii) inoculation with fungal (*Trichoderma asperellum*) and bacterial (*Bacillus* sp., *Pseudomonas* sp. and *Enterobacter* spp.) elicitors of systemic resistance in young cacao plants. After three months, cocoa leaves treated with *T. harzianum* exhibited a significantly ($p < 0.05$) lower disease score compared to the untreated control leaves. Eight months after inoculation (2 months after transplanting seedlings to the field), both number of VSD-affected leaves per plant and number of VSD-affected leaves per branch per plant were significantly ($p < 0.05$) lower as compared to control plants following inoculation by all bacterial elicitors tested, with no significant differences in the VSD controlling effect observed between the different bacterial elicitors tested.

Since the 1980s, attempts for sex pheromone-based CPB control, were hampered by the uneconomic cost of CPB pheromone (a blend of (E,Z,Z)- and (E,E,Z)-4,6,10-hexadecatrienyl acetates and corresponding alcohols) production. By modifying the existing synthesis method, we obtained a more cost-effective pheromone blend, albeit at the expense of its purity. In a Malaysian cocoa field, we tested the relative attractiveness of pheromone blends that included different levels of non-target pheromone components [(E,Z,E)-, (Z,Z,Z)-, (Z,E,Z)- and (Z,E,E)-4,6,10-hexadecatrienyl acetates] in a Malaysian cocoa field using Delta traps. Male captures were not significantly different among traps baited with pheromone blends containing 5% to 47% of non-target components, indicating that *C. cramerella* males did not discriminate among the pheromone components and other geometric isomers in the blends. The modified synthetic pathway offers the prospect of more economical production of CPB sex pheromone and its use in integrated pest management strategies such as attract-and-kill, in which lured males are killed by pyrethroid insecticides applied only to the traps instead of being sprayed on all cocoa trees.

Key words: systemic resistance, elicitors, *Ceratobasidium theobromae, Conopomorpha cramerella*, Malaysia

Posters
Special session: Biocontrol of plant diseases
S07

EFFICACY OF *OCIMUM BASILICUM* L. AGAINST *FUSARIUM OXYSPORUM* OF TOMATO IN BURKINA FASO

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Fusarium oxysporum f.sp. radicis-lycopersici is the causal agent of tomato stem and root rot. It is a disease of worldwide economic importance. In Burkina Faso, to protect their crops against this fungus, producers are over-using chemical pesticides without achieving the expected results, with consequences for human health and environment. As part of research for healthy and effective alternative control methods against this fungus, efficacy of basil extracts to reduce mycelial growth of *F. oxysporum* was evaluate in vitro. Four concentrations (15, 20, 25 and 30%) out of the six tested (5% to 30%), completely inhibited the mycelial growth of the fungus compared to the control consisting of deionized water + malt-agar.

In seed treatments, those treated with basil aqueous extract and chemical fungicide: Calthio C 50 WS (250 g/l of thiram + 250 g/l of chlorpyrifos-ethyl) used at a dose of 20 g per 5 kg of seeds were completely disinfected (100%) from the main target fungus (*F. oxysporum*) compared to the untreated control where the infection rate was 92.5%.

Regarding seedling germination, emergence rates ranged from 53% (untreated seed) to 74% (seeds treated with basil extract at 20% concentration) at 14 days post seedling. As for plant growth, seeds treated with basil extract at 20% concentration showed a 14% growth rate compared to untreated seed, 3% compared to those treated with deionized water and 12% compared to those treated with a chemical fungicide.

Owing to these interesting results basils could be used for the development of biological control measures against Fusarium wilt of tomato in Burkina Faso.

Key words: Biological control, *Fusarium oxysporum*, Tomato, *Ocimum basilicum*

Posters
Special session: Biocontrol of plant diseases
S08

SCREENING IN VITRO OF NATURAL PRODUCTS AGAINST THE PLANT PATHOGENIC BACTERIUM *PECTOBACTERIUM ATROSEPTICUM*

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Blackleg symptoms of rot of the stem and soft rot of potato tubers in field and in postharvest caused by *Pectobacterium atrosepticum* are an economically diseases in potato. Some chemical control such as cupper compounds used for the potato pest management are not efficient. So, it become essential to search for new alternative methods to control potato disease. Then, thirteen natural products (NP) were tested for their antimicrobial property against the plant pathogenic bacterium *P. atrosepticum* (CFBP 6276). The screening was carried out in vitro on agar plates following two methods: the horizontal agar diffusion method and vapour diffusion method. The vapour diffusion method screening has been supplemented by potato tubers assays for only the 3 natural products that strongly inhibited the bacteria. Results of the horizontal agar diffusion method showed that NP numbers 2, 3, 6, 10, 11, 12 exhibited a dose effect and have a significant antibacterial activity against *P. atrosepticum*. Results obtained from vapour diffusion agar and artificial infestation of potato tubers assays showed that only the NP 3 reduced the diameter of necrosis in comparison with the positive control (tuber infected by the bacterium). Thus, from all these results, NP number 3 seems very promising in vegetable protection postharvest.

Key words: *Pectobacterium atrosepticum*; diseases of potato; Natural products; screening; horizontal agar diffusion; vapour diffusion method; potato tubers assay

Posters
Special session: Biocontrol of plant diseases
S09

INTRODUCING NEXT-GENERATION PHAGE THERAPY IN AGRICULTURE

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Bacterial infections in agriculture are estimated to cause losses up to 10%. Relevant crops for agriculture in Belgium (Flanders) are *Pseudomonas syringae* pv. *porri* (*Pspo*) and *Xanthomonas campestris* pv. campestris (*Xcc*). These bacteria are known to cause bacterial blight in leek and black rot in Brassicaceae spp., respectively. Until recently, bacterial infections were treated using copper-based chemicals and antibiotics like streptomycin. The use of these compounds is however unfavourable since they are prone to resistance development and can have a detrimental impact on the environment. A valuable alternative is the use of biocontrol agents like bacteriophages to fight bacterial infections.
In this regard, different phages have been isolated to tackle both *Pspo* and *Xcc*. In the case of *Pspo*, five different phages, KIL1-KIL5 (KULeuven-ILVO), along with one host range mutant KIL3b, were found that can collapse 88% of *Pspo* strains found in Flanders1,2. For Xcc, we isolated eight different phages that can lyse 71% of the strains relevant for agriculture in Belgium (SoPhi1-7 and Phibonacci). These phages are being investigated both genetically and microbiologically to determine their host range, infection efficiency, biosafety, and potential to be used in phage therapy. Preliminary field trials have shown that the KIL cocktail is able to reduce the amount of symptomatic plants from 63% to 38,5%, which is a promising result.
Further research is needed to optimize the cocktail and its production to tackle both *Pspo* and *Xcc*. Screenings and evolution experiments are being performed to select for phages with expanded host and stability ranges. Also tests to evaluate phage biosafety are performed. In terms of production, progress has been made using a novel system for phage purification based on anion exchange chromatography using CIM® Disk (BIA Separations) FPLC. In the case of the KIL cocktail, a recovery of 95% of the loaded phages was obtained.

Key words: Phage therapy, bacterial infections, biopesticide

Posters
Special session: Biocontrol of plant diseases
S10

IN VIVO ANTIFUNGAL POTENTIAL OF ESSENTIAL OILS BASED NATURAL PREPARATIONS AND THEIR EFFECT ON WHEAT QUALITY

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All wheat varieties are subject to pathogen attacks during vegetation causing, among others, foliar disease such as *Septoria tritici*, *Erysiphe graminis*, *Fusarium* spp., *Puccinia recondita*, *Tilletia* spp. or *Pyrenophora gramineum*, *Blumeria graminis*, *Erysiphe*, *Ascochyta*, *Puccinia graminis punctiformis*, which leads to premature and permanent damage of the foliar tissue, and this in turn, causes reduction in the photosynthesis process. Alternative to use of synthetic antifungal products are natural preparations based on essential oils that have proven effective in in vitro studies against *Fusarium*, *Penicillium* or *Aspergillus*.

The aim of this paper is to study the in vivo effect of two natural emulsions based on *Anethum graveolens* in combination with *Thymus vulgaris* (I) and *Foeniculum vulgare* in combination with *Thymus vulgaris* (II), respectively, as curative, or protective agents against fungal diseases developed during wheat vegetation. Also, the impact on Fusarium mycotoxins development (DON and Fumonisins) and the effect on wheat production and quality was observed.

The natural emulsions preparations were tested on different winter wheat varieties, both as protective agents, prior to the onset of the disease, and as curative agents after the disease has been installed. The treatment applied was repetitive in 3 stages, in different phases of vegetation. The general results highlight that the natural mixtures do not show curative action against fungal and bacterial diseases ready installed but can be applied with protective effect before the disease is installed.

Concerning the mycotoxin content (DON and Fumonisins) in the wheat culture, they were not identified above the minimum detection limit of 0.08 ppm in any of the analyzed samples. In view of the low rainfall of the spring of 2017, no conditions favouring the development of fungus species such as Fusarium have been created so that the contamination with Fumonisins or DON mycotoxins has been reduced, both in the case of the control and experimental variants with antifungal treatments.

Regarding the quality indices, the humidity is not influenced by the treatment with the natural preparations, being correlated with the climatic conditions during harvesting. The moisture content of the samples ranges from 10.98-13.96%, the humidity below 14% being optimal for the preservation of cereals without biochemical and microbiological degradation. The protein content varies between 13.69-19.14%. The protein profile of the analyzed samples is like the gluten profile and suggests an increase in protein content in the experimental variants treated with preparations I and II.

In conclusion, considering the fungistatic effect and the absence of toxicity, natural preparation based on essential oils can be recommended as protective agents in wheat protection.

Key words: winter wheat, essential oils, antifungal effect

IN VITRO AND IN PLANTA ANTIFUNGAL ACTIVITY OF COGNAC OIL ON *ZYMOSEPTORIA TRITICI*

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Septoria tritici blotch is caused by the fungus *Zymoseptoria tritici* and is the most devastating foliar diseases on wheat, causing great yield losses. In intensive farming, the disease is treated via chemical fungicides. Because chemical fungicides are raising questions about the environment and health, alternative methods for crop protection are being considered. In this perspective, cognac oil (CO), an essential oil obtained by distilling wine lees during wine making, was tested on *Z. tritici*. CO, ethyl decanoate (C10) and ethyl dodecanoate (C12), its two main components identified via GC-MS, were tested three times in vitro in liquid medium using 96-well microtiter plates. CO, C10 and C12 were tested crude as well as combined with Tween 80. IC_{50} (half inhibitory concentrations) with 95% confidence intervals were obtained. CO, C10 and C12 showed antifungal properties on *Z. tritici*. Observed under the microscope, the morphology of spores was indeed altered when in contact with CO, C10 and C12. In planta, CO was tested combined with Heliosol as preventive and curative treatments on wheat var. Alixan in a greenhouse. The percentage of infected leaf area was then measured. ANOVA analysis shows that CO combined with Heliosol provides the plant some protection as a preventive treatment but had no effect as a curative treatment.

Key words: *Septoria tritici* blotch (STB), *Zymoseptoria tritici*, cognac oil, biopesticides

Posters
Special session: Biocontrol of plant diseases
S12

ULVANS ARE EFFECTIVE IN CONTROLLING POWDERY MILDEW IN BREAD WHEAT

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Ulvans are water-soluble sulphated heteropolysaccharides extracted from the cell walls of the green seaweeds *Ulva* spp. These polysaccharides are known to protect plants against a broad range of pathogens, including biotrophic, necrotrophic and hemibiotrophic fungi, such as, *Erysiphe polygoni*, *Alternaria brassicicola* and *Colletotrichum fructicola*, respectively. The first objective of this work was to evaluate the direct activity of ulvans on conidial germination of *Blumeria graminis* f. sp. *tritici* (Bgt) and, the second one, was to estimate the efficiency of ulvans to control powdery mildew on bread wheat. To evaluate conidial germination in vitro, two types of ulvan precipitates (U1P and U2P) were amended in water-agar medium at 1 and 10 mg.mL^{-1}. The two ulvans at both concentrations (1 and 10 mg.mL^{-1}) enhanced conidial germination of Bgt and stimulated the formation of multiple germ tubes. Regarding the effect of ulvan treatments on powdery mildew disease expression, U1P and U2P were sprayed on wheat leaves at 1 and 10 mg.mL^{-1} on ten-day old plantlets (cv. Pakito). After 48 hours, plants were inoculated by a spore's solution of Bgt (5x10^5 conidia.mL^{-1}) and the disease symptoms were estimated after 10 days. U1P and U2P significantly reduced the severity of powdery mildew by 42-48% and 42-40% at 1 and 10 mg.mL^{-1}, respectively, when compared to control plants sprayed with water. Our results suggest that ulvans had no direct effect on fungal in vitro and the efficacy of these polysaccharides could be attributed to plant resistance induction. Further studies are being conducted (i) to elucidate the induced plant defence mechanisms involved in wheat protection and (ii) to characterize the biochemical composition of the both ulvan precipitates. This study provides new insights into the development of ulvan-based biocontrol products to control powdery mildew in wheat plants.

Key words: algal polysaccharides, *Blumeria graminis*, seaweeds, *Triticum aestivum* L., ulvan

Posters
Special session: Biocontrol of plant diseases
S13

BIOPROTECTION OF WHEAT AGAINST *ZYMOSEPTORIA TRITICI* USING ALGAL POLYSACCHARIDES

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Zymoseptoria tritici is the most frequent and damaging foliar pathogen on wheat crops, especially in North-Western Europe, where environmental conditions are suitable for the *Septoria tritici* blotch disease development. Although the use of resistance inducers to control plant diseases is encouraged, their potential effectiveness on the wheat-*Z. tritici* pathosystem remains both poorly studied and often non-significant at the field level. Sulphated heteropolysaccharides, extracted from cell walls of *Ulva* spp., are known to protect plants against broad-spectrum of diseases caused by biotrophic, necrotrophic and hemibiotrophic pathogens. The aim of this study was to evaluate the efficacy of algal polysaccharides on bread wheat plants against *Z. tritici*. First, direct activity of ulvans on conidial germination and mycelial growth were examined at concentrations of 0.5, 1 and 10 mg/mL in vitro assays. No effect of these polysaccharides on conidial germination was detected, but at 10 mg/mL ulvan slightly increased mycelial growth of *Z. tritici*. Furthermore, protection efficacy of ulvans was tested in the greenhouse by spraying three-week-old wheat plants (0.5, 1 and 10 mg/mL; cv. Alixan) two days before fungal inoculation (1 x 10^6 conidia/mL). Disease evaluations 21 days later showed that ulvans can protect wheat plants against *Z. tritici*. As no direct effect on fungus was observed, the wheat protection obtained with foliar application of ulvans may be related to the stimulation of wheat defense mechanisms. Further assays are ongoing in order to characterize the plant defence reactions potentially induced by ulvans.

Key words: *Triticum aestivum* L., *Septoria tritici* blotch, seaweeds, ulvans.

Posters
Special session: Biocontrol of plant diseases
S14

CONTROLLING POWDERY MILDEW ON LATE PLANTING COURGETTE WITH BIOPESTICIDES

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Powdery mildew is an important foliar disease in courgette caused by the fungus *Erysiphe cichoracearum*. When the disease progresses, the white powdery spots on the leaves lead to defoliation and consequently to yield loss. Powdery mildew is typically more damaging on courgette from mid-august on. Four active compounds (sulphur, potassium bicarbonate, laminarin and *Bacillus subtilis*) are currently permitted for use in organic agriculture in Belgium. The use of Agricole, a product composed of natural polysaccharides extracted from algae, is also permitted due to its sole physical action. Most products only have a preventive effect against powdery mildew. Only sulphur and potassium bicarbonate have a limited curative mode of action. Sulphur is traditionally used most frequently but leaves a residue on harvested fruits. Little is known about the suitability of the other products as alternatives for sulphur and their effectiveness for use in courgette.
The five products mentioned above have been tested in two consecutive years in a late season crop of courgette at the organic trial farm in Beitem, Belgium. In addition, new products, which are not yet authorized for use, were added to the trial (one in 2016 and another two in 2017). The trials were planted on the 20th of July and products were applied in the period August – September. All products were applied three times in 2016. In 2017, laminarin, *Bacillus subtilis*, and the new products were applied six times, ensuring a minimum of two applications before the first symptoms occurred. Sulphur and potassium bicarbonate was used suboptimal, with four applications in total, starting when the first symptoms were detected. Of the tested active compounds, only plants treated with sulphur were significantly less affected in both trials. Despite the better condition of the plants treated with sulphur, the yield and quality were not significantly better in comparison to the untreated control. In these specific conditions the disease severity seems to be less important than other factors influencing the yield such as decreasing activity of insect pollinators, decreasing temperatures at the end of the harvesting period, senescence of plants and other influencing diseases. Our results suggest that the new products tested in these trials are not suitable as alternatives for sulphur to control powdery mildew on courgette and that suboptimal use of sulphur treatments in late planting courgette do not lead to higher yield or to better fruit quality.

Key words: *Erysiphe cichoracearum*, Courgette, Biopesticides, Sulphur

Posters
Special session: Biocontrol of plant diseases
S15

EVALUATION OF GENOTYPIC AND PHENOTYPIC DIVERSITY OF *RHIZOGENIC AGROBACTERIUM* ISOLATES FROM HYDROPONIC TOMATO GREENHOUSES IN EUROPE

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Rhizogenic *Agrobacterium biovar* 1 is the etiological agent of crazy root disease, also known as hairy root disease (HRD), affecting tomato, cucumber, and eggplant grown in hydroponic greenhouses. During the infection process, a segment of the Ri (Root-inducing) plasmid, known as T-DNA or transferred DNA, is exported from *Agrobacterium* into the plant cell nucleus, where it integrates itself into the chromosomal DNA and starts being expressed. The expression of the T-DNA segment produces the distinctive symptom of the disease, which is excessive root proliferation, that subsequently impedes irrigation in the substrate and results in a strong vegetative growth. Altogether, HRD causes yield and profit losses of more than 10%.

A previous study was conducted aiming for the characterization of rhizogenic *Agrobacterium biovar* 1 isolates originating from Belgium. Building on that basis, the present study aimed to evaluate the genotypic and phenotypic diversity of 72 rhizogenic *Agrobacterium* isolates from Belgium, the Netherlands, France, Germany, the United Kingdom, Poland, Russia, and Canada, to obtain a comprehensive view of the diversity of rhizogenic Agrobacterium isolates throughout Europe and beyond.

From each isolate, four chromosomal genes (16S, recA, rpoB, and trpE) and two plasmid-borne genes (virD and rolB) were PCR-amplified and subsequently partially sequenced. Concatamers were generated consisting of either the chromosomal genes or the plasmid-borne genes and aligned using the MUSCLE algorithm. Subsequently a Maximum-Likelihood tree was constructed with MEGA7 using default parameters and 500 bootstrap replications. Genetic distance analysis was conducted using the Tamura-3 parameter substitution model. Most of the isolates studied clustered according to genomospecies, which is the ultimate criterion used by bacterial taxonomists to define bona fide species. It is worth mentioning that four isolates clustered separately from the rest of the isolates studied.

The aforementioned genotypic data was complemented with phenotypic data obtained from biofilm forming capacity, catalase activity, and hydrogen peroxide half maximal effective concentration (EC_{50}) assays. Biofilm formation capacity was evaluated by the crystal violet method, catalase activity was evaluated by bubble production in a 3% (v/v) hydrogen peroxide solution, and hydrogen peroxide half maximal effective concentration (EC50) was evaluated in 96-well plates containing Luria broth supplemented with different concentrations of hydrogen peroxide. No clear correlation was found between the origin of the isolates, plant host, or phenotypic characteristics and their positions in the phylogenetic tree.

Key words: MLSA, rhizogenic *Agrobacterium*, hairy root disease, phenotypic diversity, genetic diversity

Posters
Special session: Biocontrol of plant diseases
S16

HIGH-THROUGHPUT SCREENING AND MODES OF ACTION OF NEW BIOCONTROL PRODUCTS ON THE WHEAT-*ZYMOSEPTORIA-TRITICI* PATHOSYSTEM

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Zymoseptoria tritici is a hemibiotrophic fungus that causes Septoria tritici blotch of wheat, a disease responsible for yield losses which can reach 50%. Nowadays, protection against this pathogen relies mainly on the use of conventional pesticides. Nevertheless, because of the resistances developed by the fungus against these products and a social context claiming a decrease of the use of chemical inputs in agriculture, new crop protection methods must be developed.
The goals of my thesis project are to identify new biocontrol products on the wheat-*Z. tritici* pathosystem and to characterize their modes of action. This project should follow three steps:

- A high-throughput screening of biomolecules of diverse origins and natures (beneficial endophytes, bacterial and fungal extracts, glycolipids, rhamnolipids …) will be performed, the potential elicitor activity of biomolecules will be tested on wheat cell culture and leaves and their direct activity will be evaluated on in vitro fungal culture (work in progress).
- The biomolecules with direct and/or indirect effect will then be tested in planta in the greenhouse to assess their protection efficacy. An optimization of the application conditions will also be realized.
- The mode of action of the most effective bio-products will finally be studied by (1) a global approach using "omics" tools and (2) a targeted one using different cytological, biochemical, and molecular tools to confirm the results obtained with the previous approach.

Key words: *Zymoseptoria tritici*, wheat, biocontrol, cell culture, elicitors

Posters
Special session: Biocontrol of plant diseases
S17

SACCHARIN INDUCES RESISTANCE IN WHEAT AGAINST *ZYMOSEPTORIA TRITICI*

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Elicitors of plant resistance are among the most considered biocontrol tools. Saccharin, one of the metabolites of probenazole, has been tested on a broad range of pathosystems, but not yet on bread wheat against *Zymoseptoria tritici*, despite of the economical incidence of this pathogen and the urgent need for a reduced use of conventional fungicides. In this study, we assessed the effect of saccharin on the susceptible wheat cultivar Alixan challenged with the pathogenic *Z. tritici* strain T02596. Wheat seedlings were sprayed with 5 mM or 15 mM saccharin mixed with Heliosol® or Cantor® as adjuvants, respectively. Two days post-treatment (dpt), plants were inoculated with a spore suspension (10^6 spores.mL^{-1}) amended with 0.05 % Tween 20. Both saccharin concentrations conferred significant protection levels against the pathogen. Saccharin reduced the percentage of diseased leaf area by 55% and 77%, at the 5 mM and 15 mM concentrations, respectively, while no protection was conferred by the two tested adjuvants when used alone. For the highest 15 mM concentration , in vitro and in planta investigations did not show any direct effect of saccharin on fungal spore germination and mycelium growth, suggesting that the protection is due to an elicitation of wheat defences. To elucidate plant defense reactions involved in this protection, relative expression of four genes encoding for several defense markers, lipoxygenase (lox), phenylalanine ammonia lyase (pal), pathogenesis-related protein 1 (pr1) and a peroxidase (pox2) was monitored on a time-course experiment (from 2 to 7 dpt). Gene expression analysis demonstrated an upregulation of lox at all studied time-points, while pr1 was only induced at 7 dpt in leaf tissues of wheat treated with saccharin. Neither pal nor pox2 expression showed any marked changes at the studied time-points when compared to the control. Taken together, our findings report for the first time the ability of saccharin to protect wheat against *Z. tritici*, probably through the activation of plant defense reactions. Further analyses would provide a better characterization of the mechanisms involved in the highlighted induced resistance.

Key words: saccharin, elicitors, *Zymoseptoria tritici*, wheat

Posters
Special session: Biocontrol of plant diseases
S18

POTENTIAL OF *TRICHODERMA* IN THE BIOCONTROL OF FUSARIUM WILT IN THE ORNAMENTAL CROP BEGONIA

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As in other crops, ornamental producers can only use a limited number of chemical products to control pathogenic fungi. IPM strategies need to be developed, including biological control as an important component of this approach. The application of antagonistic fungi is a promising alternative. *Trichoderma* has been recognized for a long time as such an antagonist, known for its biocontrol capacity and growth promoting effect. However, not all *Trichoderma* species and isolates have similar capacities. In addition, their interactions with different plant hosts and pathogens can lead to different outcomes. *Trichoderma asperellum* strain T34 has shown effective control of Fusarium wilt of tomato and carnation, but its potential against *Fusarium foetens* in begonia has not been investigated. *F. foetens* is on the EPPO A2 list (recommended for regulation) and is a big issue for Hiemalis begonia growers. Rooted cuttings of begonia cultivar Baladin were transplanted to pots filled with potting soil mixed with Asperello, a commercial product containing spores of *T. asperellum* T34 (10^4 spores/ml of potting soil). One month after transplanting, *F. foetens* was inoculated by adding 10 ml of a spore suspension of 5 x 10^3 CFU/ml around each plant. The fungicide Topsin was applied one week before pathogen inoculation as a chemical control. Most of the pathogen-inoculated plants (without Asperello or Topsin treatments) showed growth reduction. Only in a few plants, strong symptoms developed, causing the death of the plants. The stem and leaves of these plants wilted, and even the stem bent. In the worst cases, we could see *Fusarium* sporulation on the surface of the infected plants. During the trial, flower development was evaluated. At the end of the trial, shoot fresh weight and the level of root development were determined. The results of this experiment showed that the *Trichoderma* treatment could prevent plant growth reduction due to *Fusarium* inoculation. The plants inoculated with both *Trichoderma* and *Fusarium* showed a higher average fresh weight and improved flowering compared to plants inoculated with *Fusarium* only. The level of disease control was higher with *Trichoderma* than with Topsin. When only *Trichoderma* was inoculated, flower stimulation was not observed compared to the non-inoculated control. Therefore, it seems that *Trichoderma* stimulated flowering only when plants were stressed. We conclude that there are opportunities for using Asperello as a biological control agent against *F. foetens*.

Key words: biological control, *Fusarium foetens*, flowering

WHEAT SEED TANNING WITH *CLONOSTACHYS ROSEA* F.SP. *ROSEA* FOR THE CONTROL OF TAKE-ALL DISEASE OF WHEAT AND FUSARIUM HEAD BLIGHT

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Fungal diseases of wheat can lead to losses of yield and nutritional quality through production of mycotoxins. The most important diseases are: take all disease, caused by *Microdochium nivale* and some members of the Fusarium complex, and head blight, caused by *Fusarium* species. A mixture of the wild types and of two fungicide-resistant mutant strains of *Clonostachys rosea* was used for seed coating and tested on the durum wheat cultivar Quadrato. The coating markedly improved resistance to take all disease, outperforming chemical seed coating, improving crop yield and the marketable quality of grains. The treatment was less effective against head blight.

Key words: seed tanning, wheat, *Clonostachys rosea*, take all disease, Fusarium head blight

Posters
PHYTOPATHOLOGY

Posters
Phytopathology
P01

EFFECTS OF SEED TREATMENT FUNGICIDES ON IMPORTANT ROOT ROT DISEASES OF SUGAR BEET IN TURKEY

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About one third of sugar beet production of Turkey is obtained from Konya province. This region not only provides materials to four sugar beet refineries in Konya but also to Amasya, Adapazarı and Kütahya sugar beet refineries. Sugar beet root rots in Konya province was investigated during 2015-2017 with a project funded by Turkish Scientific and Technical Research Foundation and four root rot pathogens were found widespread and more aggressive. These are Aphanomyces cochlioides, *Phoma betae*, *Pythium* spp. and *Rhizoctonia solani*. Effectiveness of 11 seed treatment fungicide mixtures and one insecticide (imidacloprid) against the above mentioned fungal pathogens was determined by in vitro studies carried out in climatically controlled chambers. The highest rate of protection (84%) against *Aphanomyces cochlioides* was provided by Boscalid+pyraclostrobin+metalaxyl mixture. Against *Pythium* sp., *Phoma betae* and *Rhizoctonia solani*; Thiram+metalaxyl+hymexazol+pyraclastrobin, Thiram+hymexazol+pyraclostrobin, and Thiram+tolchlofos-methyl mixtures provided 78%, 72%, and 37% effectiveness respectively. Field performances of these fungicide mixtures are in progress.

The work presented, which was funded by TUBITAK (Turkish Scientific and Technical Research Foundation) (grant number: TOVAG 115O562).

Key words: Sugar beet, root rot, fungicide mixtures, effectiveness

Posters
Phytopathology
P02

EVALUATING THE EFFECTIVENESS OF CERTAIN FUNGICIDES APPLIED PREHARVEST TO CONTROL POST-HARVEST DISEASES OF PEACH AND NECTARINE FRUITS

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In this work, the effectiveness of fungicide treatments on the development of post-harvest rot of fruit was evaluated on 3 cultivars of peach Alexandra, Pecher-Louata and Ryan Sun, and a cultivar of nectarine Orion, located in the Sefrou region of Morocco. Five preharvest treatments were applied to the trees 15 days before harvesting according to a complete random block design with 4 replicates [Tebuconazole (62.5 g ai/ha), cyproconazole (12 g ai/ha), a mixture of tebuconazole (31.25 g ai/ha) and cyproconazole (6g ai/ha), a mixture of captan (1000 g ai/ha) and cyproconazole (12 g ai/ha), a mixture of tebuconazole (62.5 g ai/ha) and thiophanate-methyl (700 g/ha) and a control without fungicide treatment]. Fruits were harvested, packaged, placed in ambient air, and arranged according to the experimental design with 4 replicates. Infected fruits were counted daily, and the causative agent was identified accordingly. Results showed that only *Rhizopus* spp. was responsible for the spoilage of fruit in post-harvest conditions. On the control packing cases, the spoiled fruit were varied from 50 to 90% and from 80 to 100% respectively after 6 and 9 days after harvesting and depending on the cultivars. The fungicidal treatments showed a good control of this disease relative to the control. the least effective treatment was the tebuconazole and thiophanate-methyl mixture with a protective period of 6 to 9 days, and the most effective treatment was the mixture of captan and cyproconazole, which had extended the shelf life of the fruit to more than 12 days. In addition, the residue analysis was in accordance with European regulations and showed no excess of the maximum residue limit (MRL) for the used active ingredients in this study.

Key words: Peach, Nectarine, rot, fungicides, Rhizopus spp., Postharvest

Posters
Phytopathology
P03

DIAPORTHE VACCINII IN BLUEBERRY IN BELGIUM: STATUS AND FUNGICIDE EFFICACY

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The economically most important *Vaccinium* species in Europe are *V. myrtillus* (wild blueberry), *V. corymbosum* (American blueberry) and to a lesser extent *V. oxycoccus, V. macrocarpon, V. uliginosum* and *V. vitis-idaea*. In Belgium, commercial cultivation is focused on *V. corymbosum* while *V. myrtillus* is endemic in forests. The fungus *Diaporthe* includes species that cause twig lesions and mortality on Vaccinium, of which *Diaporthe vaccinii* is one of the most important ones. It is classified as a quarantine organism in the EU. To determine the status of *D. vaccinii* on *Vaccinium species* in Belgium, a federally-funded project (Gaphannex) was started. A two year survey is being conducted throughout Belgian forests as well as in the most important blueberry production regions in the country. Samples were collected and analyzed for the presence of *Diaporthe* species via traditional plating on nutrient media but also using novel multiplexed qPCR assays for *D. vaccinii, D. eres* and *D. viticola*. During the first year of the survey, no *D. vaccinii* was detected, while *D. eres* and *D. viticola* were relatively common. The qPCR assays were more sensitive than the traditional plating technique. The project also includes research towards the efficacy of plant protection products for the control of *D. vaccinii* on blueberry. So far, different in vitro fungicide trials indicate the potential of some of the products to control spore germination and/or mycelial growth of the fungus.

Key words: *Diaporthe vaccinii*, blueberry, Vaccinium, qPCR, fungicides

Posters
Phytopathology
P04

EFFICACY EVALUATION OF SEED TREATMENT AGAINST COMMON BUNT IN WINTER WHEAT

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There are two fungal species of *Tilletia* genus which occur in wheat in Latvia - *Tilletia controversa* J.G. Kühn, Hedwigia and *Tilletia caries* (DC.) Tul. & C. Tul. (syn. *T. tritici* (Bjerk.) Wint.). *T. controversa* is causal agent of dwarf bunt, and *T. caries* - common bunt. According to I. Priekule *T. caries* in Latvia could be up to 75%.

Field trial was carried out by Latvian Plant Protection Research Center in Study and Research farm "Peterlauki" in two growing seasons - 2015-2016 and 2016-2017 to investigate efficacy of fungicides against *T. caries* in winter wheat (*Triticum aestivum* L.) under artificial infection. In season 2015-2016 winter wheat cultivar 'Zentos', and in season 2016-2017 cultivar - 'Skagen' were used. *T. carries* spores were obtained from local population. Contamination of seed at rate 2 g chlamydospores per 1 kg of seed was done before seed treatment. Seed was treated with fungicides (e.g. "Seedron" with active ingredients: fludioxonil 25 g L^{-1}, tebuconazole 10 g L^{-1}) using equipment HEGE 11 right after contamination.

To evaluate common bunt incidence in winter wheat, infected wheat ears were counted in each plot at BBCH 83. In control variant disease incidence was 1.28% (71 infected ears per 15 m^2), 9.85% (546 infected ears per 15 m^2) in years 2016 and 2017 respectively. Low temperature in autumn 2016 was favorable for common bunt development in compare with autumn 2015. There was no infection of *T. caries* detected in plots where seed treatment was used, that means all seed treatments had 100% efficiency in control of *T. caries* in both years. Assessments on crop germination were made in autumn at BBCH 9 and overwintering in spring at BBCH 15-21. Root rot (*Fusarium* spp.) was assessed at BBCH 9, and pink snow mold (*Microdochium nivale*) at BBCH 12-21. There was no significant ($p>0.05$) difference in germination between treated and untreated variants. Severity of root rot in untreated variants in autumn 2016 was 10%, in autumn 2017 - 13%. Severity of pink snow mold in spring 2016 was 2%, in spring 2017 - 4%. There was no significant difference in root rot severity in 2016 but in 2017 it was significantly ($p<0.05$) lower in treated variants. In 2016 pink snow mold severity was significantly ($p<0.05$) lower in treated variants compare to control variant but there was no significant difference in 2017.

Key words: winter wheat, *Tilletia caries*, seed treatment

Posters
Phytopathology
P05

EVALUATION OF INHIBITORY EFFECT OF ORGANIC AND INORGANIC SALTS AGAINST CARROT SOUR ROT AGENT *GEOTRICHUM CANDIDUM*

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Geotrichum candidum Link. is one of the most common postharvest pathogen that causes sour rot of carrots, tomatoes, potatoes, cucumbers, melons, citrus fruits, and other vegetables and fruits. In Turkey, there are currently no registered commercial fungicides approved for the control of sour rot, therefore, alternative strategies are needed for the pathogen control. In the present study, efficacy of 28 organic and inorganic salt against *G. candidum* were evaluated under in vitro and in vivo conditions. In vitro studies indicated that ammonium (bicarbonate and carbonate), calcium (oxide), potassium (benzoate, carbonate, and sorbate), and sodium (benzoate, carbonate, fluoride and metabisulphite) salts (2%) inhibited completely mycelial growth of *G. candidum*. Except for potassium benzoate, potassium carbonate and sodium benzoate, seven other salts used in this study completely also inhibited spore germination of the fungus; however, the difference among the inhibitory effects of potassium benzoate and sodium benzoate, and the other salts mentioned above was not statistically significant ($P<0.05$). The ED_{50}, minimum inhibition concentration (MIC), and minimum fungicidal concentration (MFC) values indicated that sodium metabisulphite and fluoride, ammonium carbonate and bicarbonate, and calcium oxide was more toxic to *G. candidum* than all the other salts. In curative and preventive applications in vivo, calcium (acetate, chloride, citrate, formate, lactate, oxide, propionate, and silicate), potassium (bicarbonate) and sodium (acetate, chloride, and fluoride) salts significantly reduced the development of sour rot in carrot slices when compared to pathogen-inoculated control ($P<0.05$). The results of present study demonstrate that these salts may be used to control of carrot sour rot.

Key words: Carrot, *Geotrichum candidum*, organic and inorganic salts, alternative control

Posters
Phytopathology
P06

EVALUATION OF ANTIFUNGAL ACTIVITY OF SODIUM SALTS AGAINST FUSARIUM WILT CAUSED BY *FUSARIUM OXYSPORUM* F.SP. *MELONGENAE*[1]

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Fusarium wilt caused by *Fusarium oxysporum* (Schlechtend.: Fr.) f.sp. *melongenae* (Fomg) is one of the most important diseases of eggplant (*Solanum melongena* L.) in Turkey. Methods used to control vascular wilt are either not very efficient or are difficult to apply. In this study, the efficacy of nine concentrations (0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, and 2.0% (w/v) of five sodium salts (carbonate, bicarbonate, benzoate, citrate dihydrate and metabisulfite) as possible alternatives to methods used for the control of *F. oxysporum* f.sp. *melongenae was* evaluated in 2016. In vitro trials indicated that there were significant differences between the inhibitory effects of the sodium salts on the mycelial growth ($P<0.05$), and 2% (w/v) concentrations of sodium carbonate, benzoate and metabisulfite completely inhibited mycelial growth of the fungus, while two other sodium salts did not. Except for sodium citrate dihydrate, however, the differences in inhibitory effects of those on the spore germination of the fungus were not statistically significant ($P<0.05$). The ED_{50}, minimum inhibition concentration (MIC), and minimum fungicidal concentration (MFC) values showed sodium metabisulfite to be more toxic to the fungus than four other sodium salts. In soil tests, although concentrations of 0.75% sodium metabisulfite and benzoate completely inhibited the mycelial growth of the fungus, three other sodium salts reduced mycelial growth to some extent (50.59-83.41%) even at the highest concentration ($P<0.05$). The study results showed that the sodium metabisulfite and benzoate treatment may be applied as an alternative chemical for the control of the Fusarium wilt on eggplant.

Key words: *Solanum melongena*, Fusarium wilt, sodium salts, inhibitory effect, alternative control

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Posters
Phytopathology
P08

NEW AND FAST DETECTION OF LITTLE CHERRY VIRUS 1 USING ONE-STEP REVERSE TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP)

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Little cherry virus 1 (LChV-1, Velarivirus, Closteroviridae) is an economically important pathogen affecting mainly cherry around the world emphasizing the impetus for its efficient and accurate on-site detection. This study describes the development of a reliable diagnostic protocol of LChV-1 based on a fast, sensitive, and easy-to-use one-step reverse-transcription loop-mediated isothermal amplification (RT-LAMP). The protocol detects all LChV-1 isolates in less than 10 min by fluorescence monitoring using a mobile detection device (GENIE, OptiGene) and is most optimal when incubation is performed at 67 °C. Sharp melting curves and unique melting temperatures (Tm) were obtained for the positive samples. Both the LAMP and classical RT-PCR methods are capable of specifically detecting LChV-1 in infected leaf tissues, but this validated leaf-to-result assay has remarkable advantages in comparison to RT-PCR. It is at least hundred fold more sensitive, significantly faster, and efficient at minimal cost, and is ready for on-field applications. In conclusion, this innovative LAMP approach can contribute to implementation of sustainable integrated management strategies for on-site detection of LChV-1 in commercial orchards or for horticultural research stations. It is also suitable for decision support in phytosanitary epidemiological programs.

Posters
Phytopathology
P09

OCCURENCE OF '*CANDIDATUS PHYTOPLASMA SOLANI*' IN ASSOCIATION TO A NEW HOST PLANT PLUM (*PRUNUS DOMESTICA* L.) IN JORDAN

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Plum (*Prunus domestica* L.) is among the most important stone fruit species grown in Jordan, especially in the northern part of the country. In September 2017, orchards of *P. domestica* L. cv. Golden Plum, in the Mafraq region North East of Jordan, showed typical phytoplasma symptoms, such as yellowing and reddening of the leaves, stunted growth and witches'-broomed branches. Additionally, these fields also contained bindweed (*Convolvulus arvensis* L.) showing stunting, leaf malformation and chromatic alteration. Leaf samples were collected from 20 symptomatic and asymptomatic plum trees and 3 symptomatic bindweed plants. Total genomic DNA was extracted, and samples were analyzed by PCR using the phytoplasma universal primer pairs P1/P7 followed by nested PCR with primer pair R16F2n and R16R2. DNA samples of five out of twenty P. domestica and the three bindweed samples together with the positive controls were tested positive for phytoplasma infection, yielding the expected PCR amplicons at 1,25 kbp. No phytoplasma was detected in symptomless Prunus trees that were sampled from the same orchard and the negative controls. Sequencing was done for each gel-purified PCR amplicon. BLASTn similarity analysis of sequences derived from the plum and the bindweed host plants revealed that the sequences of phytoplasma infecting *P. domestica* and *C. arvensis* in Jordan were highly similar (>99%) to the in NCBI GenBank® deposited *Vitis vinifera* L. sequences of '*Candidatus Phytoplasma solani*' from Jordan (KC835139) and sequences from Serbia (AF248959). In silico RFLP analyses and phylogenetic analysis confirmed the affiliation of these phytoplasma strains identified in plum and bindweed in Jordan to the species '*Ca. Phytoplasma solani'* (subgroup 16SrXII-A). Taken together, these results confirm the unique presence of '*Ca. Phytoplasma solani'* associated to plum in the stone fruit growing area of Mafraq. The presence of the bindweed as a well-known phytoplasma reservoir and potential insect vectors may have contributed to the plum infection. To the best of our knowledge, this is the first detection of '*Candidatus Phytoplasma solani'* infecting *P. domestica* so far.

Posters
Phytopathology
P10

SYTRANSPOM: DEVELOPMENT OF COLLABORATIVE AND INNOVATIVE ALERT AND DECISION SYSTEMS PROMOTING INTEGRATED PROTECTION AGAINST FUNGAL POTATO DISEASES

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Potatoes are economically important crop in Belgium and France. Fungal potato pathogens cause many damages in crops. The most important fungal pathogens are *Phytophthora infestans* and *Alternaria solani*, responsible of late blight and early blight respectively. Other pathogenic fungi, such as *Botrytis cinerea, Sclerotinia sclerotiorum, Erysiphe cichoracearum* or *Phoma exigua*, may also be incriminated and require a monitoring, although they are less problematic. To develop and/or improve alert and decision systems for potato crop, SYTRANSPOM (Interreg V Program FrWVl), a collaborative cross-border research project, was set up. It started in April 2018 thanks to the combination of multidisciplinary expertise of 4 partners in each of the three regions: ARVALIS (Fr), CARAH (W), PCA and INAGRO (Vl).

SYTRANSPOM is articulated around 4 main research axes:

(i) Development of a cross-border collaborative platform to centralize known data (as climatic and soil data, …) and register new data collected from weather forecasting, precision farming, field trials and laboratory experiments
(ii) Development of laboratory qualitative and quantitative molecular methods to characterize fungal pathogens
(iii) Implementation of experimental field trials, with the support of molecular tools, to obtain early diagnosis of infections, monitoring the development of diseases and improving methods of protection
(iv) Development of complete decision support systems for potato crop incorporating alert/warning systems for several foliar pathogens

Key words: Potato crop, Fungal pathogens, collaborative platform, molecular tools, decision support systems in crop protection

Posters
Phytopathology
P11

DEVELOPMENT OF FAST AND SENSITIVE ON-SITE DIAGNOSIS, REVERSE TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP), FOR PEPPER CHAT FRUIT VIROID (PCFVD)

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Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is a fast and reliable RNA diagnostic assay, outcompeting distinctly conventional reverse transcription polymerase chain reaction (RT-PCR) in robustness, analytical sensitivity, and specificity. In addition, it is also highly cost-effective, and the procedure is far less time consuming. In this work, a PCFVd (Pepper chat fruit viroid) specific RT-LAMP detection assay was developed, based on a set of six primers. PCFVd, one of the most important tomato and pepper diseases in Thailand, can be detected within 15 minutes with a sensitivity that is comparable as a probe-based RT-qPCR and 10-100 times higher than the available endpoint RT-PCR methods. No cross-amplification with other viroids and tomato viruses was observed. The on-site diagnostic assay was adapted for on-site applications with a simple plant lysis procedure and SYTO-9, fluorescence dye, for visualization under both visible and UV lights. In the closed-tube reaction mix, positive reactions displayed color change from yellow to lime green under visible light and fluorescence under UV light. The results indicate that this PCFVd RT-LAMP assay will be useful for any in-field application of the method.

Posters
Phytopathology
P12

SCREENING FOR RESISTANCE TO FUSARIUM WILT IN TUNISIAN MELON CULTIVARS USING MOLECULAR MARKERS

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Fusarium wilt of melon caused by *Fusarium oxysporum* f.sp. *melonis* (FOM) is one of the most threatening fungal diseases of melon crops. Four races (0, 1, 2, and 1-2) of the pathogen are defined. Resistance to race 1 and race 2 is conferred by a single dominant gene Fom-2 and Fom-1, respectively. Both genes also confer resistance to race 0. Host-plant resistance is one of the management strategies that can be used to control this pathogen. In this study, we screened 15 Tunisian melon cultivars to identify new sources of resistance to Fusarium wilt using phenotypic data from melon plants inoculation by FOM (races 0 and 1) and further molecular markers validation. Melon plants (at the 1st true leaf stage) were inoculated by FOM conidial suspension (10^6 conidia/ml) and symptoms were evaluated. Following inoculation phenotyping, DNA analysis was carried out to amplify resistant and susceptible alleles. For PCR assays, two markers previously reported to be linked to Fom-2 gene were used. The Spanish accession Tortuga and the Korean accession PI161375 were used as negative and positive controls, respectively, for the Fom-2 gene. Results revealed that among the Tunisian melon cultivars evaluated, four genotypes showed resistance to races 0 and 1 by inoculation method. All genotypes were further screened for presence of alleles conferring resistance to FOM. Molecular and phenotypic screening revealed that two genotypes (TUN-5 and TUN-12) have a strong resistance to FOM races 0 and 1. These genotypes should be explored as potential source of resistance genes that can be used in breeding programs to develop new resistant melon cultivars.

Key words: Melon, Disease resistance, Fusarium wilt, Molecular marker

Posters
Phytopathology
P13

FIRST REPORT OF ERYSIPHE ELEVATA CAUSING POWDERY MILDEW ON *CATALPA BIGNONIOIDES* IN TURKEY

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Catalpa bignonioides Walter which is a member of the family Bignoniaceae, known as Southern catalpa, Cigar tree or Indian bean is a medium-sized, is planted as street and garden tree in Turkey. In autumn 2017, during a survey of ornamental trees diseases, typical powdery mildew symptoms on several leaves of *C. bignonioides* were observed in Samsun province of Black Sea region, Turkey. Symptoms of the disease included white superficial mycelium with abundant sporulation and *Chasmothecia* on almost upper surfaces of the broad Catalpa leaves. Sporulating fungal structures were dissected from leaves and examined microscopically for morphological characters. Conidia, obovoid-ellipsoid to rarely cylindrical were produced singly on the conidiophores and measured 25–30 x 11–14 µm. The cylindrical conidiophores containing a foot-cell usually equal to or shorter (occasionally somewhat longer) than the 1-2 cells above it. *Chasmothecia* were produced in abundance on the upper leaf surfaces, and they were yellow when young and turned black or dark brown at maturity. These were 80–140 µm in diameter with appendages, bore six to eight, 115–340- µm in length. The appendages, mainly equatorial, terminated in dichotomously branched, knob-like or slightly recurved tips. Each chasmothecium contained 4 to 7 asci, 49-61 x 28-43 mm, short-stalked or sessile, containing 4-5 ascospores. The ascospores were 14–25 mm x 10–15 mm, broadly ellipsoid, sometimes oblong ovoid. Based on the asexual and sexual characteristics, the fungus was identified as *Erysiphe elevata* (Burrill) U. Braun & S. Takam. (syn. *Microsphaera elevata* Burrill). To confirm the morphological identification, the internal transcribed spacer (ITS) region of rDNA of a representative isolate (Samsun_02) was amplified with primers ITS5 and P3 and sequenced. The resulting 691-bp ITS sequence was deposited in GenBank (Accession No. MG601548). A GenBank BLAST search of the ITS sequence showed >99% similarity with that of *Erysiphe elevata* (AY587014). In addition, pathogenicity test was performed on the leaves of *C. bignonioides.* To our knowledge this is the first report of *E. elevata* causing powdery mildew disease on *C. bignonioides* in Turkey. Specimens were preserved in the Herbarium of the Mycology Laboratory of the Plant Protection Department, Ondokuz Mayis University, Samsun, Turkey.

Key words: Erysiphales, new record, *Erysiphe elevata*, southern catalpa

Posters
Phytopathology
P14

IMPORTANCE AND DISTRIBUTION OF ZUCCHINI YELLOW MOSAIC VIRUS (ZYMV) INFECTING ZUCCHINI SQUASH (*CUCURBITA PEPO* L.) IN MOROCCO

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Zucchini squash, *Cucurbita pepo* L., is an important vegetable crop in Morocco. Zucchini yellow mosaic virus (ZYMV) is a potyvirus with a worldwide distribution that causes serious economic losses to multiple cultivated plant species mainly belonging to Cucurbitaceae family. To assess the occurrence and distribution of ZYMV in Morocco, large-scale surveys were conducted, between 2015 and 2017, in the major zucchini squash production areas (Bouznika, Azemmour, Doukkala, Gharb, Loukkos and Souss Massa). A total of 316 leaf samples, belonging to 3 leading varieties in Morocco, were randomly collected from 35 commercial fields and were tested by ELISA using a commercial kit. The obtained results showed that ZYMV is widely distributed in the country with an infection rate of 44.8%, especially in the region of Loukkos where 53.6% of the tested plants were infected. These results should be considered for the development of control strategies for ZYMV in the country including cross-protection and breeding for resistance technologies. To the best of our knowledge, this is the first study on the occurrence and geographical distribution of ZYMV infecting zucchini squash in Morocco.

Key words: Zucchini squash, Zucchini yellow mosaic virus (ZYMV), occurrence, distribution, Morocco.

Posters
Phytopathology
P15

A SURVEY OF APPLE STORAGE CONDITIONS IN MOROCCO AND PREVALENT FUNGAL PATHOGENS ASSOCIATED WITH SPOILT FRUIT AND AIR ATMOSPHERE STORAGE

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To fulfil the increased need of consumers for apples along the entire year, a big portion of Moroccan fruit production is commonly stored. However, during the storage period, apples are subjected to many physiological and pathological disorders. Accordingly, this study aimed at investigating the storage conditions of apple fruit, identifying postharvest fungal pathogens associated with spoilt fruit, and determining the contamination of air atmosphere storage by fungal spores. Four regions were surveyed Meknes, Elhajeb-Ifrane, Imouzzer-Kander, and Midelt. Data analysis showed that the storage temperature varies from 0 to 4 °C and nearly 50% of the surveyed refrigeration stations used traditional wooden boxes, while other 50% opted for plastic boxes. In addition, apple fruit were mostly harvested in September and stored for no long than six months. There were 50% of surveyed stations that cleaned and disinfected their pallets and boxes, whereas 96% of these stations opted for regular cleaning and disinfection of storage rooms. Furthermore, the pre-harvest treatments of apple were carried out with thiophanate methyl, carbendazim and difenoconazole. According to this survey, the highest apple losses were occurred in the region of Midelt (10%) followed respectively by Elhajeb-Ifrane (9%), Meknes (5%), and Imouzzer-Kander (3.2%). The highest density of fungal spores was 464 spores/m^3 and it was recorded in a refrigeration station of Midelt. Moreover, the postharvest fungal pathogens associated with 138 spoilt fruit from 23 surveyed refrigeration stations highlight the predominance of Penicillium expansum (62%) followed by *Alternaria tenuisima* (33%) and *Alternaria chartarum* (5%) respectively. Therefore, this study is the first report underlying a substantial variability within the Moroccan apple producing regions in term of apple storage conditions, the prevalence of postharvest diseases associated with spilt fruit, and the contamination of the storage atmosphere.

Key words: apple storage, refrigeration stations, postharvest diseases of apples, Morocco

Posters
Phytopathology
P16

TRICHOTHECENE GENOTYPES OF *FUSARIUM GRAMINEARUM* ISOLATED FROM *TRITICUM MONOCOCCUM* IN POLAND

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Triticum monococcum was the staple food several millennia ago, but presently the species is grown only in small areas of the Mediterranean Region and continental Europe. Two spring cultivars of einkorn have been researched in Poland since 2016. Einkorn grain is a rich source of proteins, lipids (containing mostly unsaturated fatty acids), lutein and antioxidants such as carotenoids, tocols, conjugated polyphenols, alkylresorcinols and phytosterols. A few studies have pointed to the possible contamination of einkorn grain with fungi of the genus *Fusarium* and Fusarium mycotoxins. The aim of this study was to: (a) analyze the species composition of Fusarium fungi isolated from the grain and glumes of several breeding lines of einkorn grown in north-eastern Poland, (b) analyze the trichothecene genotypes of Fusarium graminearum strains, and (c) determine the phylogenetic relationships between selected Fusarium species from own collection. Fungi of the genus *Fusarium* were isolated from disinfected and non-disinfected einkorn kernels and glumes onto potato glucose agar. They were identified to species level with the use of microscopic and molecular techniques. A phylogenetic tree was developed based on partial sequencing of the small 18S rDNA unit, the 5.8S rDNA region, the large 28S rDNA subunit and conserved non-coding regions (ITS). All isolates identified as F. graminearum were screened for their potential ability to produce trichothecenes by analyzing the amplification of the Tri5 gene with a specific primer pair. The glumes and kernels of common wheat were the reference material. Colonies of filamentous fungi were isolated from non-disinfected glumes of several einkorn lines, and 27.61% of the colonies on average were identified as *Fusarium* species. *Fusarium* species accounted for 9.82% of the colonies isolated from non-disinfected kernels and for 7.41% of the colonies obtained from disinfected kernels. Glumes prevented *F. graminearum* from penetrating into the kernel tissue. Two NIV chemotypes, one 3-ADON chemotype and one 15-ADON chemotype were identified in a molecular analysis of five *F. graminearum* isolates.

Key words: *Fusarium graminearum, Triticum monococcum*, trichothecene

Posters
Phytopathology
P17

IDENTIFICATION OF MARKERS LINKED TO POWDERY MILDEW AND LEAF RUST RESISTANCE GENES IN BREAD WHEAT-SPELT HYBRIDS AND THEIR PARENTAL COMPONENTS

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Phylogenetic studies of genus *Triticum* suggest that bread wheat (*Triticum aestivum* L. ssp. *aestivum*) has been arisen from multiple mutations in hexaploid spelt (*Triticum aesti*vum L. ssp. *spelta*) what implies their close relationship. Bread wheat differ little from spelt in genetic as well as in phenotypic features, especially in spike morphology and its physicochemical and nutritional properties. High genetic diversity among spelt can be the result of Asian and European gene pools existence. This makes *T. aestivum* ssp. *spelta* a potential donor of genetic material for bread wheat which genetic variation is reduced due to long-standing selection breeding.

The stability of whole food security may be at risk under fungal diseases development in wheat fields. Powdery mildew (the disease caused by *Blumeria graminis* (DC.) E.O. Speer f.sp. *tritici*) and leaf rust (*Puccinia recondita* Rob. ex Desm f.sp. *tritici* Eriks. et Henn.) are one of the most dangerous pathogens of wheat. Their presence in cultivation area can lead to yield loss reaching even 14-60%. Growing interest in resistant cultivars of wheat is considered as the most economical and environmentally safe approach to eliminate the use of chemical plant protection products and, consequently, to reduce yield losses due to fungal diseases. Genetic similarity resulting from the same chromosomes number (2n=6x=42) and considerable homology between bread and spelt chromosomes gives the chance to develop stabile, high-yielding hybrids among these species. The research concerned single crosses between bread wheat and spelt of F7 generation. The aim of the study was the identification of markers linked to powdery mildew (Pm) and leaf rust (Lr) resistance genes in parental components and their hybrids.

DNA of analyzed wheat parental components and their hybrids was isolated using specific kit Genomic Micro AX Plant Gravity (A&A Biotechnology). PCR reactions contained 30 ng of template DNA, 0.4 µM of primers (specific to selected Pm and Lr genes), 0.2 mM of dNTPs, 2.5 mM of $MgCl_2$, 2.5 µl of 10x PCR buffer and 1U of TaqDNA polymerase in a total volume of 25 µl. The amplifications were carried out in a thermocycler (Mastercycler Ep Gradient, Eppendorf). Individual PCR reactions were conducted with special attention to annealing temperatures of specific markers of powdery mildew and leaf rust resistance genes.

The results showed variability in Pm4b, Pm6 and Lr1, Lr10 and Lr28 genes markers presence in analyzed hybrids and parental components.

Key words: bread wheat-spelt hybrids, hybrids, spelt, bread wheat, resistance genes

Posters
Phytopathology
P18

MOLECULAR CHARACTERIZATION OF BNYVV ISOLATES BASED ON P25 CODING REGION IN CENTRAL PART OF TURKEY

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Beet necrotic yellow vein virus (BNYVV) causes rhizomania which is one of the most devastating diseases in sugar beet fields worldwide. In this study, 168 soil samples were collected from sugar beet growing areas of Ankara, Afyonkarahisar, Aksaray, Nigde and Karaman provinces in central part of Turkey in 2016. The plant samples grown in these soils tested by ELISA and bait plant method. Out of the samples, 53.6% (90) were found to be infected with BNYVV. Twenty of them were selected according to their geographic origins to be used in molecular studies. The partial nucleotide sequences of RNA-3 segment of BNYVV was amplified by RT-PCR, and the nucleotide sequences involving the p25 coding region were obtained. The deduced amino acid (aa) sequences of the p25 protein indicated that 19 BNYVV populations had 'ACHG', 'AHHG', 'AFHG', 'ALHG', 'VHPG', 'VHHG' and 'AHPG' residues at position 67-70. In addition, a population containing three nucleotide deletions, not shifting the reading frame but affecting the aa at position 67 (-FHG) or 68 (F-HG), was identified. 'ACHG' (40%) was found to be the most prevalent motif among BNYVV isolates. 'AHPG' motif is the first in the world according to the data available so far. Also, Rz1 gene-carrying cultivar was used in bait plant test to investigate pathogenicity of these BNYVV variants under controlled conditions. The roots of sugar beets were tested by DAS-ELISA of the BNYVV isolates containing 'ACHG' and 'VHPG' motifs, 87.5% and 50% were able to overcome Rz1 resistance respectively, whereas 100% of the isolates with all other motifs did break resistance. Furthermore, these results seem to indicate that the mutant of BNYVV with a deletion is aggressive, as are the isolates without deletion. The current study revealed that BNYVV isolates in central part of Turkey have different pathogenicity, and aa positions at 67-70 does not seem to be directly associated with resistance-breaking.

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Key words: RNA-3, resistance-breaking, Rz1

Posters
Phytopathology
P19

IDENTIFICATION AND CHARACTERIZATION OF MOROCCAN *ERWINIA AMYLOVORA* STRAINS BY RANDOM AMPLIFIED POLYMORPHIC DNA FRAGMENTS (RAPD)

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Erwinia amylovora, the causative agent of fire blight, was detected for the first time in Morocco in 2006. Since then, the disease has widespread throughout the Sais area causing important losses in pear, apple, and quince crops. Currently, fire blight affects the orchards of the entire area of the Middle Atlas, which is the stronghold of the Moroccan apple and pear production. The genetic diversity of *E. amylovora* strains isolated from different host plants and locations was investigated by random amplified polymorphism DNA (RAPD) fragment analysis. One strain of *Pantoea agglomerans* was used as out-group. One hundred-fourteen different fragments were produced by polymerase chain reaction amplification with four different 10-mer primers. Cluster analyses of the fingerprints obtained was performed following Unweighted Pair Group Method with Arithmetic mean (UPGMA) with Jaccard similarity index. This molecular method showed high polymorphism of within the isolates and resulted in several separate subgroups, producing the same amplification patterns. No correlation was found between the genetic diversity of isolates and their geographical origin or the plant from which they were isolated. The sensitivity to streptomycin bioassay showed that all strains of *E. amylovora* are sensitive. Therefore, our findings imply that some fire blight outbreaks were caused by the introduction in Morocco of infected plant material or other inoculum sources from different countries where streptomycin was not authorized for control of fire blight.

Key words: *Erwinia amylovora*, molecular characterization, RAPD analysis, polymorphism, and sensitivity to streptomycin

Posters
AGRICULTURAL ENTOMOLOGY AND ACAROLOGY

Posters
Agricultural Entomology and Acarology
E01

SCREENING FOR RESISTANCE TO *APHIS GOSSYPII* COLINISATION IN TUNISIAN MELON GENOTYPES

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Aphis gossypii (Glover), is one of the major pests of cucurbits especially melon as well as an efficient vector of plant viruses such as Cucumber Mosaic Virus among others. Host-plant resistance is one of the management strategies that can be used to control this pest. In this study, 15 Tunisian melon genotypes were screened to identify new sources of resistance to aphid colonization in melon using both, bioassays, and molecular marker validations. Two markers previously reported to be linked to the resistance gene Vat (Virus aphid transmission) were used. The Spanish melon cultivar 'Bola de oro' and the Indian melon cultivar 'PI414723' were used as negative and positive controls, respectively, for the Vat gene. Results revealed that the Vat-gene was detected in only one genotype (TUN-7) and this genotype should be explored as a potential source of resistance that can be used in breeding programs to develop new aphid resistant melon cultivars.

Key words: cotton aphid, resistance, molecular markers, Vat gene

Posters
Agricultural Entomology and Acarology
E02

BRASSICOGETHES AENEUS PEST MANAGEMENT IN RAPE CROPS: IS THERE A COMMERCIALLY VIABLE FUTURE?

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Rape (*Brassica napus*) is being cultivated as a major oil and protein crop throughout Europe and used for meeting our demand for oil, both for human and animal nutrition as well as biodiesel production. Unfortunately, the rape pest *Brassicogethes aeneus* (Coleoptera: Nitidulidae) is gaining the status of an established pest in central European rape crop fields. What can be sensibly done for its management without harming the environment? With this basic question in mind we reviewed the literature and did some preliminary olfactometer experiments in the laboratory. Pretty soon it turned out that *B. aeneus* is one of the more difficult candidates for employing olfactory cues for mating disruption. Unlike nocturnal Lepidoptera which are much dependent on mating activities catalyzed by olfaction and pheromones, diurnal beetles use several cues like sound, light, and mobile silhouettes for orientation, with semiochemicals being one but not the major sensory channel guiding orientation to food plants. The question raised in the title must be answered one way or the other if *B. aeneus* management is going to succeed in the future without the use of poisonous pesticides. After all, *B. aeneus* causes damages at an increasing level. Close cooperation between entomologists, applied ecologists, pest managers, and engineers is mandatory. So are sponsors of such research. We see major future benefits for non-toxic, environmentally compatible rape crop management.

Three separate considerations influence our view on the problem and our decisions:

1. Monetary value of damage which must be avoided vs. monetary value of treatment and environmental costs
2. Acceptance in view of indirect decline and loss of biodiversity recently are important considerations to not treat with pesticides
3. Insecticide resistance is a vexing problem for about a century. In intensive agriculture, nobody so far found a radical solution.

Posters
Agricultural Entomology and Acarology
E03

TOWARDS SMARTER IPM: HOW PHEROMONE DISPENSER TECHNOLOGY DEVELOPED WITHIN THE LAST FIFTY YEARS

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From small and seemingly insignificant beginnings, pheromone applications gradually developed into a branch of agricultural technology. Now, at the beginning of the 21st century, the field is gaining the status of a complementary endeavour rivalling in sophistication with pheromone science itself.
In the author's own experience, dispensers of various types have reached a stage of development that is remarkable. Yet by no means exhausted are their future possibilities and promises.
Early examples were simple ceramic, metal, cork, glass, and filter paper surfaces suitable for evaporating signal compounds useful for IPM. Recent examples, however, extend further into the fields of quantitative physical chemistry and material sciences. Specifically, designed polymer chips, pastes, ropes, puffer systems with valves electrically turned on and off, biodegradable hollow fibers as well as ultrathin mesofibers all have in common that they attempt the task of quantifiably dispensing relatively expensive volatile signal compounds in a predictable way into the environment, ideally with mechanical or even automatic equipment. Emphasis is focussed on the requirement of treatment without leaving any residues and thus being effective for a certain time without the need of later recollection of the dispensing medium. One further requirement is that the new devices be affordable, biodegradable, and employing renewable resources. Hopefully gone forever are the days of simplistic toxicological interventions with inorganic poisons (e.g. of the lead arsente type) against a variety of insect, microbial and plant pests.

In the presentation, a selection of examples will be highlighted.

Posters
Agricultural Entomology and Acarology
E04

FIRST ATTEMPTS TO REPEL SCALE INSECTS USING BOTANICAL PRODUCTS, CASE OF THE DATE PALM SCALE *PARLATORIA BLANCHARDI*

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Date palm, (*Phoenix dactylifera* L.), is a crop that plays a central role in the economy and social life in the Middle East and North Africa. One of the most dangerous insect enemies threatening this culture is the date palm scale (*Parlatoria blanchardi*). The aim of this study is test the repellent activity of some spontaneous plants that grow in the Algerian desert against the crawlers of the first instar larvae of *Parlatoria blanchardi*, under laboratory conditions. Methanol extracts of Castor bean *Ricinus communis*, Bitter apple *Citrullus colocynthis* and Syrian rue *Peganum harmala* were tested and compared with distilled water as a control. The results from the repellent bioassay have shown that the mean number of crawlers found in negative control leaflets was significantly higher ($P<0.05$) than most of concentrations of extracts. However, some concentrations of *C. colocynthis* and *P. harmala* lost their efficacy after 11 days of treatment. Overall results may contribute to develop new strategies to control the scale insect's populations not only by toxicity but also by repellency.

Key words: Repellent, Biopesticide, Date palm scale, Extracts

Posters
Agricultural Entomology and Acarology
E05

CHEMICAL COMPOSITIONS AND EFFICACY OF BETEL PIPER ESSENTIAL OIL AGAINST RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST)

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Essential oil from fresh leaves of betel piper, (*Piper betel* Linn.) consists of many chemical components that have properties for protecting plants from insect pests by killing them. This research was aimed to study the chemical compositions of essential oil of betel piper leaves extracted from steam distillation identified using Gas Chromatograph-Mass Spectrometer technique and study the efficacy of this essential oil against red flour beetle (*Tribolium castaneum* (Herbst)), using impregnated filter paper and vapor-phase tests. Experiments were performed under Completely Randomized Design (CRD) with 4 replications. Killing contact activity towards red flour beetle was investigated at 5 concentrations of essential oil 0; 8,000; 16,000; 24,000 and 32,000 ppm and killing fumigant activity was performed using bioassay at 5 concentrations including 0, 100, 200, 300 and 400 µL/L air. Data were recorded as number of dead red flour beetle from 24 to 168 hours. Major components of essential oil from fresh leaves of betel piper were found to be eugenol (34.86%), caryophyllene (10.56%) and ethyl methylphenyl glycidate (10.057%). Betel piper essential oil at 32,000 ppm was the most effective in killing contact red flour beetle (100% adult mortality) at 168 hours and 400 µL/L air exhibited the highest efficacy in killing fumigant maize weevil (100% insect repellency) at 168 hours. It indicated that the use of betel piper essential oil was considered as the effective and another alternative approach against red flour beetle for avoiding the risk of agricultural chemicals used in stored insect pest protection.

Key words: stored insect pest, essential oils, *Piper* spp., chemical compositions

Posters
Agricultural Entomology and Acarology
E06

SIMULATING THE POPULATION GROWTH AND DISPERSAL OF POTENTIAL OUTBREAKS OF *ANOPLOPHORA* SPP. IN BELGIUM

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The Asian long horned beetle (ALB), *Anoplophora glabripennis* (Motchulsky), and the citrus longhorn beetle (CLB), *Anoplophora chinensis* (Forster), are two non-native pests that are extremely damaging to a wide range of broadleaved trees and shrubs. Both are quarantine pests for the European Union. The natural range of the beetles includes China, Japan, and other countries in southeast Asia. ALB has been introduced in North America, Europe, and Japan on several occasions, most probably via trade of goods in infested wood packaging material. The main pathway of introduction of CLB into Europe has been with ornamental trees (bonsai) from Asia. To date, no outbreaks have been observed in Belgium, but two interceptions, in 2004 and 2008, and several outbreaks in neighbouring countries have shown that ALB and CLB both pose a serious threat to horticulture, forestry and native trees in Belgium.
Because eradication requires drastic measures and will have a serious economic impact (especially on tree nurseries or fruit farms, but also on public green spaces), it is essential to thoroughly consider which control techniques should be applied. A decision-support tool indicating the most suitable (preventive) control strategy against these pests is now being developed based on a simulation model. The main user will be the government, but the tool is also appropriate for use by nursery growers.
The first step in the model development is to simulate the population growth of the introduced pest individuals, followed by the dispersal of the adults. Biological features on the development, survival, reproduction, and dispersal of the two beetle species were obtained from scientific literature. A degree-day approach was applied to model the duration of the life cycle, which lasts more than 2 years based on the average daily temperatures in Belgium. Adult dispersal was simulated based on a leptokurtic probability function, with an average migration of 30 and 70 m per life cycle for CLB and ALB, respectively, and a maximum dispersal of 2 km. The outbreak simulation part of the model is up and running, and can be launched by entering the outbreak coordinates, the duration of the simulation (in years) and the pest situation (species (ALB or CLB), stage and number) in a user-friendly interface. The resulting map presents the simulated number of pest individuals per stage and their dispersal.
The next step is to simulate control measures and add them to the model to determine the control level and cost analysis of each strategy.

Key words: control strategy, Asian long horned beetle, citrus longhorn beetle, decision support model, quarantine pests

Posters
Agricultural Entomology and Acarology
E07

CROP MANAGEMENT WITH A CRYSTAL BALL: A DAMAGE PREDICTION MODEL FOR WIREWORMS (COLEOPTERA: ELATERIDAE)

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Wireworms, the polyphagous larvae of click beetles, can cause severe economic damage to a variety of crops. In Flanders (Belgium), potatoes, maize, and Belgian endive (witloof chicory) are among the most susceptible to damage by this pest, which is hard to control with the currently authorized soil insecticides. As a first line of pest control, Integrated Pest Management (IPM) programs work to manage the crop to prevent pests from becoming a threat. Given the long-life cycle (3-4 years) of the most common damaging wireworm species, rotating between different crops can be an important preventive measure. In this regard, it would be useful to know whether the crop choice for the next growing season would result in wireworm damage or not. To achieve this, a model to predict the likelihood of damage by wireworms on a specific field during the successive growing season is being developed.
Data for the development of this decision support model were collected by setting up a region-wide and long-term monitoring campaign in Flanders. This campaign, started in 2015, will continue through 2019. After 3 years of monitoring, information on more than 100 fields with susceptible crops has been collected. First, these data will be used to estimate the variation in wireworm species and their abundance on various agricultural fields. It has already become clear that the most common harmful wireworm species in this region are *Agriotes lineatus*, *A. obscurus*, *A. sputator* and *Hemicripidius niger*. As each species has its own biological features, a prediction model is being developed for all 4 damaging wireworm species. To better understand the factors determining the risk of damage, field data is being analyzed in relation to several field specific parameters: soil characteristics, management practices and crop history. Machine learning algorithms (e.g. logistic regression, decision tree, random forest, support vector machine, naive bayes and neural network) are being used to develop the predictive model. Preliminary analysis showed that the crop history from the last 5 years (main crops and number of years with Poaceae crops), soil type, pH and organic matter level are the determining factors in the prediction model. Monitoring data from 2018 should make it possible to verify the model parameters and refine the model prediction. Finally, this model will help farmers to make future management decisions for wireworm control. Field tests evaluating the potential of cultural, biological, and chemical control methods will further support the farmer in choosing the most effective IPM strategy.

Key words: preventive control, *Agriotes* spp., *Hemicrepidius niger*, decision support model, damage prediction

Posters
Agricultural Entomology and Acarology
E08

SEMIOCHEMICALS TO CONTROL THE WALNUT HUSK FLY *RHAGOLETIS COMPLETA*

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France, like most European walnut producers, must deal with the recent introduction of the Walnut Husk Fly, *Rhagoletis completa* (Diptera, Tephritidae), that causes severe economic losses, especially in biological productions. In orchards where *R. completa* is present and uncontrolled, 100% of walnut trees can be infested, causing losses in walnut yields of up to 80%. Therefore, there is a need for developing environment-friendly methods of control.
In this research, we hypothesised that associating walnut fruit volatiles with sticky traps enhances the fly trapping and reduces insect damages.
We identified and quantified the volatile organic chemicals (VOCs) released by walnut husks; -pinene, -pinene, linalool, (s)-3-ethyl-4-methylpentanol-1, 1-8 cineole, nonanal, tetradecane, and undecane. Then, during summer 2015 and 2016, we conducted two field assays to compare the efficiency of two R. completa monitoring traps: (1) a chromatic yellow trap (Bug-Scan®, Biobest, Westerlo, Belgium) and (2) a chromatic yellow trap associated with a walnut husk VOC dispenser releasing the previously identified compounds. The two traps were placed, in two orchards in the south east of France (SENuRA, Chatte, France).
The number of fly captures for the two different traps were, however, similar during both field seasons. This may be since the odours emitted by the dispensers do not overcome the general smell of the walnut orchard. We now aim at evaluating the potential of mating disruption, using the recently discovered *R. completa* sex pheromone.

Key words: Fruit flies, Volatile organic compounds, Integrated pest management, Walnuts.

Posters
Agricultural Entomology and Acarology
E09

DETERMINATION OF THE SUSCEPTIBILITY FOR SPOTTED WING DROSOPHILA OF DIFFERENT RIPENESS STAGES OF SWEET CHERRY, STRAWBERRY AND BLACKBERRY

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The objective of this paper was to determine the exact ripening stage at which soft fruits become susceptible to spotted wing drosophila (SWD). Tested fruits were sweet cherries (*Prunus avium*, cultivars Kordia and Regina), strawberries (*Fragaria x ananassa*, cultivar Verity) and blackberries (*Rubus fruticosus*, cultivar Loch Ness).
The method used to determine this for sweet cherry and blackberry involved producing unsprayed and uninfested fruits. These were picked during maturation and sorted in different ripening stages (4 stages for blackberry and 6 stages for cherry). The fruits were placed in a bugdorm-6 rearing cage (60 x 60 x 60 cm) containing a mass rearing of SWD for 24 hours. Then the infested fruits were incubated in a climate chamber at 22 °C for 6 days to give the larvae the time to develop. Next, they were immersed in a 10% NaCl solution for 24 hours and the larvae were sieved out prior to counting with a binocular. For the strawberries an alternative method was used: on a heavily infested parcel 5 replicates of 10 fruits were picked for each of the 6 ripening stages and the larvae were immediately counted using the same “immersion” method.
The results of the three tests show an increasing infestation as the ripening stages progress. There is a clear correlation between the intensity of the red color of the fruits and the number of larvae counted in each trial. The ripest stages are always the most susceptible to SWD. For the cherries we can pinpoint the exact stage at which they become susceptible to SWD: when the first red spot appears on a yellow or green cherry. For the blackberries it’s similar: they become susceptible when the green color shifts to red. The strawberries become susceptible much earlier, when they turn from green to white even before any red color is visible.
In conclusion, sweet cherries, blackberries and especially strawberries tend to become susceptible to SWD much earlier than previously expected. From the moment the first red coloration starts to appear (and for strawberries even earlier) they become prone to some egg laying and subsequent larval development. As red coloration increases, the liability rises as well, and the completely red coloured fruits showed high larval infestation in each test. Because of the zero tolerance for SWD infestation in soft fruits it is recommended to start the (chemical or ecological) control early, before the first red coloration is present.

Key words: Spotted wing drosophila (SWD), ripeness, cherry, blackberry, strawberry

Posters
Agricultural Entomology and Acarology
E10

DISTRIBUTED DELAY MODELS AND ROOT: BUILDING A SOFTWARE TO SIMULATE PEST INSECTS' LIFE CYCLE USING THE DAILY TEMPERATURE

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ROOT is a software package developed from a CERN project, started in 1994 by René Brun for statistical analysis in high-energy physics. Nevertheless, its potentialities can be used also in the field of plant protection against pest insects. Since long time, indeed, there is a large use of the Distributed-Delay Model (Manetsch, 1976), but to date it is not available a specific software useful to follow the research from the first step, to simulations and field validations.
During a simulation process, we can individuate two main phases: at the beginning, there is a "laboratory phase" in which entomologists must estimate a series of specie's parameters thru constant-temperature farms in climatic cells, and then a simulation can be done, with validations using field data. Once completed this work, distributed delay model equations can help farmers to forecast the peaks of pest insects, helping them to individuate the best period for treatments.
The ROOT's main feature is the use of C++ language, and to have not only a graphical interface, but also specific packages for statistical analysis, fitting and minimization of functions, multivariate analysis of data, and other tools which allow solutions for mathematical problems.
The aim of this work is to build, through the ROOT's libraries, a series of macros that consent to do non-linear fits with functions like Erlang PDF, linear-rate, Logan, Briére, Sharpe and De Michele, giving, first, support to the parameters-estimate step in laboratory sessions, and then, to solve numerically the Distributed-Delay Model equations. The results will be given both in graphical and numerical way.
Concerning the validation in open field, it was developed another macro which puts in relation the simulations results, with field data, and choose the nearest to reality. Every macro is developed individually, and can be used independently from the others, even if they are linked by the logical thread of the actions in estimation process. This helped us to check the software operation and is helpful for users that can call only the specific part that they need. Furthermore, for completion, all these tools are contained in a main (now at version 1.0) managed from command line, and with the selection possibility.

Key words: Plant Protection Modelling, Forecasting Software, Integrated Pest Management, Rate-function, Population dynamics

Posters
Agricultural Entomology and Acarology
E11

CONTROL OF ELATERIDAE AND SCARABAEIDAE IN SUGAR BEET BY SEED TREATMENTS

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To obtain high and stable yields of sugar beet, one of the important factors is successful protection in the initial stage of the growing period from harmful insect species Elateridae and Scarabaeidae, which is achieved by insecticide seed treatments. The aim of the paper was to test the efficacy of insecticides clothianidin + imidacloprid and imidacloprid in sugar beet protection against mentioned pests. The field trials were conducted in 2017 in the region of Vojvodina province at the localities Savino Selo (SS) and Rimski Šančevi (RŠ) on sugar beet; variety Nora, according to standard OEPP methods (PP 1/46; 1/152; 1/135). Insecticides based on clothianidin+imidacloprid (150+375 g a.i./L) at rate of 66 and 99 ml/seed unit and imidacloprid (600 g a.i./L) at rate of 150 ml/seed unit, were applied. Plot size was 240 m^2 per variant and consisted of four replications. Immediately before sowing, germination of untreated seeds was performed, soil analyses were done to determine the number of soil pests, as well as physical-chemical analysis of the soil. The effects of insecticides applied to the sugar beet seed were derived from the number of shot plants at distance of 10 m and the number of damaged plants from Elateridae and Scarabaeidae larvae. Two evaluations were performed, the first after shooting of crops and the second one in the phase of 4-6 leaves. Results are presented as means and significance of differences (LSD 5%) by ANOVA. In laboratory, the germination of sugar beet seed was 91%. Before sowing number of Elateridae larvae at the locality SS was 4/m^2, and Scarabaeidae larvae was 2.5/m^2, while in the locality RŠ only the presence of 1.5/m^2 Elateridae larvae was determined. At the locality SS soil is neutral, with high content of phosphorus, optimally supplied with potassium with high humus content and it is classified into highly carbonated soils, while the soil at the RŠ is neutral, optimally phosphorus sufficient, with a high content of potassium, with low humus content and it is classified as medium carbonate soil. At the locality SS, in the phase after the shooting, insecticide treatment of seeds provided 55.3 to 67.4% higher number of shot plants in comparison to the control. Number of plants damaged by Elateridae and Scarabaeidae larvae was significantly reduced by insecticide use (1.4-2.2%) regarding the control (39.5%). In 4-6 leaves phase, the studied products provided from 65.8 to 78.4% higher number of shot plants in comparison to the control. At the locality RŠ, in the phase after the shooting, insecticide seed treatments provided significantly higher number of shot plants (48.4 – 59.3%) regarding the control. Significant reduction in the number of plants damaged by Elateridae larvae regarding the control (29.4%) was established after use of insecticides (0.7-1.4%). At the locality RŠ seed treatments in the phase of 4-6 leaves increased planting density regarding the control for about 50%.

Key words: sugar beet, seed treatment insecticides, Elateridae, Scarabaeidae

Posters
Agricultural Entomology and Acarology
E12

EFFECTS OF ELEVATED CO_2 ON OLFACTORY BEHAVIORS FOR DIFFERENT MORPHS OF CORN LEAF APHID, *RHOPALOSIPHUM MAIDIS*

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Climate change, especially the rise in greenhouse gas concentration, has caused great concern around the world. The increasing concentration of carbon dioxide (CO_2) not only affects the plant growth and development, but also changes plant volatile organic compounds (VOCs), which indirect impacts the herbivore insect. In this study, we compared the olfactory behaviours of three morphs of corn leaf aphids, the choice of nymph, wingless and winged adults to clean air, ambient CO_2 (aCO_2) and elevated CO_2 (eCO_2) barley seedlings in Y-tube olfactometer. The results significantly showed that more aphids chose the odour of aCO_2 barley seedlings when tested against eCO_2 barley seedlings or clean air, eCO_2 barley seedlings was chosen more often when tested next to clean air. The wingless and winged aphids spent more time on eCO_2 barley seedlings than on aCO_2 barley seedlings, but average fecundity on aCO_2 barley seedlings was higher than on eCO_2 barley seedlings at the same time periods. Our findings lay foundation for the behavior response of different morphs under changing environment.

Key words: Climate change, corn leaf aphid, elevated CO_2, olfactory, behaviour

Posters
Agricultural Entomology and Acarology
E13

IS THE VIRUS VECTOR *MYZUS PERSICAE* SULZER (HOMOPTERA: APHIDIDAE) LIFE HISTORY AFFECTED BY ENDOPHYTIC ENTOMOPATHOGENIC FUNGI PRESENCE IN TOBACCO PLANTS?

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Entomopathogenic fungi (EPF) are biological control agents used against crop pests. Thanks to their ability to infect their hosts transcutaneously, currently, the mode of action by contact remains the most common strategy. Recent studies highlight the successful way of inoculating endophytic strains in plants for systemic action. These endophytic entomopathogenic fungi (EEPF) can live in their host plants according to a symbiotic interaction, acting as a plant "bodyguard" against their natural enemies. Thus, they interact with phytopathogenic agents such as viruses and their insect vectors. In this study, we investigated the impact of two commercial strains of Entomopathogenic fungus (Beauveria bassiana GHA from Botanigard® and *Metarhizium acridum* ARSEF 7486 from Green Muscle®) on the *Myzus persicae* Sulzer life history and on the transmission of Potato leaf roll virus (PLRV) on several Solanaceae family plants, including tobacco. EEPFs inoculation was successfully performed for both strains on tobacco plants. We found that the development cycle length, the survival, and reproduction rates are altered on plants colonised by EEPFs compared to EEPF-free-plants. We suggest that these life history modifications may have an impact on the spread of the persistently transmitted virus, such as PLRV.

Key words: Endophytic entomopathogenic fungi, *Myzus persicae*, PLRV, Life history, interactions

Posters
Agricultural Entomology and Acarology
E14

TROPICAL BASIL EFFECT ON *TUTA ABSOLUTA* MEYRICK (LEPIDOPTERA: GELECHIIDAE) OVIPOSITION RESPONSE

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Tuta absoluta Meyrick (Lepidoptera: Gelechiidae) is one of the most important pests on tomato justifying the use of synthetic insecticides for its management around the world. Because synthetic insecticides lead to resistance and have adverse effects on producers' health and natural enemies, there is a need for alternatives. In this study, we assess the oviposition deterring effect of tropical basil (*O. gratissimum*) using dual-choice behavioural assays performed in flight tunnels. Two modalities were tested, i.e. (1) a tomato plant associated either with an *O. gratissimum* plant versus a tomato plant only, and (2) a tomato plant with tropical basil essential oil (EO) formulated in paraffin oil (PO) versus a tomato plant with PO only. A 1 ml cylindrical polyethylene plug loaded with 100 µl of solution (formulated EO or PO) was placed on each plant as a diffuser. Forty-eight hours after the release in the central area of the tunnel, no difference was observed both males and females distributed on both sides of the flight tunnels in the presence of tropical basil plant. However, females laid significantly more eggs on non-associated tomato plants compared to tomato plants associated with tropical basil. With EO, only 1.0 mg of EO influenced significantly female's distribution on tomato plants. Females also laid significantly more eggs on tomato plants associated with PO compared to tomato plants associated with 1.0 mg of EO as dispenser.
GC-MS analyses showed that the major components include thymol (33.3%), p-cymene (20.4%) γ-terpinene (16.9 %) and myrcene (3.9 %) in the EO and p-cymene (33.5 ± 2.3%) γ-terpinene (23.6 ± 1.6 %) α-terpinene (7.2 ± 0.5) α-thujene (6.7 ± 0.8 %) in volatiles collected with solid-phase micro-extraction method on plants.
These results suggest a valuable potential of topical basil as component of an integrated management strategy against *T. absoluta*.

Key words: *Ocimum gratissimum*, volatiles, Behavioural, Eggs laid, Tomato leafminer

Posters
Agricultural Entomology and Acarology
E15

DIET-MEDIATED INDIRECT INTERACTIONS ALTER HOST RANGE OF PARASITOIDS

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Parasitoids are important natural enemies of aphids in fruit orchards of Belgium, and interest in them has increased in recent years. Little is known about how the bottom-up forces influence the natural enemy population. How parasitoid populations respond to habitat manipulations is a central topic in biological control agent selection. Aphids like many other insect's harbour facultative protective symbionts, some of which have been shown to influence the nature of interactions with their natural enemies. The ecological impact of such defence mechanism on parasitoid community still needs to be investigated. Here, a field-based approach to study the effect of plant-mediated indirect interactions on the parasitoid food web was conducted. We visually assessed the diversity of parasitoids associated with common polyphagous aphid species on cropping and non-cropping vegetation within horticultural landscapes in Limburg Province, the center of fruit production in eastern Belgium. Three generalist aphids, *Aphis fabae*, *Brachycaudus cardui* and *B. helichrysi* were selected to determine how host plant species influence indirectly the structure of parasitoid community. In total *A. fabae*, *B. cardui* and *B. helichrysi* hosted 16, 15 and 8 parasitoid species on 19, 12 and 8 host plant species respectively were recorded. We found that each aphid species was attacked differently by parasitoids according to host plant sheltering these biological interactions. From the bottom-up, plants can impact parasitoid success and then parasitoid community structure by changing host defense function. Our results show that the potential indirect plant-parasitoid interactions mediated by changes in plant-aphid associations could help explain the failing or successful biological control provided by parasitoids.

Key words: *Aphis fabae, Brachycaudus cardui, Brachycaudus helichrysi*, plant-mediated indirect interactions, parasitoid diversity

Posters
Agricultural Entomology and Acarology
E16

REVIEW OF A GROWING DEGREE DAY APPROACH TO PREDICT CEREAL LEAF BEETLE

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Cereal leaf beetles (CLBs) are an economically important pest of small grain cereals in many regions worldwide. Prediction models aimed to prevent yield losses caused by these feeding insects have been developed by researchers all over the world. As a foundation for many of these prediction models, it is known that a specific number of heat units, or growing degree days (GDDs), is required for an insect to complete a certain physiological process. In this poster we overview the existing growing degree day models for CLBs. Furthermore, we used our Belgian input data to compare model predictions with our own field observations. This validation showed that the existing models were not able to predict the seasonal trends present in our data: the occurrence of various life stages were monitored earlier then the model predicted. Hence, a weighted GDD model was tested on the data as well: the accumulated GDD during certain periods were balanced according to the significance of this period for the insect. Also, parameters such as rainfall and/or relative humidity were included in the model as well. We noticed that, using techniques such as multiple linear regression, ridge regression and regression trees, this approach performed significantly better compared to the simple accumulation of GDD. Although these models performed considerably better, after validation (using cross-year cross-location validation), the accuracy of the models were still too low to serve as a proper warning tool.

Key words: cereal leaf beetles, growing degree days, modelling, winter wheat

Posters
Agricultural Entomology and Acarology
E17

PHOTOPERIOD EFFECT ON DIAPAUSE INDUCTION IN WOLBACHIA INFECTED *TRICHOGRAMMA BRASSICAE*

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The direct and indirect (grand maternal and maternal) photoperiod influence on the diapause percentage of Wolbachia-infected and uninfected *Trichogramma brassicae* Bezdenko (Hym: Trichogrammatidae) were investigated under laboratory conditions. To study the grand maternal effect of photoperiod on diapause induction, grand maternal and maternal generation developed at 20 °C and day length of 0, 3, 6, 9, 12, 15, 18, 21 and 24 h and 20 °C and day length of 10 h, respectively and progeny generation developed at 10 °C and 14 °C and absolute darkness. To study the effect of maternal photoperiod on diapause induction, maternal generation developed at 20 °C and day length of 0, 3, 6, 9, 12, 15, 18, 21 and 24 h and progeny generation developed at 10 °C and 14 °C and absolute darkness. To determine the direct effect of photoperiod on diapause induction, maternal generation developed at 20 °C and day length of 10 h and progeny generation developed at 10 °C and 14 °C and day length of 0, 3, 6, 9, 12, 15, 18, 21 and 24 h. The results revealed significant influence of Wolbachia infection, photoperiod, temperature, and their interactions on the percentage of diapausing individuals in all cases (grand maternal, maternal, and direct effect of photoperiod). Experiments showed that under the short days (9 and 12) and permanent light, the percentage of diapausing individuals was significantly higher than under the ultra-short, long, and ultra-long days. A somewhat similar pattern of photoperiodic reaction (indirect and direct photoperiod reaction) was observed in both infected and uninfected *T. brassicae* however the results showed that uninfected population was more successful in diapause entering than infected one which might be the result of Wolbachia infection. The results indicated that exposure to diapause induction conditions for more than one generation increased the percentage of diapausing individuals.

Key words: diapause, photoperiod, *Trichogramma brassicae*, *Wolbachia*, temperature.

Posters
Agricultural Entomology and Acarology
E18

ENTOMOPATHOGENIC AND SOIL FUNGI AGAINST SPOTTED WING DROSOPHILA (*DROSOPHILA SUZUKII*)

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Spotted wing drosophila (*Drosophila suzukii*; SWD; Diptera: Drosophilidae) management is very challenging for the most part due to its brief generation time, polyphagy and serrated ovipositor, which allows it to infest undamaged, ripening fruit, but also because its larvae can pupate in the orchard soil and are thus protected from aerial insecticide applications. Usually there is zero tolerance for SWD larvae in fresh or processed fruit products, thus fruit producers rely primarily on frequent insecticide applications, to prevent infestation. However, many insecticides are not allowed in organic fruit production and may be disruptive to beneficial agroecosystem services and human health. Therefore, we investigated interactions between entomopathogenic (EPF) and insect-associated soil fungi and SWD in soil and aboveground environment to offer soft fruit producers a viable biocontrol management solution. We performed several laboratory experiments evaluating different fungal deployment strategies utilizing uncommercialized fungal isolates and bioinsecticides against SWD in blueberries. We hypothesized that EPF and soil fungi would express different pathogenicity against SWD pupae in soil environment. Hence, in addition to known EPF (*Metarhizium brunneum* and *Beauveria bassiana*), also a soil fungus (*Trichoderma atroviride*), was tested against pupae in conidia-spiked soil and via direct conidial applications against pupae. Further, we hypothesized that different SWD life stages would vary in their susceptibility to infection. Therefore, a selection of most pathogenic strains from pupal exposure experiments was also tested against imago's via spray application. Within these experiments also horizontal transmission of fungal infection was evaluated. Additionally, we tested an attract and infect strategy, in which the flies were exposed to EPF growing on SWD artificial food. *M. brunneum* (strain 1154) significantly reduced fly emergence in conidia spiked soil, and bioinsecticide Naturalis (based on *B. bassiana*) in direct pupal exposure tests. Several strains caused significant mortality of sprayed flies: The lowest LT_{50} among uncommercialized fungi was 9.4 d for *M. brunneum* (1154). Naturalis (24.6 d) was at par with negative control (29.2 d). Insecticide Laser (a.i. spinosad) worked best with LT50 0.9 d. Higher virulence was recorded in the attract and infect strategy: LT50 was 4.5 d for *M. brunneum* (1154), 5.6 d for *M. brunneum* (1868), 2.2 d for B. bassiana (2121) and 21.6 d for negative control. Horizontal transmission of fungal infection was observed rarely. We conclude that the imago's were generally more susceptible to fungal infection than pupae. Most likely the pupal stage is too brief to allow entomopathogens to cause a significant reduction of fly emergence. On the other hand, the high virulence obtained in direct spraying and attract and infect experiments shows promise in a potential EPF-based SWD management strategy.

Key words: biological control, integrated pest management, insect, organic, soft fruit

Posters
Agricultural Entomology and Acarology
E19

TOWARDS AN INTEGRATED PEST MANAGEMENT STRATEGY TO COMBAT *DROSOPHILA SUZUKII*

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Since a few years, the vinegar fly *Drosophila suzukii*, which originally originates from Southeast Asia, has become a major pest species in America and Europe, causing huge economic losses in the soft and stone fruit sector. With their enlarged sclerotised ovipositor *D. suzukii* females can penetrate ripening soft fruits such as cherry, strawberry, raspberry and grape early in the season. Hatched larvae consume the fruits from the inside out, rendering infested fruits unmarketable. To control *D. suzukii* many growers routinely apply calendar spraying, which is not in line with current promotions of sustainable use of pesticides and the implementation of an integrated pest management (IPM). To develop an effective IPM strategy, the objectives of this study were two-fold. First, we evaluated the efficacy of several pesticides in field trials over a period of three years. Furthermore, an extensive survey was performed aiming at the identification of main parasitoids of *D. suzukii* in Flanders. Among several insecticides tested, cyantraniliprole and lambda cyhalothrin were the only pesticides protecting the fruits up to 14 days post application. In addition, we identified two *Drosophila* parasitoids belonging to the families of Figitidae (*Leptopilina heterotoma*) and *Pteromalidae* (unidentified species). Although both species were able to parasitize the common fruit fly *Drosophila melanogaster*, parasitism efficacy on *D. suzukii* was poor. Further research efforts are needed to achieve sustainable management of *D. suzukii*.

Key words: *Drosophila suzukii*, IPM, insecticides, parasitoids

Posters
Agricultural Entomology and Acarology
E20

IDENTIFICATION OF *AGRIOTES* SPP. (COLEOPTERA: ELATERIDAE) BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

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The larvae of *Agriotes* spp. (Coleoptera: Elateridae), also known as wireworms, are abundant soil-dwelling insects and can be a serious pest of a wide range of crops, such as potatoes, maize, onions, ... When wireworm numbers rise above the economic threshold of a crop, the grower needs to take control measures. To monitor the number of wireworms in a field, bait traps are being used. As *Agriotes* look-alikes often occur in these traps, a correct identification of the captured insects is needed. Morphological identification of wireworms to species level is extremely difficult, even for experts. Identification via multiplex PCR or barcoding is a reliable solution but can only be performed by well-trained staff in laboratories equipped with adequate molecular facilities. Hence, a cheaper, highly specific, sensitive, and faster detection tool, based on a loop-mediated isothermal amplification technology (LAMP) is being developed. In this study, we will discuss the LAMP-primer design, the validation parameters such as specificity, sensitivity, and robustness for the three most important species for Belgium viz. *Agriotes lineatus*, *A. obscurus* and *A. sputator*. All tests were performed using the portable and user-friendly Genie II (OptiGene®). Primer design is based on mitochondrial COI sequences from Genbank combined with the COI sequencing obtained from our own ILVO *Agriotes* collection. The selectivity was tested on 13 different wireworms species collected in the bait traps during a three-years monitoring campaign. In addition, larvae from Carabidae and Staphylinidae found in these bait traps were also considered to test the species selectivity. To find out how robust the primers are, we run the LAMP-PCR on more than 200 wireworm specimens from different countries (France, Germany, Austria, and Switzerland). The LAMP primers for *A. sputator* are highly specific and result in a reliable detection and their applicability will be further investigated in the field. The LAMP primers designed for A. obscurus and *A. lineatus* are not sufficiently species-specific, so adjustment of the primers and further validation is required to distinguish *A. lineatus* from *A. obscurus*.

Key words: wireworms, molecular detection, LAMP, GenieII

Posters
Agricultural Entomology and Acarology
E21

PANTHEON (PRECISION FARMING IN HAZELNUT ORCHARDS) FOR THE IMPROVEMENT OF INTEGRATED PEST MANAGEMENT (IPM) EFFECTIVENESS

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We are presenting PANtHEOn, a project funded by the H2020-SFS-2016-2017 Call, Research and Innovation Action, Topic SFS-05-2017, which is aimed at the development of the agricultural equivalent of an industrial Supervisory Control and Data Acquisition (SCADA) system to be used for the precision farming of large orchards of hazelnut (*Corylus avellana* L.).
The project will be focused on the develop of unmanned aerial platform (UAV) and ground vehicles able to navigate in the orchard and to perform autonomously required operations. These instruments are useful to collect data on the plant growth habits, yield and on its phytosanitary status. All data will be sent to a central unit where an expert system will be able to assess the plant's health and to suggest the decision-making process concerning the required agronomic interventions. The latter are based on the measured indices and on the historical data of each plant. The crop protection action will consist in the monitoring of pests and diseases incidence in the orchard and in the quantification of the intervention threshold for each damage. The field data will be also used to calibrate the indicators, based on remote sensing data, and to optimize the quantities and the typology of pesticides used for insect pests and disease control. The considered biotic stress factors are: *Phytoptus avellanae; Curculio nucum*; true bugs (*Halyomorpha halys, Gonocerus acuteangulatus, Palomena prasina, Piezodorus lituratus, Raphygaster nebulosa, Nezara viridula and Dolycorum baccarum*) involved in economic detriment due to the quality losses by kernel abortion, malformation and emergence of unpleasant flavor; Cytospora canker, caused by the ascomycete *Cytospora curricula; Fusarium lateritium*; the bacteria *Pseudomonas avellanae* and *Xanthomonas arborol* pv. *corylina*.
In conclusion, one goal of this project is to improve the average health condition of the orchard, and to increase the effectiveness of the IPM techniques for a more sustainable management of the ecosystem.

Key words: Robotics, Crop protection, Stink bugs, phytosanitary status

Posters
Agricultural Entomology and Acarology
E22

FITNESS OF A GREGARIOUS PARASITOID: A THRESHOLD BETWEEN LARVAL COMPETITION AND HOST IMMUNITY

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Parasitized hosts are considered a low-quality resource and might result in its rejection or affect the number of oviposition's made by parasitoids. On the other hand, the host immune response needs to be suppressed and it depends on the number of eggs laid by the parasitoid. Thus, a trade-off between spending energy for suppressing the host immune response and obtain better offspring fitness is a matter involved. This study had the objective of finding the number of oviposition's from *Palmistichus elaeisis* (Hymenoptera: Eulophidae) allowing optimal offspring fitness and to analyze if immune response the melon worm *Diaphania hyalinata* L. (Lepidoptera: Crambidae) is correlated with the density of posture by the parasitoid. Oviposition's of *P. elaeisis* in pupae of *D. hyalinata* varied from one to five times. The immature parasitoid developmental, total number of offspring produced, sex ratio, and biomass produced per parasitized pupae were recorded for the different oviposition densities. Incidence of unviable parasitoid larvae was also recorded. The survivorship of *P. elaeisis* was daily assessed. Hemocyte dynamics and encapsulation capacity of *D. hyalinata* against *P. elaeisis* was evaluated for each number of oviposition's, from one to five. The Parasitoid developmental time decreased with increased oviposition density and three oviposition's provided higher offspring and particularly female production, and optimal larval fitness. Female and male survived longer with one oviposition of the female parasitoid. Parasitoid emergence increased with the number of parasitoid oviposition's and 100% parasitism and corresponding 100% host pupa mortality were achieved with all oviposition densities. Increased number of oviposition's decreased the number of total hemocytes, and of granulocytes and plasmatocytes in the circulating host hemolymph. The melanisation and encapsulation rates decreased with the number of oviposition's by *P. elaeisis*. Three oviposition's by the parasitoid female allowed optimal progeny production and parasitoid performance. Super parasitism is a strategy of *P. elaeisis* for optimal progeny fitness balancing optimal progeny performance with amelioration of host immune response.

Key words: Fitness, host immunity, larval competition, Parasitoid

Posters
Agricultural Entomology and Acarology
E23

POTATO VIRUS Y TRANSMISSION AND QUANTITATIVE DETECTION IN THE STYLET OF APHID SPECIES

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Potato virus Y (PVY), the type species of potyviruses is a destructive and a worldwide distributed virus causing important damage in different crops, particularly in potato. PVY is transmitted in a non-persistent manner by more than 50 different aphid species and presumably, the distal part of the stylet plays an important role in the virus transmission by these aphids. Transmission efficiency is influenced by the aphid species, the aphid feeding behavior, the virus type, strain, or isolate and environmental factors. Moreover, the type of host plant on which the aphids are feeding is also a factor that can also affect transmission efficiency yet has not been studied well so far. In this respect, the quantity of potyvirus uptake by aphid species, through probing a specific infected host plant may be correlated with the transmission efficiency. Several reports have been published reporting on the presence or even the quantity of potyviruses in individual vectors. However, they mainly report on detection or quantitation in whole aphid bodies, and only very few studies focus on virus transmission efficiency, linked to virion concentration in the stylet. An even more precise index that correlates to vector efficiency in non-persistent transmitted viruses is the virion concentration in the stylet tip, which is in close contact with the host tissue during the probing process. In this study, the transmission efficiency of four aphid species was evaluated. Additionally, the influence of five different host plants for rearing aphids on PVY transmission was assessed. The correlation between these two bioassays and virus quantity in whole aphid bodies, stylets and stylet tips was assessed using a serial dilution of PVY transcripts in a one-step real-time RT-PCR. The results confirmed that *Myzus persicae* transmitted PVY most efficiently (39%) and that aphids which were reared on faba bean transmitted PVY less efficient than those which were reared on Brussels sprouts, Chinese cabbage, sweet pepper, or potato. qRT-PCR tests showed that quantity of virus in stylets are a better index to evaluate vector transmission efficiency,

Posters
Agricultural Entomology and Acarology
E24

CUTICLE PROTEINS ARE INVOLVED IN POTATO VIRUS Y TRANSMISSION IN THE GREEN PEACH APHID (*MYZUS PERSICAE* SULZER)

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Potato virus Y (PVY) is a destructive and widespread virus in several important crops, including potato. Aphid vectors spread this viral infection between plants in a non-persistent manner and *Myzus persicae* is the most efficient one. In this research, we investigated the importance of aphid cuticle proteins, which are present in the stylet, in the transmission process of this virus. Using RNA interference (RNAi), we investigated the role of several of these cuticle proteins in *Myzus persicae* in the transmission of PVY from potato to tobacco plants. Feeding dsRNA, specific for two cuticle proteins (RR2CP2 and CPUNKN2) to the aphids led to a gene knockdown of 63% and 75% on transcript level, respectively. While silencing CPUNKN2 did not reduce PVY transmission by the aphids significantly, knockdown of RR2CP2 led to a significant reduction of PVY-transmission (27%) compared to the GFP control. These results indicate that cuticle proteins in these aphids are indeed involved in viral transmission.

Posters
Agricultural Entomology and Acarology
E25

EFFECT OF STARVATION ON THE COLD TOLERANCE OF ADULT *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE)

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The spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), is an invasive pest that poses a major threat to soft and stone fruit production worldwide. Various studies have been performed on the overwintering capacity of this insect, but none of them have focused on the effect of food deprivation on the cold tolerance of SWD. In this laboratory study, different cold tolerance metrics were measured for *D. suzukii* adults: (1) critical thermal minimum (CTmin), (2) acute cold tolerance (ACT) (exposure to -5 °C for 1 h) and (3) chronic cold tolerance (CCT) (exposure to 0 °C for 8 h). Three- to 4-day-old flies of a French population (Rennes, Brittany) were subjected to 4 experimental treatments in which they were either given ad libitum access to an artificial medium (= control group) or deprived of food for various periods of time (12, 24 or 36 h). In addition, body mass, and water, lipid, and sugar content of individuals from all treatment groups were determined.
The CTmin (i.e., the temperature at which the insect enters chill coma) of starved male and female SWD flies was significantly higher than that of non-starved individuals. Although survival (after 0, 24 and 48 h) of D. suzukii was high in all treatments, and this for both ACT and CCT experiments, statistical differences among treatments were present. Adults that were starved for over 24 h, appeared to be more resistant to acute cold exposure than those with 0 or 12 h of food restriction. Females from different starvation regimes showed a higher survival rate than those fed ad libitum, after being exposed to 0 °C for 8 hours. Overall, females had a significantly higher weight, lipid, and sugar content (in %) than males. The concentrations of dietary triglycerides, glucose and glycerol were negatively correlated with the period of food restriction in both males and females. Our results suggest that starved females of SWD might have a higher acute and chronic cold tolerance than well-fed females. Further research is needed to elucidate the significance of these results for the overwintering strategy and the invasive potential of *D. suzukii*.

Key words: spotted wing drosophila, critical thermal minimum, acute cold tolerance, chronic cold tolerance, food deprivation

Posters
Agricultural Entomology and Acarology
E26

EFFECTS OF TWO INSECTICIDES ON ENERGY METABOLISM OF MUSTARD APHID *LIPAPHIS ERYSIMI* (KAL.)

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Mustard aphid *Lipaphis erysimi* (Kal.) (Hemiptera: Aphididae) is the main pest of canola crops in Khuzestan province in Iran. In this study, the lethal and sub-lethal effects of pirimicarb and thiamethoxam, two common insecticides for controlling this aphid were assessed using leaf dip method. Lethal concentrations and their 95% confidence limits were estimated using Probit analysis procedure. The lethal dose ratio of thiamethoxam to pirimicarb based on LC_{50} values for the first, second, third, fourth nymph instars and the adult were 1.72, 2.15, 0.92, 0.76 and 0.79, respectively. To assess the effects of pollutants on organisms, the investigation in sub-cellular levels can be used as a suitable replacement for expensive and time-consuming studies in population levels. According to the metabolic cost hypothesis, Cellular Energy Allocation (CEA) can act as a potential biomarker. Different components of energy reserves including lipid, protein, sugar, and glycogen were assessed in LC_{20}, LC_{30} and LC_{50} of both insecticides in different time intervals (0-24, 24-48 and 48-96 hours), based on CEA methodology. In all periods there was a significant reduction in all energy sources in treated aphids compared to control except for sugar and glycogen in LC_{20} of pirimicarb (0-24 hours), sugar in LC_{20} of thiamethoxam (0-24 hours) and lipid in LC_{20} of pirimicarb (48-96 hours). The rate of energy consumption (Ec) was measured by electron transform activity which indicated a significant increase in all insecticide treatments. As the net energy budget is calculated from the difference between energy available (Ea) and energy consumption, significant depletion in all insecticide treatments was observed. These results can be caused by increased metabolic activity to cope with toxicant stress and/or decreased in energy absorption (food intake). Based on these findings, further investigations seem to be necessary to find out the possible correlation between the net energy budget and the effects emerging at the population and community levels.

Key words: *Lipaphis erysimi*, thiamethoxam, Pirimicarb, Cellular Energy Allocation.

Posters
Agricultural Entomology and Acarology
E27

2-DODECANONE: A POTENTIAL ALTERNATIVE TO SYNTHETIC INSECTICIDES?

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Glandular trichomes are specialized epidermal protuberances found on the surface of about 30% of all vascular plants, that produce and secrete structurally diverse specialized metabolites. Among the many substances synthesized by trichomes for plant defense purposes, methyl ketones are a widely-produced group of chemicals and their insecticidal efficacy has been described against some arthropods, such as aphids or spider mites. However, information about the mode of action and molecular effects of these compounds on insects are still very scarce. In the present study, the toxicity of 2-dodecanone (CAS 6175-49-1) was investigated in *Chironomus riparius* aquatic larvae, an insect model species commonly used in ecotoxicity studies. The effects of acute 24-h and 96-h exposures to 0.05 µg/L, 5 µg/L and 500 µg/L 2-dodecanone, were evaluated at the molecular level by analysing changes in the transcriptional rate of genes related to the endocrine system (EcR, ERR, Vtg and Cyp18a1), detoxication pathways (Cyp4G, GST and GPx) and the energy metabolism (GAPDH). Ribosomal gene 26S, actin and ribosomal protein L13 were used as reference genes. Our results showed that 2-dodecanone caused a clear dose- and time-dependent toxicity in most of the selected genetic biomarkers. While significant effects were detected even after 24-h acute exposures, longer treatments (96h) triggered a general transcriptional repression in most of the analysed genes. This study provides novel and interesting results in *C. riparius* on the toxic effects of an isolated secondary metabolite, naturally present in plants, and highlights the potential suitability of this organism to delve into the molecular effects of plant defences in insects. These findings provide new insights into insecticidal efficacy of 2-dodecanone, which might be explored under field conditions for plant protection and pest management, to reduce reliance on synthetic pesticides.

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Key words: genetic biomarkers; *Chironomus riparius*; toxic effects; plant defense; pest management

Posters
Agricultural Entomology and Acarology
E28

THE IMPACT OF LAND-USE TYPES ON COMMERCIAL BUMBLE-BEE COLONY FORAGE AND SUSTAINABILITY

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Agricultural intensification has led to landscape simplification, natural habitat loss and increase of the mass flowering crops, which has negatively affected natural pollinator diversity. Moreover, even in case of abundant pollinator populations, these could not provide sufficient pollination for larger crop fields because of overlapping and short flowering periods. Then, extra pollinators are needed to relieve pollination deficiency and promote yield quantity and quality. Thus, it is necessary to estimate the pollination potential and pollinator colony development of managed bee species in the context of supporting capacity of the different land-use types around insect-pollinated crops.
Our study was conducted on open field strawberry production farms during the strawberry flowering period within four different land-use types: arable land, arable land with orchards, mosaic and natural landscapes. Commercial bumblebee hives were used to evaluate the colony development and the attractiveness of the strawberry pollen to bumblebees in presence of several competing food plants and other pollinators within different land-use types.
The results showed that the average proportion of the strawberry pollen was varying significantly between different land-use types. The strawberry pollen amount foraged was highest in orchards and lowest in arable landscapes. Whereas, orchards seemed to provide sufficient food sources for the colony development, which was seen by high increase in hive biomass. Surprisingly in the orchards we found also lower competition by other pollinators. The natural landscapes showed lowest hive biomass increase, because of scarce food availabilities and high competition by other pollinators.

Posters
Agricultural Entomology and Acarology
E29

ENGINEERING OF THE HALYOMORPHA HALYS VIRUS FOR RNAI IN *EUSCHISTUS HEROS*

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The Neotropical brown stink bug *Euschistus heros* (Hemiptera: Pentatomidae) is a major phytophagous insect pest causing high economic losses in soybean and cotton crops. To date, the control strategies heavily rely on the use of non-selective and highly toxic wide-spectrum chemical insecticides, which can persist in the environment and affect non-target organisms. In this project, we aim to investigate the use of the post-genomic technology of RNA interference in the development of an environmentally friendly and non-transformative virus-based approach to control this important pest insect.

Key words: VIGS, RNA interference, stink bugs

Posters
NEMATOLOGY

Posters
Nematology
N01

NEMATICIDAL ACTIVITIES OF NATURALLY-BASED PRODUCTS COMPOST AND NEMAGOLD ON ROOT-KNOT NEMATODES

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Moroccan market garden production in greenhouse conditions is threatened by numerous phytosanitary problems, the plant parasitic nematodes *Meloidogyne* spp., which becomes a real constraint for its production. Here, the main objectives of this study were to ensure first the presence of gall nematodes associated with vegetable crops in 7 tunnels and to evaluate the protective effect of naturally-based products compost and Nemagold (tagetes essential oil 80% and algae extract 20%) for its control. By using a susceptible plant to nematodes, the bioassay test showed higher values of the gall index on cucumber roots in soil of both tunnels seeded with turnip and leeks ($\geq$3), followed by soils from tomatoes and carrot tunnels (1.5). In contrast, soils of tunnel 24 and control were nematodes free (absence of the gall). In addition, the variance analysis indicates a significant effect of the gall index on the fresh root weight of cucumber ($P \leq 0.05$). Furthermore, the results underlined that compost and Nemagold reduced significantly the gall index and had, thereof a substantial nematicidal activity on *Meloidogyne* spp. Applying the compost at 5 T/ha had reduced the gall index by 80.3%, whereas the 3T/ha rate gave only 58.24% of reduction. In the case of the Nemagold product, the use of 10 L/ha allowed 80.3% reduction of gall index. Therefore, our findings highlight the possibility of using these natural compounds to control root-knot nematodes in organic greenhouse systems.

Key words: *Meloidogyne* spp., biotest, gall index, compost, Nemagold product.

Posters
Nematology
N02

EFFECT OF GRAPEFRUIT WASTE ON INHIBITION OF ROOT KNOT NEMATODE (*MELOIDOGYNE JAVANICA*) IN TOMATO PLANTS

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Waste materials from plants to soil have been proven as an alternative means of nematode control. A greenhouse study was conducted to investigate the effect of Grapefruit Waste Compost (GWC) on the inhibition of root knot nematode, *Meloidogyne javanica*, and some possible mechanisms involved in nematode suppression such as C:N ratio, total phenolic component in the tomato roots and leaf chlorophyll contents were measured. Two months before transplanting of tomato seedlings, sterile and non- sterile soil was amended with dried residues of Grapefruit at 3, 5 and 7% (w/w). Sixty days after nematode inoculation, the results showed a direct correlation between increasing GWC and the final population of nematode (Pf). At rate of 7% in non-sterile soil, Pf decreased by 65% compared to non-amended control. In this study one of the nematicidal efficacy of amendments was directly correlated with their nitrogen content and inversely related to their C:N ratios. By increasing GWC, the C:N ratio was lower than non-amended control. The most efficacious against the nematode was when soil was amended with a high GWC ratio (7%). Also, tomato grown in the compost-amended media showed the presence of higher quantity of total phenols than those grown in non-amended soil. One of the possible causes of reduction in final population of *M. javanica* should be derived from the limitation in nematode penetration rates due to high phenolic component in the root which may also affect the maturity of the female and decreasing the number of eggs. In this study, no phytotoxicity was observed and soil amendment with GWC increased the leaf chlorophyll content. In overall, the results suggested that inhibition of nematode with GWC relates to complex components and various mechanisms in the soil environment, as well as the biochemical strategy of tomato plant grown in such media.

Key words: Agro-industrial waste, Control, Root knot nematode, Tomato

Posters
Nematology
N03

CHALLENGES OF THE EUPHRESCO PROJECT MELOTROP: GLOBAL WARMING AND DISTRIBUTION OF ROOT-KNOT NEMATODE SPECIES OF THE TROPICAL GROUP

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Root-knot nematodes (RKN) *Meloidogyne* spp. are the most harmful plant parasitic nematodes. Several species belonging to the tropical RKN group (*M. incognita, M. arenaria, M. javanica*, *M. enterolobii, M. ethiopica, M. hispanica, M. paranaensis* etc.) cause substantial economic losses in agriculture particularly in vegetable production as they have a wide host range including monocotyledons, dicotyledons, including herbaceous and woody plants. Yield losses of 50-80% caused by these nematodes in vegetable crops are common and greater in tropical than in temperate regions due to more favourable environmental conditions for establishment, development, reproduction, and dispersal. However, climate changes are likely to influence the future distribution of pests and it is expected that *Meloidogyne* species, previously found in tropical and subtropical regions, will become important pests in temperate regions as well.

An intensive global trade, trends in environment friendly plant production and lack of efficient management strategies make these parasites a serious risk for the crop production in Europe. *Meloidogyne incognita* and *M. javanica* were recognised as globally the most rapidly spreading plant pests. Several tropical RKN species can survive open field winter conditions particularly in the Mediterranean countries. Open field occurrence represents additional risk for several crops, especially because of predicted climate change effects and the fact that nematode infestations at larger acreages are much more difficult to manage.

To cope with such threats, detection, and identification methods for tropical RKN species are of paramount importance. Currently, one of the most reliable methods for the group diagnostics is the isozyme phenotyping but this biochemical approach has certain disadvantages and difficulties. Furthermore, while several molecular methods have been developed, selecting appropriate methods for reliable molecular diagnostic is still a major challenge.

The recognition by several EPPO countries of tropical RKNs as an emerging phytosanitary problem and their enhanced damage potential due to climate changes led to the Euphresco initiative entitled 'Global warming and distribution of root-knot nematode species of the tropical group (MeloTrop)'. Six research partners joint forces in the consortium to reach the following objectives: to generate distribution maps of the tropical RKNs in some European countries; to assess the survival ability of *M. incognita* and *M. arenaria* in continental climate conditions; to validate biochemical and molecular methods for the detection of tropical RKNs, and to generate geographical maps of the possible field distribution for each tropical RKN species occurring in Europe.

Key words: *Meloidogyne*, root-knot nematodes, global warming, distribution, identification

Posters
Nematology
N04

THE FIRST RECORD OF ENTOMOPATHOGENIC NEMATODES (HETERORHABDITIDAE AND STEINERNEMATIDAE) IN MOROCCO AND THEIR USE IN BIOLOGICAL CONTROL AGAINST SOIL-DWELLING PESTS

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A survey was carried out in three Moroccan regions: Saïs, Middle Atlas and Gharb. The aim of the survey was the demonstration for the first time, the natural occurrence of entomopathogenic nematodes (EPN) in Morocco. 70 soil samples were tested for the presence of EPN by the technique Galleria baiting technique. 5 soil samples were positives for the presence of EPN (7.14 %): 3 samples contained Heterorhabditidae nematodes (4.28 %) and 2 samples contained Steinernematidae nematodes (2.86 %). Soils where EPN were found differ about some characters (texture, pH, and organic matter content). Virulence of the five EPN isolates against white grubs, was tested. Only isolate 5 (*Steinernema* sp.2) was virulent against white grubs with a mortality reaching 77.53 %.

Key words: Morocco, entomopathogenic nematodes, Galleria baiting technique, Heterorhabditidae, Steinernematidae.

Posters
Nematology
N05

MORPHOLOGICAL VARIABILITY OF THE NORTHERN ROOT-KNOT NEMATODE (*MELOIDOGYNE HAPLA*) IN REPUBLIC OF SRPSKA

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Root-knot nematodes (RKN) are the most destructive plant-parasitic nematodes whose identification is highly demanding due to large number of species and intraspecies variability. *Meloidogyne hapla* - the northern root-knot nematode is the most common RKN in moderate climate zone and the most cytogenetically and morphologically diverse species of the genus. Presence of *M. hapla* has been reported in Republic of Srpska but detailed studies on the populations were never conducted.
Females and second stage juveniles (J2) of 44 local populations of *M. hapla* from different regions were observed and measured. Morphological identification of each population included observation of single female perineal pattern and hyaline tail terminus shape of 20 J2 and measurements of nine characteristics of J2. Measured characteristics were body length, distance from stylet base to anterior end, distance from secretory-excretory pore to anterior end, dorsal gland opening, maximum body width, body width at secretory-excretory pore, body width at anus, tail length and hyaline tail terminus length. Results showed that there are six basic shapes of perineal patterns and five most common shapes of J2 hyaline tail terminus. Different deviations of the shape of hyaline tail terminus were also recorded. Morphometric data separate populations in two groups what indicating the presence of two races (race A and B) but this should be confirmed with cytological analysis. Potential presence of two races on agricultural fields in Republic of Srpska would require race identification of each field population before implementation of control measures because of their differences in pathogenicity and host range.

Key words: Root-knot nematodes, characterization, population, morphological characteristics

Posters
Nematology
N06

THE HOST STATUS OF SOME TOMATO VARIETIES TO DIFFERENT INITIAL POPULATIONS OF *MELOIDOGYNE ARENARIA* AND *MELOIDOGYNE JAVANICA*

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The cultivation of resistant or low-host varieties provides significant advantages in terms of control and cause the reduction of the damage from root knot nematodes (*Meloidogyne* spp.). In this context, it is important to know the reactions of different plant varieties to root knot nematode species. In this study, hosts status of 4 tomato cultivars such as 'Beaufort', ''H-2274'', ''Catering'', ''Ret unto'' (*Solanum lycopersicum* Mıll.) to different initial population levels of *M. arenaria* and *M. javanica* have been researched. These varieties were infected with eggs (0, 500, 5000) of *M. arenaria* and *M. javanica* and controlled greenhouse used for pot experiments conducted with 4 replications and repeated once again. After 8 weeks from artificial inoculations, the plants were rooted and leaf number, plant height, fresh and dry plant weight, fresh root weight were determined as plant development criteria. The most important criteria for the damage of nematode was assessed by using gall index (0-10). In addition, the number of eggs in the roots and the reproductive factor (Rf=Pf/Pi) were determined. Generally, when the plant development criteria were summarized, the most affected varieties were Retinto and H 2274 and the less affected varieties was Ikram. For 2 species of root nematode, root gall index was higher in H-2274 and less in Beaufort. Again, for each of the 2 nematode species, Retinto is the varieties with the highest number of eggs per g root and Beaufort has the least. Beaufort has the lest R0 for both nematode species and H-2274 has the higher R0 for *M. arenaria* and Retinto for *M. javanica*. In general, as the population level increases, the plant development criteria are not affected in the same way, while the nematode damage increases proportionally as the population increases. Thus, in areas where these nematode species are problematic, like Beaufort varieties may be offered as a non-host or weak host tomato plant.

Key words: tomato, *Meloidogyne arenaria*, *Meloidogyne javanica*, host status

Posters
Nematology
N07

EFFECT OF INSECTICIDES ON THE SURVIVAL AND CONTROL RATES OF ENTOMOPATHOGENIC NEMATODE AGAINST CHILI FRUIT FLY PUPAE (*BACTROCERA LATIFRONS* HENDEL)

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Entomopathogenic nematode (EPN) is one of the bio-agents that is effectively used against chilli fruit fly and can be used in combination with insecticides to promote Integrated Pest Management (IPM). Therefore, the research objective was to study the effect of four different types of insecticides including imidacloprid, thiamethoxam, cypermethrin and carbosulfan on the survival and control rates of EPN. The results showed that the survival rate of EPN, which was mixed with imidacloprid, was the highest at 78.50% and found no statistically significant difference to control (96.50%) followed by EPN which was mixed with thiamethoxam (56.50%), cypermethrin (15.50%) and carbosulfan (0.75%). The EPN after mixed with imidacloprid and thiamethoxam was tested against chilli fruit fly pupae. Control (insecticide-free EPN) gave the highest mortality rate at 87.50% and there was statistically significant difference (P ‰¤0.05) between control and EPN after treated with thiamethoxam (52.50%) and imidacloprid (45%). Although imidacloprid did not affect to EPN's survival rate, it affected the control rate. Therefore, the results from this research indicated that all insecticides are not suitable to combine with EPN for the control of chilli fruit fly pupae.

Key words: entomopathogenic nematode, imidacloprid, thiamethoxam, cypermethrin, carbosulfan

Posters
FORMULATION AND APPLICATION TECHNOLOGY, PESTICIDE RESIDUES, TOXICOLOGY AND ECOTOXICOLOGY

Posters
Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
R01

MASSIVE SPREAD OF PESTICIDES IN AREAS WITH AN INTENSIVE FARMING SYSTEM: THE CASE OF DOUCEN AND AIN NAGA IN BISKRA (ALGERIA)

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The intensive farming has taken of essential place in recent years and boosted the agricultural dynamics in this region, caused a huge development of the superficies of crops in greenhouses lines in intensive from 1 370 ha in 2000 to 5767 ha in 2016. So, 60% of the needs of vegetable products produced from the area of Biskra. The adequate climatic and edaphic conditions give her a great potential, qualitative and quantitative vegetable productions. But, Pests and diseases generate big problems to vegetable production, the damage caused by them has led to farmers using a wide range of chemicals.

In fact, pesticides used extensively on vegetable farms, small or large as the frontline strategy to protect crops, and to the achievement of global food security and poverty reduction. However, pesticides can be held responsible for the threat to the environment and sustainable agricultural development in the arid region.

Our work aims to identify the most important vegetable, greenhouses in areas with an intensive farming system at the region of Biskra, a general diagnosis on the main phytosanitary problems that affect this sector and the significant pesticides in movement used and caused the contamination of environmental resources. The survey conducted from October 2016 to September 2017, among 90 producers and 20 phytosanitary product seller, randomly selected in two zones poles of the region of Biskra (Ain Naga and Doucen). The results obtained show the most efficient and specialized in vegetable production areas, where the sale of a diversity of pesticides with ranges of active substance broadcast in the soils and waters exploited such as (glyphosate, lambda cyhalothrin, deltamethrin, abamectine, chlorpyriphos–ethyl, imidacloprid, spiromesifen, mancozeb, metalaxyl-m, iprodione, bentazone, metribuzin… etc).

Key words: vegetable production areas; intensive farming; active substance; phytosanitary problems.

Posters
Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
R02

ECOTOXICOLOGICAL EFFECTS OF ORGANIC AND CONVENTIONAL RICE FIELDS ON PLANKTON COMMUNITY

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This study assessed the ecotoxicological effects of untreated organic and conventional rice field treated with herbicides, pesticides and fertilizers on phytoplankton and zooplankton communities. Plankton were monthly collected through plankton net with mesh size 20 micron during August to October. The results showed that a total of 23 taxa belonging to 5 groups of zooplankton were recorded. Among zooplankton, rotifer was the most dominant group both qualitatively as well as quantitatively. The density of zooplankton ranged between 29.93-292 unit/l and 796-1,283.34 unit/l for conventional and organic rice fields. The zooplankton communities from organic rice field were more abundance than those from conventional rice field. The overall phytoplankton analysis of the rice fields revealed the presence of 45 taxa belonging to 4 divisions. The conventional rice field, euglenophyta was the dominant phytoplankton group (57.79-90.59%). Whereas the chlorophyta (38.41-72.49%) was found to be the major group in organic rice field. Regarding the bioassessment, euglenophyta ordinary represented as a bioindicator for organic pollutants. The trophic status of all studies sites evaluated from the density of phytoplankton could be classified as meso-eutrophic or moderate-polluted water quality for conventional rice field and mesotrophic or moderate water quality for organic rice fields.

Key words: plankton community, organic rice field, conventional rice field

Posters
Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
R03

DIETARY EXPOSURE TO DICHLORVOS AND LAMBDA-CYHALOTHRIN RESIDUES IN FOOD AND RISK ASSESSMENT FOR CONSUMERS IN THE METROPOLIS OF NIAMEY

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Plant protection products are widely used by small scale farmers in the neighbourhoods of Niamey. However, their misuse can have potential adverse health effects. Dietary exposure to plant protection products residues was assessed in Niamey metropolis, using the 24-hour recall method. Portion sizes are estimated using a collection of pictures previously prepared according to the local usual diet. Thirty composite samples, representative of the most consumed vegetables in Niamey in the dry hot season were collected around and in Niamey. Samples were analysed with a multi-residue method (QuEChERS) with a limit of quantification (LOQ) of 0.01 mg/kg. Residues of lambda-cyhalothrin and dichlorvos were detected in samples (respectively in 56.6% and 6.6% of samples) and exceed several times the Maximum Residue Level (MRL) allowed for the food commodities. The highest concentrations reach 0.30 mg lambda-cyhalothrin/kg and 0.41 mg dichlorvos/kg. To evaluate the intake and to characterize the risk level for various consumer groups (adults and others), the PSD (Pesticide Safety Directorate) spreadsheet was used. Results based on consumptions at the 97.5th percentile show that the exposure values to lambda-cyhalothrin are well below the acute reference dose (ARfD) for all age groups. But for dichlorvos, the exposure values largely exceed the corresponding ARfD value with a higher exposure of youths (infants and toddlers) than the adults. Therefore, more investigation about pesticides residues in vegetables in Niger is needed and a risk assessment study should be carried out.

Key words: Dichlorvos, lambda-cyhalothrin, food-residues, risk assessment, Niamey

Posters
Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
R04

TERATOGENICITY STUDY ON KYLEO (GLYPHOSATE, 2,4-D) AND LEAD TO CHICKEN EMBRYOS

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The agrochemicals are the most important environmental pollutants within the agricultural production. The pesticides sprayed during the plant protection activities might have influence not only on mature birds but also on the embryos in the eggs. In the environment which is contaminated by pesticides, these materials can modify the chemical environment of animals and induce poisoning, respectively. The presence of lead and its effects in the environment and in living organism have been known for many years. Teratological tests carried out on avian embryos provide useful data for environmental protection and facilitate the development of environment-friendly chemical plant protection techniques. As the ecotoxicological testing methods used in the practice are mainly limited to study the toxic effect of compounds alone, data on interactions between pesticides and heavy metals can be regarded as gap-filling information especially in relation to the avian organism.
The objective of this study was to determine the individual and combined toxic effects of glyphosate and 2,4-D containing herbicide formulation (KYLEO) and lead acetate on the development of chicken embryos. The eggs were immersed in the solution or emulsion of the test materials for 30 minutes on the first day of the incubation. The applied concentration of lead acetate was 0.01% and of the herbicide KYLEO was 1% corresponding to that used in plant protection practice. Number of embryonic death, developmental abnormalities and body weight of embryos were recorded on day 19 of hatching. The body weight was statistically evaluated by one-way ANOVA and Dunnett post-test, the embryo mortality and the developmental abnormalities were analysed by Fisher exact test. Lead acetate alone, and the combination of herbicide and lead acetate reduced the body weight of embryos significantly. Single treatment of lead acetate and the combinate treatment increased the mortality of embryo significantly. Single and concomitant administration of lead acetate increased the rate of embryo mortality. Developmental abnormalities were observed sporadically due to the single and concomitant administration of the test items. Our teratogenicity study revealed, that the individual toxic effect of lead acetate and KYLEO (glyphosate, 2,4-D) were embryotoxic but not teratogenic in chicken. There is addition type toxic interaction between the lead acetate and KYLEO herbicide formulation.

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Key words: glyphosate, lead acetate, 2,4-D, ecotoxicology, chicken embryo

Posters
Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
R05

INVESTIGATION OF IRRITATION POTENCY OF PESTICIDES ON ISOLATED CHICKEN EYES

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Numberless toxicological tests are necessary before the pesticides became available on the market. The determination of eye irritation potential of the chemicals is part of this test packet. Until now the OECD 405 study in rabbits can be used to classify the irritation potential of substances. The Draize-test is the basis of the OECD 405 guideline, which is one of the most criticized in vivo methods, because of the injuries of the test animals and subjective nature of the test on recording the results.
Nowadays, several in vitro tests are available which can be partly or totally replace the in vivo eye irritation testing depending on the circumstances. The isolated chicken eye test method is one of these alternative methods. Six different pesticides were examined in the following way: (1) The eyes were collected in a slaughterhouse. At least three eyes treated with test item and, three positive and one negative control eyes were used in each test. Concurrent positive and negative controls were included. In parallel running studies with multiple test items, the same controls served as common positive and negative controls for each test item. Positive and negative controls showed the expected results. The experiments were valid. (2) The test compound was applied in a single dose onto the cornea of isolated chicken eyes to potentially classify the test compound as either 1: causing "serious eye damage" (category 1 of the Globally Harmonised System for the Classification and Labelling of chemicals (GHS)), or 2: not requiring classification for eye irritation or serious eye damage according to the GHS. (3) Tested corneas were evaluated pre-treatment and at approximately 30, 75, 120, 180, and 240 minutes after the post-treatment rinse. (4) The endpoints evaluated were corneal opacity, swelling, fluorescein retention, and morphological effects. All the endpoints, except for fluorescein retention (which was determined only at pre-treatment and 30 minutes after test substance exposure) were determined at each of the above time points.
In these in vitro eye irritation studies, using the Isolated Chicken Eye model with six different pesticides, ocular corrosion or severe irritation potential were observed. These results correspond to the available information about the tested pesticides, so these studies with isolated chicken eye are successful.

Key words: in vitro, isolated chicken eye, fluorescein retention, corneal opacity, swelling

Posters
Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
R06

EARLY EMBRYOGENESIS STUDY ON CHLORPYRIPHOS AND COPPER TO CHICKEN EMBRYOS

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Large number of chemical agents can reach into the environment, biological activity of which is still not well-known nowadays. Generally, the pesticides are investigated separately in the ecotoxicology studies. However, it can be considered that the pesticides are not alone in the environment; many other chemicals maybe present at the same time (e.g. heavy metals) which can pollute the environment. Generally, the chemical load can appear as a complex way, therefore the simultaneous toxic effect and interaction of the chemical agents can be expected in the same time. The components can modify each other's toxic effect.

The single and simultaneous fetotoxic effect of copper sulphate and CYREN EC (480 g/l chlorpyriphos) were investigated on chicken embryos at early embryonic development.

The chicken eggs were dipped in the solution or emulsion of the test materials for 30 minutes on the first day of incubation. The applied concentration of copper sulphate was 0.01% and of insecticide CYREN EC was 0.5%. On day 3 of incubation 15-15 fertile eggs were opened and the viability and developmental stage of the embryos were examined by preparing permanent sliders from them as follows. Above the air space the calcic eggshell and the shell membrane were removed, then the germinal disk was cut around and stained with 0.1% osmium tetroxide solution. The stained germinal disk was placed into avian physiological saline solution and it was floated on a slide and fixed with DPX histological adhesive. Finally, the slide was covered with coverslip. The permanent sliders were examined by light microscope. The statistical analysis of the results of embryo mortality and developmental abnormalities were performed by Fisher's exact test. The incidences of embryo mortality were observed sporadically due to the single and combined administration. Single treatment of both items and their combination increased the rate of developmental anomalies, but the combined treatment of insecticide and copper caused significant changes. The single toxic effect of chlorpyriphos containing insecticide formulation was embryotoxic in chicken embryos. The combined administration of CYREN EC and copper sulphate increased the embryotoxic effect and the type of toxic interaction may be additive.

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Key words: chlorpyriphos, copper sulphate, toxic interaction, ecotoxicology, chicken embryo

Posters
Formulation and Application Technology,
Pesticide Residues, Toxicology and Ecotoxicology
R07

METHOD FOR THE DETERMINATION OF THIOPHANATE-METHYL AND PROTHIOCONAZOLE IN PPPS

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In this study, an isocratic reversed-phase high-performance liquid chromatography method with diode array detection (HPLC-DAD) for the simultaneous determination of thiophanate-methyl and prothioconazole in plant protection products (PPPs) was developed and validated. Thiophanate-methyl (dimethyl 4,4'-(o-phenylene)bis (3-thioallophanate)) and prothioconazole ((RS)-2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazole-3-thione) are fungicides with preventive, curative and eradicative effects, widely used to control fungal diseases on crops. For the determination of thiophanate-methyl in formulated products, the validated method is available, while according to the available data for the determination of prothioconazole, as well for simultaneous determination of these active ingredients in PPPs formulations, there is no standard method, which demands development and validation of the corresponding one. For the analysis, an Agilent Technologies 1100 Series LC system with Zorbax SB-C18 column (5 µm, 250 mm x 3 mm internal diameter) was used. Analytes were diluted in acetonitrile and ultrasonically dissolved. For determination of thiophanate-methyl and prothioconazole different wavelengths and different components in mobile phase have been tested. The best separation was obtained with a mobile phase consisting of acetonitrile and deionized water (80/20, v/v). The flow rate was 1.0 ml/min, column temperature 25 °C, injected volume was 1 µl and the most suitable detection was at the wavelength of 254 nm. Separation of the peaks of the analyzed active ingredients was satisfactory, with retention times for thiophanate-methyl and prothioconazole of 0.674 min and 1.415 min, respectively and with separation coefficient (Rs) of 1.5. The linearity of detector response was determined at five concentration levels. The values of correlation coefficients (0.998 for thiophanate-methyl and 0.999 for prothioconazole) suggest that the increase of concentration is linearly followed by increase in peak area. High precision in determination of these compounds was confirmed by RSD values of 0.65% and 0.52%. Based on Horwitz equations, RSDr for peak area are significantly below modified values of Horwitz limit of 1.75% for intra-laboratory repeatability of determination of formulated products with the 19.03% of active ingredient. The accuracy was determined by the standard addition method. Recovery values of 89-90.3% confirm the accuracy of the applied method for determination of thiophanate-methyl and prothioconazole in formulated products. The performance characteristics of the method completely fulfil international criteria.

Key words: thiophanate-methyl; prothioconazole; PPPs; HPLC-DAD