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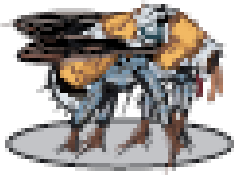

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Biological tools and technologies

AMPELOMYCES QUISQUALIS FOR CONTROL OF POWDERY MILDEW

D. Angeli[1], K. Saharan[2], C. Gessler[3], I. Pertot[1]

[1] Department of Sustainable Agro-Ecosystems and Bioresources (DASB), Research and Innovation Centre
(FEM), 38010 S. Michele all'Adige, Italy

[2] Institute of Botany, University of Basel (UNIBAS), 4056 Basel, Switzerland

[3] Department of Environmental Systems Science (D-USYS), Swiss Federal Institute of Technology (ETH),
8092 Zurich, Switzerland

dario.angeli@fmach.it

Ampelomyces quisqualis is an intracellular mycoparasite of powdery mildew and the active ingredient of the oldest biofungicide but the new isolated *A. quisqualis* strain ITA3 showed an higher efficacy in controlling powdery mildews. Research is currently underway towards to develop a suitable conidial formulation strategies for use as an effective biocontrol agent against powdery mildew in agronomical field applications. The development of production and formulation processes for biocontrol fungi has primarily focused on reducing costs by maximizing the yield of infective propagules, increasing storage stability, and improving product form for an ease application. To enable commercial production of the conidia of ITA3, the medium composition and culture conditions were optimized in the current study in order to obtain maximum biomass during growth phase and maximum conidia yield during subsequent conidiation phase. We also considered stability as a dry preparation and biocontrol efficacy of the conidial formulation. Many statistical experimental design methods have been employed in bioprocess optimization. Among them, response surface methodology (RSM) is the one suitable method for identifying the effect of individual variables and for seeking the optimum conditions for a multivariable system efficiently. Here, the RSM approach was adopted to locate optimum levels of substrate concentration. In the present study, yield of the strain ITA3 vary much depending on the substrates, cultivation methods and conditions. Cultivating conditions such as higher surface aeration (220 rpm) and lower initial pH value (pH 6.5) were demonstrated to produce good yields of conidia in this fungus. The optimized jaggery broth medium has resulted in significant enhancement in the productivity of the biomass and conidial yield. An increase of 23.2 – fold in biomass and 1.4×10^6 – fold in conidial yield were obtained with optimized jaggery broth at a reduction of 43% in time in comparison to the base jaggery broth. Other aspects of this work regarded the scale-up of *A. quisqualis* inoculums for biocontrol purposes which was one of the crucial steps towards its commercialization and practical use in plant protection. In our experiments conidia of ITA3 were formulated and then sprayed on powdery mildew colonies. Solid based silicagel formulation with 25 ml of conidial suspensions (10^7 conidia g ml^{-1}) and 50 grams of silicagel powder gave the best powdery mildew control. The high conidial yield and the good biocontrol results achieved in the present study with silicagel formulated conidia seems to be economically sustainable for commercial production of *A. quisqualis.*

TRICHODERMA ATROVIRIDE FOR CONTROL SOIL-BORNE PATHOGENS

A. Pellegrini[1], D. Prodorutti[2], I. Pertot[1]

[1] Department of sustainable agro-ecosystems and bioresources, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via Mach 1, 38010 San Michele all'Adige, TN, Italy

[2] Centre for technology transfer, Fondazione Edmund Mach (FEM), Via Mach 1, 38010 San Michele all'Adige,
TN, Italy

ilaria.pertot@fmach.it

Trichoderma atroviride SC1 has biocontrol properties against several soil-borne plant pathogens. However its persistence in soils is often very short, thus the efficacy in reducing pathogen's inoculum can be rather limited. This is particularly true for soil-borne pathogens as *Armillaria mellea*, which can survive several years in root residues in soil. Bark mulch is commonly used to control weeds and preserve soil fertility. However the use of *Armillaria*-infected coniferous bark can led to the spread of *Armillaria* root rot in orchards. We demonstrated that coniferous bark can be used as a carrier to distribute and maintain high levels of *T. atroviride* SC1 inoculum in soil. The different types of bark examined did not have any differential effects on the level of biocontrol activity against *Armillaria gallica*. *T. atroviride* SC1 can persist and grow on bark for a long period after treatment (up to 16 weeks). The incidence of disease on plants that were mulched with bark pre-inoculated with SC1 and then infected with *A. gallica* was significantly lower than that observed on the untreated control (25 and 70%, respectively).

THE NEW METHOD OF INSECT MATING DISRUPTION BY VIBRATIONAL NOISE

Valerio Mazzoni, Jernej Polajnar

Fondazione Mach, Via Marconi 2, 38010 San Michele all'Adige, Italy

valerio.mazzoni@fmach.it

Scaphoideus titanus is a leafhopper insect, vector of the grapevine FD phytoplasma. Since FD is recognized as a quarantine disease, compulsory control measures include the large-scale treatments of vineyards with insecticides, including neurotoxic compounds and chitin synthesis inhibitors. The concerns about detrimental effects of pesticides on biodiversity and human health are leading to an increasingly restricted use of chemicals in agriculture. One of the main challenge in increasing the safety of the global food production is to identify appropriate alternative approaches for the numerous insect pests that do not rely on chemical communication. Since the first successful field trial, pheromone based mating disruption enabled sustainable insect control, which resulted in reduced levels of pesticide use. However, in *S. titanus* the mating behaviour is driven by substrate-borne signals while pheromones are absent. In fact, vibrational signals are crucial for the species mating success in that a male and a female establish a stereotyped vibrational duet that enables partner recognition and localization. In the present contribution, we show that effective mating disruption based on substrate-borne vibrational signals can be achieved in the field. When the device which transmitted disruptive vibrational signals to grapevine plants through a supporting wire, mating frequency of the leafhopper pest dropped to less than 10% in a mature vineyard. The underlying mechanism of this environmentally friendly pest-control tactic is a masking of the vibrational signals emitted by *S. titanus* during pair formation. Because vibrational communication is widespread in insects, mating disruption using substrate vibrations can transform many open field and greenhouse based farming systems.

INTEGRATED MANAGEMENT OF THE INVASIVE TOMATO LEAFMINER *TUTA ABSOLUTA* IN FLANDERS (BELGIUM)

Veerle Van Damme[1,2], Rob Moerkens[3], Els Berckmoes[4], Lieve Wittemans[4], Nick Berkvens[2], Bert Beck[2], David Nuyttens[2], Raf De Vis[4], Hans Casteels[2], Luc Tirry[1], Patrick De Clercq[1]

[1] Ghent University, Faculty of Bioscience Engineering, Department of Crop Protection, Laboratory of Agrozoology, Coupure Links 693, B-9000 Gent, Belgium

[2] Institute for Agricultural and Fisheries Research (ILVO), Burgemeester Van Gansberghelaan 96, B-9820 Merelbeke, Belgium

[3] Tomato Research, Proefcentrum Hoogstraten, Voort 71, B-2328 Hoogstraten, Belgium

[4] Research Station for Vegetable Production, Duffelsesteenweg 101, B-2860 Sint-Katelijne-Waver, Belgium

veerle.vandamme@ugent.be

Since its first detection in Flanders (Belgium) in 2009, the South American tomato leafminer *Tuta absoluta* is now frequently encountered in tomato greenhouses in this region. In the framework of the European Directive 2009/128/EC on the sustainable use of pesticides, an integrated control strategy against this pest in protected areas is being developed.

It was assumed that the low temperatures commonly associated with Belgian winters would prevent the exotic species from successful overwintering. Experiments on cold-hardiness, however, demonstrated that the adults and pupae of this leafminer can overwinter in empty but frost-free greenhouses for at least two weeks. Therefore there should be aimed for an as low as possible population level of *T. absoluta* towards the end of the production cycle. This minimizes the number of overwintering adults and pupae, thus allowing the new crop to start in a clean environment which is the first step in an integrated pest management approach.

Currently *Macrolophus pygmaeus* is the most used commercial natural enemy against *T. absoluta*. Population buildup of this predatory bug is slow, making it difficult to control *T. absoluta* at the beginning of the production cycle or when pest levels are high. The population growth and distribution of *M. pygmaeus* in the greenhouse should be optimized by releasing the predator as soon as possible after planting in combination with the provision of food supplements. Laboratory experiments demonstrated that the predatory bug prefers the eggs of *T. absoluta* and preys only to a limited extent on the larvae.

To complement the limited control potential of *M. pygmaeus* towards *T. absoluta* larvae, the efficacy of three commercially available entomopathogenic nematodes (EPN) *Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora* was tested against the four larval stages of *T. absoluta*. Control of the larger larval instars L3 and L4 was most successful, resulting in 71.8–97.4% mortality. The efficacy of the selected EPN was tested at 18°C and 25°C. At both temperatures, *S. feltiae* was most effective, killing 100% of the larvae after three days. The above findings show that EPN might be implemented in an IPM strategy to control this pest but appropriate spray application techniques need to be developed to obtain an optimal deposition of EPN infective juveniles on tomato leaves.

THE EFFECT OF β-AMINOBUTYRIC ACID (BABA) ON CROP PLANT CAPABILITY TO MANAGE HERBIVOROUS PEST ATTACK

Sylwia Karolczyk, Małgorzata Kiełkiewicz

Department of Applied Entomology, Warsaw University of Life Sciences-SGGW,
Nowoursynowska, 02-776 Warsaw, Poland

sylwia.karolczyk@wp.pl

According to the Directive 2009/128/EC on integrated pest management (IPM), non-chemical methods of pest control should be used as an alternative to chemical plant protection. Recently, more and more chemical elicitors are studied and recommended to modify plant capability (either hormonal signalling pathways or metabolic events) to effectively alleviate negative effects of biotic/abiotic stresses.

This review presents the recent findings on the effectiveness of plant priming by BABA (non-protein amino acid) against different taxa of phytophagous pests, and aims to provide a better insight into the potential of BABA-induced plant defence as an alternative or supplementary method for pest control.

Our updated review shows the following:

(1) BABA-induced plant resistance against herbivorous pests representing various life-styles (aphids, psyllids, caterpillars, root-knot nematodes) has been demonstrated in 21 plant species belonging to 7 botanical families;

(2) The evaluation of BABA efficiency in plant protection against pests has mostly been carried out by using young plants or seedlings. The preferred method of BABA application was 'soil drench' at 25 mM or 50 mM concentration;

(3) Generally, BABA applied as 'soil drench' at 25 mM or 50 mM concentration has no impact on plant fitness (e.g. plant growth, fresh and dry matter accumulation etc.);

(4) BABA treatment at 25 mM – 50 mM concentration enhances protection of tic bean, pea, broad bean, runner bean, red clover and alfalfa against the pea aphid (*Acyrthosiphon pisum*); Arabidopsis against the cabbage aphid (*Brevicoryne brassicae*), the cabbage looper (*Trichoplusia ni*) and the diamondback moth (*Plutella xylostella*); calabresce, and also of cabbage, spring cabbage, salad rape, black and white mustard against *B. brassicae*, the green peach aphid (syn. the peach-potato aphid) (*Myzus persicae*), *T. ni* and *P. xylostella*; wheat against the grain aphid (*Sitobion avenae*); soybean against the soybean aphid (*Aphis glycines* Matsumura) and citrus against the Asian citrus psyllid (*Diaphorina citri*).

(5) The most likely, decrease in pest performance, measured as r_m, adult survival or mortality, female fecundity etc., was casued by BABA-induced change in quality of the treated host-plants;

(6) In two cases, also negative impact of the prey fed on BABA treated plants on its natural enemies (parazytoid; predator) was documented.

IN VITRO INHIBITION BY NATURAL WHEAT CHEMICALS AND IDENTIFICATION OF THEIR BIOCHEMICAL TARGETS

Thomas Etzerodt[1], Kazuyuki Maeda[2], Yuichi Nakajima[2], Bente Laursen[1], Inge S. Fomsgaard[1], Makoto Kimura[2]

[1] Department of Agroecology, Aarhus Universitet, Forsøgsvej 1, DK-4200 Slagelse, Denmark

[2] Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa, Nagoya, Aichi 464-8601, Japan

Inge.Fomsgaard@agrsci.dk

Fusarium head blight (FHB) is a severe disease in wheat caused by mainly *F. graminearum* and *F. culmorum*. FHB results in significant yield losses and in accumulation of the trichothecene mycotoxins in grain. Susceptibility to Fusarium and mycotoxin accumulation in grain is cultivar dependent, *e.g.* Sumai-3 wheat cultivar exhibits high FHB resistance and low accumulation of trichothecenes in grain. In order to reduce the use of fungicides, various plant secondary metabolites have been studied for their ability to inhibit trichothecene biosynthesis, e.g. ferulic acid and 4-acetylbenzoxazolin-2-one.

Several secondary metabolites naturally occurring in wheat (including benzoxazinoids, phenolic acids, cinnamic acids, and flavonoids) were tested for their inhibitory activity against trichothecene production in liquid cultures with a *F. graminearum* lineage producing the trichothecene B, 15-ADON. The benzoxazinoid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) completely inhibited toxin production, while the structurally similar 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one (HMBOA), lacking of 7-methoxy and 2-hydroxy group, respectively, did not possess any inhibitory activity. Benzoxazinoids benzoxazolin-2-one (BOA) and 6-methoxy-benzoxazolin-2-one (MBOA) and the flavonoid homoorientin exhibited moderate inhibitory activity. *trans*-ferulic acid and *trans*-p-coumaric acid, stimulated toxin production several fold compared to controls.

Employing *tri6* and *tri5* gene expression and TRI5 and TRI4 trichothecene protein assays, we were able to identify targets of the trichothecene biosynthesis pathway. DIMBOA suppressed *tri6* gene expression and partially inhibited TRI4 protein activity, while BOA only temporarily suppressed *tri5* gene expression. Initial experiments for homoorientin suggested that TRI5 was the target for the flavonoid. Previous results by other authors (Ponts et al. (2011), *Phytopathol.*, 101, 929–934) revealed that ferulic acid enhances toxin production by upregulating expression of *tri5*.

To our knowledge this is the first time that the benzoxazinoids DIMBOA, DIBOA, MBOA and BOA have been tested for their effects on trichothecene production.

Our results indicate that DIMBOA plays an important role in the suppression of the production and accumulation of toxic trichothecenes in wheat grain. Further studies of these wheat metabolites are highly desired to test the effect of these wheat secondary metabolites with other lineages of *F. graminearum*.

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OVERVIEW OF EU-FUNDED PROJECT BIOCOMES

Jürgen Köhl

Wageningen UR – Plant Research International, P.O. Box 69, 6700 AB Wageningen, The Netherlands
jurgen.kohl@wur.nl

The EU emphasizes the role of integrated pest management as an important approach to reduce dependency on pesticides use (Directive 2009/128/EC). Biological control of pests and diseases can be a very effective, sustainable and environmentally friendly strategy for crop and forest protection as part of integrated pest management (IPM) practices. The availability of sufficient biological control products is important for an effective IPM strategy. Unfortunately, biological control alternatives against a range of important pests and pathogens – causing high economic losses to agriculture and forestry – are not or not sufficiently available at this moment. The objective of the EU project BIOCOMES is to develop 11 new biological control agents (BCAs) and 2 production technologies for key markets in European agriculture and forestry. BCAs were identified through market analysis by six manufactures of biological control products. BCAs will primarily be for use in open field crops of vegetables (3), of which 2 are also for use in protected crops, arable crops (3), fruit crops (3), and three different types of forests (2). Primary targeted pests are: gypsy moth (*Lymantria dispar*), pine weevil (*Hylobius abietis*), tomato leaf miner (*Tuta absoluta*), white flies, aphids of fruit tree crops and *Mamestra brassicae*. Primary targeted pathogens are: damping-off diseases in forest nurseries, soilborne pathogens of oilseed rape and cereals, brown rot (*Monilinia* spp.) of stone fruit, and powdery mildew of cereals (*Blumeria graminis*). The economic sustainability during the entire development process will be assessed by the responsible industrial partners. The environmental sustainability will be quantified for each BCA by means of the Sustainable Process Index method. The entire developmental process for each of the 11 BCA products is guided by a consultancy partner specialized and leading in (bio) pesticide registration including risk assessments for European (bio) pesticide industries.

ELICITRA – INTEGRATED FRENCH NETWORK PROMOTING THE STRATEGY OF PLANT RESISTANCE INDUCTION BY ELICITORS THROUGH RESEARCH, TRAINING AND DEVELOPMENT

R. Berthelot[1], M. Turner[2], X. Daire[3], M. Ponchet[4]

[1] ARVALIS-Institut du végétal, Station expérimentale, 91 720 Boigneville, France

[2] Vegenov-BBV, Penn ar Prat, 29250 St Pol de Léon, France

[3] INRA, Centre de Dijon 17 rue Sully, BP 86510, 21065 DIJON, France

[4] INRA, 400 route des Chappes, BP 167, 06903 Sophia Antipolis, France

r.berthelot@arvalisinstitutduvegetal.fr

Agriculture must face challenging and apparently contradictory issues: becoming both competitive and sustainable. The current reduction plan of pesticide use, occurring throughout Europe, must therefore be accompanied by the development of efficient environmentally friendly methods in crop protection. Among them, the enhancement of plant defence mechanisms by elicitor treatments is one of the most promising strategies and has become a major topic of current research.

Elicitra is a French network co-animated by ARVALIS-Institut du vegetal, Vegenov and INRA. Its main mission consists in promoting plant protection by induced resistance through research, training and development. This network is dedicated to a large range of plant productions: field crops, vegetable, fruits, vine, ornamental plants and medicinal plants. It includes partners from public research, technical institutes, universities, agricultural colleges, various actors of the crop industries and competitive clusters. By bringing together various partners with different skills ranging from field to lab and from research to training, the understanding and development of this alternative approach is accelerated.

Elicitra is supported and financed by the French ministry of agriculture. It was launched in 2011 and will work until 2018.

Main Results:

Network & communication

- An active community about elicitor
- A web site : www.elicitra.org (in French)
- A 2 days meeting in 2013 (150 participants)
- A scientific & technical monitoring : **Elicitr'Actu** (4 times a year)

Scientific & technical results

- A definition of what is an elicitor
- A data base with the main experimental results
- A guide for experimental practices

Impact on research

- Reorientation of public research priority
- A list of research priority (based on an exhaustive work about plants diseases and elicitation potential progress)

– New research projects

During the next 4 years, Elicitra will keep on working about sharing results, communication and research with 5 main axes: assessment of new elicitors, plant response to elicitor, application condition, elicitor in IPM and agro ecology, unintended effects.

Elicitra is an open network: contribution and exchange with elicitor actor (in France and abroad) are welcome.

EVALUATION OF ELICITOR'S ABILITY TO REDUCE THE USE OF FUNGICIDES TO CONTROL POTATO LATE BLIGHT AND WHEAT SEPTORIA LEAF BLOTCH 15

D. Gaucher, C. Maumene

ARVALIS-Institut du végétal, Station expérimentale, 91 720 Boigneville, France

Due to environmental and human health concerns, French authorities have planned to reduce the use of pesticides in agriculture (Ecophyto plan). In parallel, according to an ECPA studies, the number of active ingredients registered and available for use, continues to decrease dramatically like the number of new molecules in the pipeline, which has been divided per two in 10 years. Consequently there is an increasing need to innovate, and especially for the development of low risk products or technics, to maintain the competitiveness of agriculture.

So, ARVALIS tests new systems or technology to control diseases with fewer amounts of chemicals, such as decision support systems, cultivars resistance, plant defenses stimulators (elicitors) or reduced doses of fungicides.

Intensive fungicide use is the most common way to control potato late blight (average number of fungicide treatments is about 14 to 15 per year in France). Different elicitors have been tested in potato late blight field trials, in semi-controlled conditions (misting system + inoculation), based on weekly applications from beginning of June to end of July at Boigneville (2012, France).

Some alternative products show promising results especially in combination with a reduced dose of fungicides. In trials with different elicitors, it was found that phosphites based products show a good efficacy to control late blight. However, they are not enough effective to be used alone. The use of 750 g / ha of PO3 in combination with a half dose of fungicide, was nearly as effective as the full dose of the same fungicide. The dose of 1000 g / ha, again in mixture, with a half dose of fungicide, would have been probably slightly better, more stable and safer for users.

Another product has so far shown some very interesting results. This product contains only plant extracts (saponin, glutathion and oligosacharin) without any phosphites and could be used in organic farming (or as biocontrol product).

As a conclusion for potatoes, a 50% reduction in use of fungicide (Ecophyto French plan goal) on potatoes seems possible, facilitated by the large number of treatments, the good efficacy of fungicide half-doses, and with the help of elicitors in mixture.

Researches on septoria (*Zimoseptoria tritici*) are far less advanced. Little attention has been paid until now to cereals, in term of elicitors, despite the fact the wheat surface represents in France about 5 millions ha cultivated and is sprayed on average twice a year.

First trials show that replacing a half dose of the first treatment (conventional chemical) by a double application of one elicitor is a reasonable goal. Among the different elicitors tested, with this protocol, in the field at Boigneville on three cultivars (France, 2012), (microorganisms extract, *Trichoderma harzanium* extract, phosphite based product, chitosan) only a chitosan based product, has given regular and acceptable results.

DOES BABA CAN MITIGATE THE EFFECT OF ARTHROPOD 19

PEST INFESTATION ON EGGPLANT?

Sylwia Karolczyk[1], Małgorzata Kiełkiewicz[1], Katarzyna Kowalczyk[2]

[1] Department of Applied Entomology,

[2] Department of Vegetable and Medicinal Plants Warsaw University of Life Sciences-SGGW, Nowoursynowska, 02-776 Warsaw, Poland

sylwia.karolczyk@wp.pl

Although the eggplant fruits are known to be rich in antioxidant secondary metabolites, they still remain a niche crop in Poland. They are largely cultivated under cover, and require chemical protection, because frequently are infested by thrips, mites, white flies, aphids and many other herbivores.

Suppression of pest herbivores to low densities with non-chemical, biological methods combined with simultaneous strengthening eggplant self-defence, could result in effective protection, compliant with the EU Directive (2009/128/EC).

It is known that a non-protein β-aminobutyric acid (BABA), applied as a priming agent, may confer protection against a broad spectrum of biotic and abiotic stresses. It can sensitize plant by the activation of so called 'primed state' in which the plant is able to respond more effectively to pest attack than other non-treated plants.

The eggplants (*Solanum melongena* L. ScorpioF_1) were grown on soilless media in greenhouse, to mimic the usual production conditions. The plants were treated with water (control) or with BABA at 25 mM or 50 mM concentrations applied as seed imbibition (SI) or soil drench (SD). BABA-untreated and treated plants were naturally infested by a mixed population of thrips and two-spotted spider mites. Following the infestation, eggplant growth, development and leaf chlorophyll fluorescence (Fv/Fm – maximum quantum efficiency of PSII, PI – performance index, øPSII – quantum efficiency of PSII), as well as pest performance, were assessed.

In the present study, simultaneously feeding thrips and mites caused only week changes in eggplant growth and development. Measurements of chlorophyll fluorescence parameters suggest that BABA has the capacity to mitigate negative effects of these pests, at least to some extent. However, its effectiveness depend on BABA mode of application, concentration and pest density.

REQUIREMENTS FOR EFFECTIVE MATING DISRUPTION OF A LEAFHOPPER WITH VIBRATIONAL NOISE

22

Jernej Polajnar, Valerio Mazzoni

Fondazione Mach, Via Mach 1, 38010 San Michele all'Adige, Italy

jernej.polajnar@fmach.it

After the success of preliminary trials with the bioacoustic method for mating disruption of the leafhopper *Scaphoideus titanus* Ball, we set out to investigate requirements for efficiency of this method in small-scale trials. Artificially induced vibrational noise is, in principle, able to prevent mating by obscuring information about the emitter's location, identity and quality, contained in vibrational signals and necessary for mating success in leafhoppers. The next step is to examine technical issues, such as minimal amplitude and time of activation, as related to energy consumption and equipment wear. We used a pre-recorded disturbance signal, such as it is used by males of this species in rival interactions with other males, and transmitted it into plants using electromechanical transducers. We tested efficiency and limitations of the method in laboratory and semi-natural conditions, focusing on amplitude, diel pattern of activation and the method of transmitting vibrational noise to host plants of *S. titanus*. Necessary amplitude for efficient disruption was determined in laboratory trials in a simplified localization task, while diel pattern of activation and attenuation were examined in the field. As expected, hours when the disruption must be active overlap the most active periods of male calling, but it is possible to switch off the playback at least for a part of the day without losing efficiency. Attenuation, on the other hand, is proving to be a major issue with prototype transducers in the field.

IN VITRO STUDIES ON THE POTENTIAL FOR BIOLOGICAL CONTROL OF *RHIZOCTONIA SOLANI* ON PEPPER BY BINUCLEATE *RHIZOCTONIA*

Cafer Eken[1], Serdar Tuncer[2]

[1] Süleyman Demirel University, Faculty of Agriculture, Department of Agricultural Biotechnology, Isparta, Turkey

[2]Horticultural Research Station, Erzincan, Turkey

cafereken@sdu.edu.tr

Pepper (*Capsicum annuum* L.) belongs to the family Solanaceae, which is an important group of vegetables cultivated extensively in Turkey and also widely cultivated in almost every country of the world. *Rhizoctonia solani* is associated with root and hypocotyl rot of pepper. Protection against root and hypocotyl rot diseases caused by *R. solani* has been reported in many crops treated with nonpathogenic binucleate Rhizoctonia (BNR). Three isolates of BNR from pepper were evaluated for their in vitro for control of *R. solani* anastomosis groups AG-2 type 1 (B418), AG-4 (B92) and AG-6 (B93). BNR isolates B82 (AG-K), B84 (AG-A) and B360 (AG-G) provided protection of 76,7 to 100% against isolate B418 of AG-2 type 1 and 11,6 to 23,3 protection against isolate B92 of AG-4. The BNR isolates did not provide protection to the pepper (cv. Demre sivrisi) seedling when tested against B93 (AG-6). The water agar method was useful for evaluating the potential of the nonpathogenic BNR as biocontrol agents. These BNR isolates may have potential use in management of *R. solani* in pepper, but will require rigorous testing under greenhouse and field conditions.

The work was supported by the Ministry of Food, Agriculture and Livestock, the General Directorate of Agricultural Research and Policies (TAGEM), Project No. TAGEM-BS-07/10-01/02-02, and the Scientific Research Fund of Atatürk University, Grant No. 2007/25.

LABORATORY TESTING OF ENTOMOPATHOGENIC OR INSECT ASSOCIATED FUNGI FOR THE CONTROL OF CABBAGE FLEA BEETLES (*PHYLLOTRETA* SPP.)

Jaka Razinger, Špela Modic, Hans-Josef Schroers

Agricultural Institute of Slovenia, Hacquetova ulica 17, Ljubljana, SI-1000, Slovenia

jaka.razinger@kis.si

Cabbage flea beetles of the genus *Phyllotreta* (CFB) present an increasing problem specifically in Slovenia and have the potential to even overshadow pest significance of the *Delia radicum* cabbage root fly that is considered the major threat to brassica crops in Europe. Due to new regulations, integrated pest management strategies are mandatory in the EU since 1st January 2014. In the framework of the project PURE, such strategies involving the biological control against CFB with entomopathogenic or insect-associated fungi were explored in *in-vitro* laboratory bioassays. Experimental systems consisted of a 450 ml plastic cup into which a fresh leaf of kale and 10 field-collected *Phyllotreta* spp. beetles were added. The pot cover and pot base was perforated to insure air circulation. Our test fungi were isolated from various substrata in Slovenia and included *Metarhizium anisopliae*, *M. brunneum*, *Clonostachys rosea* and *Trichoderma atroviride*. Each species was represented by a single isolate. *Beauveria bassiana* from product Naturalis served as a positive reference strain in the experiments. Conidial suspensions were prepared in 0.1% Tween 80 at a concentration of $1x10^8$ conidia ml^{-1} and sprayed three times on the upper and lower side of kale leaves using a hand-held 10 mL dispenser. Naturalis was similarly applied using the recommended concentration of 0.05% and the negative control consisted of 0.1% Tween 80. Four replicates per treatment were made. The experiment was repeated twice independently. Experiments were evaluated after 7 and 14 days. The number of living, dead and mycotic beetles and the kale leaf feeding rate was recorded. All fungal strains tested and the product Naturalis were infective to CFB imagos and significantly increased the mortality rate already after 7 days. Abbott's corrected mortality at day 14 ranged from 31.6±5.5 to 84.7±6.8%. *Metarhizium anisopliae*, *M. brunneum* and *B. bassiana* (product Naturalis) significantly reduced also the CFBs' feeding rates and caused highest rates of mycoses. The two new fungal isolates of *M. anisopliae* and *M. brunneum* were identified as highly virulent against CFB. Because of their potential as biological control agents, they will be further evaluated in field experiments.

MATING DISRUPTION AGAINST *LOBESIA BOTRANA* (DEN. & SCHIFF) USING ISONET-LTT DISPENSERS IN THE DOURO WINE REGION (PORTUGAL)

52

Cristina Carlos[1,2], Fátima Gonçalves[2], Maria Do Carmo Val[1], Susana Sousa[2], Márcio Nobrega[3],
José Manso[1], Rui Soares[4], Álvaro Martinho[4], Sérgio Soares[4,] Laura Torres[2]

[1] ADVID – Association for the Development of Viticulture in the Douro Region, Quinta de Santa Maria, Apt. 137, 5050-106 Godim, Portugal

[2] Centre for the Research and Technology of Agro-Environmental and Biological Sciences, CITAB, University of Trás-os-Montes and Alto Douro, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal

[3] SOGEVINUS FINE WINES S.A., 4400-111 Vila Nova de Gaia, Portugal

[4] Real Companhia Velha- R. Azevedo Magalhães, n.º 314 Oliveira do Douro, 4430-022 Vila Nova de Gaia, Portugal

cristina.carlos@advid.pt

The European grapevine moth, *Lobesia botrana* (Den & Schiff.) is the most important pest in the Douro Wine Region (DWR) where, typically develops three generations per year, being the third one, the most damaging to grapes. The use of mating disruption (MD), an environmentally friendly method to control this pest, is widely recommended in IPM strategies. Mating disruption have been registered in Portugal since 2000, with ISONET-L (Shin-Etsu Chemical Co. Ltd., Tokyo, Japan), loaded with 172 mg of E,Z-7,9-dodecadienyl acetate. The application of this dispenser over the years (2000–2010) allowed the identification of some major constraints to the use of MD in DWR, namely: the high biotic potential of the moth; the climate conditions, particularly the high summer temperatures; the effect of the winds on the distribution of the pheromone on the hill; the impact of slope; the fragmentation of many vineyards and the size of the treated area. Under ECOVITIS project, since 2011, two wine companies (Real Companhia Velha and Sogevinus Quintas SA.) have used a new pheromone dispenser, Isonet-LTT, developed and produced by the same company (Shin-Etsu Chemical Co. Ltd.), and applied in 180 hectares of vineyards, under the technical supervision of a winegrower organization (Associação para o Desenvolvimento da Viticultura Duriense- ADVID) and a research center (Centre for the Research and Technology of Agro-Environmental and Biological Sciences-CITAB). In the period of 2011–2013, the dispensers used were loaded with 300 mg of pheromone, and applied at the rate of 400 dispensers/ha, while in 2014 they were loaded with 400 mg of pheromone, and applied at the rate of 300 dispensers/ha, yielding in both cases 120 g of pheromone per ha. The main goal was to investigate the effectiveness of these two dispensers in the control of *L. botrana,* having in mind the specificity of each wine farm and year. The results obtained are critically discussed with the aim of improving the effectiveness of the application of this technique in DWR. In general MD was most effective in years of low pest population density, when applied in large areas, with more dispensers per hectare, and after consecutive seasons.

THE INFLUENCE OF NEEM BASED EXTRACTS ON THE MORTALITY, OVIPOSITION AND FEEDING BEHAVIOUR OF ADULT BLACK VINE WEEVIL (*OTIORHYNCHUS SULCATUS*)

Michael Gaffney[1], Gordon Purvis[2]

[1] Horticultural Development Department, Teagasc Research Centre, Ashtown, Dublin 15, Ireland

[2] School of Agriculture and Food Science, Agriculture and Food Science Centre, University College Dublin,
Belfield, Dublin 4, Ireland

Michael.Gaffney@Teagasc.ie

There is a growing need to identify and better understand plant extracts which can be used to affect an insect's lifecycle, feeding habits and overall fitness, rather than relying on a quick 'knock down effect' as an indicator of efficacy. Adult black vine weevils (*Otiorhynchus sulcatus*) were offered foliage treated with two solutions, each containing azadirachtin at concentrations ranging from 0–100 p.p.m. One solution (Aza1) was a commercial formulation, containing only azadirachtin (4.5%), while the second was directly extracted from neem kernels containing a known amount of Azadirachtin (1%), but also other phytochemicals (Azadirex). Foliage of *Euonymus europaeus* was treated with these solutions and offered to adult weevils who were commencing oviposition, at fourteen day intervals for a period of 8 weeks. The initial effect after two weeks with the Aza1 product reduced egg laying significantly across all concentrations, ranging from 70% (10 p.p.m), 90%(50 p.p.m) to 92% (100 p.p.m) compared to the untreated control. Similarly, the egg hatch rate decreased from 73.5% in the untreated controls to 34.4% at the 100 p.p.m application rate. A similar effect was observed with the azadirex solution with egg laying significantly reduced by 90% (50 p.p.m) to 100% (100 p.p.m) compared to the untreated control. Similarly, the egg hatch rate decreased from 73.5% in the untreated controls to 57.9% at the 50 p.p.m application rate. These trends were largely repeated over the next two application periods (4 weeks). After 6 weeks, all remaining weevils were offered untreated foliage to observe if there was any recovery in oviposition. Adults previously fed aza1 at 100 p.p.m did not recover and laid significantly less eggs than the untreated control (Aza1 $p = 0.007$; Azadirex $p = 0.006$). Overall there was no statistical difference in the number of vine weevil adults surviving to the end of the experiment and up to concentrations of 100 p.p.m of aza1 no statistical decrease in foliage consumption was observed. Overall there was a dose dependent decrease in the total cumulative number of viable eggs laid by adult weevils following consumption of foliage treated with both aza1 and azadirex. Similarly the pooled viability of any eggs laid decreased in a direct dose dependent manner. All data was combined and a binary logistic regression was performed to establish the statistical likelihood ('odds ratio') of egg laying under each treatment. The analysis indicated that for every 1000 eggs laid by adults fed untreated foliage, 13 eggs would be laid by adults treated with azadirex (100 p.p.m) and 34 by adults treated with aza1 (100 p.p.m). The persistent impairment of BVW reproduction following ingestion of azadirachtin treated foliage is an important observation in the development of grower friendly IPM approaches in the soft fruit and nursery stock sectors.

ENHANCING THE SOIL FOOD WEB TO HELP CONTROL SOIL DWELLING PESTS OF FIELD VEGETABLES

Celine Delabre[1], Andy Evans[2], Michael Gaffney[3], Julia Cooper[4], Bryan Griffiths[1]

[1] Crop and Soil Systems, Scottish Rural Colleges, United Kingdom

[2] Applied Practice, Scottish Rural Colleges, United Kingdom

[3] Horticulture Development Unit, TEAGASC, Republic of Ireland

[4] Nafferton Ecological Farming Group, Newcastle University, United Kingdom

celine.delabre@sruc.ac.uk

With changing EU legislation and consumer pressure leading to a reduction in the use of pesticides, new sustainable solutions are urgently needed to control the effects of field vegetable pests. Evidence suggests that soils with long and complex food webs, with more trophic links and more abundant predatory fauna, can effectively suppress plant pathogenic organisms. The project aim is to test this hypothesis for the damaging Brassica pest *Delia radicum* (cabbage root fly, CabRF) at two established fully factorial field trials, in Kinsealy (Ireland) and Newcastle (UK). At both sites the soil food web will be quantified over at least a two year period and key soil parameters also determined. The abundance of entomopathogenic fungi, nematodes, predators and parasitoids will be specifically monitored and abundance and damage from CabRF will be assessed on site. First growing season data in Kinsealy indicates that agronomic practices such as soil fertility and crop protection treatments, as well as variety choice, clearly influence soil biology and impact the target pest at different life stages. Soil respiration is affected by crop protection methods, with soils sampled from organic crop protection plots (OP) respiring more than soils from the conventional crop protection (CP) soils (on log scale, CP=1.39±0.039, OP=1.46±0.035, $F=13.44$, $p=0.035$). Soil fertility treatments affect nematode community composition, as organically fertilised soil communities are less disturbed than the conventionally fertilised (maturity index OS=1.66±0.16, CS=1.27±0.15, $p<0.001$). This constitutes the first indication of agronomic practices having an impact on the soil food web in this study. Field monitoring data show that at the beginning of the season more eggs can be found in the organically fertilised plots (OS=8.53±0.78 eggs per 40mL soil sample, CS=5.05±0.56 eggs per 40mL soil sample, $F=27.11$, $p<0.001$), however the 2nd generation egg count seems to be more affected by the type of crop protection used than the soil fertility treatments (OP=9.01±0.72, CP=12.21±0.87, $F=12.55$, $p<0.001$), hinting at possibly different influences of practices over the growing season . Pupae count and feeding damage at the end of the 1st generation are mainly influenced by protection treatments, but crop variety choice also matters. Invertebrate activity and parasitism data are being analysed to determine possible impacts of those practices on natural enemies. Detailed laboratory experiments will consequently be designed to determine the effects of different soil food web complexity on the cabbage root fly egg laying, growth and development in soils taken from field sites. Experimental results, together with input from growers to better understand the interactions between soil and pest, could lead to enhanced control field conditions.

BIOSTIMULANTS – THE USEFUL TOOLS FOR IPM SOLUTIONS 55

Kinga Matysiak, Sylwia Kaczmarek

Institute of Plant Protection – National Research Institute, Węgorka 20, 60-318 Poznań, Poland
ior.poznan.kinga@gmail.com

The main objective of IPM strategies is the use of various agricultural techniques to reduce pesticide input and to decrease the cost of production and to increase the yield per area unit. Lower pesticide input however advantageous for humans and environment can enhance biotic stress of plants. Moreover, combining different methods of plant protection, under some specific conditions, can lead to increased crop abiotic stress sensitivity. This is especially clear with respect to integrated weed management when intensification of non-chemical methods of weed control may impair crop and change soil conditions in the longer term.

In recent years a growing interest has been observed with natural biostimulating substances and several materials that may regulate growth and production have been placed on the market. Biostimulants are products that can modify physiological functions of plants, strengthen plant defenses against different biotic and abiotic stresses and improve nutrition efficiency. Usually they are known as 'plant conditioners' which help plants to adapt to unfavorable conditions. These plant conditioners suppress or eliminate plant growth-limiting factors affecting plant during its life. They protect plants and work differently from other plant protection products. They are also not fertilizers because their main function is not to deliver nutrients to the plant.

In the last few years the importance of preparations containing marine algae (seaweeds) for biostimulation of cultivated plants has been growing. Seaweeds constitute the most essential live organisms used as biostimulants on a wide scale commercially, and extracts from seaweeds are commonly called seaweed liquid fertilizer. They have been mostly reported to increase resistance to diseases and environmental stresses. Phytohormones contained in algae help plants adapt to stress conditions mainly by stimulation of the root system development and maintaining constant cell hydration. It has been demonstrated that algae effect greatly depends on used dose, frequency of application and species of the treated plant. Seaweeds promote root and shoot growth, nutrient uptake and photosynthesis efficiency.

The second group of more and more widely used biostimulants are humic substances including humic and fulvic acids. They are major components of humus which contains most of known trace minerals. Humic substances exhibit the action similar to extracts from seaweeds, although a bigger importance in the development of the plant root system are ascribed to them. Humic acids are considered to be compounds increasing permeability of cellular membranes in plants, and recent studies prove that these substances significantly increase the seed germination energy, the intensification of the seedling growth and the biomass development of roots and shoots. The suppressing effect of humic and fulvic acids on the development of some pathogens is also known.

For over 10 years in the Institute of Plant Protection – National Research Institute in Poznan (Poland) there are conducted glasshouse and field experiments with several groups of biostimulants, particularly seaweeds and humic substances but also mineral biostimulants (caolin clay). The aim of the experiments is evaluation of influence of these substances on growth, development and yield of some the most important crops as oilseed rape, wheat and maize depending on technique and

frequency of treatments. The main object of the all studies is determination of such conditions of biostimulants application that would be the most advantageous for crops.

EFFECTS ON NATURAL DERIVED PRODUCTS ON THE TOMATO RUSSET MITE *ACULOPS LYCOPERSICI* 64

Penelope Zanolli[1], Beatrice Fabris[2], Alberto Pozzebon[2], Carlo Duso[1,2]

[1] National Research Council – IBAF, 35020 Legnaro (Padova), Italy

[2] University of Padova, Department DAFNAE, 35020 Legnaro (Padova), Italy

carlo.duso@unipd.it

The tomato russet mite *Aculops lycopersici* is an important pest of commercially grown tomato *Lycopersicon esculentum*, but its host range includes other worldwide important vegetable and ornamental crops (e.g., eggplant, potato). Effective biological control strategies against this pest have not been developed and its control is based on acaricide use. Among potential alternatives to conventional pesticides, the use of natural enemies is limited by tomato glandular trichomes that hamper the activity and the establishment of predators on plants. Other alternatives are represented by products based on entomopathogens and plant-derived substances. We evaluated the effects of three products based on entomophathogenic fungi (*Beauveria bassiana*, *Paecilomyces fumosoroseus* and *Lecanicillium muscarium*) and two products based on plant-derived substances (pyrethrins and azadirachtin) on *A. lycopersici* in the laboratory. The experiment was performed by exposing tomato russet mite adults to dry residues of the tested products. A water treated control was also included for comparison. The effects of products were evaluated in terms of survival and escaping rate after 72 and 168 hours from application. The survival of *A. lycopersici* was reduced by pyrethrins and azadirachtin but especially by *B. bassiana*. Escaping rate of russet mites was also influenced by treatments. Escaping rate was higher where azadirachtin, pyrethrins, *L. muscarium* and *P. fumosoroseus* were applied as compared to the control and *B. bassiana* treatments. Results are discussed in the framework of Integrated Pest Management on tomato.

DIMETHYL DISULFIDE (DMDS) IN THE SUSTAINABLE CONTROL OF THE CYST NEMATODE *HETERODERA CAROTAE* AND THE ROOT-KNOT NEMATODE *MELOIDOGYNE JAVANICA* ON CARROT IN SOUTHERN ITALY

C. Dongiovanni[1], N. Sasanelli[2], M. Di Carolo[1], G. Fumarola[1], A. Santori[3], A. Myrta[4]

[1] Centro di Ricerca, Sperimentazione e Formazione in Agricoltura "Basile Caramia", Locorotondo (Ba), Italy

[2] Istituto per la Protezione Sostenibile delle Piante, CNR, Bari, Italy

[3] Certis Europe B.V., Saronno (VA), Italy

[4] Certis Europe B.V., Brussels, Belgium

n.sasanelli@ba.ipp.cnr.it

Two field trials were carried out in Apulia region (Southern Italy) in the years 2012 and 2013 to verify the efficacy of dimethyl disulfide (DMDS) in shank application against the carrot cyst nematode *Heterodera carotae* and the root-knot nematode *Meloidogyne javanica*, respectively. DMDS is an innovative soil fumigant for a modern and sustainable soil management. In both trials soil was subdivided in 4 x 50 m plots for shank application of the fumigant. Each plot was subdivided in 5 sub-plots to provide replications for each treatment. DMDS was applied at rates of 180, 280 and 370 L/ha and then covering the soil with VIF plastic film. Untreated soils were used as controls. As chemical controls were considered 1,3 dichloropropene (140 L/ha) in the year 2012 and oxamyl (50 Kg/ha) in the year 2013. In the second trial regarding the control of *M. javanica*, for the lowest dose of DMDS (180 L/ha) the soil was covered after shank fumigation not only with VIF film but also with TIF film. Films were removed in both trials 15 days later their applications and the soil aired for further 15 days. After soil aeration carrot (cv Bolero in 2012 and cv Exelso in 2013) was sown. In the first year before sowing, soil samples were collected in each plot to extract cysts by the Fenwick can. Cysts, collected from treated and untreated soils, were subjected to a hatching test to verify vitality of their eggs. Hatch percentage in untreated control was significantly higher (35.2%) than those observed in all other treatments (< 0.36%) that were not significantly different each other (P = 0.01). At the end of the experiment no significant differences were observed in the number of cysts among the different treatment included the untreated control (P = 0.01). The higher and significant marketable carrot yield was recorded in plots treated with DMDS at 370L/ha (575 q/ha) (P = 0.01). Other treatments were significantly lower than DMDS at 370 L/ha. In the untreated control no marketable yield was harvested. In the second trial on *M. javanica*, at the end of the trial, soil samples were collected to extract eggs and juveniles by the Coolen's method. The lowest soil nematode population was observed in soils of plots treated with DMDS at rate of 370 L/ha and it was significant lower than those observed in oxamyl and untreated plots (P = 0.01). However no statistical differences were observed among the different rates of DMDS and between the two plastic films VIF and TIF (P = 0.01). The highest carrot marketable yield was observed in all three applied doses of DMDS covered with VIF (P = 0.01). In untreated control marketable yield was significantly lower than those observed in all other treatments (P = 0.01). Results providing evidence that DMDS represents a

sustainable technical solution for controlling root-knot and cyst carrot nematodes. Further trials to control nematodes, combining DMDS with not chemical solutions, are ongoing for a sustainable soil management.