ABSTRACTS

61st International Symposium on Crop Protection

May 19, 2009
Gent
Belgium

HONORARY CHAIRMEN	**D. DEGHEELE (†), W. DEJONCKHEERE (†), A. GILLARD (†), R.H. KIPS (†), C. PELERENTS, J. POPPE, J. STRYCKERS (†) J. VAN DEN BRANDE (†), W. WELVAERT**
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Tel. no. + 32 9 264.60.09 (P. Spanoghe)
Fax. no : + 32 9 264.62.49
E-mail : iscp@ugent.be
Website: http://www.iscp.ugent.be

GENERAL PROGRAMME

May, 18	15.00-18.00	**REGISTRATION**
May, 19	08.00	**REGISTRATION**
	09.30-11.00	**PLENARY SESSION**
	11.00-13.00	**ORAL SESSIONS**
	13.00-14.00	**LUNCH**
	14.00-15.00	**POSTER SESSION**
	15.00-17.20	**ORAL SESSIONS**
	17.30	**RECEPTION**
	19.30	**BANQUET** **Het Pand** **Ghent University** **Onderbergen 1, 9000 Gent**

THE SYMPOSIUM VENUE

Section	Topic	Blok (Building)	Floor	Room No
Session				
PS	Plenary Session	E	first	1.002
SP	Special Session on Drift	A	first	1.015
1	Application Technology	A	first	1.015
2	Insecticides Host Plant Resistance	E	first	1.012
3	Agricultural Entomology Side-Effects	E	first	1.015
4	Herbology	A	ground	0.030
5	Nematology	A	second	2.097
6	Phytopathology and Integrated Control of Plant Diseases (1)	E	second	2.009
7	Phytopathology and Integrated Control of Plant Diseases (2)	E	second	2.010
8	Fungicides	E	first	1.015
Posters				
Posters A01-A05	Application Technology	A	ground	0.041
Posters R01-R28	Pesticide Residues, Toxicology and Ecotoxicology	A	ground	0.041
Posters E01-E43	Agricultural Entomology and Acarology	A	ground	0.041
Poster N01-N21	Nematology	A	ground	0.041
Posters H01-H13	Herbology	A	ground	0.041
Posters P01-P57	Phytopathology	A	ground	0.041

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Plenary session

Plenary session

ASSESSMENT OF THE IMPACT ON CROP PROTECTION OF THE "CUT-OFF CRITERIA" IN A NEW REGULATION FOR AUTHORISATION OF PLANT PROTECTION PRODUCTS

D.M. RICHARDSON
Pesticides Safety Directorate
York, YO1 7PX, UK

Plant Protection Products have been regulated under Directive 91/414/EEC which required a comprehensive risk assessment to ensure acceptable levels of safety before an active substance was authorised. In January 2009, European Parliament agreed a draft Regulation to replace 91/414. This Regulation, originally proposed in June 2006, maintained risk assessment but also introduced the concept of hazard assessment or 'cut-off criteria' for the approval of active substances. In addition it proposed the concept of comparative assessment and substitution for those active substances meeting certain 'less stringent' hazard criteria.

The Commission's impact assessment for the proposed Regulation did not consider the likely consequences of the hazard criteria and substitution provisions; nor did the Parliament provide an assessment of the implications of the amendments proposed in its first reading report. PSD therefore conducted and published an assessment of the impact of the proposals both on active substance availability and on agronomic aspects. This raised a number of serious concerns, particularly due to the likely loss of most triazole fungicides. At their most stringent, the proposals would have seriously undermined the viability of current commercial agriculture, with major impacts on yield and quality. Since publication of that assessment and through the second reading, there has been a substantive amelioration of many of the proposals. A revised PSD assessment indicated that up to 14% of active substances are likely to be lost as a result of the agreed (but not yet adopted) Regulation. The presentation will outline the concerns from the UK and provide an indication of the agronomic impact of the proposals.

Plenary session

HOW DOES COVERED CROP TYPE INFLUENCE RISK FROM PESTICIDES TO THE ENVIRONMENT, CONSUMER, OPERATOR AND BYSTANDERS ? A REVIEW OF TRUE AND FALSE CONCERNS

Ettore CAPRI
Istituto di Chimica Agraria ed Ambientale
Università Cattolica del Sacro Cuore, Piacenza, Italy

The goal of this paper is to report the status of the art of the relative impacts of covered crop systems such as greenhouses, glasshouses, tunnels, plastic shelters, based on current literature and discussion on going in different institutions and stakeholders associations. Relative impacts have been shown highly dependent on the selection of specific pesticides, crop management, water and soil management in the different systems placed in different climatic conditions. The lack of data on the covered crop distribution Europe, on the pesticide fate measurement and on tools such as scenario and models make difficult a reliable quantitative assessment although in theory appear clear the increment or decrement of the impact case by case. This means that the risk assessment could be improved by a careful selection of agronomic, climatic and pesticide parameters. Missing information might be available in the next future but in order to improve the relative impact calculations, future research in pesticide transfer are needed. The latter will benefit the implementation of the new regulation such the sustainable use of pesticide, the authorisation of the plant protection product and the land management regulations.

Special session on drift

Special session on drift

DEVELOPMENT OF INDICATORS THAT ALLOWS TO EVALUATE IN REAL TIME RISKS OF THE SPRAY DRIFT

Marie-France DESTAIN[1], Frédéric LEBEAU[1], Bruno SCHIFFERS[2] & Arnaud VERSTRAETE[1]

[1] Department of Engineering and Construction, FUSAGx, Belgium
[2] Laboratory of phytopharmacy, FUSAGx, Belgium

During the spray application, the drift is considered as the most important secondary effect. To limit this negative impact on the environment, the final goal of this study is to develop indicators that evaluate in real conditions of spray the percentages of exceeding or of reduction of the drift in relation to the reference data (Ganzelmeier curves). The originality of this study is to make the tests on cultures, with a real mixture in order to come closer to the conditions really met by farmers. To collect data, a chain of sensors is mounted on a sprayer to measure boom height, sprayer speed, pressure, flow, boom movements, global position, wind speed, wind direction. What is more, a meteorological station is placed during the tests to collect data on temperature, hygrometry, wind speed, wind direction, turbulences. During tests, collectors in veil of glass are placed at vegetation level around the sprayed field at different distances. In the mixture, a tracer, in this case, the fluorescein, is added to quantify the drift. After the spray, collectors are analysed in a laboratory with a fluorimeter to quantify indirectly the quantity of plant-care products that are drifted. In a second phase, the signals stemming from the sensors are treated. From these data, the drift is predicted by the model RTDrift. This model is an adaptation of the Gaussian model used in atmospheric pollution to the drift of pesticides. It supposes that the panache disperses along a central line depending on dispersion coefficients. The result of the modelling is then compared to the results obtained in the field and parameters of the model are adjusted if it is necessary. The modelling will allow to map the spray drift and to see the areas where the drift is more important. According to that, farmers decide to spray or to put their spray off until later. At term, an electromechanical device on the sprayer will stop the spray if the meteorological conditions are bad.

Special session on drift

THE WIND TUNNEL AS SUCCESSFUL TOOL TO EXAMINE DRIFT-AFFECTING FACTORS

Mieke DE SCHAMPHELEIRE[1], David NUYTTENS[2], Donald DEKEYSER[2], Pieter VERBOVEN[3], Pieter SPANOGHE[1], Wim CORNELIS[4], Donald GABRIELS[4], Walter STEURBAUT[1]

[1] Ghent University, Laboratory of Crop Protection Chemistry
Coupure links 653, BE-9000 Gent, Belgium
[2] ILVO - Technology and Food, Agricultural Engineering
Burg. Van Gansberghelaan 115, BE-9820 Merelbeke, Belgium
[3] Catholic University of Leuven
De Croylaan 42, BE-3001 Leuven, Belgium
[4] Ghent University, Department of Soil Management and Soil Care
Coupure links 653, BE-Gent, Belgium

Spray drift is influenced by many factors, like spraying technique, weather conditions, crop type, surrounding characteristics and physicochemical properties of the spray liquid.
Field experiments are laborious, time-consuming and costly. Wind tunnel under semi-realistic conditions can be used as a screening method, priory to field work.
During the project "The efficiency of drift-mitigating measures to protect the Flemish environment", numerous wind tunnel experiments were performed.
In a first part, several spray nozzles types were measured at various spray pressures (factor 'spraying technique'). Secondly, the effect of formulation type (EC, SC, WP, WG and SL) and the addition of a polymeric drift-retardant were evaluated (factor 'physicochemistry'). Finally, the interception potentials of various artificial border structures and natural crops were determined (factor 'surrounding characteristics').
The wind tunnel proved to be a successful tool to examine drift-affecting factors, and to evaluate drift-mitigation measures.

PREDICTIVE MODELLING OF DRIFT FROM GROUND BOOM SPRAYS: THEORY TO PRACTICE

Pieter VERBOVEN[1], David NUYTTENS[2], Mieke DE SCHAMPHELEIRE[3], Katrijn BAETENS[1], Herman RAMON[1] & Bart NICOLAÏ[1]

[1] BIOSYST-MeBioS, K.U.Leuven, Belgium
[2] Agricultural engineering, ILVO, Belgium
[3] Laboratory of Crop Protection Chemistry, Ghent University, Belgium

Correct field drift prediction is a key element in environmental risk assessment of spraying applications. A Computational Fluids Dynamics (CFD) and a reduced order drift prediction model based on the diffusion-advection equation are presented. The CFD model solves the spraying process comprehensively on the 3-D field geometry. The reduced model allows fast assessment of the drift potential of specific ground boom applications under specific environmental wind conditions that obey the logarithmic wind profile. The model was calibrated based on simulations with the validated CFD model. Validation of both models against carefully conducted field experiments was performed for distances up to 20 m from the field edge, for spraying on flat pasture land. Both models succeeded to predict drift for different nozzle types, wind velocities, boom heights and spray pressures. The reduced order model can be solved more than 4 orders of magnitude faster than the comprehensive CFD model.

OFF-TARGET LOSS IN ORNAMENTAL NURSERIES WITH DIFFERENT SPRAY TECHNIQUES

H. ZHU[1], R.C. DERKSEN[1], C.R. KRAUSE[1] & R.H. ZONDAG[2]
[1] USDA/ARS Application Technology Research Unit, Wooster, Ohio, USA
Corresponding author E-mail: heping.zhu@ars.usda.gov
[2] The Ohio State University Lake County Extension, Painesville, Ohio, USA

Information is lacking on spray techniques to reduce off-target loss on the ground and via spray drift from the treated area in nursery applications. Airborne deposits at three elevations on sampling towers and on the ground at several distances from the sprayer were investigated with the three spray treatments in an open field without crops. Tests were conducted with an air blast sprayer equipped with conventional hollow cone nozzles (HC), low drift nozzles (AI), and conventional hollow cone nozzles with a drift retardant (HCDR) in an open field without crops. To compare field test results, wind tunnel experiments were conducted to assess spray deposits on the floor beyond 0.4 m downwind distance from the nozzles and airborne deposits at 2.1 m downwind from the spray discharge point with the three spray techniques. Droplet size distributions across spray patterns were measured with a laser particle/droplet image analysis system. There was no significant difference in airborne deposits for the three elevations at both 15 and 30 m downwind from the sprayer between AI and HC methods except for 3.05 m elevation at the 15 m distance although the average airborne deposits with AI were lower than that with HC. The downwind spray deposits on the ground at 15 and 30 m from the sprayer with AI were higher than that with HC and HCDR. Compared with conventional hollow cone nozzles, drift reduction from air induction nozzles or the spray mixture with drift retardant was significant in wind tunnel tests but was not significant in field tests.

POLLUTION OF THE ATMOSPHERE BY EVAPORATION OF PESTICIDE ACTIVE INGREDIENTS

P. SPANOGHE[1], M. DE SCHAMPHELEIRE[1] & D. NUYTTENS[2]

[1] Department of Crop Protection, Ghent University
Coupure links 653, BE-9000 Gent, Belgium
E-mail: Pieter.Spanoghe@Ugent.be

[2] Institute for Agricultural and Fisheries Research (ILVO), Technology and Food Sciences Unit, Agricultural Engineering, Merelbeke, Belgium
E-mail: david.nuyttens@ilvo.vlaanderen.be

Pesticide active ingredients (a.i.) can evaporate from crop and soil surfaces, during or after the application, and enter into the atmosphere. The evaporation depends on crop type, weather conditions and physicochemical properties of the active ingredients.

In this research, experiments were performed in various crop types with various pesticide formulations to determine the rate of evaporative fluxes of pesticide a.i. under realistic spray scenarios.

Grassland was sprayed with tolylfluanid, cyprodinil and pyrimethanil. A barley field was sprayed with chlorothalonil, diflufenican and trifloxystrobin. A potato field was sprayed with dimethomorph, propyzamide and fluazinam.

The sprayings were performed with a Hardi Twin force trailed sprayer with a 27 m boom length. ISO 110 03 standard flat fan nozzles were mounted with a nozzle spacing 0.5 m and boom height of 0.5 m above the canopy surface. Spray pressure was set at 0.3 MPa and forward driving speed at 8 km/h, resulting in an application rate of about 180 l/ha.

Air sampling was performed at intervals of 2 hours above the canopy with Tenax TA® sorbent tubes, connected to a pump with constant air sucking rate of 1.5 l/min. After sampling, the Tenax TA® sorbent tubes were analysed with a GC-MS, equipped with a Thermal Desorption injection System (TDS).

A Campbell Scientific weather station was used to monitor the following meteorological conditions every minute: air temperature, relative air humidity, net radiation, atmospheric pressure, rainfall, wind speed and wind direction, canopy wetness, canopy temperature, soil temperature, soil water content and soil heat flux.

The obtained data seem to suggest that volatilisation in grass is much higher than in potato canopies and barley. Volatilization from leaf surfaces was better explained by the Henry coefficient (H) compared to the vapor pressure V_P.

THE CONCENTRATIONS OF TWO FUNGICIDES IN AIR FOR 24 HOURS FOLLOWING APPLICATION TO A CEREAL CROP

Clare BUTLER ELLIS, Andrew LANE, Christine O'SULLIVAN & Paul MILLER
Silsoe Spray Application Unit, The Arable Group, Wrest Park, Silsoe, Bedford, UK

Losses of pesticide to the air following application are important to quantify both for understanding environmental fate and for undertaking risk assessment for non-target species. In the UK, the exposure of rural residents and bystanders has become a politically important issue, and therefore work is being undertaken to investigate levels of exposure of people near to treated fields from pesticide vapours. A combination of modelling and experimental approaches are being used to estimate airborne concentrations, and an experiment was undertaken in June 2008 to determine concentrations of epoxiconazole and fenpropidin in air for 24 hours following application in a single tank mix to a cereal crop. Measurements were also made, using a cascade impactor, to determine whether any of the measured pesticide in air was from airborne particles, rather than volatilized active ingredient. This paper presents the results of this experiment and discusses the implications for exposure assessment.

DRIFT-REDUCING NOZZLES AND THEIR BIOLOGICAL EFFICACY

D. NUYTTENS[1], M. D'HOOP[1], V. DE BLAUWER[2], O. HERMANN[3], W. HUBRECHTS[4], I. MESTDAGH[2] & D. DEKEYSER[1]

[1] Institute for Agricultural and Fisheries Research (ILVO), Technology and Food Sciences Unit, Agricultural Engineering, Merelbeke, Belgium
E-mail: david.nuyttens@ilvo.vlaanderen.be
[2] Research and Advisory centre for Agri- and Horticulture (POVLT), Rumbeke, Belgium
[3] Royal Belgian Institute for Sugarbeet Improvement (IRBAB-KBIVB), Tienen, Belgium
[4] National Chicory Research Centre (NPW), Herent, Belgium

In 2007 and 2008, field trials were carried out with different standard and drift reducing nozzles in sugar beet, maize, chicory, Belgian endive (all herbicide applications), wheat (fungicide application) and potatoes (Haulm killing herbicide application). The effect of nozzle type (standard flat fan, low-drift flat fan, air injection), nozzle size (ISO 02, 03 and 04) and application volume on the biological efficacy was investigated. All applications were done using a plot sprayer with volume rates ranging from 160 to 320 $l.ha^{-1}$ at recommended dose rates with commonly used (mix of) plant protection products. For each crop, the experiments included four replicates in a randomized block design.

Depending on the type of application, the efficacy was measured in terms of weed control, disease and yield level, percentage dead leaf and stem, etc. In a previous research, drift and droplet characteristics of the different techniques were measured..

In general no important effect of application technique on biological efficacy was observed for the tested herbicide and fungicide applications within the interval of volume rates and droplet size tested. Drift-reducing nozzles performed similar as conventional nozzles under good spraying conditions and using a correct spray application technique.

Special session on drift

Session 1
Application Technology

THE MANAGEMENT OF PESTICIDE APPLICATION DOSE IN LARGE TREE CROP CANOPIES IN AUSTRALIAN ORCHARDS

Henry J. Drew
Growing Greener Growers, Queensland, Australia formerly
CRC for Tropical Pest Management,The University of Queensland, Brisbane, Australia

The management of pesticide application dose is relevant to all facets of pesticide use efficiency and pesticide risk management. Very large canopies are a characteristic of Australian avocado and macadamia orchards, in some cases exceeding 45,000 m^3 of canopy per hectare. These crops are generally sprayed for a range of pests and diseases using air-assisted sprayers.

An in-depth survey of avocado and macadamia growers on the Sunshine Coast of Queensland showed that the application practices, pesticide mixing rates and resulting applied doses varied widely. The data showed that the legal limits on application rates set by the product labels did not effectively control the dose applied per unit of tree canopy. The results showed a wide range of endosulfan doses from 2.3 ml to 28.7 ml per 100 m^3 of canopy. While the dose of endosulfan applied per unit canopy fell as orchard canopy size increased, the rate per hectare rose. The analysis also showed that while almost all growers were using low volume spray application (below point-of-runoff) and were applying less than the equivalent high volume dose (based on an estimated point-of-runoff of 7.5 litres per 100 m^3 of canopy) many were not using rates which were strictly in accordance with product labels or permits. In some cases growers complying with legal directions were using doses 2-3 times higher than other growers who were not complying with legal directions. This situation had arisen because of outdated label directions that were not appropriate for low volume spraying and because growers generally had a poor understanding of the target dose concept. A Forum in 1998 involving selected Australian Commonwealth, State and industry players suggested that commercial and organisational issues had impeded the changes in product labelling required to meet grower's needs despite apparent consensus on many technological issues. In 2002 the Australian national registration authority adopted a new model label for tree crops introducing the dilute and concentrate rate concepts and giving greater flexibility in mixing rates to permit the adoption of a "best practice", target dose model of spray application. Recent training programs to explain the application dose concepts in the Australian avocado, macadamia and mango industries are still to be fully evaluated.

DEVELOPMENT OF A CROP HEALTH SENSOR (CHS) TO MINIMIZE SPRAY APPLICATIONS IN ORCHARDS

J.C. VAN DE ZANDE[1], J. MEULEMAN[1] & M. WENNEKER[2]
[1] Wageningen University & Research Centre - Plant Research International B.V.
P.O. Box 16, NL-6700 AA Wageningen, The Netherlands
E-mail: jan.vandezande@wur.nl
[2] Wageningen University & Research Centre - Applied Plant Research, Research Unit Fruit
P.O. Box 200, NL-6670 AE Zetten, the Netherlands
E-mail: marcel.wenneker@wur.nl

In 2006 the project ISAFRUIT (www.isafruit.org) – "Increasing fruit consumption through a trans disciplinary approach leading to high quality produce from environmentally safe, sustainable methods" – was launched within the 6th Framework Program of the EC. Within the project's work package ECOFRUIT (WP5.1 – Safe European fruit from a healthy environment) a Crop Adapted Spray Application (CASA) system is developed. This system is developed to ensure efficient and safe spray application in orchards according to actual needs and with respect to the environment. It consists of three components:

- Crop Identification System – CIS (Balsari *et al*., 2008)
- Environmentally Dependent Application System – EDAS (Doruchowski *et al*., 2008)
- Crop Health Sensor – CHS (Van de Zande *et al*., 2008)

To develop the CHS, novel technologies will be used to quantify tree health conditions in the orchard, based on the developments in crop sensing techniques for grassland and arable crop production. By adapting the sensors for fruit production, dose and timing of chemicals will be determined based on the crop health status of the fruit tree. A measuring tool developed for characterizing grass-swards has been adapted to measure picked single apple leaves placed on the floor in the laboratory. With two hyper spectral line cameras the reflection in the band-widths 400-900nm and 900-1600nm are measured. Spectral analysis measurements were performed on individual apple leaves from several apple varieties. For the varieties Elstar and Jonagold apple scab and powdery mildew infected leaves were also measured. In an experiment healthy and artificially infected leaves (inoculated with conidia of apple scab) were compared, and evaluated 2, 4, 8 and 24 hours, 2, 14 and 28 days after infection. First results showed a difference between infected and healthy leaves. Based on the typical wavelength identified for apple scab on apple leaves the development of an apple scab specific sensor will start.

COMBINATIONS OF SOIL FUMIGANTS FOR METHYL BROMIDE REPLACEMENT

ETIENNE VAN WAMBEKE[1], AN CEUSTERMANS[2], ANKE DE LANDTSHEER[2] & JOZEF COOSEMANS[2]

[1] Certis Europe, Brussels, Belgium

[2] Laboratory for Phytopathology and Plant Protection, KU Leuven, Belgium

The development programme synergistically combining dazomet and 1,3-dichloropropene at reduced dose rates for broad spectrum soil disinfestation was pursued with the simultaneous application of dazomet and the potential dimethyl disulfide (DMDS). Combinations were compared to the separately applied fumigant through soil column experiments allowing comparison of treatments, the study of soil conditions (soil type, soil moisture content and soil temperature), and dose rates.

Efficacy over depth profile in experimental soil columns was studied with packed soil buried target organisms: fungi, nematodes and seeds.

It was shown that the combination of 20 g Basamid (98% dazomet) and 40 ml DMDS/m², even at short fumigation period (1 week), under well controlled conditions performs very well both on the level of broad activity as on depth activity. Factors determining fumigant performance were also studied, such as soil temperature, soil moisture content and the presence of organic matter.

It was shown that weaker performance of full rate separate compounds could become compensated by simultaneous application at half of the recommended dose rates of each. Appropriate soil conditions were confirmed as well as the necessity to avoid high organic matter content in soil or to increase dose rate at high content for successful soil disinfestation.

It is also emphasized that both methyl isothiocyanate (MITC), that is the active generated in soil from dazomet, and DMDS are naturally occurring compounds similar to the actives generated by 'biofumigation' practices The advantage of synthetic generators is the better control upon effective dose rate.

OPTIMIZATION OF THE SPRAY APPLICATION TECHNOLOGY IN BAY LAUREL *(LAURUS NOBILIS)*

D. NUYTTENS, P. BRAEKMAN & D. FOQUE
Institute for Agricultural and Fisheries Research (ILVO), Technology and Food Sciences Unit, Agricultural Engineering, Merelbeke, Belgium
E-mail: david.nuyttens@ilvo.vlaanderen.be

Bay laurel is an evergreen, commercially grown and expensive ornamental pot plant, which is susceptible to different pests like aphids, scale and lerp insects, thrips, caterpillars of codling moth and sooty moulds. Recently, caterpillars of the mediterranean carnation leafroller (*Cacoecimorpha pronubana*) cause more and more problems. Pests can lead to important financial losses for the growers.
During summer the individual pot plants are placed on a field-container in a fairly dense configuration. Crop protection is traditionally done by moving with a spray lance between the rows of pot plants and treating each individual plant from bottom to top. Good penetration and a relatively uniform deposition are clearly important advantages for this spray technique but it is very time-consuming, unhealthy and laborious. Some other growers use a 'spray platform' on a high-clearance tractor. Plants sprayed from this platform are exclusively approached from above resulting in an inferior spray deposition on the lower parts of the plants. To overcome the disadvantages of both available techniques, the potential of an automated tunnel sprayer was investigated.
Five different nozzle types were evaluated under laboratory conditions i.e. hollow cone, standard flat fan, air inclusion flat fan, deflector flat fan and twin air inclusion flat fan at spray pressures varying from 3.0 to 7.0 bar depending on the type of nozzle. For each nozzle type, three nozzle sizes were included in the experiments which resulted in 15 different spray application techniques. All experiments were done at a speed of 2.5 $km.h^{-1}$. This resulted in three different application volumes: 2450, 4900 and 7300 $l.ha^{-1}$.
After optimizing the nozzle configuration (distance and orientation) using water-sensitive paper, deposition tests with five different mineral chelates as tracer elements were performed. Filter papers were used as collectors at 20 different positions to measure spray deposition, distribution and penetration in the canopy. For each application technique, four plants were selected as repetitions.
Irrespective of the nozzle type and spray pressure, 4900 $l.ha^{-1}$ was found to be the optimal spray volume with deposition rates varying from about 50 to 70% depending on the nozzle type. The best results were found for the hollow cone, the standard flat fan and the air inclusion flat fan nozzles. Nozzle type and pressure and the corresponding droplet characteristics were closely related with the penetration and deposition results. With this automated tunnel system, it is possible to obtain a good spray result in combination with an increase in the productivity and a reduction in operator exposure.

INVOLVEMENT OF MICROORGANISMS OTHER THAN PSEUDOMONADS ON THE DEGRADATION OF THE NON-FUMIGANT ORGANOPHOSPHATE NEMATICIDE FENAMIPHOS

J. Alfonso CABRERA, Richard A. SIKORA & Alexander SCHOUTEN
Department of Phytopathology in Soil Ecosystems & Nematology, Institute for Crop Science and Resource Conservation, University of Bonn. Nussallee 9, DE-53115 Bonn, Germany

Fenamiphos is a broad spectrum, non-volatile, systemic, organophosphorus nematicide extensively used throughout the world to control plant-parasitic nematodes. The efficacy of this nematicide can decrease in soils where microorganisms accumulate that are capable of rapidly degrading the active ingredient. Among the documented microorganisms to degrade organophosphate compounds, *Pseudomonas* spp. was frequently identified. However, it still not clear whether or not this bacterial genus is the major responsible one in the biodegradation process. Our objective was to study the roll of *Pseudomonas* spp. and other soil bacteria on the degradation of fenamiphos in soils with different nematicide application history. In some of these soils fenamiphos metabolizing micro organisms were found, whereas in others not. For example, a soil with 42 fenamiphos applications during 16 years neither contained *Pseudomonas* spp. nor biodegrading microorganisms. In two different soils, both with 25 fenamiphos applications in 12 years and containing metabolizing microorganisms, only one of them contained *Psuedomonas* spp., demonstrating that the nematicide was rapidly metabolized by microorganisms other than *Pseudomonas* spp. Conversely, a control soil, with no previous nematicide application history, contained the highest number of *Pseudomonas* spp. of all soils analyzed. The number of bacteria of this genus could be increased when compost was added, although this did not alter the lack of the fenamiphos degradation process, even after six weeks and three consecutive nematicide treatments. The *Pseudomonas* diversity of the non-degrading control soil was composed of *P. putida* (50%), *P. fluorescens* (31%), *P. syringae* (13%) and *P. chlororaphis* (6%) according to gas chromatography identification. Individual analysis of the *Pseudomonas* spp. bacteria showed that none were capable of metabolizing fenamiphos *in vitro*. In conclusion, we demonstrated that *Pseudomonas* spp. are not intrinsically capable of fenamiphos metabolization. We also found that *Pseudomonas* spp. may not always accumulate upon fenamiphos treatment and that there are other microorganisms able to metabolize this nematicide.

PROPOSAL ON A SUSTAINABLE STRATEGY TO AVOID POINT SOURCE POLLUTION OF WATER WITH PLANT PROTECTION PRODUCTS

Inge MESTDAGH[1], Bernard BONICELLI[2], Ramon LAPLANA[2] & Manfred ROETTELE[3]

[1] Provinciaal Onderzoeks- en Voorlichtingscentrum voor Land- en Tuinbouw, Belgium
[2] Cemagref, France
[3] Better Decisions, Germany

Based on the results and lessons learned form the TOPPS project (Training the Operators to prevent Pollution from Point Sources), a proposal on a sustainable strategy to avoid point source pollution from Plant Protection Products (PPPs) was made. Within this TOPPS project (2005-2008), stakeholders were interviewed and research and analysis were done in 6 pilot catchments areas (BE, FR, DE, DK, IT, PL). Next, there was a repeated survey on operators' perception and opinion to measure changes resulting from TOPPS activities and good and bad practices were defined based on the Best Management Practices (risk analysis).
Aim of the proposal is to suggest a strategy considering the differences between countries which can be implemented on Member State level in order to avoid PPP pollution of water through point sources. The methodology used for the up-scaling proposal consists of the analysis of the current situation, a gap analysis, a consistency analysis and organisational structures for implementation. The up-scaling proposal focuses on the behaviour of the operators, on the equipment and infrastructure available with the operators. The proposal defines implementation structures to support correct behaviour through the development and updating of Best Management Practices (BMPs) and through the transfer and the implementation of these BMPs. Next, the proposal also defines requirements for the improvement of equipment and infrastructure based on the defined key factors related to point source pollution. It also contains cost estimates for technical and infrastructure upgrades to comply with BMPs.

Session 2
Insecticides
Host plant resistance

NEONICOTINOIDS AS SEED POTATO TREATMENTS TO CONTROL WIREWORM (*AGRIOTES* SPP.) DAMAGE IN A POTATO CROP

H.F. HUITING & A. ESTER
Applied Plant Research, Wageningen UR
P.O. Box 430, NL-8200 AK Lelystad, The Netherlands

Damage to potatoes caused by wireworms steadily increases over the years. Unlike cereals, potatoes mostly suffer quality loss as a result of wireworms attacking instead of plant loss. Even low population densities of wireworms can render potatoes unfit for sale. The withdrawal of broad-spectrum insecticides and the varying efficacy of the currently available insecticides ethoprophos and fosthiazate have brought into focus the need for new ways of controlling wireworms. A series of field trials was carried out, comparing several insecticides of the neonicotinoid class, applied as seed potato treatments at planting, with ethoprophos and chlorpyrifos soil treatments as references. Test compounds were imidacloprid at rates of 175, 88, 70, 35 and 4 g; thiamethoxam at 140, 70, 50, 35 and 18 g; and thiacloprid at 144 and 72 g a.i. per tonne seed potatoes. Seed potato treatment application rates of imidacloprid and thiamethoxam showed significant control of wireworms at harvest, in September, whereas thiacloprid treatments showed insufficient protection of the crop. The effective insecticides imidacloprid and thiamethoxam showed no phytotoxicity to the crop, in terms of a decreased emergence or yield. Prospects and benefits of seed potato treatments are discussed, including lowering of the amount of insecticide needed for adequate protection of the yield.

COMPARATIVE TOXICITY OF SPINOSAD AND SPINETORAM AGAINST KEY PESTS IN VEGETABLES

Jahangir KHAJEHALI, Thomas VAN LEEUWEN & Luc TIRRY
Laboratory of Agrozoology, Ghent University, Belgium

Spinosad is a mixture of different spinosyns. These are fermentation-derived compounds produced by the actinomycete *Saccharopolyspora spinosa*. Recently, spinetoram was developed by Dow AgroSciences by chemically modifying these fermentation products. Acting through a novel target site in the nicotinic receptor in the nervous system, spinetoram and spinosad have demonstrated activity against a wide range of pest species.
Using laboratory bioassays, we compared insecticidal and acaricidal properties of spinetoram and spinosad against some key pests of vegetable crops: the two-spotted spider mite *Tetranychus urticae*, the cotton leafworm *Spodoptera littoralis*, the Colorado potato beetle *Leptinotarsa decemlineata* and the western flower thrips *Frankliniella occidentalis*. Previous reports indicated that spinosad acts systemically through the roots when applied to rockwool, an artifical substrate used in tomato culture. We tested whether spinetoram had similar systemic properties.

EFFICACY RESULTS OF INDOXACARB AGAINST WESTERN CORN ROOTWORM ADULTS (*DIABROTICA VIRGIFERA VIRGIFERA*, LECONTE 1868) IN HUNGARY

G. RIPKA[1], L. VASAS[1], I. MOLNÁR[2], E. TÓTH[2], I. SOMLYAY[2], A. BASSI[3], T. HEGYI[4] & T. BARASITS[4]

[1] Agricultural Admin. Central Office Plant, Soil and Agroenvironmental Protection Directorate, HU-1118 Budapest, Hungary

[2] DuPont Hungary Ltd. HU-2040 Budaörs, Hungary

[3] DuPont Italia Srl., IT-20063 Cernusco sul Naviglio, Italy

[4] Syntech Research Hungary HU-9700 Szombathely, Hungary

The western corn rootworm WCR (*Diabrotica virgifera virgifera* LeConte 1868) is a new endemic insect in Europe since the last decade of the 20th century. In Hungary the economic damage in corn/maize has been drastically increased in the last three consecutive years and reached the level when the adoption of effective control measures is needed to avoid significant economic losses.

There are many different management practices aimed at the control of corn rootworms. These practices include corn variety selection, early planting, insecticides, crop rotation and transgenic corn varieties. The insecticide solutions can be targeted to the larvae –using granular or liquid soil insecticides- or to the adult beetles, using foliar sprays.

Our objective was to test the indoxacarb containing insecticide preparation (STEWARD® 30DF) against the WCR adults under Hungarian field circumstances.

The test material STEWARD® 30 DF (30 % indoxacarb) was applied in seven experimental plots of corn hybrids situated countrywide. The infestation level was determined using yellow sticky cups and the number of adult WCR (alive and dead) was counted on 5x10 plants.

The dosages of STEWARD® 30 DF applied were as follows: 85, 125, 150 and 170 g/ha. Additionally a tank-mix combination with a cucurbitacin containing pumpkin seed oil was tested to check the possible attractant effect of this mixture.

In conclusion foliarly applied Steward®30DF from the rate of 125 g/ha provided a good control of WCR adults, in the same range as the registered reference product (5% lambda cyhalotrine) .

EFFICACY OF ACARICIDES AND PREDATORY MITES AGAINST *POLYPHAGOTARSONEMUS LATUS*, THE BEGONIA MITE, ON *HEDERA HELIX* (2008)

Joachim AUDENAERT[1], Marc VISSERS[1], Bart HALEYDT[1], Ruth VERHOEVEN[1], Frans GOOSSENS[2] & Bruno GOBIN[1]

[1] pcs Destelbergen, Division Potted Plants
Schaessestraat 18, BE-9070 Destelbergen, Belgium
[2] Vlaamse Overheid - Department Landbouw & Visserij
Burg. Van Gansberghelaan 115 A, BE 9820 Merelbeke, Belgium

In recent years, the begonia mite (Polyphagotarsonemus latus) has become an important threat to different ornamental cultures in warm greenhouses. At present there is not a single plant protection product registered in Belgium for the control of mites of the Tarsonemidae family. In a first screening test, we evaluated the efficacy of a range of acaricides (abamectin, milbemectin, pyridaben, spirodiclofen, bifenazate, azocyclotin, flufenoxuron, fenbutatin oxide, flonicamid, acequinocyl, etoxazole) registered against mites in various other crops, as well as a few insecticides with potential acaricidal side effects (dimethoate, teflubenzuron, spiromesifen KLOPT DIT? Based on the results of the screening test XX products were selected for a full efficacy trial following EPPO guidelines. The best control results were obtained with two products from the avermectine group: abamectine and milbemectine.

As growers currently have to rely solely on the use of natural enemies there is a strong need for practical evaluation of efficacies of the various predatory mite species (*Amblyseius swirskii*, *Amblyseius cucumeris*, *Amblyseius andersoni*) used in biological mite control.

In a series of experiments, we screened the use of different species of predatory mites. The first efficacy tests on heavily infested plants at different rates of dosage and under different circumstances (temperature, dose rate, application technique) were started in May 2008. In these experiments *Amblyseius swirskii* showed good efficacy. But temperature was the limiting factor: the predatory mite needed a minimal temperature of 18°C to obtain good results. Further research is necessary to search for predatory mites that can be used in winter conditions (lower temperatures, less light).

THE MULTIPLE TARGET USE OF ENVIDOR IN IPM POMEFRUIT IN BELGIUM

Luk DE MAEYER & Rik GEERINCK
Bayer CropScience, BE-1050 Brussels, Belgium

Envidor, active ingredient spirodiclofen is a broad spectrum acaricide acting via lipid biosynthesis inhibition (LBI) with no cross resistance to currently available acaricides and with additional insecticidal properties. Envidor is positive IPM listed but in frame of resistance management limited to one application per season. In pear growing Envidor constitutes an important tool in pear sucker control in a tandem strategy with abamectine. Being totally selective on predatory bugs *Anthocoris nemoralis,* Envidor installs a favourable low prey/predator ratio avoiding any damage of further 3d or 4th Psylla generations. The application of Envidor on the second Psylla generation coincides with migration of *Lepidosaphes ulmi* scales and with spread of rustmites *Epitremerus pyri* on which season long control is achieved.
On apples, Envidor settled as an important tool for scales control especially on the wood but recently its acaricidal use revivals. This is induced by the withdrawal of tolylfluanid with its regulating mite control and with the further efficacy decline of registered METI -acaricides. Even with full introduction of *Typhlodromus pyri,* mites and rustmites may unexpectedly flear up.
On *Typhlodromus pyri*, Envidor is IOBC 2 classified (slightly harmful) but the favourable low prey/predator ratio and within season recovery make it full IPM compatible.
On *Aculus schlechtendali*, apple rustmite with a short generation cyclus, the larval and fecundity effects of Envidor lead to an explicit knock down efficacy. On mixed populations of *Panonychus ulmi* with longer generation cyclus , Envidor shows a slow initial effect with culminating efficacy after 2-3 weeks and a long lasting efficacy of more than 100 days, avoiding any leaf or consequent fruit damage. High rainfastness and the right positioning at 30- 50 % hatching of winter eggs make of Envidor a very consistent correction acaride. The addition of a surfactant is not recommended for mite control. Up to now, no shifting in susceptibility of Envidor on *Panonychus ulmi* or *Tetranychus urticae* is found in Belgian orchards.
Since Envidor combines both in apples and pears an efficacy on multiple pests occurring simultaneously after flowering, with a good selectivity profile on predators, parasitoïds, syrphids, lacewings and earwigs, it remains a most important tool in modern IPM pomefruit.

MUTATIONS IN THE MITOCHONDRIAL CYTOCHROME B OF *TETRANYCHUS URTICAE* KOCH (ACARI: TETRANYCHIDAE) CONFER CROSS-RESISTANCE BETWEEN BIFENAZATE AND ACEQUINOCYL

Pieter VAN NIEUWENHUYSE[1], Thomas VAN LEEUWEN[1], Jahangir KHAJEHALI[1], Bartel VANHOLME[2] & Luc TIRRY[1]
[1]Laboratory of Agrozoology, Department of Crop Protection
[2]Laboratory of Applied Molecular Genetics, Department of Molecular Biotechnology
Faculty of Bioscience Engineering, Ghent University
Coupure links 653, BE-9000 Gent, Belgium

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most important phytophagous mites worldwide. One of the major problems in the control of *T. urticae* is their ability to quickly develop resistance to many important acaricides.
Resistance of *T. urticae* to bifenazate was recently linked with mutations in the mitochondrial cytochrome b Q_o pocket, suggesting that bifenazate acts as a Q_o inhibitor. Since these mutations might cause cross-resistance to the known acaricidal Q_o inhibitors acequinocyl and fluacrypyrim, resistance levels and inheritance patterns were investigated in several bifenazate susceptible and resistant strains with different mutations in the cd1- and ef-helix aligning the Q_o pocket.
Cross-resistance to acequinocyl in two bifenazate resistant strains was shown to be maternally inherited and caused by the combination of 2 specific mutations in the cytochrome b Q_o pocket. Although most investigated strains were resistant to fluacrypyrim, resistance was not inherited maternally, but as a monogenic autosomal highly dominant trait. As a consequence, there was no correlation between cytochrome b genotype and fluacrypyrim resistance.
Although there is no absolute cross-resistance between bifenazate, acequinocyl and fluacrypyrim, some bifenazate resistance mutations confer cross-resistance to acequinocyl. In the light of resistance development and management, high prudence is called when alternating bifenazate and acequinocyl in the same crop. Maternally inherited cross-resistance between bifenazate and acequinocyl reinforces the likelihood of bifenazate acting as a mitochondrial complex III inhibitor at the Q_o site.

NICTABA PROTECTS TOBACCO PLANTS AGAINST INSECT HERBIVORY

Gianni VANDENBORRE[1,2], Els J.M. VAN DAMME[2] & Guy SMAGGHE[1]
[1] Laboratory of Agrozoology, Ghent University, Belgium
[2] Laboratory of Biochemistry and Glycobiology, Ghent University, Belgium

Since the mobility of plants is restricted, they are hampered in avoiding hostile visits by pest insects. To protect themselves against these insect attacks, plants have developed a wide range of chemical defence mechanisms. The induced defence response in plants towards chewing insects is characterized by a jasmonate-dependent alteration of the gene expression profile, which leads to accumulation of newly synthesized proteins called jasmonate-induced proteins (JIPs).
Recently, a lectin called *Nicotiana tabacum* agglutinin or NICTABA has been discovered in the leaves of tobacco *Nicotiana tabacum* cv Samsun NN (Chen *et al.*, 2002). Plant lectins are carbohydrate-binding proteins with a putative defensive function. Under normal environmental conditions NICTABA is not present in the leaves, but after treatment with certain jasmonates, the tobacco lectin starts to accumulate. Since treatment of tobacco leaves with other plant hormones does not result in lectin accumulation, NICTABA can be considered as a genuine JIP. In order to understand the functional role of NICTABA in tobacco leaves, a detailed study was carried out on the accumulation of different jasmonates after *Spodoptera littoralis* herbivory and its correlation to the accumulation of NICTABA. The expression profile of NICTABA is similar to several other known defence proteins, in time as well as in cellular localization and can clearly be distinguished from jasmonate-responsive genes that are responsible for signalling amplification. In addition, NICTABA accumulation was not only observed locally at the site of attack but also in systemic leaves.
To investigate the insecticidal activity of NICTABA towards the cotton leafworm *S. littoralis*, transgenic tobacco plants were constructed with a constitutive NICTABA expression and subsequently analyzed for successful transformation. Feeding trials whereby *S. littoralis* larvae were fed on different transgenic tobacco lines, showed a clear insecticidal effect by NICTABA.
In conclusion, our results show that NICTABA is induced in *N. tabacum* leaves by *S. littoralis* herbivory and this response can be considered as part of the inducible defence system to protect tobacco against pest insects.

RESISTANCE OF TRANSGENIC PLANTS EXPRESSING TYPE-2 RIBOSOME-INACTIVATING PROTEIN AGAINST SEVERAL PEST INSECTS AND IMPORTANCE OF THE CARBOHYDRATE-BINDING ACTIVITY FOR INSECTICIDAL ACTIVITY

S. SHAHIDI-NOGHABI, Els J.M. VAN DAMME & Guy SMAGGHE
[1] Laboratory of Agrozoology, Department of Crop Protection
[2]Laboratory of Biochemistry and Glycobiology, Department of Molecular Biotechnology
Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Type-2 ribosome-inactivating proteins (RIPs) are a group of chimeric proteins built up of an A-chain with RNA N-glycosidase activity and a B-chain with lectin activity. Elderberry bark (*Sambucus nigra*) contains two NeuAc(α-2,6)Gal/GalNAc-specific agglutinins referred to as *Sambucus nigra* agglutinin I (SNA-I) and I' (SNA-I'). Both proteins have been recognized as type-2 RIPs and share 77% sequence similarity.
In a first part of this project we evaluated if constitutive expression of SNA-I and SNA-I' in tobacco can enhance the plant's resistance against pest insects. The results revealed significant increases in mortality for aphids (*Myzus nicotianae*) and beet armyworm (*Spodoptera exigua*) fed on the transgenic lines as compared to wild type plants. In addition, significant effects were observed on *M. nicotianae* life parameters, such as survival, intrinsic rate of increase, net reproductive rate, mean generation time and mean daily offspring, suggesting that a population of aphids would build up significantly slower on transformed plants. Significant reductions in fresh weight of *S. exigua* as well as a retardation in development were also observed when larvae were fed on transgenic plants overexpressing SNA-I' or SNA-I. This information provides further support for RIPs having a role in plant resistance to insect pest species.
In a second part, we demonstrated the importance of the carbohydrate-binding activity of SNA-I for insecticidal activity. Here transgenic lines expressing a mutant RIP in which one or both carbohydrate-binding sites were altered were tested against *M. nicotianae*. Interestingly there was a relationship between the degree of carbohydrate-binding activity and insecticidal potency.

APHID RESISTANCE IN WHEAT VARIETIES

Henriett ELEK[1], Peter WERNER[1], Lesley SMART[2], Ruth GORDON WEEKS[2], John PICKETT[2] & Miklós NÁDASY[3]
[1] KWS UK Limited, Thriplow, UK
[2] Rothamsted Research, Harpenden, UK
[3]Pannon University Georgikon Faculty of Agriculture, Keszthely, Hungary

Aphids are able to cause severe damage worldwide in cereal crops through direct feeding and by transferring plant pathogenic viruses e.g. Barley yellow dwarf virus (BYDV). Insecticides currently give protection but are expensive, environmentally undesirable and may not be effective in the future because of the development of insecticide resistance. Furthermore, within the EU serious plans are in preparation to greally reduce the range and quantity of insecticide products applied to the wheat crop. Therefore, our objectives are to help plant breeders to produce marketable aphid resistant wheat varieties. We have chosen to work with the cereal aphid *Rhopalosiphum padi* because this is one of the main vectors of the BYDV.

We tested 56 wheat varieties including diploid, tetraploid and hexaploid varieties using a settling test. In general we found the hexaploid and tetraploid wheat varieties were more attractive for aphids than the diploid varieties. For most of the varieties we also measured aphid fecundity. The test used determined how long it takes an aphid from birth to produce the first nymph and how many nymphs were produced over an equivalent time on the test varieties. From the data we calculated the intrinsic rate of population increase. This showed that nymphs were produced in greater numbers on the hexaploid varieties than diploid varieties.

To further investigate these results we took samples of leaf tissue to check the level of Hydroxamic acids e.g. DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one). Hydroxamic acids have been suggested to play an important role in bacterial, fungal and insect resistance, and have been shown to deter aphid feeding and to reduce reproduction. They are present in plants in a non toxic form as the glucoside, but in response to stress or injury this may converted to the toxic aglucone.

We tested the leaf tissues by HPLC and found DIMBOA and in some cases DIBOA in the hexaploid varieties, but we could not detect either of these Hydroxamic acids in the diploid varieties. In an experiment to determine whether levels of Hydroxamic acids are affected by aphid feeding, we recorded higher levels of DIMBOA in plants with aphids compared to plants without aphids for both tetraploid and hexaploid varieties.

These results cast doubt on the hypothesis that Hydroxamic acids reduce nymph production and have a feeding deterrent effect on *Rhopalosiphum padi*. In the future we would like to do further tests to more fully challenge this hypothesis.

CHEMICAL HOST PLANT RESISTANCE TO THRIPS IN WILD AND CULTIVATED TOMATO PLANTS

Mohammad MIRNEZHAD[1] , Roman R. ROMERO GONZÁLEZ[2], Kirsten A. LEISS[1], Young HAE CHOI[2], Peter G.L. KLINKHAMER[1] & Robert VERPOORTE[2]

[1] Plant Ecology, Institute of Biology, Leiden University
Kaiserstraat 63, NL-2311 GP, Leiden, The Netherland
[2] Metsabolomics, Institute of Biology, Leiden University
Einsteinweg 55, NL-2333 CC, Leiden, The Netherlands.

The western flower thrips, *Frankliniella occidentalis*, is one of the most serious pest problems in many agricultural and horticultural glasshouse crops. Little is known about host plant resistance to thrips. In this study we investigated thrips resistance of wild and cultivated tomatoes. We subjected 10 wild and 10 cultivated tomato lines to a non-choice thrips bioassay to identify thrips resistant and susceptible tomatoes. On these we subsequently applied NMR (nuclear magnetic resonance spectroscopy) to study the metabolomic basis of thrips resistance. NMR is a tool allowing the simultaneous detection of a wide range of metabolites, the identity of which are, *a priori*, unknown. We show that cultivated tomatoes had significantly more feeding damage by thrips compared to the wild tomatoes. While Lycopersicon *pennellii* was the most resistant line within the wild tomatoes, there were no differences in feeding damage within the cultivated lines. We did not detect a relationship between morphological characteristics and thrips resistance. Instead thrips resistance was chemically based. The metabolomic profiles of thrips resistant and susceptible tomatoes were significantly different. Resistant tomatoes had higher amounts of malic acid, citric acid and a newly described isomer of α-tomatine. Our results prove NMR a promising tool in identifying metabolites involved in thrips resistance, which provides key information for herbivore resistance breeding.

A NEW RAPID METHOD OF SENSITIVITY STUDY VIS-À-VIS THE GREEN MITE (*MONONYCHELLUS TANAJOA* BONDAR) AND ITS APPLICATION ON SOME CASSAVA (*MANIHOT ESCULENTA* CRANTZ) CULTIVARS

A.M. BADEGANA
University of Dschang, Faculty of Agronomy and Agricultural Sciences
Department of Plant Protection, P.O. Box 96, Dschang, Cameroon

Cassava is a highly cultivated crop in Sub-Saharan Africa for the consumption of both its tubers, young shoots and leaves (Pynaert, 1951). However, this crop is affected by a great number of pests. Among them, the green mite (*Mononychellzis tanajoa* Bondar) remains the major pest. The method we present here facilitates a rapid study of the sensitivity of different cassava cultivars and selected varieties vis-a-vis the green mite. This method can substitute long and expensive field studies. It consists of laboratory study of the fecundity on leaves of the same age of different cultivars of *M. tanajoa* through the floating discs technique (Badegana, 2000). The leaf age has an influence on *M. tanajoa* fecundity (Badegana, 2002). By this method, the fecundity study is not done throughout the oviposition period but on the first half of the period. This should be the case because by the end of the first half of the oviposition period, 79.7% of all the descendants of which 84.1% female descendants are already laid (Badegana, 2003). The sensitivity classification of the different cultivars according to the number of eggs laid by the end of the first half of the oviposition period is not different to that obtained at the end of oviposition. The calculation of the intrinsic rate of increase of mite population often carried out at the end of the oviposition period according to
the Birch (1948) formula:

$$\sum_{i-1}^{i-c} 1_x m_x e^{-rm.x}$$

(mx = number of female eggs per female/day; 1_x= percentage of surviving females, x the age of females in days, the first day of oviposition and which is the end of oviposition period) can already be obtained at the end of the first half of the oviposition period. To obtain the rm value at this point we write

$$\sum_{i-1}^{i-b} 1_x m_x e^{-rm.x}$$

=0,955 instead of 1. (b = the end of the first half of oviposition period). This is a simplified and new Birch formula for the gTeen mite. This method of study of sensitivity of *M. tanajoa* was experimented on four cassava cultivars: two local cultivars (Dschang and Njombé) and two improved varieties (IITA8017 and IITA82516). Results obtained enabled the following classification of the cultivars: From the least sensitive to the most sensitive (fmj being the mean daily fecundity rate during the first half of oviposition; Dschang (fmj = 1.6; mi = 0.133), IITA8017 (fmj = 1.9; rm = 0.134), IITA82516 (fmj = 2.6; rm = 0.143), Njombé (fmj = '2.8; mi= 0.152). These results are the same as those obtained with the sauté cultivars in field studies (Badegana, 2001). This simple and rapid method can therefore replace field experiments.

Session 3
Agricultural Entomology
Side-effects

IMPACT OF SUBLETHAL DOSES OF CROP PROTECTION AGENTS ON HONEY BEES: ABERRANT ONSET OF FORAGING AND ITS INFLUENCE ON GLOBAL COLONY VITALITY

Tim BELIËN[1], Jeroen KELLERS[1,2], Kevin HEYLEN[3], Wannes KEULEMANS[2], Johan BILLEN[3], Lut ARCKENS[3], Roger HUYBRECHTS[3] & Bruno GOBIN[1*]

[1]Zoology department, pcfruit vzw
[2]Division of Crop Biotechnics, KULeuven
[3]Zoological Institute, KULeuven
*Present address: PCS Research Centre for Ornamental Plants
E-mail: tim.belien@pcfruit.be

Honey bees (*Apis mellifera*) are the most economically valuable pollinators of fruit crops worldwide. Taking into account bees' contributions to other flowering agricultural crops, about one-third of our total diet comes directly or indirectly from bee-pollinated plants. However, in recent years there increasingly have been worrisome alarm sounds on serious bee mortalities and mysterious disappearance of bees from beehives. Among several environmental factors (e.g. climate and bee pathogens), stress factors arising from agricultural practices can potentially play a role in bee losses. Detailed knowledge on the effects of plant protection products is essential to improve usage with minimal risks.

The goal of the present study was to assess potential effects of some common plant protection products on foraging activity of adult honey bees following worst case exposure. More specifically, our objective was to evaluate treatment effect on the timing of a bee's duty transition from nursing to foraging activities. Hereto, a large-scale experiment was conducted in which at four distinct locations (in the Limburg region of Belgium) strictly age-controlled bees from four different bee hives (representing three different contaminations –imidacloprid, fenoxycarb, indoxacarb- and a non-contaminated control hive) were analysed for their phototactic movements. As foraging necessitates positive phototaxis, this allows for an evaluation of bee's shift from in-hive nursing activities to outside foraging activities. In a supplementary experiment, foraging activity of age-marked bees from different colonies (again undergoing distinct pesticide treatments) was followed up by filming the hive entrance 12 hours a day, in a time frame that extensively covers the expected transition from nurse to forager. Our observations indicate differences in foraging onset between bees belonging to distinct hives. These results were analysed in the light of several carefully assessed parameters indicating overall colony vitality, like total surface of capped brood, total amount of active and dead bees, colony weight, etc. The implications of these outcomes are discussed in terms of potential short-term and long-term consequences of disturbed foraging behaviour triggered by exaggerated exposure to sublethal doses of crop protection chemicals.

POTENTIAL COTTON POLLINATORS ARE NOT AFFECTED WITH BT COTTON IN SOUTH AFRICA

J-L. HOFS[1*], M. VAISSAYRE[1], A.S. SCHOEMAN[2], M.A. MELLET[3], W.D. HOLTZHAUSEN[2] & J. PIERRE[4]

[1] CIRAD, UPR Cotton Farming Systems, F-34398 Montpellier, France
[2] Department of Zoology & Entomology, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria 0002, South Africa
[3] Bayer CropScience, Johannesburg, South Africa
[4] INRA, UMR 1099 Biologie des Organismes et des Populations Appliquée à la Protection des Plantes, F-35650 Le Rheu, France
* E-Mail: jean-luc.hofs@cirad.fr

Bt cotton was introduced in South Africa ten years ago. In 2003-2004, two preliminary field studies were carried out in existing experimental or commercial plots, in Marble Hall (Mpumalanga Province) and Rustenburg (North-West Province), in order to assess the effect of the Cry1Ac toxin on potential pollinators (flower visiting insects) of cotton flowers.
Insect counting was carried out by bagging open flowers. The flower bags were placed in a freezer to kill the insects, which were later identified and preserved in 70% alcohol. *Astylus atromaculatus* Blanchard was the most abundant species in cotton flowers susceptible to carry pollen grain, at both sites. Wild bees (Apoidae) was the second group followed by Honey bees (*Apis mellifera* L.). The study pointed out the possibility for *A. atromaculatus* to be the main cotton pollinator in SA and showed that Bt toxin didn't impact flower visiting insect abundance. Pollinating insect activity should thus be taken into account in gene flow studies between GM and non-GM cotton fields.

THE SELECTIVITY IN THE TIME OF THIACLOPRID (CALYPSO 480 SC®) APPLIED AT PREBLOSSOM ON THE FURTHER *ANTHOCORIS NEMORALIS* POPULATION BUILD UP IN PEARS

E. BANGELS, C. SCHOEVAERTS, T. BELIËN, L. DE MAEYER & B. GOBIN
Pcfruit vzw, Sint-Truiden, Belgium
KULeuven, Leuven, Belgium

The study investigates if thiacloprid (Calypso 480 SC) + surfactant, applied at preblossom for the control of the first generation pear sucker *Psylla pyri,* is selective for the further population build up of *Anthocoris nemoralis* later in the season. For registration purposes and positive IPM listing, Belgian authorities request compulsory selectivity studies on *Anthocoris nemoralis,* the main predator for *Psylla pyri*. As a direct spray thiacloprid shows an acute toxic effect for Anthocorids (IOBC 3-4). However, besides the additional approach of prey/predator ratio an essential step in the IPM compatibility evaluation is the acceptance and implementation of the principle of selectivity in the time.

Therefore five large scale trials on pear Conference with randomized block design and four replicates were conducted and sprayed with a calibrated praxis sprayer. Two types of orchards were included: the first type is surrounded by abundant vegetation from which Anthocorids easily can fly in when attracted by the presence of prey (*Psylla pyri*); the second type has no "reservoir" vegetation around the orchard and here it is considered that mostly the residential population of Anthocorids constitutes the predation.

From preblossom till August the Anthocoridae larvae and adults were monitored by a beating method. At similar timings also the pear sucker *Psylla pyri* young and older larvae were assessed. The preflowering application with Calypso 480 SC + Trend, triggered at threshold crossing of more than 30 % of flower clusters with pear sucker L1 larvae, was compared with a pyrethtroid considered as the "toxic" reference at this application timing. As a "non toxic" reference either kaolin or oil was included.

In both type of orchards, thiacloprid (Calypso 480 SC) controls the first generation of pear sucker avoiding economical loss. At this timing Anthocoridae presence is minimal and the residual effect of this application does not harm the build up of the predatory Anthocoridae population later in the season.

The right positioning of thiacloprid at preblossom in pears shows complete selectivity in the time on *Anthocoris nemoralis* and fits with Integrated Pest Management.

WESTERN CORN ROOTWORM *DIABROTICA V.VIRGIFERA* IN EUROPE: STATUS, AND OPTIONS FOR FUTURE MANAGEMENT

Hans E. HUMMEL[1,2] & A. DEUKER[1]
[1] Justus-Liebig University, Organic Agriculture
Karl-Gloeckner-Str. 21 C, DE-35394 Giessen, Germany
[2] Illinois Natural History Survey, Biodiversity and Ecological Entomology
Champaign, Ill. 61820, USA

Introduction: *Diabrotica v.virgifera* (Col.:Chysomelidae) (WCR), unfortunately is now an established European maize pest without indigenous natural enemies. Twenty years after its repeated introduction into Europe by traffic and trade, eradication is wishful thinking and not longer a viable option. Rather, European legislation calls for toxicological emergency measures on a case by case basis where and when new infestations occur. Nevertheless, biological, biotechnical and cultural management approaches should occupy some priority as sustainable methods. Prominent among them are trapping and survey with attractants in conjunction with traditional crop rotation and phytosanitation.
Methods: Suitable survey traps and pheromone/kairomone lures have been described on numerous occasions in the past (for review see Hummel 2007).
Results and discussion: In an attempt to summarize the present status of WCR in Europe, its natural advances and its man-aided spreading act in synergy. In southeastern Europe, a solid block of infestation with partial economic damage by WCR appears on the distribution maps. "Jumping advances" in all directions can be seen as spotty colonies from which WCR tries to consolidate its territory of invasion into central (Austria, Germany and eastern France) and eastern Europe (Poland, Romania, Bulgaria and Ukraine). Examples of our research from Southern Germany, Slovenia, Romania and Ticino/Switzerland are presented. The picture thus emerging is by no means uniform but is characterized by much variation. A trend, caused by the peculiar geographic, topographic and prevailing climatic conditions, indicates a steady expansion of WCR into new European territory, coupled with the constant need and cost for future monitoring and timely intervention. A match between high-risk areas, previously identified by models of Baufeld and Enzian (2005), and actual invasion by WCR can be confirmed for Southern Germany. –Crop rotation alone, as practiced successfully in the Ticino canton for the last 5 years, could establish a seemingly steady WCR population and a quasi-equilibrium. It is noteworthy for its relative simplicity, low cost, environmental and ecological sustainability.
Conclusions: 1. A welcome transfer of this strategy, however, to Germany and adjacent central European States is impeded by significant agricultural differences and constraints. 2. Yet, the successful management example in the Ticino is a role model and a sign of hope. 3. New introductions from North America into Europe and intra-European cross-invasions should be strictly avoided by permanent surveillance. 4. Gradual changes of agricultural policy away from maize monoculture and towards increased sustainability should be the preferred future management strategy.

OCCURRENCE, ECOLOGY, IMPACT AND MANAGEMENT OF *NYSIUS HUTTONI* IN BELGIUM (NYSHUT)

J. BONTE[1,2], H. CASTEELS[1], P. DE CLERCQ[2] & M. MAES[1]
[1] Department of Crop Protection, Institute for Agricultural and Fisheries Research
Burg. Van Gansberghelaan 96, BE-9820 Merelbeke, Belgium
[2] Department of Crop Protection, Ghent University
Coupure Links 653, BE-9000 Ghent, Belgium

In 2002 the exotic lygaeid bug *Nysius huttoni* White (originating from New Zealand) was observed for the first time in the Netherlands and Belgium, on weeds. In 2006 this polyphagous bug was added to the EPPO Alert List, however, at present no regulation exists in Belgium managing its import and control. In New Zealand the pest species has been noted to be capable of causing severe economical damage to various agricultural crops, including *Triticum* and *Brassica* spp.
The introduction of *N. huttoni* to our regions presumably occurred via overseas transport of apple and kiwi fruits originating from New Zealand. Laboratory experiments determined that both adults and eggs of the New Zealand wheat bug were capable of surviving cold conditions similar to those in overseas transportation.
The bug has presently been able to spread over the entire region of Flanders, with the exception of Limburg, and over the provinces Hainaut and Brabant Walloon in the Walloon region. Despite its advanced distribution in Belgium, only 19.1% of the collected adults were capable of flying (macropterous). Thus, only for a small fraction of the population spread can be attributed to flight capacity of adults and other methods of disperse may have been involved.
Ecosystems in which *N. huttoni* have been located include dry habitats containing mosses, pebble substrates and habitats with limited ground coverage containing common host plants.
Several arguments support *N. huttoni* not being a threat for agricultural crops in Belgium at present:

- Only 14% of the locations where *N. huttoni* was found were situated nearby agricultural fields;
- Arable land does not belong to its preferred habitats;
- In laboratory experiments mangel-wurzel, rapeseed (canola) and wheat only suffered damaged at high densities of *N. huttoni* which are unlikely to occur in Belgium.
- In summer the species can acquire sufficient food when solely exploiting common weeds.

Our conclusions only apply to the current population densities of *N. huttoni* in Belgium and the Netherlands. Shifts in soil usage and climate change could influence future abundance and distribution of the bug.
Recommendations for efficient chemical control programs of *N. huttoni* could not be formulated based on the performed toxicity tests. Alternative control methods such as biological control, are not an option at this time due to the lack of known natural enemies. As long as no effective chemical controls can be recommended, it is advisable not to remove weedy host plants in the field margins.

COMPARATIVE ANALYSIS OF THREE SCALE INSECT SPECIES POPULATIONS (HEMIPTERA: COCCOIDEA: DIASPIDIDAE) AND THEIR PARASITOID, *APHYTIS DIASPIDIS* (HOWARD) ON PEAR TREES IN BURG EL-ARAB AREA, ALEXANDRIA, EGYPT

H.A. MESBAH[2], A.K. MOURAD[2], S. MOURSI KHADIGA[1], A.S. FATA AZIZA[1], O.A. ZAGHLOUL[2] & I.S. ABDEL-RAZAK[1]
[1] Agricultural Research Centre, Alexandria, Egypt
[2] Faculty of Agriculture, Saba- Basha, Dept. of Plant Protection
Alexandria University, Alexandria, Egypt

The infestation abundance of each of the inspected scale insect species: San Jose scale, *Diaspidiotus perniciosus* (Comstock), Greedy scale, *Hemiberlisia rapax* (Comstock) and Olive scale, *Parlatoria oleae* (Cloveé) on pear trees, more or less varied as the months of each growing season differed.
The comparative abundance percentages in both seasons of 2004-2005 and 2005-2006 were (51.80 and 51.40), (41.20 and 40.90) and (7.10 and 7.70) of the counted individuals of the grand total of *H. rapax*, *D. perniciosus* and *P. oleae*, in respect. In other words, the Greedy scale (GS) was the highly abundant scale followed by the San Jose scale (SJS), but the olive scale represented the lowest one.
The more or less increased and/or decreased abundance of each of the investigated scale insect species pointed to the existence of three overlapping generations throughout the season which were characterized by fluctuating peaks of population densities of the studied pests during the months of each annual season: autumn, winter, spring and summer.

Session 4
Herbology

WEED FLORA IN PAVED AREAS IN RELATION TO PAVEMENT TYPE, WEED CONTROL AND ENVIRONMENT

Maureen FAGOT[1], Benny DE CAUWER[1], Dirk REHEUL[2], Robert BULCKE[1] & Anne BEELDENS[3]

[1] Weed science Unit, Department of Plant Production, Ghent University, Belgium
[2] Unit of plant breeding and sustainable crop production, Department of Plant Production, Ghent University, Belgium
[3] Technical Committee Road Pavements, Belgian Road Research Centre, Belgium

To be proactive in minimizing pesticide use, Flemish authorities agreed to phase out the use of herbicides on public areas by the end of 2015. This agreement highlights the need for more research into weed control on paved areas. Indeed, weed growth and applied weed control strategies on paved areas have not extensively been studied in Flanders up till now. The objectives of this study were (1) to explore the species composition of paved areas in relation to various environmental conditions and (2) to determine the impact of different weed control methods on vegetation composition. In autumn 2008, a vegetation survey was conducted on 163 public pavements at 34 locations across Flanders. On each of these pavements, botanical composition of the weed flora in paving joints was determined in 50 random sampling quadrates of 25 x 25 cm. Species importance (%) was calculated according to the combined frequency-rank method of De Vries. A score for picture quality for weed growth was calculated based on average weed coverage of joints and vegetation height. In addition, for each pavement a set of environmental conditions was determined: placement, function, intensity of use, pavement type, pavement width, paving element size, joint width, … Data on the applied weed control methods (e.g. chemical, mechanical or thermal) were obtained through direct personal interviews with the pavements administrators.

Apart from mosses (Musci), the 5 most important plant species in paving joints were *Poa annua*, *Sagina procumbens*, *Erigeron canadensis*, *Taraxacum officinale* and *Plantago major*. This seems well in accordance with studies in other European regions. The applied weed control technique, pavement type, intensity of use, light regime and joint width, all had significant effects on the botanical composition of the vegetation and/or on the picture quality of the pavement. Furthermore, shifts in weed flora composition were observed caused by repeated use of any weed control method. Continuous application of thermal weed control methods favored annual and biennial monocotyledonous species, in particular *Poa annua*. The importance of mosses was significantly higher in pavements treated with chemical herbicides (mostly glyphosate). These preliminary results suggest that a more optimal weed control on pavements might be achieved by alternating weed control methods possessing different modes of action. These opportunities will be further investigated under more controlled conditions during future in situ experiments.

PYROXSULAM: A NEW HERBICIDE TO CONTROL GRASS AND BROADLEAVED WEEDS IN CEREALS

H. EELEN[1], D. LARELLE[2] & R. GAST[3]

[1] Dow AgroSciences B.V., Prins Boudewijnlaan 41, BE-2650 Edegem, Belgium
[2] Dow AgroSciences, 6, Rue Jean-Pierre Timbaud, FR-78067 St Quentin Yvelines, France
[3] Dow AgroSciences LLC, 9330 Zionsville Rd, Indianapolis, IN 46268, USA

Pyroxsulam is a new herbicide from Dow AgroSciences developed for the post-emergence control of grass and broadleaved weeds in selected wintercereals. The active ingredient belongs to the triazolopyrimidine sulfonamide family and acts as an acetolactate synthase inhibitor. It is a systemic herbicide, absorbed via leaves and roots and transferred to the growing points through phloem and xylem. The straight pyroxsulam formulation (GF-1274) developed in Europe is a water dispersible granule (WG), containing 75 g/kg pyroxsulam plus 75 g/kg of the safener cloquintocet-mexyl. Brand name is Capri*. The product can be safely applied in spring from the three-leaf stage to the second node in winterwheat, triticale, spelt, and rye at rates upto 250 g fp/ha plus adjuvant. It controls a broad spectrum of grass and broadleaved weeds, amongst which *Alopecurus myosuroides*, *Apera spica-venti*, *Avena* sp., *Lolium* sp., *Bromus* sp., *Poa annua*, *Veronica* sp., *Viola arvensis*, *Stellaria media* and cruciferous weed species. Pyroxsulam is fastly degraded in the soil. Due to this short half-life all rotational crops can be cultivated without any restrictions.

* Trademark of Dow AgroSciences

HERBICIDE RESISTANT WEEDS AS A THREAT TO CEREAL CROPS IN THE MEDITERRANEAN REGION

B. RUBIN
R.H. Smith Institute of Plant Sciences & Genetics in Agriculture, Faculty of Agriculture
Food and Environment, The Hebrew University of Jerusalem
Rehovot 76100, Israel
E-mail: rubin@agri.huji.ac.il

The evolution of herbicide-resistant weeds in dry land farming is a continuous process which endangers the profitability of arable crops in several Mediterranean countries. The region is characterized by long dry summers followed by a short rainfall season (November-March) with low annual precipitation (150-300 mm), and frequent drought years. Chemical weed control in non-irrigated arable crops is crucial due to the strong competition employed by a wide range of broadleaved and grass weeds. Lack of alternative crops imposes cereal monoculture interrupted with fallow years, reduced tillage and frequent use of herbicides. This selection pressure resulted in the evolution of a large numbers of herbicide-resistant weeds. An altered target site resistance to ALS-inhibiting herbicides was identified in *Chrysanthemum coronarium*, *Conyza canadensis* and *C. bonariensis*, *Capsella bursa-pastoris, Diplotaxis erucoides* and *Erucaria hispanica* and *Lolium rigidum* in wheat and hay crops. At least three different point mutations were identified in the ALS gene in these weeds. Similarly, post-emergence applied ACCase inhibiting herbicides against grass weeds resulted in an altered target site resistance in *Lolium rigidum*, *Phalaris minor*, *P. paradoxa* and *Avena sterilis*. These grass weed populations exposed to a high selection pressure by ACCase inhibitors conferred various alterations of the target enzyme endowing different resistance response to these herbicides. The recent evolution and spread of glyphosate-resistant *C. bonariensis* and *C. canadensis* populations is a most dangerous phenomenon which jeopardizes the adoption of minimum tillage practices and may also pose a threat to irrigated crops due to the easy seed dissemination by wind. These findings emphasize the need for integrated weed management approach that combines residual herbicides in the rotation, more frequent and better timed soil tillage and more competitive crop plants.

BLACK-GRASS RESISTANCE TO HERBICIDES: THREE YEARS OF MONITORING IN BELGIUM

François HENRIET[1] & Pierre-Yves MARÉCHAL[2]
[1] CRA-W, Pesticides Research Department, Rue du Bordia 11, BE-5030 Gembloux, Belgium
[2] FUSAGx, Unité de Phytotechnie des Régions tempérées
Passage des Déportés 2, BE-5030 Gembloux, Belgium
E-mail: henriet@cra.wallonie.be

Black-grass (*Alopecurus myosuroides* HUDS.) is a common weed of cereal crops widely spread in Northern Europe. Even if the first Belgian case of resistance was reported in 1996, until now, Belgium was quite spared of this problem and only a few restricted areas were concerned: the Polders, the marshland of the Escaut River and the Fosses-la-Ville region.
About 90 seed samples were collected trough the South part of Belgium and in the Polders during July 2006, 2007 and 2008. These populations were tested in greenhouse conditions by spraying plantlets with herbicides of three modes of action. The herbicides used were photosynthesis inhibitor, ACCase inhibitors and ALS inhibitors. Susceptible and resistant standard populations (Rothamsted and Peldon) were included in the test in order to validate it and to permit wild populations classification according to "R" rating system.
Populations showed differences of susceptibility to photosynthesis inhibitor, ACCase inhibitors and ALS inhibitors. For each herbicide mode of action, it was possible to find at least one population in each resistance class of the "R" rating system. Furthermore, it appeared that resistance was not confined to restricted areas listed above anymore.

CONTROL OF *ALOPECURUS MYOSUROIDES* (BLACK-GRASS) RESISTANT TO MESOSULFURON+IODOSULFURON

Stephen MOSS, Richard HULL & Ron MARSHALL
Rothamsted Research, Harpenden, Herts, United Kingdom

Resistance to the ALS inhibitor mesosulfuron+iodosulfuron ('Atlantis') has been identified in 133 populations of *A. myosuroides* in the UK and the presence of ALS target site resistance (Pro197Thr & Trp574Leu) has been confirmed. Two field trials were conducted in winter wheat crops, sown in early October, where ALS target site resistance (Pro197Thr) had been proven (Town Mead 2006/07, Long Covert 2007/08). The aim was to evaluate the efficacy of different herbicides and study the effects on continued selection for ALS target site resistance.

The pre-emergence herbicide flufenacet+pendimethalin (240+1200 g a.i.ha^{-1}) gave 85% and 68% reductions in *A. myosuroides* plant numbers in the two trials. Flufenacet+diflufenican (240+60 g a.i. ha^{-1}) and prosulfocarb+pendimethalin (3200+1200 g a.i. ha^{-1}), both used on Long Covert only, gave slightly poorer control at 65% and 56% respectively. Mesosulfuron+iodosulfuron (12 + 2.4 g a.i. ha^{-1}) gave a 79% and 73% reduction in *A. myosuroides* head numbers when applied in November and January in Town Mead, but only -7% and 5% reductions from January and April timings in Long Covert. This indicated that a low proportion of plants in Town Mead and a high proportion in Long Covert were ALS target site resistant.

Mixtures and sequences improved overall control levels. Pre-emergence flufenacet+pendimethalin followed by mesosulfuron+iodosulfuron plus pendimethalin (1320 g a.i. ha^{-1}) or clodinafop+trifluralin (30 + 960 g a.i. ha^{-1}) gave 93 – 98% reductions in Town Mead but only 60 – 73% reductions in Long Covert. A non-ALS treatment of pre-emergence flufenacet+pendimethalin followed by isoproturon+pendimethalin (1500+1320 g a.i. ha^{-1}) in late October and clodinafop+trifluralin in November or February achieved 97% and 93% reductions in the two trials.

Seed samples collected from surviving plants were evaluated in glasshouse assays to quantify any changes in the incidence of resistance. There was a substantial increase in the proportion of plants resistant to mesosulfuron+iodosulfuron regardless of whether it was used alone, in mixture or sequence. No such changes occurred with non-ALS treatments.

The results confirmed that ALS target site resistance can seriously reduce the efficacy of mesosulfuron+iodosulfuron against *Alopecurus myosroides*. The use of non-ALS herbicides in mixture or sequence with mesosulfuron+iodosulfuron can improve overall control in the short term, but does not reduce ALS selection pressure. The trials highlight the difficulty of achieving adequate control with alternative herbicides, especially as isoproturon and trifluralin will not be available for use in the UK after 2009. Greater use of non-chemical control measures will be essential to reduce the reliance on herbicides, especially where resistance occurs.

RESPONSE OF AN ALS SENSITIVE POPULATION OF CATCHWEED BEDSTRAW (*GALIUM APARINE*) TO POST-EMERGENCE HERBICIDES APPLICATION IN WINTER WHEAT (*TRITICUM AESTIVUM*)

Husrev MENNAN[1], Mathieu NGOUAJIO[2], Jens C. STREIBIG[3] & Soner CANKAYA[4]

[1]Ondokuz Mayıs University, Agriculture Faculty, Department of Plant Protection TR-55139 Samsun, Turkey

[2]Michigan State University Department of Horticulture, East Lansing, MI 48824, USA

[3]The University of Copenhagen, Faculty of Life Sciences, Dept. Agriculture and Ecology (Crop Science) DK-2630 Taastrup Denmark

[4]Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science Biometry and Genetics Unit, TR-55139 Samsun, Turkey

Catchweed bedstraw is an annual troublesome broadleaved weed dispersed throughout Eurasia and North America. It can cause severe problems in a wide range of climate and habitat but is mainly problematic in winter wheat fields and other winter sown crops. Field experiments were conducted from 2005 to 2008 in wheat fields in Samsun, Turkey, to determine the response of catchweed bedstraw to chlorsulfuron, tribenuron-methyl, tribenuron-methyl plus thifensulfuron-methyl, dicamba plus triasulfuron and mesosulfuron-methyl plus iodosulfuron-methyl-sodium and possible resistance to these herbicides. Experiments had a randomized complete block design with a factorial arrangement and four replicates in each year. The herbicides were applied at the 2-4, 4-6, and 6-8 true leaves stages of catchweed bedstraw. The Weibull curve, an asymmetric sigmoide curve, was fitted to obtain regression parameters ED_{50}, ED_{90} and ED at the absolute biomass of 0.05 g ($ED_{0.05}$). There were large differences among the upper limits of the curves and consequently the response levels of the commonly used relative EDx response levels differ among curves for all herbicides. When $ED_{0.05}$ values were evaluated for all herbicides at different application time the patterns were slightly different from the other ED-levels. None of the herbicides reduced catchweed bedstraw biomass or populations satisfactorily when applied at recommended field rate. Twofold recommended field rate was required to achieve acceptable reduction in biomass. Overall, inadequate control of this weed cannot be attributed to either ALS resistance or improper application methods. It is likely due to a slow and progressive development of ALS tolerant populations by sheer selection of less sensitive biotypes.

Session 5
Nematology

EXPLOITING THE *MELOIDOGYNE HAPLA* GENOME SEQUENCE TO UNDERSTAND PARASITISM

David Mc K. BIRD, Charles H. OPPERMAN, Elizabeth SCHOLL, John CROMER, Peter DIGENNARO, Michael GOSHE & Dahlia NIELSEN
Centre for the Biology of Nematode Parasitism, NC State University
Raleigh NC 27608, USA

The recently completed 54 Mbp genome sequence of the diploid root-knot nematode, *Meloidogyne hapla* (Opperman *et al*., 2008. *PNAS*, 105:14802-7) is providing a research platform to begin to study the biochemical basis for parasitism. In particular we are focusing on genome-wide changes in transcription profiles during important biological transitions of the parasitic life-cycle, and are endeavoring to integrate expression data with the computationally- and experimentally-deduced proteome.

FLAVONOIDS AND EARLY DEFENCE RESPONSES IN CYST NEMATODE INFECTION OF *ARABIDOPSIS THALIANA*

Joachim JACOB, Peter MELIS, Bartel VANHOLME, Bernard CANNOOT & Godelieve GHEYSEN
Department of Molecular Biotechnology, Faculty of Bioscience Engineering
Ghent University, Belgium

Chorismate is one of the key branch-point metabolites in plants, transferring an estimated 20% of the plant's fixed carbon flux into diverse pathways with roles in growth, development and plant defence. The major pool of chorismate is the plastid, where the plant's chorismate mutase (CM) enzymes catalyze the first steps to phenylalanine (F) and tyrosine (Y) synthesis. However, the finding of a cytosolic CM enzyme is enigmatic, suggestive of a cytosolic pathway of F and Y synthesis. During nematode infection of plant roots, a cocktail of proteins is secreted by the nematode. Some are injected by sedentary nematodes into a selected root cell to transform them into giant nematode feeding sites – a structure on which the nematode relies for the completion of its life cycle. A few of those proteins are identified, one of which encodes a secreted CM. This enzyme potentially provides the nematode with a molecular tool to directly interfere with a core metabolic pathway of the plant. However, due to its cytoplasmatic localization, its function is still a matter of debate. The proposed link with flavonoids is not supported by our data from infection tests with the cyst nematode *Heterodera schachtii* on flavonoid-deficient mutants of *Arabidopsis thaliana*. Moreover, detailed investigation could not reveal accumulation of flavonoids in cyst nematode feeding sites – contrary to previous reports. Recent results concerning the role of CM point into the direction of manipulation of defence responses in the plant. We measured early plant defence responses using Q-PCR, 1 and 2 days after infection. Roots and shoots behave completely different. Negligible alterations in SA are measured in roots at both time points, while in shoots only a strong upregulation of SA (x100) is seen from 2 dpi on. In contrast, the strong upregulation (x100) of jasmonic acid (JA) seen in the roots during 1dpi, is completely vanished already 2 dpi, while no sign of JA signalling is observed in shoots. The possible link of these responses with nematode CM has yet to be confirmed – but findings of similar secreted CM in plant- and animal-pathogenic bacteria strongly indicates a general role in pathogenesis.

GENETIC DIVERSITY OF THE POTATO CYST NEMATODE *GLOBODERA PALLIDA* IN SOUTH AMERICA: PHYLOGEOGRAPHIC HISTORY AND CONSEQUENCES FOR MANAGEMENT OF THIS PEST

S. FOURNET[1], D. FOUVILLE[1], G. ANTHOINE[2], A.M. CHAPPÉ[2], Flores E. HENRIQUEZ[3] & E. GRENIER[1]

1 INRA, Agrocampus Rennes, Université Rennes 1, UMR1099 BiO3P FE-35653 Le Rheu, France

2 Laboratoire National de la Protection des Végétaux – Unité de nématologie – Domaine de la Motte – BP 35327 – F-35653 LE RHEU cedex – France ;

3 Gobierno de Chile, Servicio Agricola y Ganadero, Laboratorio y Estacion Cuarentenaria Agricola, Ruta 68, km 22, Santiago, Chile.

Coevolution of hosts and parasites is a well-known phenomenon, and plant nematodes make no exception. The cyst-nematode parasites of wild potatoes coevolved with their hosts and thus originated in the Andes. A phylogeographic study of the variability of *G. pallida* was conducted in Peru. Phylogenies based on the mitochondrial cytochrome b marker and on 8 microsatellite loci exhibit a clear south-to-north phylogeographic pattern. This phylogeographic structure belongs to a well-known type and implies colonization of progressively emerging favourable areas. We have been able to identify the origin of European populations with high accuracy. They are all derived from a single restricted area in the extreme south of Peru, located between the north shore of the Lake Titicaca and Cusco. The allelic richness at seven microsatellite loci observed in the European populations, although only one third of that observed in this part of southern Peru, is comparable to the allelic richness observed in the northern region of Peru. This result could be explained by the fact that most of the genetic variability observed at the scale of a field or even of a region is already observed at the scale of a single plant within a field (see also the seminar of the 20th May). Thus, even introduction via a single infected potato plant could result in the relatively high genetic variability observed in Europe.

In order to get insights on the detection and consequences of a novel introduction of PCN into Europe, we have tested - against the variability of the parasite in its native area - the reliability of different molecular diagnostic tools and the efficiency of three different oligogenic potato resistances worked at INRA. Even if *G. pallida* populations were correctly identified, our results highlight (1) the strong consequences that a new introduction could have on the durability of *G. pallida* resistances developed in Europe, (2) the need of both a deep validation of molecular tools before use for official analysis and further scientific investigations regarding the diversity of PCN southwards to lake Titicaca and the taxonomic status of particular populations.

WILL *MELOIDOGYNE MINOR* BECOMES A THREAT TO OUR AGRICULTURE?

Gerard KORTHALS[1], Wianda VAN GASTEL[1], Johnny VISSER[1], Anton VAN DER SOMMEN[2] & Loes DEN NIJS[2]

[1] Applied Plant Research PPO AGV, Wageningen University, The Netherlands

[2] Plant Protection Service, Wageningen, The Netherlands

Meloidogyne minor is found very recently in the Netherlands and the United Kingdom (Karssen, G., Bolk, R.J., Aelst, A.C. van, Beld, I. van den, Kox, L.F.F., Korthals, G.W., Molendijk, L.P.G., Zijlstra, C., Hoof, R. van and Cook, R. Description of Meloidogyne minor n.sp. (Nematoda: Meloidogynidae), a root-knot nematode associated with yellow patch disease in golf courses. Nematology, 2004, Vol. 6(1), 59-72.) In the Netherlands, this species was found in a potato-field, therefore it can be expected that this new root knot nematode may give risks. At this stage it is important to get more information on host suitability and damage levels for this nematode and some important arable crops. To get this kind of information a field trial started in 2008. At this field site a natural population of *M. minor* and *M. naasi* is present. During 2008 five crops (maize, rye, Italian ryegrass, potatoes and sugar beet) were grown, to get information on host suitability of these crops. In 2009 two potato cultivars will be grown to investigate damage levels of *M. minor* for potato and to estimate the potential risk of *M. minor*. The poster will highlight some aspects of the design of the field trial and some first results on host suitability.

AN APPROACH TO MONITORING CYST NEMATODE POPULATIONS

Milan RADIVOJEVIĆ, Dobrivoje GVOZDENOVIĆ & Nikola GUJIĆ
Institute for Phytomedicine, Faculty of Agriculture, Beograd, Serbia

Horizontal distribution of the plant root parasitic cyst nematodes (Heteroderinae) in fields is rather patchy, making it difficult to provide good estimates of the average number of their larvae (L2) enclosed in cuticular cysts in soil. Soil sampling and subsequent analysis are costly, so providing reliable results with minimum expense is essential. The two potato cyst nematode species have been quarantined, which involves measures to eradicate them and need to monitor the effects of these measures on nematode populations using feasible methods. Such a methodology, suitable for fields with moderately to highly infested parts is being developed. The concept is to mark 50 sampling points in a highly infested part of the field and to always collect soil cores very close to these points to reduce the effects of spatial variation. The sampling points are arranged along a 50 m straight line transect, which is subdivided into10 sampling units 5m long, with each unit having 5 sampling points 1 m apart. Five cores of c. 20 g soil each, from 5 - 20 cm depth from each sampling unit are mixed to create c. 100 g soil unit samples, and ten of these unit samples from 10 respective sampling units constitute one sample. Cysts are extracted from dried unit samples by Fenwick's can. Vivid contents of 50 randomly selected cysts from the samples are examined seasonally from individually dissected cysts to estimate number of larvae. In 2008, seven samples were taken from transect I with *Globodera rostochiensis* and six from transect II in field infested with *G. pallida*. Transect I yielded an average population density of 47.1 L2/g soil and 43.5 cysts/100g soil ± 8.8 cysts, i.e. C.V. 20.4 % variation between sample means. Variation between sampling units, which reflects variation of the density along the transect, averaged 77.9 %, and the variation within a sampling unit averaged 45.9 %. In the transect II variation between and within sampling units was similar to that in the first transect: the population densities were 44.5 L2 /g soil and 33.2 cysts/100 g soil ± 5.4 cysts, i.e. C.V. 16.3 %, variation between sample means. Differences between samples were not significant and correlations between samples within a transect were high, at 0.8 - 0.9. Precision of average seasonal cyst density estimates of 15 - 20 % is tolerable, given the amount of the soil analyzed. The approach presented here where samples are taken in densely infested parts of the field with precisely marked sampling points and several samples are taken annually, is promising, though there certainly remains room for improvements.

PATHOGENESIS OF *RADOPHOLUS SIMILIS* IN AN IN-COMPATIBLE INTERACTION WITH *MUSA* SPP.

Dhakshinamoorthy SUGANTHAGUNTHALAM, Dirk DE WAELE & Annemie ELSEN

Laboratory of Tropical Crop Improvement, Department of Biosystems
Faculty of Bioscience Engineering, Katholieke Universiteit Leuven (KULeuven)
Kasteelpark Arenberg 13, BE-3001-Leuven, Belgium.
E-mail: Suganthagunthalam.Dhakshinamoorthy@biw.kuleuven.be

In an in-compatible plant-nematode interaction, nematode resistance of the plant can occur towards one or more stages of nematode infection namely attraction towards the host, penetration of the host cell wall, development and reproduction inside the host root system. Identifying these stages wherein the host resistance occur is the first step in revealing the resistance mechanisms. With this aim, three *Musa* genotypes originating from the centre of origin and resistant to the burrowing nematode *Radopholus similis* were studied in comparison with the susceptible genotype Grande Naine. Nematode attraction, penetration and developmental studies were conducted in the greenhouse as well as under well controlled *in vitro* autotrophic conditions. In each experiment, observations on nematode numbers were made over time. The results show that there are no differences in the number of nematodes penetrated in the susceptible and resistant genotypes up to 12 days after nematode inoculation. However, significant differences were observed in the number of eggs laid by the penetrated females at the same time of observation. Early observations on the nematode development and reproduction indicate that the resistance reaction is strongly expressed at these two levels of *R. similis* pathogenesis in the tested *Musa* genotypes.

SCREENING OF COMMON BEAN (*PHASEOLUS VULGARIS*) FOR RESISTANCE AGAINST TEMPERATE ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.)

Wim WESEMAEL[1] & Maurice MOENS[1,2]
[1] Institute for Agricultural and Fisheries Research, Merelbeke, Belgium
[2] Laboratory for Agrozoology, Ghent University, Belgium

In Belgium, common bean (*Phaseolus vulgaris*) is mainly grown for the deep freezing or canning industry. An important part of the production area is located on the sandy soils of the provinces Antwerp and Limburg where *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* are widespread. De host plant status of 10 bean cultivars (Cantare, Flagrano, Fulvio, Jamaica, Lipsos, Masai, Mercana, Polder, Proton and Verbano) for root-knot nematodes was determined by examining the presence of egg masses eight weeks after inoculation with second-stage juveniles. The plants were grown in plastic tubes and kept in a temperature-controlled glasshouse. The tested cultivars were poor to good hosts for *M. chitwoodi*, no or bad hosts for *M. fallax* and excellent hosts for *M. hapla*. The differences between *Meloidogyne* spp. stress the importance of a correct diagnosis of the nematode problem. The penetration and development of *M. chitwoodi*, *M. fallax* and *M. hapla* inside the roots of cvs Masai, Mercana, Polder and Verbano were monitored two, four and six weeks after inoculation. These three species of root-knot nematodes were able to penetrate, install a feeding site and develop into mature females in the four bean cultivars. Compared to *M. chitwoodi* and *M. hapla*, significantly less *M. fallax* were found in the roots. Moreover, the development of *M. fallax* was delayed. The total number of *M. hapla* found inside the roots four and six weeks after inoculation was significantly higher than that two weeks after inoculation. Vermiform juveniles of *M. hapla* were still found after four weeks indicating that penetration took place over a longer period than that of *M. chitwoodi* and *M. fallax*. The number of mature females of *M. chitwoodi* in cv. Polder six weeks after inoculation was not different from that in other cultivars although less egg masses were found on this cultivar in the screening test. Therefore cv. Polder might be a potential trap crop. However, increasing the time between inoculation and screening to 10 weeks resulted in egg masses of *M. chitwoodi* on all the tested plants of cv. Polder. The pros and cons of the used screening technique will be discussed.

IMPROVEMENT AND MONITORING SOIL HEALTH

Gerard KORTHALS, Johnny VISSER & Leendert MOLENDIJK
Applied Plant Research PPO AGV, Wageningen University, The Netherlands

Since the application of pesticides and artificial fertilizers the appreciation for the soil as the foundation for agricultural production diminished. Recently attention is paid to the possible negative influences of these aspects on the microbial population in the soil. The withdrawal of most soil fumigants enhanced the interest for soil aspects like root disease incidence, soil resistance and plant health.
Recently PPO has started a long-term field experiment to develop strategies to achieve and establish soil health. Methods applied are crop frequency, crop rotation, use of resistant varieties of economic and green manure crops, anaerobic biological soil disinfestation, the application of compost or chitine. The effect of these measurements will be established by monitoring changes in nematode community structure and the effects upon arable crops, such as yield and disease incidence.
Results showed that anaerobic biological soil disinfestation is effective in decreasing the nematode populations of free living *Trichodorid* nematodes and root lesion nematodes *Pratylenchus penetrans*. The green manure crop *Tagetes patula* decreased *Pratylenchus penetrans*, but increased *Trichodorids*. Chitine application resulted in a lower count of *Trichodorids*. The development of crops after the different treatments was inversely proportional to the nematode counts. Within this project bio assays with the root knot nematode *Meloidogyne* and the fungi *Verticillium dahliae* and *Rhizoctonia solani* were performed. Furthermore the effect of the treatments on several soil parameters was measured. Nematodes proved to be very good bioindicators of soil health.

SUITABILITY OF SOIL - COVER CROPS COMBINATION FOR ECO-FRIENDLY MANAGEMENT OF ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) IN TOBACCO

N. THARSHANI & G. MIKUNTHAN
Department of Agricultural biology
Faculty of Agriculture University of Jaffna, Sri Lanka

Meloidogyne incognita, soil borne nematode, a causal agent of tobacco root-knot. About 61-100 per cent loss in yield of tobacco leaves was recorded in Jaffna, Sri Lanka and on par with the yield loss in the South Asia. Fields were selected from two different great soil groups namely Calcic Red Yellow Latersol (CRYL) and Regasol with two popular tobacco cultivars 'Pani' and 'Sotu". The effects of Sesamum (*Sesamum indicum*), cassava (*Manihot esculentum*) and foxtail millet (*Eleusine corocana*) as cover crops on gall density and leaves yield were evaluated. A randomized complete block design was used and treatments were replicated three times. The results exhibited significant difference among the two groups of soils, that Regasol soil group reduced nematode incidence by 63% in "Sotu" tobacco and no nematode incidences in "Pani" tobacco. 'Sotu" supported higher density of gall than "Pani" in both soil groups. The maximum reduction of the density of gall by *Sesamum indicum* (56%) and *Eleusine* sp (43%) was recorded in some sampling occasions at CRYL. No significant reduction of gall was detected by the two cover crops at Regasol. Manihot did not reduce the gall density in most cases and up to 52% higher densities of galls were recorded in the Manihot compared to control at CRYL. 'Pani" tobacco yielded higher than "Sotu" tobacco at Regasol and similarly "Sotu" tobacco recorded higher yield in CRYL than Regasol. Sesamum sown at the time of transplanting of tobacco reduced "Sotu" yield by 10% in CRYL and 21% in Regasol. Eleusine reduced 11% in CRYL and 25% in Regasol. Manihot reduced 72% of tobacco yield in CRYL and 18% in Regasol. Cover crops established prior to tobacco transplanting did not significantly reduce the yield of tobacco except in CRYL which recorded higher yield with sesamum as companion crop. The results revealed that early planting of cover crops in CRYL reduced nematode incidence. Manihot is not a best cover crop with tobacco in either soil. Regasol was suited for "Pani" tobacco and similarly CRYL was suited for "Sotu" tobacco. *M. incognita* showed location specific and cultivar based resistance. These findings are very useful for planning the tobacco- crop rotation for the effective management of root knot nematode with the use of suitable cover crop combinations.

FUNGI ASSOCIATED WITH EGG MASSES, FEMALES AND SECOND STAGE JUVENILES OF ROOT-KNOT NEMATODES IN SAMSUN GREENHOUSES, TURKEY

Sevilhan MENNAN[1], Tuba KATI[1], Gökhan AYDINLI[1] & Ismail ERPER[1]
[1] Ondokuz Mayıs University, Agricultural Faculty, Plant Protection Department
55139, Samsun, Turkey

Microbial agents found in agricultural ecosystems naturally have potential for control of plant parasitic nematodes. Especially using fungal parasites of eggs has increased rapidly. Many fungi have been isolated from females and egg masses of *Meloidogyne* spp. throughout the world. It was searched naturally occurring fungal parasites of second stage juveniles, females and egg masses of root knot nematodes in vegetable growing greenhouses in Samsun (Middle Black See Region, Turkey). Totally 130 vegetable growing greenhouses were surveyed to examine infestation level and distribution of root knot nematodes and 85 greenhouses (65.38%) were found infected with root knot nematodes with a different infestation level. The most infested plant was cucumber (81%) and tomatoes (74%). After isolation from egg masses, females and second stage juveniles, 345 fungal isolates were found. Among this, 50.7 % isolated from females, 41.8% from egg masses and just only 7.5% from second stage juveniles, thus females were the most infested stage with fungi. A diversity of fungal species was identified including *Fusarium* spp., *Pythium oligandrum, Phythium* sp., *Rhizoctonia* spp. *Alternaria* spp. *Trichoderma* sp. and unidentified 18 isolates. *Fusarium* spp. was the most founding fungi, 253 isolates were identified as *Fusarium*, including *Fusarium oxysporum* (40.3%) *Fusarium moniliforme* (13.2%), *Fusarium culmorum* (0.7%) and *Fusarium* spp. (45.8%). Infested females, egg masses and second stage juveniles were photographed and morphological changes were observed.

MOLECULAR DETECTION OF NEMATICIDAL CRYSTALLIFEROUS *BACILLUS THURINGIENSIS* STRAINS OF IRAN AND EVALUATION OF THEIR TOXICITY ON FREE LIVING AND PLANT PARASITIC NEMATODES

Gholamreza Salehi JOUZANI[1], Ali SEIFINEJAD[1], Ayatollah SAEEDIZADEH[2], Mehdi YAZDANI[3], Amin NAZARIAN[1], Rosa JAHANGIRI[1] & Reza MAALI AMIRI[3]

[1] Department of Microbial Biotechnology and Biosafety
Agricultural Biotechnology Research Institute of Iran (ABRII)
[2] Department of Plant Protection, Faculty of Agriculture, Shahed University, Tehran, Iran
[3] Department of Agronomy and Plant Breeding, Faculty of Agronomy and Animal Sciences, University College of Agriculture and Natural Resources, University of Tehran

The characterization of nematode-effective strains and *cry* genes in the Iranian *Bt* collection (70 isolates) is presented. Characterization was based on PCR analysis using 12 specific primers for *cry5*, *cry6*, *cry12*, *cry13*, *cry14*, *cry21* genes encoding proteins active against nematodes, crystal morphology, and protein band patterns as well as their nematicidal activity on root knot nematode (*Meloidogyne incognita*) and two free living nematodes (*Chiloplacus tenuis* and *Acrobeloides enoplus*). PCR results with primers for these genes showed that twenty two isolates (31.5%) contain minimum one nematode-active *cry* gene. Strains containing *cry6* gene were the most abundant and represent 22.8% of the isolates. *Bt* strains harbouring *cry14* genes were also abundant (14.2 %). *cry21* and *cry5* genes were less abundant, found in 4.2 and 2.8% of the strains, respectively. Totally, six different nematode-active *cry* gene profiles were detected in this collection. Four isolates did not show expected PCR product size for *cry5, cry6* and *cry21* genes, they might contain potentially novel insecticidal crystal protein genes. Twenty two *Bt* isolates containing nematode active *cry* genes were selected for preliminary bioassays on *M. incognita*. Based on these bioassays, four isolates were selected for detailed bioassays. Isolates YD5 and KON4 at $2x10^8$ CFU ml^{-1} concentrations showed 77 and 81% toxicity on *M. incognita*, respectively. The free-living nematodes, *C. tenuis* and *A. enoplus* were more susceptible and the most mortality was observed within 48 hours of incubation at all the concentrations tested. Maximum mortality was recorded for isolates SN1 and KON4 at $2x10^8$ CFU ml^{-1} concentrations and resulted in 68 and 77% adults' deaths of *C. tenuis*, and 68 and 72% for *A. enoplus*, respectively. Our results showed that PCR is useful technique for toxicity prediction of nematicidal *Bt* isolates.

DEVELOPMENT OF AN EFFICIENT APPLICATION OF ENTOMOPATHOGENIC NEMATODES IN VEGETABLES

Eva BRUSSELMAN[1], Bert BECK[2], Femke TEMMERMAN[3], Pieter SPANOGHE[2], Maurice MOENS[4,5] & David NUYTTENS[1]

[1] Institute for Agricultural and Fisheries Research (ILVO), Technology and Food Sciences Unit, Agricultural Engineering, Merelbeke, Belgium

[2] Laboratory of Crop Protection Chemistry, Ghent University, Belgium

[3] Provincial Research and Advisory Centre for Agriculture and Horticulture (POVLT) Beitem, Belgium

[4] Institute for Agricultural and Fisheries Research, Merelbeke, Belgium

[5] Laboratory for Agrozoology, Ghent University, Belgium

The withdrawal of approvals for agrochemicals on many food crops within Europe is likely to generate an increasing market for bio-pesticide products, including entomopathogenic nematodes (EPN). However, for the EPN to be a success, a cost-effective and reliable usage should be assured. Therefore, progress in areas like application and formulation technology are needed, next to the development of optimal application strategies. A new research project will focus on optimizing the field application of EPN in vegetables. Three model pests, i.e. caterpillars and cabbage fly in cabbage and thrips in leek, will be used in the study. First, both application and formulation technologies will be optimized. Then, these technologies will be tested under semi-field conditions with special attention for the effect of weather conditions and timing of application. All the acquired knowledge will be implemented and evaluated in field experiments. A presentation of the project together with some preliminary results will be presented.

Session 6
Phytopathology and integrated control of plant diseases (1)

Phytopathology and integrated control of plant diseases (1)

Session 6

VIRAL DISEASES IN ZUCCHINI (*CUCURBITA PEPO*) IN FLANDERS: OCCURRENCE AND IDENTIFICATION

Kris DE JONGHE[1], Luc DE ROOSTER[2], Dominiek GOOSSENS[2], Raf DE VIS[2] & Martine MAES[1]

[1] Institute for Agricultural and Fisheries Research, Merelbeke, Belgium
[2] Research Station for Vegetable Production, Sint-Katelijne-Waver, Belgium

Several viruses are the cause of some of the most common diseases affecting cucurbits in many growing areas. They cause growth and yield reduction but also affect the fruit quality, often making the product unmarketable. The Flemish region has known a vast expansion in zucchini cultivation over the last 15 years. This is well-illustrated by the cultivated area which quadrupled from 1993 (115 ha) to 2007 (460 ha). Occasionally, this has led to problems with viruses, but the last 5 years incidence and damage dramatically increased leading to serious agronomic impact threatening the zucchini industry. Virus screenings in 2006, 2007 and 2008 indicated a predominance of Watermelon mosaic virus (WMV), especially in the first part of the season. Late in the season, Cucumber mosaic virus (CMV) becomes more dominant. Zucchini yellow mosaic virus (ZYMV) and Papaya ringspot virus (PRSV) are found to a very limited extend. Additionally, symptoms tend to develop earlier in the season causing severe damage and loss. In 2007, for the first time, damage in a greenhouse culture was reported. However, compared to field-grown zucchini, the virus problem remained limited in greenhouse crops. Virus screening experiments in zucchini cultivars showed important differences in viral presence and symptom development. In addition, identification of ZYMV was not reliable by means of commercial ELISA kits. A DAS-ELISA with antibodies from 2 different companies resulted in a number of positive ZYMV test samples which could not be confirmed by means of RT-PCR or real-time PCR. These positive samples also tested positive for WMV of which the presence was confirmed by means of RT-PCR. This indicates cross-reaction in the DAS-ELISA kits for these two viruses.
To be able to offer the growers a control strategy, more research is needed to elucidate the virus problem in the zucchini cultivation in Flanders, including a reliable and sensitive detection of the three potyviruses (WMV, ZYMV and PRSV) and CMV.

NEW INSIGHTS ON THE MOLECULAR HOST-PATHOGEN RELATIONSHIP BETWEEN PEACH TREES AND PEACH LATENT MOSAIC VIROID

O. PARISI, S. LEPAIR, P. LEPOIVRE & M.H. JIJAKLI
Plant Pathology Unit, Faculté Universitaire des Sciences Agronomiques
Passage des Déportés 2, BE-5030 Gembloux, Belgium

Viroids are circular single-stranded RNA molecule that do not code for any protein. The Peach Latent Mosaic Viroid ranges in size from 335 to 351 nucleotides and presents a higly branched secondary structure consisting in stems-loop and pseudoknots. It mainly infects peach.

The mechanisms involved in viroids pathogenicity are poorly understood and several hypothesis are postulated: (1) the pathogenic effect of viroid can be induced by an interaction with host proteins, (2) the gene silencing which protect the plant genome against virus and viroid can be the target of the viroid action. In this latter case two ways can be borrowed: in the first one, a hypothetical degradation of the viroid by this defence mechanism produces small RNA which can silence host genes; in the second, the silencing machinery of the host can be affected during the viroid infection by an unknown mechanism interfering with the normal host genes regulation.

Our study aimed to characterize the molecular relationship between peach trees and the PLMVd. To explore the mechanisms involved by the infection a cDNA-AFLP analysis on total RNA peach extract was performed. This technique offers an open strategy which does not need any knowledge of the host genome to determine the transcriptional modifications induced by the infection.

Previously studied pathogenic variants of the PLMVd were isolated and induced different intensities of mosaic and chlorosis. One of these variants showed partial chlorosis of the foliage giving rise to symptomatic and asymptomatic leaves on the same plant which were harvested to compare their expression profiles while another one induces a latent infection.

Our protocol compares their variants as well as healthy plant. This permit to compare (1) the effect of variant inducing different symptoms as well as the effect of a latent variant and (2) the transcriptional modification between a healthy and an infected plant.

We have obtained differentially expressed bands corresponding to genes whose expression is modified by the infection. From them we found genes implicated in photosynthesis and mRNA translation. The modification of the gene expression also showed differences between the inoculated PLMVd variants. All of our results will be discussed during the meeting.

MONITORING AND OCCURRENCE OF NEW RACES OF *VENTURIA INAEQUALIS* ON APPLE IN BELGIUM

B. LEFRANCQ & M. LATEUR
Department of Biological Control & Genetic Resources
Walloon Agricultural Centre, Gembloux, Belgium.

Since 1999, inside the framework of an European research project ('Durable Apple Resistance in Europe'), a monitoring of scab infection on leaves on 24 differential apple cultivars (cvs) chosen for their different resistance genes against *Venturia inaequalis* was carried on trees in an unsprayed scab trap orchard at Gembloux, Belgium. The occurrence of scab races was determined by the use of a global assessment scale from 1 (no symptom) to 9 (maximum susceptibility) taking into account of both incidence and severity of the disease. Since 2001, scab was also scored according to a second evaluation scale based on qualitative symptoms. On the basis of the occurrence of symptoms on the various differential cvs, five described physiological races of *V. inaequalis* were progressively identified in the orchard. During this period, important differences in incidence and severity of scab were observed on the different cvs. The susceptible cvs 'Golden Delicious', 'Gala' and 'Fiesta' were the most infected. On 13 polygenic varieties sporulation was more or less important according to the disease pressure and their degree of susceptibility. The presence of race 7 on trees of *Malus floribunda* 821 has already been reported since 2000 year. Races 5, 6 and 7 sporulated rather abundantly on their specific hosts according to the season's conditions although races 2 and 4 incited limited infection on their differential hosts and only under high disease pressure. Up to now, scab race 3 was never observed in our conditions.

DROUGHT AND OXIDATIVE STRESS DETERMINE THE SENSITIVITY OF THE PEAR TOWARDS BROWN SPOT INFECTIONS

S. VAN LAER[1], M. HÖFTE[2] & P. CREEMERS[1]
[1] Proefcentrum Fruitteelt v.z.w.
Fruittuinweg 1, BE-3800 Sint Truiden, Belgium
E-mail: stijn.vanlaer@pcfruit.be
[2] University Gent, Laboratory of Phytopathology
Faculty of Agricultural and Applied Biological Sciences
Coupure Links 653, BE-9000 Gent, Belgium

A survey among Belgian fruit growers carried out in 2006 revealed that the Brown Spot is not equally spread in Belgium. The absence of Brown Spot is linked to the presence of loam. It is thought that the specific drainage properties of a loam soil are responsible for the absence of Brown Spot. An epidemiological study carried out in 2005 and 2006 supports this idea. In this study different orchards were looked upon in which an infestation gradient occurred. An analysis of the soils in those orchards reveals that soil drainage conditions play a role in determining the sensitivity of the tree for *Stemphylium* infections. During the growth season of 2005, 2006 and 2007 actual *Stemphylium* infection risk was determined by means of window treatment experiments and fruit encapsulating experiments. An in-dept analysis of the occurrence of the actual *Stemphylium* infection risk moments revealed a close relation with the occurrence of drought stress during these growth seasons. The sensitivity of the pear towards brown spot infections is not only linked to drought stress, but also to high radiation, ozone and temperature. In the growth season of 2008 chlorophyll fluorescence measurements were performed to determine the relative importance of the different factors that contribute to the oxidative stress on pears during the growth season. Non photochemical quenching (NPQ) was used as a measure of oxidative stress damage and protection against this type of stress. A correlation analysis indicates that ozone is probably the largest contributor to oxidative stress damage on pear.

PRESENCE AND DYNAMICS OF *COLLETOTRICHUM ACUTATUM* ON WEEDS

Wendy VAN HEMELRIJCK[1], Jane DEBODE[2], Kurt HEUNGENS[2], Martine MAES[2] & Piet CREEMERS[1]

[1] Research centre for fruit cultivation
Fruittuinweg 1, BE-3800 Sint-Truiden, Belgium
[2] Institute for Agricultural and Fisheries Research
Burg. van Gansberghelaan 96, BE-9820 Merelbeke, Belgium

Colletotrichum acutatum is the main causal agent of anthracnose on strawberry fields in Belgium. Typical symptoms of the disease are brown/black sunken spots on ripe strawberries and elliptical brown sunken lesions which often girdle runners. *Colletotrichum* is generally introduced into new areas by symptomless infected planting material. Localised spread occurs by means of water splash. The pathogen overwinters on infected strawberry plants or on crop debris in the soil. It is also reported to be present, proliferate and overwinter on alternate host plants like weeds. Therefore, weed plants can act as a source of inoculum for new infections. Furthermore, there are indications that paraquat-containing herbicides encourage sporulation of *C. acutatum* on the weeds, resulting in an increase of the inoculum at the start of the season.

The purpose of this research is to unravel the possible role of weeds in the epidemiology of anthracnose disease in Belgium. The presence and the dynamics of *C. acutatum* on different weeds commonly present in Belgian strawberry fields and the role of herbicides as stimulator of *Colletotrichum* sporulation and disease spread are investigated.

EFFECT OF CANOPY MANIPULATION ON CANE AND FRUIT BOTRYTIS IN PROTECTED RASPBERRY

Tim O'NEILL[1], Angela BERRIE[2], Erika WEDGWOOD[1], Janet ALLEN[1] & Xiangming XU[2]

[1]ADAS Boxworth, Boxworth, Cambs, CB23 4NN, UK

[2]Plant Pathology, East Malling Research, West Malling, ME19 6BJ, UK

Botrytis cinerea causes disease on both fruit and cane of raspberry. The incidence of latent and post-harvest fruit botrytis was examined in 19 commercial open-field and protected crops. Many samples showed a high incidence of infected fruit (>50%), even on protected crops sprayed with fungicides. Differences between open-field and protected crops, between sprayed and unsprayed crops and between two varieties (Glen Ample and Tulameen) were not statistically significant. The incidence of latent infection by *B. cinerea* in unripe fruit did not correlate with the incidence of botrytis fruit rot developing on ripe fruit.

Experiments were conducted in two commercial crops to investigate whether the removal of lateral leaves and thinning of primocanes during the flowering and fruiting period could reduce the incidence of fruit and cane infection by *B. cinerea.* Canopy manipulation resulted in considerable decreases in humidity inside the canopy at one site, where the original floricane density was very high and not at the second site where cane density was lower. Canopy thinning did not significantly reduce the incidence of fruit botrytis at either site but reduced the incidence of leaf and cane infection in the dense crop. Results suggest that a significant reduction of cane infection by canopy manipulation can be realised for situations where cane density and disease pressure are high. The present studies suggest that in dense canopies in a protected crop, cane lesions are more likely to result from direct infection of canes by the pathogen, although the pathogen can readily invade wounds on canes, including de-leafing wounds.

WOUND SHAPE AND AGE DEPENDENT RESISTANCE OF TOMATO STEM WOUNDS TO *BOTRYTIS CINEREA*

Tom BEYERS[1,2,3], Rudi AERTS[2], Kathleen HEYENS[2], Liesbeth VOGELS[2], Katrijn STOFFELS[2], Janick MATHYS[1], Monica HÖFTE[3] & Bruno CAMMUE[1]

[1] Centre of Microbial and Plant Genetics (CMPG), KU Leuven, Belgium
[2] Research group Sustainable Crop Protection, KH Kempen, Belgium
[3] Laboratory of Phytopathology, Ghent University, Belgium

Every year *Botrytis cinerea* causes severe losses in heated tomato greenhouses around the world. Although this necrotrophic fungal pathogen can theoretically infect all aerial parts of tomato plants, stem wounds are the main infection sites under the actual conditions in heated tomato culture. These wounds are created mainly during leaf pruning, a common and necessary cultural practice. As most workers pay little attention to the wounds they leave behind, the shape thereof tends to vary greatly. Our research clearly shows the impact of the wound shape on resistance of tomato stem wounds to *B. cinerea*. Using both natural and artificial inoculations with conidia in commercial and trial greenhouses the susceptibility to *Botrytis* infection of different types of wounds was examined. Due to the necrotrophic nature of this pathogen, it prefers necrotic tissue. When leafs are not removed properly small tabs of petiole epidermis are left behind, or tears and rips are created in the stem epidermis. Obviously this produces a considerable amount of dead cells, which facilitates *Botrytis* infections. Smooth wounds contain a minimum of necrotic tissue, and are therefore less favourable to *B. cinerea*. Nevertheless, smooth petiole stubs, resulting from incomplete leaf removal, are highly susceptible. By contrast, complete and proper removal of the petiole almost immediately results in absolute resistance to *B. cinerea*. Additionally, experiments on the timing of plant defence activation in petiole stubs and proper stem wounds were conducted. Wounds of both types were artificially inoculated at different time points postwounding. Proper stem wounds showed a very low initial susceptibility to *B. cinerea* and a quick built up of absolute resistance during the first hours after wounding. Petiole stubs, on the other hand, were highly susceptible at first and required several days to deploy effective defences. These findings are backed by the results of extensive tests in commercial greenhouses in which the shape of thousands of naturally infected wounds was registered. *B. cinerea* was completely unable to infect proper, smooth stem wounds under the actual conditions of heated tomato greenhouses. This notorious tomato pathogen can thus be effectively controlled by fine-tuning the cultural practice of leaf pruning, a sustainable alternative for chemical control.

BACILLUS-BASED BIOCONTROL OF *FUSARIUM* DISEASE ON TOMATO CULTURES IN BURUNDI

Venant NIHORIMBERE[1,3], Marc ONGENA[1,2], PascaL KAKANA[3] & Philippe THONART[1,2]

[1] Walloon Center of Industrial Biology, Gembloux Agricultural University
Passage des déportés 2, BE-5030 Gembloux, Belgium
E-mail: venant.nihorimbere@gmail.com
[2] Laboratory of Microbial Technology, University of Liege
[3] Faculty of Agronomy, University of Burundi

Biocontrol potential of *Bacillus subtilis* S499 on tomato was studied in experimental field sites in low altitude area of the plain of Imbo, Rural-Bujumbura province, Burundi. These assays were performed in order to control a locally important fungal disease. The causing pathogen was isolated from diseased leaves of tomato plants at different locations in the fields. This pathogenic isolate was identified as *Fusarium* according to the morphology of macroconidia, microconidia and their arrangement in chains or false heads, the size and type of conidiophore, and the presence or absence of chlamydospores. Based on the cultural characteristics and morphology, the isolate mostly predominantly found in that area is closely related to *Fusarium semitectum*. It causes blight symptoms and foliar necrosis leading to plant death. This blight is characterised by a brown to black discoloration of the leaves. Such disease was not yet met in Burundi and especially on tomato where numerous diseases are hitherto routinely chemically controlled. Results of assays performed in two successive years on the same site indicate that bacterial treatment of seeds and seedlings significantly improved growth and fruit yield. It also provided a high level of protection (20 %) against the disease caused by the Fusarium pathogen. Our results also demonstrate that the bacterial strain grew well in natural field conditions and showed a good capacity of root colonization. This is the first reported study on that disease that is significantly impacting tomato plantings in Burundi. These results suggest *that B. subtilis* S499 can be considered as an effective solution to that disease because it may work as biocontrol agent where many other options have failed.

SUSCEPTIBILITY OF CHRYSANTHEMUM AND PARIS DAISY VARIETIES TO SEVERAL ISOLATES OF *FUSARIUM OXYSPORUM* F.SP. *CHRYSANTHEMI*

Angelo GARIBALDI, Domenico BERTETTI & Maria Lodovica GULLINO
Centre of Competence for the Innovation in the Agro-environmental Sector (AGROINNOVA), University of Turin
Via Leonardo da Vinci 44, IT-10095 Grugliasco (TO), Italy

Fusarium oxyporum f.sp. *chrysanthemi* is a pathogen recently reported in Italy on four economically important species belonging to the Compositae family: chrysanthemum (*Chrysanthemum morifolium*), Paris daisy (*Argyranthemum frutescens*), African daisy (*Osteospermum* sp.) and gerbera (*Gerbera jamesonii*). The risk of transmission of the pathogen among these species is really dangerous because the hosts are frequently cultivated in the same nursery.
Several trials were carried out to evaluate the susceptibility of 24 Paris daisy and 12 chrysanthemum cultivars to several isolates of *F. oxyporum* f.sp. *chrysanthemi*. Paris daisy and chrysanthemum 45-days-old plants were respectively inoculated with 4 and 10 strains isolated from chrysanthemum, Paris daisy, African daisy and gerbera. Plants were inoculated with conidial suspensions at the concentration of 1 x 10^7 colony forming units/ml and cultivated in greenhouse, with temperatures ranging from 25 to 28°C. After the appearance of symptoms, plants were checked weekly, using a 0-100 scale (0 = absence of symptoms; 100 = dead plant).
All the isolates used on Paris daisy showed high virulence. Among the 24 tested cultivars, only "Sole mio", "Butterfly" and "Maria" were resistant to all isolates of *F. oxyporum* f.sp. *chrysanthemi*. Among the tested chrysanthemum cultivars, "Menthise bianco", "Cottonball", "Super Yellow" and "Meribell" were resistant to all the tested strains. The isolates used in the trials showed a different virulence towards the tested cultivars and this aspect need to be investigated further. The strain isolated from gerbera in Brazil had a different host range, showing that it probably belongs to a different *forma specialis*.

APPLICATION OF MOLECULAR DETECTION TECHNOLOGY IN PEST RISK ANALYSIS OF *FUSARIUM FOETENS,* A PATHOGEN OF *BEGONIA X HIEMALIS*

Hanneke HUVENNE, Jane DEBODE, Martine MAES & Kurt HEUNGENS
Institute for Agricultural and Fisheries Research (ILVO)
Plant Crop Protection, Merelbeke, Belgium
E-mail: kurt.heungens@ilvo.vlaanderen.be

Fusarium foetens is a recently (2004) described *Fusarium* species that causes an aggressive vascular disease in *Begonia x hiemalis*. Based on its aggressiveness and exotic nature, it has been added to the EPPO alert list. The recent description of real-time PCR primers for specific and quantitative detection of *F. foetens* (De Weerdt *et al*, 2006) has opened possibilities for additional research in the pathways of spread and the host range of this pathogen.
Sample type, sample size, and DNA extraction techniques were optimized to detect the pathogen in samples of practical size with sufficient sensitivity. These optimized protocols allow detection of as little as 50 spore equivalents in samples of up to 1 litre drain water and detection of less than 1500 spore equivalents per entire plant root system. Using these protocols, the presence of the pathogen was determined in drain water samples from commercial ebb and flow irrigation systems and in plants of various ages, including starting material. We also determined the accumulation rate of pathogen DNA in the plant after artificial inoculation. Based on these combined data, likely pathways of spread into and within commercial nurseries have been deducted. Fifteen plant species that are cultivated in the same niche as *Begonia x hiemalis* were inoculated and subjected to symptom analysis and PCR-mediated detection after a 12 week cultivation period. This revealed that several plant species may be latent carriers of the disease. The data contributed to an adjusted pest risk analysis.

EFFECTIVENESS OF ESSENTIAL OILS ON THE IN VITRO GROWTH OF POSTHARVEST FUNGI

K.H.S. KOUASSI[1,2], M. BAJJI[1], Y. BROSTAUX[3], A. ZHIRI[4], D. BAUDOUX[4], A. SAMB[2], P. LEPOIVRE[1] & M.H. JIJAKLI[1]

[1] Plant pathology Unit, Gembloux Agricultural University
Passage des Déportés 2, BE-5030 Gembloux , Belgium
[2] Laboratory of chemistry and biochemistry of natural products
Cheikh Anta Diop University of Dakar, Senegal,
[3] Statistics, Science and Applied Mathematics Unit, Gembloux Agricultural University
[4] Pranarôm International S.A.
E-mail: kouassi.k@fsagx.ac.be

Plants extract, such as essential oils, which have long been used in traditional preparations, are currently used in modern formulations adapted to the industrial manufacturing of consumer products. In agriculture, it was reported that essential oils could be an interesting alternative to chemical fungicides and could be used as biofungicides in postharvest biological control. The finally main objective of the present work is the identification and characterisation of effective essential oils against postharvest pathogens. The study began with the selection of thirty species of essential oils selected according to intrinsic (yield, phytotoxicity) and extrinsic (availability, cost, popularity) criteria. Then, we have developed a protocol based on a microscale bioassay Elisa plate using the optical density for a rapid quantitative and qualitative assessment of biofungicidal activity of essential oils. The preliminary study of optimal conditions for growth of fungi tested in absence and presence of alcohol has permitted to set the negative control (10.4 conidia/ml; 0.01 v/v orange juice; 0.5% alcohol). Evaluation of the biological effectiveness of essential oils (positive control) against the three postharvest phytopathogenic fungi (*Penicillium digitatum, Penicillium italicum*, and *Colletotrichum musea*) and the determination of the MIC and MCF is currently in progress. Some essential oils tested until now present high percentage fungus growth inhibition. Three of them were effective against all fungi at 100 ppm. This is a first encouraging study for the development of biofungicides based on essential oils as an alternative to chemical fungicides.

ABIOTIC STRESSES SEQUENTIALLY APPLIED ENHANCE NATURAL RESISTANCE AND REDUCE POSTHARVEST DECAY

G. D'HALLEWIN[1], G.M. MOLINU[1], A. DORE[1], T. VENDITTI[1] & V. RODOV[2]

[1] Consiglio Nazionale delle Ricerche, ISPA - Unità di Sassari
Traversa La Crucca 3, IT-07040 Sassari, Italy
[2] Institute for Technology and Storage of Agricultural Products
Dept. of Postharvest Science of Fresh Produce A.R.O.
The Volcani Center, P.O. Box 6, IL-50250 Bet Dagan, Israel

The demand for alternatives to synthetic postharvest fungicides has implemented the researches on biological control agents, generally recognized as save compounds (GRAS), physical methods along with studies focused at enhancing fruit natural resistance. In this direction, natural resistance of citrus fruit (oranges and lemons) was studied following sequential treatments with heat and ultraviolet light C (254 nm, UV-C). Heat treatment (HT) was performed by keeping fruits in a humid saturated room at 36 °C for 36 h, while, UV-C light treatment by using 3W lamps (254 nm) providing 3 or 6 kJm^{-2}. The physical treatments were sequentially and reversely applied to 24 h old inoculated and not inoculated fruit with *Penicillium digitatum* followed by 30 days of storage at 8 or 5 °C with 95% relative humidity (RH) and a 6 day simulated marketing period at 20 °C and 75% RH. Natural resistance was monitored in terms of preformed (cuticle and epicuticular wax) and induced (scoparone biosynthesis) resistance and correlated to decay development. HT influenced positively the mechanical barrier to pathogen penetration by containing cuticular cracks and by remodelling the epicuticular wax layer. HT induced scoparone synthesis only in wounds, especially when inoculated. In UV-C treated fruit scoparone accumulated rapidly in the outer rind (flavedo) but created, particularly with 6 kJm^{-2}, cuticle ruptures. Compared to treatments applied separately, combined ones resulted significantly more effective in controlling decay on either, inoculated and non inoculated fruit. Between the sequential treatments the best result was obtained when HT preceded UV-C lightening. Compared to treatments performed singularly, the combination of the two reduced the decay in uninoculated lemon fruit by 34 and 52%, respectively, and in oranges by 49 and 45%. The same trend was observed with inoculated fruits but to a much greater extend. The involvement of induced and preformed natural resistance will be discussed and evidence will be provided to support the role of these factors in the control of decay.

USE OF CDNA-AFLP TO STUDY THE DEFENCE-RELATED GENE EXPRESSION IN BANANAS (*MUSA* SPP.), INOCULATED WITH *COLLETOTRICHUM MUSAE* RESPONSIBLE OF CROWN ROT

Ludivine LASSOIS[1], Luc DE LAPEYRE DE BELLAIRE[2] & M. Haïssam JIJAKLI[1]
[1] Plant Pathology unit, Gembloux Agricultural University, Belgium
[2] CIRAD-PERSYST, Montpellier, France

Crown rot disease of bananas is widespread in producing countries and is considered as the most important post-harvest disease of exported bananas. Variations of susceptibility to the disease have been noted between bananas but the origins still unknown. The biological responses of the fruit, including physiological change and disease susceptibility are controlled and regulated by gene expression. One way to understanding the reactions involved in variation of banana susceptibility to the disease in relation to their physiological state, is to study the expression of genes involved in these processes. To this purpose, crown sample previously inoculated with *C. musae* and showing 2 levels of susceptibility (very high and very low) were collected to be compared. Crown sample of each susceptibility level was collected at two different maturity stages: at harvest and 13 days after harvest (3 days after ripening). Collected crowns were immediately freeze-dried, an original method to conserve gene expression. cDNA-AFLP was applied on these 4 cell populations in order to highlight the differential transcription of genes whose function is "a priori" unknown. The cDNA-AFLP result was confirmed using quantitative real-time reverse transcription PCR. Various defence-related genes were identified and will be presented.

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DICKEYA SPP. (SYN. *ERWINIA CHRYSANTHEMI*) – A NEW AND SERIOUS THREAT FOR SEED POTATO PRODUCTION IN EUROPE

Robert CZAJKOWSKI & Jan M. VAN DER WOLF
Plant Research International
P.O. Box 16, NL-6700 AA, Wageningen, The Netherlands
E-mail: robert.czajkowski@wur.nl, jan.vanderwolf.@wur.nl

Potato blackleg caused by *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium atrosepticum* and *Dickeya* species is a worldwide disease creating serious economical losses in seed potato production. Effective management to control blackleg in the field is lacking and validated detection methods for blackleg-causing bacteria do not exist. Simultaneously, the knowledge of the ecology of blackleg pathogens is incomplete.

In the past *P. atrosepticum* was responsible for the majority of blackleg infections in Europe. Recently, however an increasing frequency of *Dickeya* spp. in seed potatoes is observed.

Dickeya spp. have been recently grouped into six species. Up till five years ago, *D. dianthicola* (*E. chrysanthemi* biovar 1 and 7) was associated with potato in temperate climates in Europe.

Recently a new strain belonging to biovar 3, but not classified within the described six *Dickeya* species appeared in Europe. The new biovar 3 strain, called *D. solani* possesses a higher growth temperature optimum than the biovar 1 and 7 strains. Exclusively *D. solani* was isolated from seed potatoes in The Netherlands in last three years. The same species was isolated in, Finland, Poland and Israel indicating that it is widely spread in Europe. All these isolates were clonal, demonstrating its common origin.

Studies on the distribution of a *D. solani* strain in seed potatoes revealed that the pathogen was located mainly inside tubers at stolon ends and rarely in the peel and deeper located tissue indicating a vascular origin of the pathogen. In line, we found that a GFP-tagged biovar 3 strain systemically colonized progeny tubers via the roots after soil infestation and was able to produce blackleg symptoms. Systemic colonization of plants including roots, stolons and progeny tubers, was also found after injection of the strain into intact potato stems. A biovar 7 strain was not able to colonize the plants effectively after root or stem inoculation. Potato leaves inoculation with a GFP-tagged biovar 3 strain, showed degradation of the inoculated plant material and spreading of the internal inoculum to the petiole and axil and finally to the main stem.

In conclusion, biovar 3 *Dickeya solani* was proven to be the major blackleg pathogen nowadays in Europe. It efficiently colonizes various plant tissues and seems to displace *D. dianthicola* and *P. atrosepticum* from the potato ecosystem.

GENETIC AND PATHOGENIC DIVERSITY OF *ERWINIA CHRYSANTHEMI* (*DICKEYA* SP.) IN SEED POTATOES FROM FLANDERS

Rachid TAHZIMA, Annemie HOEDEKIE, Brigitte DE PAEPE, Martine MAES & Johan VAN VAERENBERGH

Institute for Agricultural and Fisheries Research, Plant Unit: Crop Protection Burgemeesters
Van Gansberghelaan 96 bus 2, NL-9820 Merelbeke, Belgium
E-mail: johan.vanvaerenbergh@ilvo.vlaanderen.be

Incidence of *Erwinia chrysanthemi* (Ech) has significantly increased in seed potatoes in Flanders and other regions in Western Europe. Ech isolates were collected over a 15-year period (1993-2008) and investigated by comparing genetic and pathogenic properties. Ech isolates were characterized based on the pectate lyase ADE gene cluster amplicon and genetic variability was assessed by producing RFLPs of the *mdh*, *suf*A and *rec*A genes and of several virulence-related genes, including six pectate lyase genes, the pectate lyase regulatory gene *kdg*R, the pectin methylesterase gene, the type III secretion gene *hrp*N and the indigoidin and flagella synthesis genes *ind*C and *fli*C. The isolates were consistently classified in two gene polymorphism groups. PCR-RFLP of the 16S-23S rDNA spacer and ERIC-PCR profiling revealed a greater genotypic diversity.

The isolates displayed temperature dependent pectinolytic activity and variable reactions in tobacco and chicory leaves, in potato tubers and potato plantlets. The study indicates that the Ech population type with weakly macerating, HR-minus properties and a pectinolytic optimum at 25-28°C has not been recovered from field samples in the past five years. This current population consists of a strongly macerating, HR-positive type with a pectinolytic optimum of 32-36°C. These isolates clustered in one polymorphism group and one specific ERIC profile type.

TOWARDS DISEASE RESISTANCE IN POTATOES USING INTRAGENIC APPROACHES

Sathiyamoorthy MEIYALAGHAN[1,2,*], Sara MOHAN[1,2], Julie M. PRINGLE[1], Jeanne M.E. JACOBS[1,2] & Anthony J. CONNER[1,2]

[1] New Zealand Institute for Plant & Food Research Limited
Private Bag 4704, Christchurch, New Zealand
[2] Bio-Protection Research Centre, Lincoln University
P.O. Box 84, 7647 Canterbury, New Zealand
[*] E-mail: sathiyamoorthym@crop.cri.nz

Disease resistance is an important objective of global potato breeding programmes. The use of resistant cultivars is a significance tool for disease management. Recent advances in plant molecular genetics have allowed the identification and cloning of numerous genes for resistance to potato diseases from within the germplasm pool available to potato breeders. Antimicrobial peptides, such as the Snakin-1 (*StSN1*) and Snakin-2 (*StSN2*), have been recently isolated from potato tubers. Over-expression of the *StSN1* and *StSN2* genes is known to provide broad spectrum activity against a wide range of bacterial and fungal pathogens in potato. *RB* or *Rpi-blb1* gene from *Solanum bulbocastanum* is known to confer resistance against the pathogen *Phytophthora infestans* causing late blight disease. *StSN1*, *StSN2* and *RB* are well known to provide effective disease resistance in transgenic potato cultivars. However, development of transgenic potato with non-potato DNA has raised public concerns, especially when the transferred genes are expressed in the plant components utilised as food. To circumvent this issue we are investigating the use of intragenic gene transfer technology to transfer disease resistance in potato without the incorporation of foreign DNA.

To date an expression cassette was constructed with the 5' promoter and 3' terminator regions from the potato chlorophyll a/b binding gene (*StLhca3*). The coding regions of the *StSN1*, *StSN2* and *RB* genes were carefully cloned individually between the promoter and terminator regions to avoid the incorporation of any non-potato nucleotides. The resulting chimeric genes (intragenes) were individually cloned into the potato-derived T-DNA-like region for potato transformation. The potato cultivar Iwa has been co-cultivated with *Agrobacterium* harbouring binary vectors with the *StSN1* and *StSN2* genes. Putatively transformed potato plants with the *StSN1* and *StSN2* genes have been regenerated and PCR screening is being performed to identify plants transformed with these disease resistance genes.

IRRIGATION WATER AS INOCULUM SOURCE FOR *PSEUDOMONAS CICHORII,* THE CAUSAL AGENT OF MIDRIB ROT IN GREENHOUSE BUTTERHEAD LETTUCE

E. PAUWELYN[1,2], B. COTTYN[3], K. VANHOUTEGHEM[2], M. HÖFTE[1], P. DE VOS[4], M. MAES[3] & P. BLEYAERT[2]

[1] Laboratory for Phytopathology, Department Crop protection, Ghent University, Ghent, Belgium
[1, 2] Provincial Research and Advisory Center for Agriculture and Horticulture Rumbeke, Belgium
[3] Unit Plant – section Plant Protection, Institute for Agricultural and Fisheries Research Merelbeke, Belgium
[4] Laboratory for Microbiology, Department WE10, Ghent University Ghent, Belgium
E-mail: ellen.pauwelyn@Ugent.be

During the past 10 years, bacterial midrib rot caused by *P. cichorii* has become a serious threat for greenhouse lettuce (*Lactuca sativa* L. var. *capitata*) production in Flanders, Belgium. Bacterial midrib rot is characterised by a dark brown rot of the midrib of one or more inner crop leaves. The emergence of this disease coincided with the transition of the lettuce production from open field to sheltered cultivation in greenhouses, where the crop depends on overhead irrigation. This suggests the irrigation water to be a possible source of inoculum. The search for inoculum sources and the evaluation of their impact on the disease epidemiology are essential steps in developing control strategies.

Field experiments showed that a single irrigation with water containing 10^2 CFU/ml *P. cichorii* on nearly mature plants can result in serious midrib rot infections. This emphasises the importance of detecting low amounts of *P. cichorii* in irrigation water samples. Therefore, a real-time PCR system with detection sensitivity of 1 CFU/ml was developed. PCR primers and a MGB-probe targeting sequences of the conserved hypersensitive reaction/pathogenicity (*hrp*)-genes were designed.

To monitor *P. cichorii* in irrigation water, samples from either surface or well-water sources of seven commercial lettuce growers were collected over a year. *P. cichorii* was detected more frequently in surface water samples than in well water samples. Particularly in surface water samples, temporal fluctuations in concentration of *P. cichorii* populations were observed with increasing recurrence during warmer periods. Concentrations of *P. cichorii* in irrigation water ranged from <1 to 10^3 CFU/ml with the latter concentration highly exceeding the infection threshold.

Our results have shown that *P. cichorii* can survive in irrigation water at low concentrations and that this water is probably the most important inoculum source in the epidemiology of bacterial midrib rot of lettuce in Belgian greenhouses. Monitoring *P. cichorii* populations in irrigation water is a first step towards a better management of this disease.

THE USE OF FLOW CYTOMETRY FOR THE DETECTION OF *PSEUDOMONAS CICHORII*

Liesbet D'HONDT[1], Leen LEUS[1], Johan VAN VAERENBERGH[1], Bart COTTYN[1], Erik VAN BOCKSTAELE[1,2] & Monica HÖFTE[2]

[1] Institute for Agricultural and Fisheries Research, Plant Unit
Caritasstraat 21, BE-9090 Melle, Belgium
[2] Ghent University, Phytopathology lab, Coupure links 653, BE-9000 Ghent, Belgium
E-mail: liesbet.dhondt@ilvo.vlaanderen.be

Pseudomonas cichorii is the causal agent of midrib rot of greenhouse-grown lettuce leading to severe losses and reduced market values. This pathogen is able to survive and multiply in the irrigation water and can cause infection at concentrations as low as 100 CFU/ml. Pathogen concentration can rapidly rise after a warm period. To be sure that irrigation water is free of *P. cichorii,* at least four water samples, each taken after a warm period, should be analysed every year for each grower. A real-time PCR protocol was developed for detection of *P. cichorii*. This method is very sensitive, having a detection limit of 1 CFU/ml, but it also has high labour and material costs.

Flow cytometry (FCM) was originally developed as a rapid technique to analyze blood samples for medical diagnosis and is still used for countless medical applications. In plant science, flow cytometry is known for ploidy analysis. Although flow cytometry can be a promising tool for the detection of plant pathogens as it is rapid, sensitive and quantitative, applications for plant pathology are almost non-existent. Additionally, FCM can discriminate living cells from dead ones and does not rely on the ability of bacteria to grow on culture media.

We evaluated whether flow cytometry can be used as an alternative for the detection of *P. cichorii* in irrigation water from greenhouse lettuce production. Immunomagnetic separation was used to selectively capture and concentrate *P. cichorii* in water samples, followed by viability staining and flow cytometric analysis. Paramagnetic beads were coated with antiserum against *P. cichorii* and added to the water samples. Captured bacteria were magnetically isolated and washed, followed by live/dead staining with syto9 and propidium iodide. The samples were analysed with the flow cytometer and bead-bound bacteria were identified by their fluorescence and scatter properties. Influence of bead/bacteria ratio, sample volume, incubation time and washing method on the specificity and recovery efficiency will be discussed.

SUSTAINABLE CONTROL OF *PHYTOPHTHORA PORRI* IN LEEK (*ALLIUM PORRUM*): FINE-TUNING THE MODEL

B. DECLERCQ[1], N. CAP[2], J. DE NIES[3], S. POLLET[4] & M. HÖFTE[1]
[1] Laboratory of Phytopathology, Faculty of Bioscience Engineering, Ghent University
Coupure Links 653, BE-9000 Gent, Belgium
E-mail: Bart.Declercq@ugent.be
[2] Vegetable Research Centre (PCG)
Karreweg 6, BE-9770 Kruishoutem, Belgium
[3] Research Station for Vegetable Production (PSKW)
Duffelsesteenweg 101, BE-2860 Sint-Katelijne-Waver, Belgium
[4] Provincial Research and Advisory Center for Agriculture and Horticulture (POVLT)
Ieperseweg 87, BE-8800 Rumbeke, Belgium

Leek (*Allium porrum*) is an important crop in Europe and more specifically in Belgium. During the last fifteen years, the Belgian turnover of leek has tripled. Nowadays, the Belgian leek area is stabilizing at about 4,500 ha. *Phytophthora porri* is a major disease of leek, causing yield and quality losses in cold and humid conditions, mainly in autumn and winter. A 4 year project of Ghent University together with three Flemish vegetable research stations (PCG, PSKW and POVLT) aimed to develop a predictive model for *P. porri* which will help farmers to spray with more effective timing.

During the project various field trials and laboratory experiments have been executed to better understand the disease cycle of *P. porri.* The main factor triggering the disease turned out to be precipitation. Six different rainfall-based models were tested in the field during the season 2007-2008. All models performed statistically better than the untreated control, but no statistical significant differences were observed between the models. Although one model received 4 treatments less than the positive control which was sprayed every 2 weeks, the disease was controlled to a similar level. Besides these rainfall based models, some new models based on the difference in leaf wetness and relative humidity between two consecutive days were tested in the field during 2008-2009 without better results. The models based on rainfall outcompeted all other models and performed as well as the positive control.

All trials with model-supported spraying clearly show that rainfall is the major factor in the development and the spreading of *P. porri.* It is important to treat timely against this disease, mainly in periods of substantial rainfall. Therefore all currently registered fungicides were tested on their rain fastness.

FUNCTIONALITY OF THE *PHYTOPHTHORA RAMORUM* SEXUAL SYSTEM

Xavier BOUTET[1], Annelies VERCAUTEREN[2], Kurt HEUNGENS[2] & Anne CHANDELIER[1]

[1]Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium
[2]Institute for Agricultural and Fisheries Research (ILVO)
Plant Crop Protection, Merelbeke, Belgium

Phytophthora ramorum (Werres, De Cock, Man in't Veld) causing "Sudden Oak Death" in the United States and dieback and leaves necrosis in ornamental plants (mainly *Rhododendron* and *Viburnum)*) in Europe is a heterothallic species with two compatibility types, A1 and A2. Initial pairing studies revealed that all European isolates were of A1 type whereas all the American isolates were of A2 type. Since 2003, three Belgian isolates were designated as European A2 while some A1 isolates were reported in American nurseries, therefore, suggesting a possible crossing between both mating types. However, attempts to produce oospores *in vitro* with classical methods were difficult compared to other heterothallic species, therefore suggesting a weak functionality of the sexual system in *Phytophthora ramorum*. The European A1 strain 2299, producing very few chlamydospores, was found to be a better mating partner than other A1 strains when paired with some European (EU) or American (US) A2 strains. Moreover, sexual spores (= oospores) were produced mainly in the growth sector of the 2299 strain.

Oospores were extracted and their viability was evaluated after different incubation times (60, 110, 250, 350 and 500 days) by staining with tetrazolium bromide (MTT) and by evaluating the germination capacity. Differences were obtained by comparing these two methods, the MTT method appeared to overestimate the germination ability. Germinated oospores were observed in several cases, even after 500 days, from EU x EU and EU x US crossings.

The characterisation of oospores progenies revealed a large variability in morphological features, growth rate and pathogenicity on *Rhododendron* leaves. Furthermore, the proportion of each mating type in the progeny seemed to be linked to the original pairing (EU x EU or EU x US). All the progenies presented genetic rearrangements according to their microsatellite profile. Moreover, mitochondrial analysis on EU x US progenies gave new evidence on the important role played by the European A1 strain 2299 during mating. All these data have to be discussed in a context of Pest Risk Analysis.

EVIDENCE OF ANEUPLOID GENOMIC REARRANGEMENTS IN THE PROGENIES OF *PHYTOPHTHORA RAMORUM*

Annelies VERCAUTEREN[1], Xavier BOUTET[2], Anne CHANDELIER[2], Martine MAES[1] & Kurt HEUNGENS[1]

[1] Institute for Agricultural and Fisheries Research (ILVO)
Plant Crop Protection, Merelbeke, Belgium
[2] Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium

Phytophthora ramorum is the oomycete plant pathogen responsible for "Sudden oak death", a disease responsible for significant mortality of several oak species in North America. *P. ramorum* was also found in Europe on nursery plants (mainly *Rhododendron* and *Viburnum*), causing twig dieback and leaf necrosis, but has not been found to cause extensive damage in forests. Genetic studies have shown that North American and European populations belong to the same species but are genetically distinct. *P. ramorum* is heterothallic, having two opposite mating types A1 and A2. The A1 mating type was originally only found in Europe (EU), with the exception of three A2 isolates of the European lineage collected in Belgium in 2002 and 2003 (Werres & De Merlier, 2003). Since 2003 the occurrence of a few isolates belonging to the European A1 lineage were reported in North America. Although there is no evidence for sexual reproduction in nature, the presence of both mating types at a single site might lead to genetic recombination through sexual outcrossing. The potential for exchange of genetic material might lead to an increase in the pathogens general fitness and host range. The formation of sexual resting spores could further increase the long-term survival potential of *P. ramorum*.

To test whether the sexual cycle in *P. ramorum* was functional, germinating oospores of a cross between a North American A2 isolate and a European A1 isolate (obtained by CRA-W), were genotyped with heterozygous microsatellite markers. All crosses contained alleles of both parents confirming exchange of genetic material between the two mating type parents, however meiotic irregularities were also observed. A considerable amount of non-Mendelian inheritance events was detected across the loci, including inheritance of more than two alleles at a locus or non-inheritance of alleles from one parent at another locus. The aneuploid progeny was mitotically unstable: zoospore and hyphal tip derivatives of the progenies showed genotypic rearrangements resulting in phenotypic variation.

OLIGOGALACTURONIDES INDUCE RESISTANCE IN WHEAT AGAINST POWDERY MILDEW

B. RANDOUX[1], D. RENARD-MERLIER[1], F. DUYME[2], J. SANSSENÉ[3], J. COURTOIS[4], R. DURAND[1] & Ph. REIGNAULT[1]

[1] Mycologie-Phytopathologie-Environnement, GIS PhyNoPi
Université du Littoral Côte d'Opale, France
[2] Arvalis-Institut du Végétal, Boigneville, France
[3] Ecole d'Ingénieurs de Purpan, Purpan, France
[4] Polysaccharides Microbiens et Végétaux, Université de Picardie Jules Verne, Amiens, France

Biological activities, priming and protective effects of two oligogalacturonides fractions were assayed during a compatible wheat/*Blumeria graminis* f.sp. *tritici* interaction. These fractions were obtained from polygalacturonic acid of *Citrus*. They both consisted of oligogalacturonides with polymerisation degrees (DP) ranging from 2 to 25, and one of them was a 30% chemically acetylated fraction.
A 5 $g.L^{-1}$ solution of each fraction was infiltrated in the first leave of ten-days-old plantlets, and activities of defence-related enzymes were measured 48H post-treatment. Among them, oxalate oxidase, lipoxygenase and peroxidase activities increased, suggesting an elicitation due to both fractions of oligogalacturonides.
Some of the pre-treated plantlets were subsequently submitted to powdery mildew infection. As revealed by 3,3'-diaminobenzidine (DAB) staining, the accumulation of hydrogen peroxide (H_2O_2) at the penetration site increased to the same extent in plantlets infiltrated by both fractions. On the other hand, the intensity of fluorescence associated with papillae was higher when plantlets were pre-infiltrated with the acetylated fraction, whereas no difference was observed between control plantlets and those treated with the non-acetylated fraction. Moreover, microscopic assessment of the number of haustoria showed it was only reduced when acetylated fraction was used.
Despite different modes of action of these molecules, a similar 45% protective effect occurred in both cases when the oligogalacturonides fractions were sprayed on ten-days-old plantlets.

MECHANISMS OF RESISTANCE AND TOLERANCE TO *MYCOSPHAERELLA GRAMINICOLA* IN WHEAT

Léa EL CHARTOUNI[1], B. RANDOUX[1], F. DUYME[2], D. RENARD-MERLIER[1], B. TISSERANT[1], P. HALAMA[3] & Ph. REIGNAULT[1]

[1] Mycologie-Phytopathologie-Environnement, GIS PhyNoPi
Université du Littoral Côte d'Opale, France
[2] Arvalis-Institut du Végétal, Boigneville, France
[3] Institut Supérieur d'Agriculture, GIS PhyNoPi, Lille, France

The aim of this study was to investigate the infection process of *M. graminicola* and the defence mechanisms related to active oxygen species (AOS) in five French wheat cultivars. These cultivars exhibited various resistant levels to *M. graminicola* infection: Maxyl, Caphorn and Gen11 are susceptible cultivars, whereas Capnor and Gen23 show high levels of quantitative resistances. In addition, Capnor, Gen23 and Gen11 are tolerant cultivars, *i.e.* their yield performance was less affected by infection compared to non-tolerant cultivars. These cultivars were inoculated with the IPO323 reference *M. graminicola* strain. First wheat leaves were collected 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21 days after inoculation. The cytological and antioxidant response of the cultivars were both studied over the whole time course. Although infection occurred mainly through stomata, direct penetration attempts were also scored. Moreover, papilla formation turned out to be very rare. Assays for changes in peroxydase (PO), glutathione-S-transferase (GST) and lipoxygenase (LOX) activities allowed us to compare their levels in the five French wheat cultivars regarding to their resistance and/or tolerance towards *M. graminicola* infection. PO and GST were correlated to necrosis probably as a consequence of detoxification and LOX was related to some of the germination process steps. We also showed that significant differences for several biochemical parameters exist between the studied cultivars in non inoculated conditions but these differences were less important in the presence of the fungus.

IN PLANTA XYLANASE ACTIVITY AND PATHOGENICITY ON WHEAT-*MYCOSPHAERELLA GRAMINICOLA* PATHOSYSTEM

Ali SIAH[1], Caroline DEWEER[1], Florent DUYME[3], Jean SANSSENÉ[4], Roger DURAND[2], Philippe REIGNAULT[2] & Patrice HALAMA[1]

[1] Biotechnologie des Micro-organismes, GIS PhyNoPi
Institut Supérieur d'Agriculture, Lille, France
[2] Mycologie-Phytopathologie-Environnement, GIS PhyNoPi
Université du Littoral Côte d'Opale, Calais, France
[3] Arvalis-Institut du Végétal, Boigneville, France
[4] Institut Polytechnique Lasalle-Beauvais, GIS PhyNoPi, Beauvais cedex, France

A total of twenty four French strains and two reference strains IPO323 and IPO94269 of the hemibiotrophic fungus *Mycosphaerella graminicola* were investigated for the first time *in planta* to examine the association of the cell-wall degrading enzyme endo-*β*-1,4-xylanase (EC 3.2.1.8) with pathogenicity. The French strains were selected from a collection of 363 strains previously genotyped using microsatellites, actine and ß-tubuline markers. Disease level assessments as well as enzyme quantifications were carried out at 20 days post inoculation from the third leaves of inoculated whole plants of the susceptible wheat cultivar Scorpion. Great variability of both pathogenicity levels and endo-*β*-1,4-xylanase activity patterns was obtained among strains. Only 15 out of the 26 assessed strains including the reference strain IPO323 were able to induce lesions bearing pycnidia. The percentages of diseased leaf areas bearing pycnidia ranged from 6.2 % to 77 %, while amounts of endo-*β*-1,4-xylanase activity ranged from 0 to 399.15 mU/µg of total proteins. A Pearson correlation test revealed very high linkage between endo-*β*-1,4-xylanase activity level and lesions bearing pycnidia production within strains ($r = 0.94$). Additional cytological and enzymatic investigations every 2 days for 22 days on two strains exhibiting different pathogenicity levels highlighted that successful disease induction is not conferred by either spore germination or direct and stomatal penetration rates of the host, but by the ability of the fungus to colonize the mesophyll and to secrete endo-*β*-1,4-xylanase activity during the necrotrophic phase. This study strongly suggests the importance of mesophyll colonization and endo-*β*-1,4-xylanase activity during the infection process of *M. graminicola*.

EFFECT OF PEA CULTIVAR, PATHOGEN ISOLATE, INOCULUM CONCENTRATION AND LEAF WETNESS DURATION ON ASCOCHYTA BLIGHT CAUSED BY *MYCOSPHAERELLA PINODES*

Benali SETTI[1], Mohamed BENCHEIKH[1], JameL HENNI[2] & Claire NEEMA[3]

[1] Institut de Biologie, Université de Chlef, BP 151, DZ-02000 Chlef, Algérie

[2] Institut des Sciences, Université d'Es Senia, Oran, Algérie

[3] UMR de Pathologie Végétale, INRA/INA-PG/Université Paris VI

16 rue Claude Bernard, FR-75231 Paris Cedex, France

The effect of host leaf wetness duration, *Mycosphaerella pinodes* inoculum concentration and pathogen isolate on the latent period and the incubation period of the pathogen or disease severity were quantified on pea (*Pisum sativum* L.). Seedlings of two widely grown pea cultivars, Onward and Merveille de Kelvedon, respectively susceptible and moderately resistant to *M. pinodes* were subjected to six leaf wetness durations of 6, 12, 24, 48 and 72 h, and inoculated with fi ve inoculum concentrations, 2.5×103, 4×104, 3.5×105, 4x106, and 5.2×107, in order to determine whether the cultivars reacted differently to *M. pinodes* isolates inoculated under identical conditions. Increasing the duration of leaf wetness and inoculum concentration caused significant (P<0.001) increases in disease severity within each cultivar. Both the incubation period and the latent period decreased with increasing conidial concentration and leaf wetness duration. Generally, the cv. Onward had a significantly shorter incubation period, and latent period and higher disease severity than cv. Merveille de Kelvedon. Isolates differed in aggressiveness at higher levels of leaf wetness (48 h) duration and of inoculum concentration (4×106), but there was no significant interaction between isolates and leaf wetness duration, or between isolates and inoculum concentration. The optimum levels for obtaining a consistent infection and for readily separating the susceptible and the partially resistant cultivars were a leaf wetness of 48 h and an inoculum concentration of 4×106. The study also showed that continuous leaf wetness for 48 h was a threshold for application of fungicides to control the fungus in the susceptible cultivar.

GENETIC VARIABILITY AND POPULATION STRUCTURE OF *MYCOSPHAERELLA PINODES* IN WESTERN ALGERIA USING AFLP FINGERPRINTING

Benali SETTI[1], Mohamed BENCHEIKH[1], Jamel HENNI[2] & Claire NEEMA[3]

[1]Institut de Biologie, Université de Chlef, BP 151, DZ-02000 Chlef, Algérie

[2]Institut des Sciences, Université d'Es Senia, Oran, Algérie

[3]UMR de Pathologie Végétale, INRA/INA-PG/Université Paris VI

16 rue Claude Bernard, FR-75231 Paris Cedex, France

The genetic diversity and population structure of *Mycosphaerella pinodes* were investigated by AFLP markers. A total of 75 isolates from different collections sites corresponding to four geographic regions were analysed. Data from AFLP loci were used to estimate gene diversity, genetic distance and to make indirect measures of gene flow between population groups. Extensive diversity was detected in the *M. pinodes* population regardless of the population group. The percentage of polymorphic loci varied from 40.88 in semi arid superior region to 50.30% in subhumid region. Nei's gene diversity across loci was 0.471 and the Shannon index across loci was 0.663. A high level of differentiation (Gst=0.308) and low gene flow (Nm=1.118) among population group were revealed. Based on analysis of molecular variance, 58% of the genetic variation of *M. pinodes* was within population, 31% differences between regions and 11% among populations within region. Although UPGMA clustering established through Nei's genetics distances, separated the four populations according to their geographic group appurtenances, a cluster analyses using individual isolates failed to group them according to population. This is the first report on genetic diversity and population structure of *M. pinodes* on peas in Algeria.

Session 8
Fungicides

EFFECT OF ADJUVANTS ON THE EFFICACY OF FUNGICIDES AGAINST *FUSARIUM* HEAD BLIGHT

B. HEREMANS[1], K. DEWITTE[1], B. PYCKE[1], A. PERSYN[1], B. RYCKAERT[2], M. DE SCHAMPHELEIRE[2], P. SPANOGHE[2], W. STEURBAUT[2] & G. HAESAERT[1]

[1] University College Ghent, Faculty of Biosciences and Landscape Architecture, Voskenslaan 270, BE-9000 Gent, Belgium

[2] Ghent University, Department of Crop Protection Coupure Links 653, BE-9000 Gent, Belgium

Fusarium head blight (FHB) is a devastating disease of wheat and barley which causes extensive losses worldwide. *Fusarium avenaceum*, *Fusarium culmorum, Fusarium graminearum* (teleomorph *Gibberella zeae*), *Fusarium poae* and *Microdochium nivale* (teleomorph *Monographella nivalis*) are the species most commonly associated with the disease. Infection by ascospores of *Fusarium* species is initiated in wheat florets at anthesis, from where the fungi spread to other spikelets within the spike. Eventually, the fungi-infected spikelets become necrotic and bleached. Grain from *F. culmorum*-, *F. graminearum*- and *F. poae*-infected plants accumulate the mycotoxin deoxynivalenol (DON), a vomitoxin, which further limits grain quality. This mycotoxin causes a wide range of acute and chronic effects in humans and animals. The best control methods combine the planting of cultivars that are partially resistant to FHB with fungicide application at anthesis and rotation with non-host crops. Conflicting evidence exists regarding the effect of fungicides on the development of FHB and on the DON concentration. During three successive growing seasons the effect of combining tebuconazole with different adjuvants was investigated on the development of FBH, grain yield and DON concentration.

TOXICITY OF COPPER / DIMETHOMORPH COMBINATION FOR *LEMNA MINOR* AND DEPURATION OF THE FUNGICIDES BY AQUATIC PLANT

Smain MEGATELI[1,2], Rachel OLETTE[1], Saida SEMSARI[2] & Michel COUDERCHET[1]

[1] Laboratoire des Plantes, Pesticides et Développement Durable, URVVC-SE. UFR Sciences
Université de Reims Champagne-Ardenne
BP 1039, FR-51687 Reims, France

[2] Laboratoire de Génie Chimique, Département de Génie des Procédés, Université Saad Dahlab,
BP 270, route de Soumaa, Blida, DZ-09000, Algérie

Runoff-water of Champagne vineyards is often collected in decantation ponds where pesticides may be eliminated before the water is transferred to rivers. In order to accelerate this process, *Lemna minor* was tested for its phytoremediation capacity. In the ponds several pesticides coexist. Therefore, the cross-influence of copper and dimethomorph, two pesticides frequently used on grape to control fungal diseases, was studied on toxicity and their removal by *L. minor*. The toxicity of copper and dimethomorph alone and in combination, was assessed by growth inhibition of *L. minor* cultures after 4 and 7 days. Copper had a severe impact on growth (maximum inhibition: 90 % at 1 mg/L) while dimethomorph (as pure ingredient or formulated as Forum®) did not (inhibition < 45 % at 1 mg/L) after 7 days of treatment. When both chemicals were combined, a synergistic effect was detected after 4 days of exposure to copper and Forum. However, this interaction tented toward additivity after 7 days. Additivity was also observed when the pure active ingredient replaced Forum in the mixture of copper and dimethomorph at 96h00 and 168h00. The removal of copper depended on Forum concentration. For example, with 0.25 mg/L of dimethomorph as Forum, removal of copper increased from 36 to 65 %. Depuration of dimethomorph (as Forum) by *L. minor* varied between 15 and 44 % after 4 days. This depuration decreased in the presence of copper possibly due to the metal toxicity.

CHEMICAL CONTROL OF DOWNY MILDEW ON LETTUCE AND BASIL UNDER GREENHOUSE

Maria Lodovica GULLINO, Giovanna GILARDI & Angelo GARIBALDI
Centre of Competence for the Innovation in the Agro-environmental Sector
(AGROINNOVA), University of Turin
Via Leonardo da Vinci 44, IT-10095 Grugliasco (TO), Italy

Experiments were conducted during 2007 and 2008 to evaluate the efficacy of different fungicides against downy mildew of lettuce (*Bremia lactucae*) and of basil (*Peronospora* sp.) under greenhouse condition at temperatures ranging of 20-22°C. The mixture fluopicolide + propamocarb hydrochloride (containing a fungicide belonging to the new chemical class of acyl-picolides) was compared with metalaxyl+Cu, zoxamide+mancozeb, iprovalicarb+Cu, fenamidone+fosetyl-Al and azoxystrobin. Two treatments were carried out at 8-12 day intervals on lettuce and basil. The artificial inoculation of *B. lactucae* on lettuce (cv Cobham Green) and *Peronospora* sp. on basil (cv Genovese gigante) was carried out by using $1x10^5$ CFU/ml 24 h after the first treatment. In the presence of a medium–high disease severity, fluopicolide + propamocarb hydrochloride was at least as effective against downy mildew on lettuce and basil as the other fungicides used.

STUDY OF USE OF DIFFERENT TYPES OP HYDROGEN PEROXIDES (2006-2008)

Marc VISSERS[1], Pieter VAN PARYS[1], Joachim AUDENAERT[1], Bruno GOBIN[1], Pierrot KERGER[2], Wim DE WINDT[3] & Jan DICK[3]

[1] pcs Destelbergen, Division Potted Plants
Schaessestraat 18, BE-9070 Destelbergen, Belgium
[2] ABT, Ledebaan 86, BE-9300 Aalst, Belgium
[3]Avecom, Industrieweg 122P, BE-9032 Wondelgem, Belgium

Hydrogen peroxides are commonly used in greenhouses for cleaning purposes and disinfection of irrigation water systems, i.e. to prevent clogging by duckweed (Lemna minor), algae and other (micro)-organisms. This use contains a potential risk of involuntary contact to the plants, e.g. to roots through irrigation or to the plant leaves through accidental droplets (spraying mist). To help growers to maximize disinfection with minimal risks, we compared the efficacy and plant safety of a variety of commercially available peroxide formulations, i.e. pure peroxide products, peroxide products with Ag, peroxy formic acid, peroxy acetic acid and with sorbitol.

In a first series of experiments peroxides were screened on their stability in closed recipients with pure (without fertilizers) irrigation water (tap water, rain water) and on their preventive effect on floating green algae. Most stable and effective were the peroxides with Ag-stabilisators: with only one application of 100 ppm a.i., the peroxide remained relative stable (after 6 weeks still 25 ppm present and 100% clean water), while the pure peroxide products showed a stability of only 2 weeks. In open recipients with pure irrigation water the result was comparable (peroxide with Ag best), but to keep clean water we had to apply 100 ppm every month. For the other peroxides weekly applications with 100 ppm were necessary; they scored better than 3 applications/week with 33 ppm or 5 applications/week with 20 ppm.

In a second series of experiments peroxides were screened on their curative effect on algae in open recipients with fertilized irrigation water. All types of peroxides showed efficacy but the best results were obtained with peroxide formulations with performic acid: the green water became clean (transparent) within 2 days after application (at dose of 200 ppm a.i) and weekly applications sufficed to maintain water free of algae, as well as giving a spectacular reduction in duckweed (Lemna minor) and bacterial populations. For the pure peroxides, weekly applications with 400 ppm were necessary to get good curative results.

To assess plant safety, different peroxide products were screened by weekly spray applications on a model plant, potted Ficus benjamina, at different dose rates (0 – 10 – 100 – 250 – 500 – 1000 – 1250 – 2000 ppm); toxicity appeared from 1000 ppm and this was the same for all type of peroxide products. Furthermore peroxides were tested by weekly irrigations above or below the plants at different dose rates (0 – 200 – 2000 – 5000 – 50000 ppm); toxicity appeared from 2000 ppm with irrigations above plants; no toxicity appeared with irrigations below but the plant growth was significantly reduced. To extend the validity of our screening to other varieties, we screened spray applications around the estimated phytotoxicity level (500 en 1000 ppm) on 30 potted plant varieties.

Posters
Application technology

EFFLUENT WATER BEING AN ORGANIC FERTIFICATION AND PESTIFICATION TOOL FOR ECO FRIENDLY RICE PEST MANAGEMENT

N. THARSHANI & G. MIKUNTHAN
Department of Agricultural Biology
Faculty of Agriculture University of Jaffna, Sri Lanka

Plant health conditions play a vital role in eco friendly pest management in rice cultivation. Over use of agrochemicals both fertilizers and pesticides not only increase the cost of rice production but also lead soil and water pollution especially in rice fields. This study aimed to find out a low cost and eco-friendly method of rice cultivation for its sustainable production. A field study was conducted using most popular rice cultivar AT 401, H4 in Sri Lanka during 2007 and 2008 at Calcic Red Yellow Latersol (CRYL), where leaf folder, brown spot, weeds were the serious problem annually. This area also face problem to dispose palmyrah (*Borassus flabellifera*) toddy distillery effluent water as an industrial waste. Paddy grown in neglected field nearer to the distillery unit showed numerous tillers with luxury growth. This research was conducted to determine the effect of fertification by the effluent water from the toddy distillery unit on rice pests. A randomized complete block design was used and treatments were replicated five times. Results exhibited that, there was significant difference between effluent water treated and control in paddy fields. Effluent water treated field was produced more than 25% and 23% yield in AT 401 and H4 respectively. Leaf folder incidence was observed in AT 401-control (60%) and in AT 401-effluent water field (3.5 %) while in H4-control (67%) and in H4-effluent water (4%) in paddy field. Brown spot incidence was observed in AT 401- control (32%) and in AT 401-effluent water (5%) while H4 -control (33%) and H4-effulent water (4%). In fields treated with effluent water, cent percent reduction of broader leaved weeds was recorded. In control plots nearly 11 species of narrow leaf weeds were recorded and it was five times greater than the treated plot. Weed coverage was recorded in treated plot (0.15%) and control (4.34%). These findings would be useful in developing an integrated pest management in rice, as part of a holistic crop management approach.

EFFECTS OF OPTICAL BRIGHTENERS USED IN BIOPESTICIDE FORMULATIONS ON CROPS: REFLECTANCE, STOMATAL CONDUCTANCE, PHOTOSYNTHESIS, AND GROWTH

Ana Mabel MARTÍNEZ, Sandra P. VELASCO, Aricely MÉNDEZ, José I. FIGUEROA, María L. ESPAÑA, Raúl CÁRDENAS-NAVARRO & Samuel PINEDA

Instituto de Investigaciones Agropecuarias y Forestales
Universidad Michoacana de San Nicolás de Hidalgo
Km. 9.5 Carr. Morelia-Zinapécuaro. MX-58880 Tarímbaro, Michoacán, Mexico

Optical brighteners have attracted interest as adjuvants in baculovirus-based biological insecticides due to their ability enhance the insecticidal properties of these viruses and protect virus particles from the degrading effects of ultraviolet (UV) radiation. The effects of two types of optical brighteners, Tinopal CBS (a distyrylbiphenyl derivative) and Tinopal C1101 (an ethenediyl benzenesulfonic derivative) at 1 or 3% (wt./vol.), on growth of different crops [maize, *Zea mays* L. (var. HY-311), sorghum, *Sorghum vulgare* Pers. (var. Silo), tomato, *Lycopersicum esculentum* L. (var. Floradade IT), or pepper, *Capsicum annum* L. (var. Cal Won 300)] were examined after once a week application during four weeks. Both compounds significantly affected the growth of maize plants, whereas sorghum plants were affected only at the highest concentration of Tinopal C1101. Neither brightener had negative effects on tomato or peppers plants. Both compounds increased the percentage of reflectance of maize and tomato leaves when analyzed using laboratory and field spectrophotometers. A greenhouse experiment involving single application of 1 and 3% Tinopal C1101 indicated that the stomatal conductance and photosynthetic rate of maize and tomato plants were not significantly affected. We conclude that the effects of optical brighteners on plant growth are more likely to be influenced by differences between plant species than differences between brightener compounds.

SURVIVAL OF KAOLIN-BASSED FORMULATIONS OF THREE YEAST ISOLATES THAT CONTROL BLUE MOLD OF APPLE

Lachin MOKHTARNEJAD[1], Hasan REZA ETEBARIAN[1] & Hamid REZA FAZELI[2]
[1] Departement of Plant Protection, College of Aburaihan
University of Tehran, Iran
2 Probiotic Reserch Centre, Faculty of Farmacy
Tehran University of Medical Science, Iran

Biological control by using epiphytic yeasts against *Penicillium expansum* has been considered as an alternative method for controlling the disease. The yeast isolates *Rhodotorula mucilaginosa, Candida membranifaciens, Pichia guilliermondii* is reported as a novel antagonistic micro-organism against the pathogen *P. expansum* on the apple fruit. In this study we compared shelf life of the postharvest biological control yeasts, *R. mucilaginosa, C. membranifaciens, P. guilliermondi* to determine which have a longer storage life at kaolin-based formulation. We used cane molasses-based medium for biomass production of yeast isolates. Centrifuged yeast cells were added to kaolin as a carrier and adjuvants. Kaolin-bassed formulation, contaned kaolin and sodium alginate and glycerol. Viability of yeasts cells in the formulations stored at 4°C and 24±1°C was determined over a six month of storage period. In the both temperature yeast isolate *P. guilliermondi* had significantly higher viable yeast cells content over six month storage period. The shelf life of *R. mucilaginosa* within formulation was very short. The survival of *P. guilliermondi* yeast isolate at formulation that stored at 4±1°C was longer than those stored at 24±1°C. The shelf-life at 24±1°C was about four month for all formulations after which yeasts cell viability reduced markedly. Because that the efficacy of bioproducts is directly related to the number of viable cells, preparation of formulation that supported high number of viable antagonist cells is very important. The results obtained in this study are an approach for futher upscaling of *P. guilliermondi* production.

BIOLOGICAL CONTROL OF BLUE MOLD IN APPLE WITH DIFFERENT FORMULATION OF *CANDIDA MEMBRANIFACIENS*

Lachin MOKHTARNEJAD[1], Hasan REZA ETEBARIAN[1], Hamid REZA FAZELI[2]
[1] Departement of Plant Protection, College of Aburaihan, University of Tehran, Iran
[2] Probiotic reserch center, Facualty of farmacy, Tehran University of Medical Science, Iran

The biocontrol yeast *Candida membranifaciens* can effectively reduce blue mold of apple fruit during storage. Blue mould, caused by *Penicillium expansum* is most common pathogen during storage and shipment of apple. To produce commercial biocontrol agents (BCAs) successfully, it is important that cheap and economic substrates are used which support high numbers of good quality inoculum. In this study different formulations of *C. membranifaciens* yeast isolate that grown on a cane molasses-based medium with different carriers, talc, kaolin, rice bran and wheat bran prepared and the viability of yeast cells in formulations was determined over a six month period at two different temperatures. Formulation that containing wheat bran as carrier had significantly higher viable yeast cell content over a six month storage period. Formulations stored at 4±1°C had a longer shelf life than those stored at 24±1°C. The shelf life at 24±1°C was about 4 months for most formulations after which yeast cell viability reduced markedly. Among the formulations, wheat bran-based formulation was selected in vivo testing because had the highest number of viable yeast cells during storage. This formulation was tested on apple to control blue mold. Results indicate that this formulation effectively inhibited blue mould of apple fruits. The use of wheat bran-based formulation of strain *C. membranifaciens* at concentration of 1010 CFU/ml significantly reduced the amount of disease development in the apple fruit in the storage condition. This study shows that a suitable powder formulation for commercial application can be produced with high viability and conservation of biocontrol efficacy.

EYE IRRITATION STUDY OF SOME PESTICIDES ON CHORIOALLANTOIC MEMBRANE OF THE EGG

Éva KORMOS[1], Péter BUDAI[1], Andrea PONGRÁCZ[1] & József LEHEL[2]
[1] Pannon University, Georgikon Faculty, Keszthely
[2] Szent István University, Faculty of Veterinary Science, Hungary
Department of Pharmacology and Toxicology, Budapest, Hungary

The chorioallantoic membrane of chick embryo has been used extensively for many years in various fields of biological research, including virology, bacteriology and toxicology.
The chorioallantoic membrane (CAM) is a complete tissue that responds to injury with a complete inflammatory reaction, this process similar to that induced by chemicals in the conjunctival tissue of the rabbit eye.
A possible model for assessing the irritation potential of a chemical or product to such a vascularized tissue is the choriallantoic membrane of the embryonated hen's egg, as this is a highly vascular, thin membrane with relatively easy access for both treatment and assessment.
In recent years various in vitro methods have been developed to replace the heavily criticized Draize rabbit eye test for irritation testing. One of the most studied alternative methods is the Hen's Egg Test - Chorioallantoic membrane (HET-CAM).
In our studies a comparative screening was done with a set of pesticides to establish parallel data on in vitro (HET-CAM) and in vivo (DRAIZE) results.
In most cases good correlation was found between the HET-CAM assessment and results from the Draize rabbit eye test. The actual form of the HET-CAM test is a valuable prescreen for predicting ocular irritation potential of chemicals, and can be used to reduce the number of experimental animals. The HET-CAM test is useful as a part of a battery of tests to replace the Draize rabbit eye test.

Posters
Pesticide residues, toxicology and ecotoxicology

IMMUNE SYSTEM ON CATFISH AS BIOMARKER FOR WATER CONTAMINATION WITH MALATHION

FAHIUM A. EL-KARIUM ELKAUAL[1] AND YAHIA YOUSSEF[1,2]
[1] Omar El-Moktar University, Faculty of Agricultural, Libya
[2] Suez Canal University
Environmental Impact Assessment Unit, Ismailia, Egypt

This work was conducted to estimate malathion concentration in water, sediments and fish samples collected from different location at Ismailia canal and some agricultural drainage canals and the effect of malathion on T-lymphocyte proliferation responses was evaluated. 10 locations in Ismailia canal and agricultural drainage canals were investigated. Malathion was detected in all examined locations except two locations (one fresh and one drainage canal) for water and sediments. The mean concentrations were 0.458±0.084 µg/L, 0.234±0.0339 µg/Kg and 0.113±0.019 µg/Kg in water, sediments and fish samples collected from Ismailia canal and agricultural drainage canals, respectively. Results revealed higher malathion concentration in the following order water, sediment then fishes. Samples collected from agricultural drainage canals had higher malathion concentration as compared to Ismailia canal, where malathion means were 0.346±0.094 µg/L and 0.184±0.052 µg/Kg in drainage canals water and sediment respectively, while means were 0.218±0.161 µg/L and 0.092±0.064 µg/Kg in Ismailia canal water and sediments, respectively. Water pH had a mean of 7.838±0.110 and 7.624±0.208 in drainage canal and surface water, respectively. The alkaline pH increases the degradation of malathion in water, and because of that, we believe that the initial concentration of malathion pollution in water was higher than levels detected, so we used have of the LCD_{50} to evaluate malathion's effects on fish immune system. T-lymphocyte proliferation is a good indicator of the viability of the immune system. It was found that malathion had a significant cytotoxic effect on T-lymphocyte proliferation, which makes fishes vulnerable to viral, bacterial and parasitic diseases

THE EFFECT AND OPTIMAL DOSE OF ADJUVANTS COMBINED WITH NICOSULFURON ON THE CONTROL OF WEED GRASSES

B. MARYNISSEN[1], K. DEWITTE[1], K. COOREVITS[1], B. HEREMANS[1] & G. HAESAERT[2]

University College Ghent, Faculty of Biosciences and Landscape Architecture
Diepestraat , BE-9820 Merelbeke[1], Voskenslaan 270, BE-9000 Gent[2], Belgium

A laboratory test was set up to measure the effect of different doses of adjuvants combined with nicosulfuron for control of several weed grasses namely *Echinochloa crus galli, Digitaria sanguinalis, Digitaria ischaemum, Setaria verticilaata, Setaria viridis.*

The different weed grasses were sown in seed-trays and grown at 22°C at a day/night regime of 16h/8h. Nicosulfuron was used at a dose of 60 g/ha (Accent: 0.08 kg/ha) and applied when the weed grasses were in the 2-3 leaf stage. Treatments were carried out with a 1,5 m spray-boom equipped with XR11003 (300 l water/ha) or XR800015VS (150 l water/ha) Teejet nozzles after placing the seed-trays on a surface of 10 m².

The different weed grasses were scored on growth inhibition, chlorosis and necrosis compared to the untreated object. The addition of several adjuvants improved the efficiency of nicosulfuron towards necrosis when applied on *Echinochloa crus galli* and when applied on both *Digitaria* species. Positive effects for both growth inhibition and chlorosis were found on both *Setaria* species when adjuvants were added to Accent.

Some adjuvants had a consistent effect on all weed grasses concerning necrosis, chlorosis and growth reduction. The efficiency of the adjuvants in combination with Accent varied for different doses tested and in some cases an optimal dose could be found.

THE EFFICIENCY OF ADJUVANTS COMBINED WITH FLUPYRSULFURONMETHYL PLUS METSULFURON-METHYL (LEXUS XPE) ON THE WEED CONTROL IN WINTER WHEAT

B. MARYNISSEN[1], K. DEWITTE[1], K. COOREVITS[1], B. HEREMANS[1] & G. HAESAERT[2]

University College Ghent, Faculty of Biosciences and Landscape Architecture
Diepestraat , BE-9820 Merelbeke[1], Voskenslaan 270, BE-9000 Gent[2], Belgium

A field trial based on former laboratory tests was conducted during the growing season 2008. The purpose of the trial was to search out the efficiency of flupyrsulfuronmethyl plus metsulfuron-methyl (Lexus XPE) in combination with different adjuvants. Treatments were carried out with a 1.5 m spray-boom equipped with XR11003 (300 l water/ha) or XR800015VS (150 l water/ha) Teejet nozzles. Before and after the treatment, weed populations were counted. Partly due to prosperous climate conditions, the herbicide treatment worked out very well on all weeds, except for common windgrass (*Apera spica-venti)*. The field trial was set up on a parcel that was known for its high presence of common windgrass. Because of this fact, the standard treatment with Lexus XPE did not came out to be totally effective against the entire population of this weed. When adjuvants were added the results for common windgrass showed an improved effect of Lexus XPE.

ASSESSMENT OF PHYTOTOXICITY OF ADJUVANTS USED IN CROP PROTECTION

B. BECK & P. SPANOGHE
Laboratory of Crop Protection Chemistry
Faculty of Bioscience Engineering
Ghent University
Coupure links 653, BE-9000 Gent, Belgium

The aim of the study was to determine if and to what extent adjuvants exert a phytotoxic effect on plants. This study is meant as a first tier approach and does not deal with dose-response effects.
The adjuvants used in this study were Actirob-B, AE5, Bond, Breakthru G 850 and Silwet L-77. All adjuvants were used at a concentration of 1 g/l. To measure the phytotoxic effect of the adjuvants in combination with pesticides, two pesticides were selected: Ramrod (0.2 g/l, active ingredient: propachlor) and Kerb (0.025 g/l, active ingredient: propyzamide). The effects of the adjuvants and of the adjuvant-pesticide mixture on phytotoxicity were evaluated with Phytotoxkits®. These kits measure the effects of chemicals on root growth and germination of the seedlings of three plant species: *Sorghum saccharatum*, *Sinapis alba* and *Lepidium sativum*.
No significant differences in germination percentage were noted between the control group and the adjuvant-added groups. Root growth of the three plant species was significantly affected by some adjuvants: results point towards a differential reaction of the three plant species. While *Sorghum* plants only show minor growth inhibiting effects and for some adjuvants even a growth stimulating effect of the treatment, the growth inhibition is much more visible with the smaller *Sinapis alba* and *Lepidium sativum* seeds. The pesticide-adjuvant mixes showed an additive rather than a synergistical effect on root growth.

SIDE-EFFECTS OF FENAZAQUIN ON A CELLULAR MODEL OF *PARAMECIUM*

Houneïda BENBOUZID[1], Houria BERREBBAH[2], Mohammed-Réda DJEBAR[2] & Guy SMAGGHE[3]

[1] Department of Biology, University Centre of El Tarf, Algeria
[2] Lab Cell Toxicology, Department of Biology, Annaba University, Algeria
[3] Lab Agrozoology, Department of Crop Protection, Ghent University, Belgium

Our biodiversity has long been preserved, but the main constituents of our environment have been particularly affected by the addition of molecules resulting from agricultural and industrial activities. It is well accepted that these changes may stress some species, making them more vulnerable. In this project work, we determined the disruptive side-effects of a pesticide on several biochemical endpoints and the behaviour of a microorganism as the ciliate protist *Paramecium sp*. Here we used fenazaquin [4-(4- tert -butylphenethoxy)quinazoline] that belongs to the quinazoline class of chemicals and is a pesticide intended to control mites and insects (especially whiteflies); its route of exposure is ingestion and dermal, and its mode of action is the disruption of the biochemistry of insect mitochondria. In our experiments we recorded disturbances in protein and glutathione, in glutathione S-transferase, and a decrease in consumption of oxygen. The results are discussed in relation to potential risks and mechanisms of action. In addition, the data can be used as reference values in further ecotoxicity testing with other pesticides and chemistries.

IMPACT OF THE INSECT GROWTH REGULATOR DIFLUBENZURON ON THE BIOCHEMICAL COMPOSITION OF THE CUTICLE OF THE SHRIMP *PENAEUS KERATHURUS*

N. SOLTANI[1]*, H. LECHEKHAB[1] & G. SMAGGHE[2]
[1] Département de Biologie, Université de Annaba, DZ-23000 Annaba, Algérie
[2] Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, BE-9000 Gent, Belgium
*E-mail: nsolt@yahoo.fr

Benzoylphenyl urea (BPU) insecticides such as diflubenzuron prevent the molting process by inhibiting chitin formation, thereby causing abnormal cuticular deposition and abortive moulting in insect and crustacean species. In this project we worked with Dimilin® (25% WP) as this formulation of diflubenzuron is widely used against insect pest in forests in Algeria. Previously, we have shown in histological and ultrastructural studies that diflubenzuron is causing a modification in the lamellar appearance particularly detected in membranous layers in a non-target organism, *Penaeus kerathurus* (Forskal, 1775) (Decapoda, Peneidae). Therefore, the aim of this study was to evaluate under laboratory conditions the secondary effects of Dimilin® on the biochemical composition of the cuticle in order to explain the cuticular abnormalities reported. Here we determined the amount of chitin, protein and calcium salts in the cuticle. The compound was added to the rearing sea water at a final concentration of 1 µg AI/litre, and newly ecdysed adult shrimps were exposed for 10 days, i.e. until stage C during the moulting cycle. In the treatments, it was clear that the thickness of both principal and membranous layers was significantly reduced, and also the amounts of chitin in the cuticle were conspicuously lower. In conclusion, the results obtained in *P. kerathurus* confirm the primary mode of action of diflubenzuron against chitin biosynthesis in this non-target organism.

EFFECTS OF ARTEA, A SYSTEMIC FUNGICIDE, ON THE ANTIOXIDANT SYSTEM AND THE RESPIRATORY ACTIVITY OF DURUM WHEAT (*TRITICUM DURUM L.*)

Mohammed-Réda DJEBAR[1], Mostapha YOUBI[1], Nacira HENNOUNI[1], Rachid ROUABHI[2] & Houria BERREBBAH[1]

[1] Cellular Toxicology Laboratory, Annaba University, DZ-23000 Annaba, Algeria
[2] Department of Biology, Tebessa University Center, DZ-12000 Tebessa, Algeria

The present work is aimed at the study of Artea (a systemic fungicide) effects on durum wheat (*Triticum durum* L. CV. *Hard GTA*). Seeds were grown in a medium containing 25, 50, 75 and 100 ppm of Artea under controlled conditions. Roots of eight day old were used to determine the enzymatic activities of catalase, ascorbate-peroxydase and guaïacol-peroxydase. Root respiratory activity was also determined using a polarographic method (Clarck electrode). The results after treatment with Artea show an enhancement of respiratory activity and increased levels of antioxidative enzymes in *durum* wheat roots. Activities of catalase, ascorbate-peroxydase and guaïacol-peroxydase increased proportionally and were more meaningful at high concentrations (75 and 100 ppm). Modulations in respiratory metabolism and antioxidant system could probably be the result of Artea induced toxicity which could cause an oxidative stress state.

THE IMPACT OF NOVALURON FEEDING ON GLYCOSAMINOGLYCAN AND SULFHEMOGLOBIN BIOSYNTHESIS IN 1 DAY HATCHED CHICKS *GALLUS DOMESTICUS* (L.)

Rachid ROUABHI[1*], H. DJEBAR-BERREBBAH[2] & M.R. DJEBAR[2]
[1] Department of Biology, University Larbi Tebessi, DZ-12000, Tebessa, Algeria
E-mail: r_rouabhi@yahoo.fr
[2] Laboratory of Cellular toxicology, Department of Biology, Faculty of the Sciences
Annaba University, DZ-23000, Annaba, Algeria

Novaluron, an inhibitor of insect chitin synthesis, was fed to neo-hatched chicks in concentrations of 50 to 2000 ppm in the diet. The activity of the vertebrate hexosamine transferases responsible for glycosaminoglycan (mucopolysaccharide) formation was monitored by measuring the *in vivo* rate of incorporation of a labeled precursor into hyaluronic acid and chondroitin sulfate of skin. No inhibition of biosynthesis was noted at any concentration; indeed the insecticide appeared to stimulate the synthesis of these compounds. After 3 weeks on the diet, chicks eating 1000 and 2000 ppm novaluron showed obvious signs of cyanosis, and sulfhemoglobin was demonstrated in the blood of chicks eating more than 100 ppm. The amount of sulfhemoglobin appeared to be related to the dietary insecticide content and the highest level seen was 15% of total hemoglobin in the 2000 ppm group after 31 days. These values returned to normal within 3 weeks when novaluron was removed from the diet.

TOXICO-PATHOLOGICAL EFFECTS IN RATS INDUCED BY LAMBDA-CYHALOTHRIN

S. DAHAMNA, D. HARZALLAH & N. SEKFALI
Department of Biology; Faculty of Sciences, University Ferhat Abbas
DZ-19000, Sétif, Algeria
E-mail: dahamna_s@yahoo.fr

Pesticides are widely used chemicals making human exposure to pesticides a realistic possibility. Biomonitoring is a common and useful tool for assessing human exposure to pesticides. Pyrethroids are effective insecticides that are often used in household sprays, aerosol bombs, insect repellents, pet shampoos, and lice treatments. Using products containing these compounds will expose people to these chemicals. Since these compounds frequently are used on crops, they are often detected in fruits and vegetables. Biomonitoring of exposure is a useful tool for assessing exposure to pesticides. Biomonitoring involves the measurement of the parent pesticide, its metabolite or reaction product in biological media, typically blood or urine, to determine if an exposure has occurred and the extent of that exposure. Although not without its limitations, biomonitoring has great utility in integrating all routes of exposure allowing for one exposure measurement. Pesticides have much shorter environmental half-lives and tend not to bioaccumulate. In fact, from humans within 24 hr as the parent pesticide, a mercapturic acid detoxification product, oxidative or dealkylation metabolites, and/or glucuronide-or sulfate-bound metabolites. However, because of the heavy agricultural and residential use of these chemicals, humans are continually exposed to many of these chemicals. The objective of the present study was to explore modification in toxico-pathological responses of rats treated with lambda-cyhalothrin (commercially called karate). Rats (250 g weight), were gavaged by 1/100 LD_{50} for 4 weeks (one dose every week). Blood was collected before dosing and after 48 hours from the treatment. Enzyme activities were assayed in the plasma samples obtained. Glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminase (GPT), Alkaline phosphatase (ALPH and Glucose. The results showed a decrease in RBC; WBC and Hb. This probably explained by the effect of lambda cyhalothrine on the erythropoiesis and the distruction of cells. An increase of plasma enzyme activities in GOT and GPT were recorded, explain a high energy-generating product. The histopathological results showed alteration on the target organs such as liver and kidney, these biochemical and histological modifications are probably due to the effect of lambda cyhalothrin or their metabolites.

BIOCHEMICAL INVESTIGATION OF CYPERMETHRIN TOXICITY IN THE RABBIT

S. DAHAMNA, D. HARZALLAH & N. SEKFALI
Department of Biology, Faculty of Sciences, University Ferhat Abbas
DZ-19000, Sétif, Algeria
E-mail: harzaldaoud@yahoo.co.uk

Pyrethroids are the most frequently used pesticides in agriculture, forestry, horticulture, hospitals public health, homes and textile industry. Cypermethrin, a composite pyrethroid is moderately toxic to mammals. Exposure to the pyrethroids occurs by inhalation, dermal and oral routes both accidentally as well as from the environment. Cypemtherin and DDT have been detected in human breast milk from malaria endemic area in South Africa. The WHO has recommended that the level of permethrin in drinking water not exceed 20 micrograms per liter (µg/L). The effects of exposure to any hazardous substance depend on the dose, the duration, how you are exposed, personal traits, habits and whether other chemicals are present. Pyrethroids are often combined commercially with other chemicals called synergists, which enhance the insecticidal activity of the pyrethrins and pyrethroids. The synergists prevent some enzymes from breaking down the pyrethrins and pyrethroids, thus increasing their toxicity. Because these compounds are broken down in the body quickly, there are several ways to measure the metabolites of these chemicals in human blood and urine. In this study the pyrethroid cypermethrin Sherpa 25% (active substance 250 g/l cypermethrin) was used, rabbits (1 kg weight), were gavaged by 1/20 LD_{50} for 3 weeks (one dose every week). Blood was collected before dosing and after 24, 72, 144 hours after the treatment. Enzyme activities were assayed in the plasma samples obtained. GOT, GPT, ALPH, CREA, GGT and Glucose and Total Pro were measured. Rats showed depression, decrease in feed intake, body weight and loose faeces. Livers exhibited fatty change, necrosis, Lesions in kidney included tubular necrosis and pink homogeneous tubular casts. Serum ALT and creatinine concentrations increased while those of total proteins, albumin, serum cholesterol and triglycerides decreased. The results showed a decrease in RBC; WBC and Hb. This probably explained by the effect of cypermethrin on the erythropoiesis. An increase of plasma enzyme activities in GOT, GPT and CPK were recorded, explain a high energy-generating product. An increase, in the plasma enzyme activity in Alkaline phosphatase, related to their role in the cell permeability. The histopathological results showed lesions and morphological changes of hepato-cellular, fibrosis and appearance of inflammatory infiltrate, confirmed disturbances of the biochemical parameters. These changes were much underlines during the animal toxicity.

COMPARATIVE HISTOPATHOLOGICAL EFFECTS OF ETHYLENE BIS DITHIOCARBAMATE ON THE TESTIS IN *RABBITS* AND *RATS*

L. MALLEM[1], A. CHOUAIBIA & M.S. BOULAKOUD
Laboratory of Animal Ecophysiology, Department of Biology, Faculty of Sciences
University Badji Mokthar-Annaba
B.P 12 El Hadjar, DZ-23000, Annaba, Algeria
[1] E-mail: leilamallem@yahoo.fr

Maneb is an ethylenebisdithiocarbamate fungicide used in the control of the fungal diseases of plants. Males of domestic rabbits *Oryctolagus cuniculus* and *Wistar* rats received daily by gavage 2 and 3 mg/kg body weight of Maneb for 3 weeks.
At the end of the treatment, the animals were sacrificed and the testis's samples were weighed and placed in formol 10% solution. The histopathology changes study was made in accordance with *Martoja and Martoja,* 1967 method. The obtained results show a very considerable reduction of the weight of testes in treated animals compared to the control group especially in the rabbits. A very reduction in testosterone concentration was noticed in the treated rabbits. The histological observation showed that no effect was observed in the testes in rats. Then, the testicle of treated rabbits with 2 mg/kg body weight reveal seminal tubules in involution Sertolienne and Leydig cells are a little too visible. While rabbits treated 3 mg/kg indicates that there is no spermatozoa in the epididymis, the most of the tubules are empty as compared to control. The testis in the control show normal spermatogenesis and the mitoses are distributed to all levels and spermatozoa are observed in many tubules and epididymis. In conclusion, administration Maneb with the used doses affects histological deformation in the testis of rabbits.

AN ASSESSMENT OF CARBARYL RESIDUES IN SOIL AND ON BRINJAL CROP IN AN AGRICULTURAL FIELD IN BIKANER, RAJASTHAN (INDIA)

Stephen DHAS & Meera SRIVASTAVA
Laboratory of Entomology
P.G. Department of Zoology
Govt. Dungar College, Bikaner-334 001
Rajasthan, India.
Email:meerayk@rediffmail.com

Solanum melongena Linn. (Brinjal) is the most common, popular and principal vegetable crop grown in many geographical parts in India. This staple vegetable crop is extensively damaged by the insects and farmers use large quantities of chemical insecticides, carbaryl being one of them. The present study was undertaken to evaluate the persistence of carbaryl on brinjal (fruit) and soil in an agricultural field in Bikaner (28°N latitude and 73°18'E longitudes), Rajasthan, India.
For determination of Carbaryl residues in soil, soil samples were collected from the farm. These samples were air-dried, ground to pass through 2mm sieve and stored in glass bottles. The soil samples were found to be fortified at 15.20 µg g^{-1} and 32.50 µg g^{-1} soil. Sufficient amount of methanol was added to form slurry. The contents were thoroughly mixed and kept overnight at room temperature (35±5°C) for drying. The soil (80 g) was taken in a tubling jar along with fresh untreated soil (720 g) and mixed thoroughly. This resulted in 15.20 µg g^{-1} concentration of carbaryl in soil. Similar procedure was followed for 32.50 µg g^{-1} treatment using 160 g of treated and 640 g of fresh untreated soil. Triplicate samples of fortified soils after each treatment (25 g) were taken in 50ml beakers and distilled water (8 ml) was added to each beaker for maintaining the soil moisture at field capacity. The samples were air-dried, ground and mixed with 0.25 g of activated charcoal and 0.50 g of florisil and packed in glass column (62×1.5 cm ID) containing a 3 cm layer of Sodium sulphate over a cotton plug. The column was eluted with chloroform-diethyl ether (1:1) for a minimum period of 2 h. The organic solvent fractions from the three extractions were pooled and evaporated to dryness at room temperature.This single step extraction and clean-up technique removed most of the interfering co-extractives and eluent obtained was analyzed colorimetrically for carbaryl. The eluent so dried was dissolved in a known quantity of methanol (20 ml) i. e. two 10 ml fractions of methanol and transferred distilled water (DW) and 5ml of 0.3 % Sodium Nitrate solution were added and then 5 ml of Sulphanilic acid were also added. The contents were mixed, left for 10 minutes and 10 ml of 16% Sodium hydroxide were added and then the absorbance was recorded on colorimeter at 520nm against a blank containing all the reagents except carbaryl. On brinjal fruits Carbaryl sprays were given with hand compressor sprayer at fruiting stage in brinjal (*Solanum melongena*). Fruit samples were collected at 0, 1, 3, 5 and 7 days after spray and samples were analysed for the insecticide residues. Fifty (50 g) brinjal fruit samples were extracted with 100ml mixture of isopropanol:hexane (1:2). The samples were re-extracted with 50ml of the solvent mixture and filtered. The filtrate was washed with water to remove isopropanol. For clean-up, 0.5 g activated charcoal was added and shaken thoroughly. It was filtered through a bed of anhydrous sodium sulphate (3 cm layer). The column was eluted with chloroform-diethyl ether (1:1) for a minimum period of 2 hr. The organic solvent fractions from the three extractions were pooled and evaporated to dryness at room temperature.

The single step extraction and clean up technique removed most of the interfering co-extractives and eluent obtained was analysed by employing the colorimetric method (Yuen 1965).

The residues in soil progressively declined up to 60 days but became non-detectable during the next sampling at 75th day. The dissipation of insecticide was faster during the first 8 days as compared to latter period, while, on fruits the initial deposit of 11.47 ppm from 0.2 % Carbaryl spray was dissipated to 9.93 ppm within one day after treatment recording thereby a decrease in residue to about 13.40 %. After 25th day residues were non-detectable representing 100 % dissipation. It may be concluded from the observations of the present study that the residues of the pesticide Carbaryl in soil became non-detectable after 60 days of spraying; while, on crops it persisted up to15 days.

AN ASSESSMENT OF PHOSPHAMIDON RESIDUES ON MUSTARD CROP IN AN AGRICULTURAL FIELD IN BIKANER, RAJASTHAN (INDIA)

Stephen DHAS & Meera SRIVASTAVA
Laboratory of Entomology, P.G. Department of Zoology
Govt. Dungar College, IN-Bikaner-334 001
Rajasthan, India
E-mail: meerayk@rediffmail.com

Mustard / rapeseed cultivation is done widely throughout India. It produces around 7 million tones of it annually and occupies 3rd position in the list of this crop producing countries contributing about 11% of the world's total production. Rajasthan and Uttar Pradesh account for majority share, contributing to over 50% of the total Indian produce. It is basically a winter crop, and requires a temperate climate to prosper. The planting season or the sowing period in India is the Rabi season i.e. October to November. The harvesting period is from February to March.

As all other crops this crop is also attacked by a number of insect pests. Being a cash crop, to protect it against pest infestation, insecticides are sprayed on it, one of them being Phosphamidon. The present study was undertaken to evaluate the persistence of phosphamidon on mustard crop (foliage and siliqua) and soil in an agricultural field in Bikaner (28°N latitude and 73°18'E longitudes), Rajasthan, India.

The crop was sprayed with 0.05% phosphamidon @ 700 litre/ha before flowering and 15 days after flowering. For the study samples of soil were taken on 0, 5, 10.1, 20, 27 days, while, for foliage the samples were collected on 0, 1, 5, 10, 12, 15 days after first day and, siliqua fruits at same intervals as that of foliage after second spray. The analysis was done following the method given by Getz and Walt (1964) as modified by Jain *et al*. (1974).

The initial deposit of phosphamidon was found to be 0.60 mg/Kg of soil just after the spray, which gradually decreased with time and became non-detectable on the 27th day after spraying. The analytical results pertaining to residues on foliage and siliqua were found to be 10.83 mg/Kg and 8.53 mg/Kg respectively after first and second spray and which declined to 0.25 and 0.31 mg/Kg respectively after 15 days of spraying. On the basis of tolerance limit of 0.5mg/Kg for phosphamidon as assigned by FAO/WHO (1975) the results show that the residues which persist till the 12th day after application are more than the prescribed limits and their consumption could be harmful. The rate of dissipation of phosphamidon residues from second spray treatment followed a trend almost similar to that seen after first spray. The residues found in siliqua were less due to smaller surface area of the fruits as compared to that of leaves. It is evident from the present study that the persistence of phosphamidon on mustard foliage may be recorded until the 12th day after spraying rendering it unfit for human consumption.

THE PERSISTENCE TOXICITY OF THREE INSECTICIDES AGAINST ADULT *LYSIPHLEBUS FABARUM* (HYMENOPTERA: APHIDIIDAE)

Qodrat SABAHI[1], Arash RASEKH[1], Amir H. SANGAKI[1] & Aziz SHEIKHI GARJAN[2]

[1] Plant Protection Department, University College of Agriculture, University of Tehran
[2] Plant Pest and Disease Research Institute, Tehran

In order to investigate the effects of three insecticides on *Lysiphlebus fabarum* (Hymenoptera: Aphidiidae), the larval parasitoid of *Aphis fabae*, an experiment was carried out using IOBC/wprs method. Persistence toxicity of insecticides have been evaluated in the semi-field condition. The trials were laid out in randomized complete block design (RCBD) with 3 replicates and an untreated check. The insecticides abamectin 1.8 EC, imidacloprid 350 SC, and pymetrozine 25 WP were used at recommended field rates. The insecticides were applied on broad bean foliage using a hand sprayer, until run-off. Contact toxicity of semi field-aged residues of insecticides on adult parasitoids was evaluated using the cage-method. The mortality of adult parasitoid, after 24 h contact with 1- day old residues of abamectin, imidacloprid and pymetrozine were 53, 90 and 57%, respectively. After 5 days the effect of residues decreased so that the adult mortality diminished to 28, 77 and 18% for mentioned above insecticides. 15-day old residues lead to 9, 22 and 14%; and 30-day old residues lead to 0, 3 and 1% mortality for these insecticides, respectively.

PREVENTION OF PESTICIDE WATER CONTAMINATION USING AN IN SITU ECOLOGICAL TECHNIQUE

Raquel ROJAS, José USERO, José MORILLO & Hicham EL BAKOURI
Department of Chemical and Environmental Engineering, University of Seville
ES-41092 Seville, Spain

In this study a new technique based on the use of organic residues is proposed for their use as barriers to prevent the pesticide contamination of aquatic ecosystems, while it constitutes a new use for residues generated by urban and agro industrial activities. Organic matter can adsorb pesticides and retain them, which means a delay in their lixiviation and a chance to be degraded. Retention of pesticides depends on the structure and composition of the organic substrates, and also on the physicochemical characteristics of the pesticide. In order to optimize the efficiency of these techniques for a broad range of pesticides and organic substrates, the fate of pesticides and the effects of degradation and retention processes need to be understood. The sorption behaviour of six pesticides with varying physico-chemical characteristics was investigated. The selected pesticides were alachlor, cyproconazole, chlorpyrifos, endosulfan-sulfate, trifluralin and simazine. These pesticides were selected based on their physicochemical characteristics, solubility, frequency of detention in surface and ground waters and toxicity. Five of the studied pesticides are included in the European Water Framework Directive. Organic residues used as substrates were chick manure, rice husk, residues from cotton gins, sawdust and composted sewage sludge mixed with pruning residues. The effect of several physicochemical parameters such as shaking time, concentration of pesticide solution and temperature was evaluated by batch experiments. Pesticide determination was carried out using stir bar sorptive extraction and gas chromatography coupled with mass spectroscopy. Maximum removal efficiency was reached using 0.3 g of organic waste (< 1 mm) and 50 mL of a mixture of six pesticides (0.5 ng L^{-1}). The removal efficiency was highest for sawdust (85%), followed by chick manure (76%), cotton gins (70%), composted sewage sludge (69%) and rice husk (62%).The removal efficiency of each pesticide was also studied on each organic residue. Sawdust showed the highest removal capacity for cyproconazole (100%) and 99% for endosulfan-sulfate, while chicken manure was able to adsorb 98% of chlorpyrifos and 94% of simazine. No effect of temperature was observed. According to adsorption kinetic data, 4 h were considered as the equilibrium time for determining adsorption isotherms. The present study is supported by the Ministry of Science and Innovation (Spain) (Project Ref. CGL2006-11646/HID).

RISKS ASSESSMENT OF WATER POLLUTION BY PESTIDES AT LOCAL SCALE (PESTEAUX PROJECT): STUDY OF POLLUTING PRESSURE

Stéphanie NOEL[1] & Boubacar BILLO BAH[2]
[1] Engineering Department, Agricultural Research Centre of Wallonia (CRA-W) Gembloux, Belgium
[2] Biometry, Data management and Agrometeorology Unit Agricultural Research Centre of Wallonia (CRA-W), Gembloux, Belgium

Pollution of water resources (surface waters and ground waters) by pesticide uses is one of the key point of the European policy with the implementation of the Water Frame Work Directive (2000/60/EC) and the thematic Strategy on the Sustainable use of pesticides. According to this legislation, the Member States must initiate measures to limit environmental and toxicological effects caused by pesticide uses.

The Agricultural Research Centre of Wallonia (CRA-W) emphasized the need of a tool for spatial risk analysis and develops it within the framework of PESTEAUX project. The originality of the approach proposed by the CRA-W is to generate maps to identify the risk of pollution at locale scale (agricultural parcel). The risk will be assessed according to the study of different factors, grouped under 3 data's layers: polluting pressure, vulnerability of the environment (soil) and meteorological data.

This approach is directly based on the risk's definition which takes into account the polluting pressure, linked to the human activities, and the vulnerability of the soil, defined by factors of physical environment which characterize the water flow in the parcel. Moreover, meteorological data influence the intensity and likelihood flow of water, and indirectly pesticide by leaching or run off.

The PESTEAUX's approach to study the pollution is based on the model "source-vector-target". The source is the polluting pressure, in other words, the pesticides which could reach the target. The vector is the water which vehicles the pesticide on and trough the soil until the target which could be the surface waters or ground waters.

In this paper we introduce the factors contributing to the polluting pressure. These factors are linking to the human activities and more precisely, to the pesticide uses. The factors considered have an influence on pesticide's transport by water (in its solid state or in dissolved state by leaching, run-off, or erosion) but also on a set of process controlling pesticide behaviour in the environment such as degradation, sorption,….

A METHODOLOGY TO DETERMINE PESTICIDES POLLUTION SOURCES IN WATER CATCHMENTS: STUDY CASE (BELGIUM)

Quentin LIMBOURG[1], Stéphanie NOEL[1], Ludovic CAPET[2] & Vincent HALLET[2]
[1] Agricultural Research Centre of Wallonia (CRA-W), Engineering Department
Gembloux, Belgium
[2] University of Namur, Geology Department, Namur, Belgium
E-mail: q.limbour@cra.wallonie.be

In the Walloon Region (Belgium), a Committee of Investigation was created in 2007 to investigate and determine the potential pesticides pollution sources in drinkable water catchments. This Committee, constituted by a multidisciplinary team of expert's i.e agronomists, soil scientists, phyto-chemists, hydrogeologists, is coordinated by the Walloon Agricultural Research Centre (CRA-W) and funded by the Public body in charge of waste water (SPGE).
The diagnosis method is inspired of the AQUAPLAINE method (Arvalis, France), and is composed of four steps:

1. preparing the diagnosis using existing data,
2. diagnosis using data bank completed by field observations,
3. meeting and discussion with the pesticide users,
4. final diagnosis and remediation proposal.

In a rural district of Walloon Region, a water producer who possesses two catchments (P1 et P2) has problems with pesticides. The pollution started in 1998 with atrazin and bromacile detected in the two catchments. In 2004, 2,6-dicloro-benzamide, metabolite of dichlobenil, was also detected in the catchments. At present, all these pesticides are still found in the catchment P1 and only the 2,6-dichlorobenzamide is found in the other catchments.
These active ingredients are not used in agriculture expect atrazin. Indeed, the main user of these products is the public sector. An investigation was realised to locate the main sites which are treated with these pesticides in this commune.
The conclusion of this study is that the local authority used dichlobenil, bromacile and atrazine to weed the public areas. The pollution could be due to the presence of the cleaning area near the catchments. Furthermore the hydrogeology context lead us to think that the pesticides residues of this area can get to the groundwater.

BACTERIAL BIODEGRADATION OF ORGANOPHOSPHATE PESTICIDE CHLORPYRIFOS BY EGYPTIAN BACTERIAL ISOLATES

Reda A. BAYOUMI, Ezz AL-DEEN M. MAHMOUD & Abdullah H. ATTEIA
Botany and Microbiology Dept., Faculty of Science (Boys)
Al-Azhar University, P.O. 11884, Cairo, Egypt
E-mail: redaelbayoumi@yahoo.com

This study also concerning biodetoxification and biodegradation of organophosphate pesticide chlorpyrifos Dursban by using bacteria. Sixteen microbial isolates were isolated from three soil and activated sludge collected from different Egyptian localities could biodegraded organophosphate pesticide (Dursban). Only six bacterial isolates were capable of biodegrading of Dursban and utilizing it as only source of carbon and energy aqueous media.. The six bacterial isolates managed to grow on enrichment medium containing Dursban up to 50 ml / liter, with seven days at 25 ºC, but each one exhibited different potentiality in its growth on different Dursban concentrations. The two most potent bacterial isolates out of six isolates capable of biodegraded and utilizing Dursban as only sole source of carbon and energy, so that it was selected and identified down the species level. as following: *Pseudomonas stutzeri*-S7-B4 and *Flavobacterium* sp.-S8-B5. These two bacterial isolates were also subjected to further work including studying some environmental and nutritional parameters which affecting the biodegradation process of Dursban. These including: the optimum incubation period,(7 days); Dursban concentrations, (10 ml/l); inoculum size,(4 ml/l); incubation temperature, (35°C); optimum pH value,(7); carbon source,(fructose & ribose) respectively; nitrogen source, (urea and peptone) respectively; amino acid, (histiden); vitamin source, (yeast extract), under shaking condition. Degradation study of Chlorpyrifos by *Pseudomonas stutzeri*-S7-B4 and *Flavobacterium* sp.-S8-B5 and was examined by GC-MS; no persistence accumulated metabolite was observed. The best results in the present study two bacterial strains could completely mineralize chlorpyrifos. This two bacterial isolates will be potentially useful in biotreatment of wastewater and bioremediation of contaminated soil.

SURVEY ON THE IMPLEMENTATION OF REDUCING TECHNIQUES FOR POINT SOURCE EMISSIONS OF PESTICIDES TO SURFACE WATER IN THE POEKEBEEK WATER CATCHMENT IN FLANDERS

E. DE ROCKER[1], I. VAN DE POPULIERE[2], P. VAN PARIJS[3] & J. VERGAUWEN[4]

[1] Vegetable Research Centre East-Flanders, Kruishoutem, Belgium
[2] Province East-Flanders, Ghent, Belgium
[3] Research Centre for Ornamental Plants, Destelbergen, Belgium
[4] Potato Research Centre, Kruishoutem, Belgium

Point source emissions are considered as the most important emission sources of pesticides to surface water in Flanders. During the winter of 2007-2008 a survey has been conducted on the implementation of good agricultural practices by farmers in het Poekebeek water catchment. In the surface water of the Poekebeek catchments different pesticides (e.g. glyphosate) and pesticides residues (e.g. AMPA) are frequently detected during monitoring campaigns of the Flemish Environment Agency (VMM). Although not all of the detected chemical compounds are from pesticide applications in agriculture, the farmers have an important role in avoiding point source emissions of pesticides to surface water.

About 75% of the farmers, that contributed to the survey, take the responsibility of the pesticide applications on their farms and in only 25% of the cases the application was executed by an external operator. 16% of the farms were diary farms, 30% mixed agriculture-diary farms, 14% mixed agriculture-diary-vegetable farms and the other farms grew ornamental plants (11%), vegetable crops (8%), agricultural crops (5%) and fruit or other crops (6%). In 73% of the cases field sprayers were used. At the time of the survey about half of these sprayers were not yet equipped with a rinsing water tank. However, it can be expected that this number will diminish very soon, because of a legally bound sprayer control every 3 years in Flanders. In case of a rinsing water tank the volume is about 10% of the volume of the sprayer tank. Only about 20% of the field sprayers had already a device for external cleaning in the field. On most of the farms the external parts of the sprayer equipment are still cleaned on hard surfaces on the farm with risk of point source emission to surface waters or sewage systems. External cleaning on the field can diminish these emission considerably. When cleaning on the farm at least the rinsing or cleaning water should be collected. Research is done in Flanders to treat these effluents by bioremediation systems, but a suitable legislation in Flanders does not yet exist. The most important measure to avoid point source emissions during filling of the sprayer tank is a visual control by the farmer himself and the use of a antifoam product. Therefore it is important to promote more the use of control systems during filling and the use of absorbing materials in case of spilling. The most important restrictions for farmers to implement innovative practices to avoid point source emissions are the extra investment costs and the lack of sufficient financial support to implement them.

ADSORPTION BEHAVIOR OF PESTICIDES DETECTED IN ENVIRONMENTAL WATER SAMPLES ON MODIFIED ORGANIC WASTE RESIDUES USED IN BIOPURIFICATION SYSTEMS

Hicham EL BAKOURI[1], José USERO[1], José MORILLO[1] & Abdelhamid OUASSINI[2]

[1] Department of Chemical and Environmental Engineering, University of Seville
ES-41092 Seville, Spain

[2] Department of Chemical Engineering, University of Abdelmalek Essaadi
MA-90000, Tangier, Morocco

Numerous studies have reported that a great part of ground and surface water contamination is caused by direct losses (e.g. spills during filling operations, leakages of spray equipment, spray leftovers, spills of rinsing water from cleaning of the spraying equipment). The situation is worse when the contaminated water is thrown away sites with minimal opportunity for pesticide sorption and/or degradation.

Several recent publications report the use of low-cost and locally available adsorbents to purify water contaminated by pesticides and other hazardous chemicals. Nonetheless, the literature is still insufficient to cover this problem, and more work and studies are needed in this field to develop other locally available and economical adsorbents to eliminate pollutants from water.

The purpose of this work is to understand the adsorption and degradation behaviour of different pesticides on modified organic waste residues. The effect of several physicochemical parameters, such as sorbent particle size, contact time, temperature and pesticide concentration, was evaluated to better describe the adsorption process. The vertical mobility of the studied chemicals was performed at laboratory scale using soil columns with modified organic amendments. The vertical mobility of the studied chemicals was performed at laboratory scale using soil columns with modified organic amendments. The real efficiency of the adsorbents was evaluated using spherical biopurification backs designated to regenerate waters that can be used as irrigation water for biomass crops destined for green energy production.

TRANSPORT AND DEGRADATION OF PESTICIDES IN A BIOPURIFICATION SYSTEM UNDER VARIABLE FLUX

Tineke DE WILDE[1], Pieter SPANOGHE[1], Jaak RYCKEBOER[2], Peter JAEKEN[3] & Dirk SPRINGAEL[2]

[1] Laboratory of Crop Protection Chemistry, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, BE-9000 Gent, Belgium

[2] Division Soil and Water Management, Faculty of Bioscience Engineering, K.U. Leuven Kasteelpark Arenberg 20, BE-3001 Heverlee, Belgium

[3] PCF-Royal Research Station of Gorsem De Brede Akker 13, BE-3800 Sint-Truiden, Belgium (present address: Essenscia, A. Reyerslaan 80, BE-1030 Brussel, Belgium)

The efficiency of a biopurification system (to treat pesticide contaminated water), is for a big extent determined by the chemical and hydraulic load. A high input of pesticide contaminated water, might lead to a decreased retention and biodegradation of the pesticide. Therefore, insight into the behaviour of pesticides under different fluxes in the biopurification system is necessary. Consequently, the behaviour of metalaxyl, bentazone, linuron, isoproturon and metamitron was studied under three different flows with or without the presence of pesticide-primed soil in micro- and macrocosms (column experiments).

An increasing flux, appeared to induce non-equilibrium sorption at the highest flow for all pesticides (except linuron) in the microcosms. As the sorption process was time-dependent at the highest flows, retention of the pesticides with intermediate mobility (metalaxyl, metamitron and isoproturon) was significantly influenced by the flux, this in contrast to bentazone and linuron, respectively, a very mobile and immobile pesticide. Degradation of the intermediate mobile pesticides in microcosms was also submissive to variations in flux as sorption is strongly related to degradation. An increase in flux, leads to a decrease in retention, which in turn decreases the opportunity time for biodegradation. The presence of pesticide-primed soil was only beneficial for the degradation of metalaxyl.

Sorption of the studied pesticides in the macrocosms was considerably higher compared to the microcosms. Breakthrough of isoproturon, linuron, metamitron and metalaxyl was small or not detectable. Retention of bentazone was again not influenced by the flux, but dissipation of the studied pesticides in the effluent was slightly larger at the lowest flow. Moreover, it could be observed that incorporation of pesticide-primed soil in the macrocosms decreased the presence of pesticides in the effluent slightly.

Finally, it could be concluded that the influence of flux (within the range studied) decreased with increasing column size. This means that a biopurification system at full scale will be less influenced by variations in flux, this is however only valid within the boundaries studied in this work.

A META-ANALYSIS OF EFFECTS OF PROCESSING ON PESTICIDE RESIDUES IN FRESH PRODUCE

Boitshepo M. KEIKOTLHAILE, Pieter SPANOGHE & Walter STEURBAUT
Laboratory of Crop Protection Chemistry, Ghent University
Coupure Links 653, BE-9000 Gent, Belgium

Researchers have been concerned about the effect of food processing on pesticide residues and some studies have been dedicated to this issue. Studies investigating method like washing and peeling have concluded that there is reduction to the residues. Meta-analysis has been used to quantitatively summarise findings from different experiments addressing the same issue in various fields such as ecology, medicine and social sciences. In this paper, we apply meta-analysis of response ratios in a systematic review of effects of food processing on pesticide residues in fruits and vegetables. Response ratio quantifies proportional experimental change brought by the processing method. The pre-processing and post processing concentrations of pesticide residues together with their standard deviations were obtained from literature sources. The total numbers of samples in the investigation were also recorded. These values were divided according to different processing techniques and evaluated for homogeneity using Q-statistics. The test showed that the data was non-homogenous and therefore random effects method was used in meta-analysis calculations. The effects of food processing on pesticide residues were calculated at 95 and 99.5% confidence limits.

DEGRADATION OF ^{14}C-GLYPHOSATE IN COMPOST AMENDED SOILS

Ersilia ALEXA[1], Mihaela BRAGEA[2], Renata SUMALAN[1], Monica NEGREA[1] & Aurel LAZUREANU[1]

[1] Banat's University of Agricultural Science Timisoara
Calea Aradului 119, RO-300645, Timisoara, Romania
[2] Institute of Public Health, Timisoara, Romania

Glyphosate (N-phosphonomethyl-glycine), the active ingredient in several herbicide formulations, is a non-selective, post-emergent herbicide used in a variety of crop and non-crop situations. Glyphosate is a non-volatile herbicide that is relatively immobile in soil. Its degradation is due to microbiological processes and most laboratory studies have been conducted with 14C-glyphosate with the rate of ^{14}CO2 evolution being used as an indication of herbicide breakdown.

In this paper we have studied the glyphosate degradation in compost amendment soils using Scientilator Liquid TRIATHLER and Glyphosate-phosphonomethyl-^{14}C-labeled with specific activity 2,2mCi/mmol. Four types of soils have been taken under study: Black Chernozem, Vertisol, Gleysol and Phaeozem with different characteristics. For the each type of soil have been realized four experimental variants (glyphosate blind sample with 1,5 ppm, concentration, autoclaved soil, soil with glyphosate and addition of compost in field concentration of 40 t/ha, respectively 60 t/ha.

The mineralization curves of $^{14}CO_2$ accumulated were compared during of 40 days. All the mineralization curves for the soils exhibited same patterns, with only two phases, the initial rapid phase of degradation, for about 20 days, attributed to microbial action on the free glyphosate and the second slow phase, when the curves attained plateaus.

Compost applied with different concentrations to Vertisol and Black Chernozem did not appear to stimulate the microbial degradation of glyphosate. In Gleysol and Phaeozem with lower humus content, the mineralization curve of ^{14}C indicate the increase degradation capacity, expressed as accumulated $^{14}CO_2$ as % total ^{14}C, with the increase of compost concentration.

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STATUS QUO OF THE WESTERN CORN ROOTWORM *DIABROTICA V.VIRGIFERA*: SUCCESSFUL MANAGEMENT 2008 IN SOUTHERN SWITZERLAND BY MONITORING AND CROP ROTATION

Hans E. HUMMEL[1,2] & M. BERTOSSA[3]
[1] J. Liebig University, Organic Agriculture
Karl-Gloeckner-Str. 21C, DZ-35394 Giessen, Germany
[2] Illinois Natural History Survey, Biodiversity and Ecological Entomology
Champaign, Ill. 61820, USA
[3] Agroscope Changins-Wädenswil ACW, Centro Cadenazzo
CH-6594 Contone, Switzerland

Introduction: *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae), the western corn rootworm (WCR), is one of the more challenging alien invasive insect pests in Europe. Its eradication throughout Europe is an elusive goal and, similarly to the US, likely doomed to failure. But the management option of crop rotation for Switzerland with its special geographical conditions turns out as a realistic approach, as our research efforts in the Ticino canton over the last five years indicate.
Methods: Sticky cup traps used for survey and monitoring are described by Levine and Metcalf (1988) and are used extensively for our research (Hummel et al. 2005, Hummel et al. 2006, Bertossa and Hummel 2008).
Results and discussion: Mandatory crop rotation has been introduced in Switzerland since beginning of WCR detection in 2000 as the exclusive, permanent and sustainable IPM measure and has been practiced ever since. As a general result, WCR populations have been kept stable at a non-economic level until now. Two specific trapping and survey patterns were obtained in 2008 and plotted with resolution over 1.time and 2.space. As for time, beetle flights began in 2008 during week 30 and lasted until week 39. The flight maximum occurred during week 33 approaching a total of 100 beetles per ten pheromone baited Metcalf traps. Kairomone baited Metcalf traps and Csalomon traps showed an exactly parallel pattern, although at significantly lower levels.–When plotted over space, traps at several locations produced a higher number of beetles than the remaining locations. So far, it is undecided if this difference is explainable by a) the proximity of the respective traps to major railroad or road traffic routes or b) the annual influx by air of WCR migrating from Lombardy (Italy) through the southern to the northern Ticino as described by Bertossa et al.(2004). In the latter case b the distribution of beetles would reflect the gradient of WCR settling along their main flight path as dictated by the prevailing meteorology. Closer future analysis may reveal that the observed pattern may be a combination of a and b. A powerful indicator for the validity of b is the capture in 2008 of 2 WCR at mountain pass Mt.Ceneri where no maize is being grown but where WCR must migrate through in order to reach the maize fields in Magadino plain and adjacent side valleys connecting northward to St. Gotthard and St. Bernardino passes.
Conclusions: Results and their significance for IPM of WCR may not be directly transferable to different topographies and conditions found in central European countries. The successful principle of crop rotation, however, is tempting and efforts towards its realization should be undertaken in this direction.

MONITORING WESTERN CORN ROOTWORM (*DIABROTICA V.VIRGIFERA*) ADULTS: SITUATION IN ROMANIA 2008

Hans E.HUMMEL[1,2], S. DINNESEN[3], T. NEDELEV[3] & Ch. ULRICHS[3]
[1] J. Liebig University, Organic Agriculture
Karl-Gloeckner-Str. 21C, DE-35394 Giessen, Germany
[2] Illinois Natural History Survey, Biodiversity and Ecological Entomology
Champaign, 61820 Ill. USA
[3] Humboldt-Universität zu Berlin, Urban Ecophysiology
Lentzeallee 55, DE-14195 Berlin, Germany

Introduction: In the EU, Romania ranks first in total area of grain maize (2.5 million ha) while the country occupies rank four only in actual maize production of 7.9 million tons. Abiotic factors but also numerous insect pests are responsible for these losses along the production chain. Among the insects, *Diabrotica v. virgifera* LeConte (Col.: Chrysomelidae) (WCR) is prominent. Maize monoculture greatly facilitated the progress of this invasive pest during the last dozen years. From all indicators, WCR is there to stay and is expected to further expand from westerly to easterly direction, both naturally and with the help of mankind. We investigated the suitability of our monitoring system based on pheromone and kairomone trapping under typical Romanian growing conditions and will present first results.
Methods: Survey and monitoring traps are of the Metcalf sticky cup design and are baited with 8-methyl-decane-2-olpropanoate (pheromone) and 4-methoxy-cinnamaldehyde (kairomone) lures dispensed on heavy filter paper. In addition and for comparison, mass capacity traps of the Vario type have also been used.
Results and discussion: Regional differences exist in WCR numbers between maize field plots of Banat's University farm situated close to the town of Timisoara and commercial production sites at the village of Sag some 10 km to the south. At both locations, a major WCR population peak at the end of July and beginning of August of 2008 was observed, followed by a much smaller peak in the last week of August. Reasons for this second peak are not immediately obvious and need further research. At Sag, the peak flight is delayed by about one week compared to Timisoara, which may be attributed to the higher sum of degree days accumulated near urban areas.-In absolute numbers, 8996 WCR were trapped in maize fields near Sag with up to 90 adults/trap/day. At Timisoara, 5253 beetles were trapped, with an average ranging from 90-105 beetles/trap/day. The second minor flight peak toward the end of August was much weaker and yielded 37, resp. 20 WCR/trap/day in the maize fields. In contrast to common assumption, alternative hosts such as *Sorghum* spp., *Helianthus annuus* and melons can harbor numerous WCR and may diminish the future effectiveness of crop rotation as one of the most practicable and inexpensive pest management options available. Noteworthy were the strongly attractive kairomone baited traps in *Sorghum* at Timisoara. High capacity traps baited with pheromone are surprisingly effective and, in combination with the silica powder AL06, will be considered as future alternatives to Metcalf traps in cases where expenses for frequent monitoring are to be minimized.
Outlook: This monitoring system will be a valuable component of future WCR management in Romania and elsewhere.

MAKING SOIL CONTAINING NUMEROUS EGGS OF WCR FOR GREENHOUSE AND LABORATORY EXPERIMENTS

Tamás NÉMETH[1], Miklós NÁDASY[1], Zsolt MARCZALI[1] & József TAKÁCS[2]
[1] University of Pannonia Georgikon Faculty Institute for Plant Protection Keszthely, Hungary
[2] Kleffmann & Partner Ltd. Budapest, Hungary

In the course of our work we often faced to the problem that WCR lays its eggs unevenly so it is impossible to find soils under field circumstances which contains eggs in homogenous distribution and in large numbers. Owing to the inhomogeneous distribution and low number of eggs it is quite difficult to study the effectiveness of soil disinfectant and seed-dressing insecticides on larvae of WCR in pot experiments. Therefore, the aim of our studies was to gain soil samples with known quantity and distribution of eggs. According to our idea, numerous adults were placed into a relatively small place under ideal environmental conditions and a small quantity of soil was provided for them to lay eggs.
During summer season after a couple of weeks of the egg laying we started collecting adults. In favour of expediting our work, traps containing flower-scent as bait without moth-killer cases (Csalomon KLPflor+) were placed to the experimental field at Hetes, Somogy County. The great advantage of this type of trap is that it attracts both female and male adults. The traps were emptying regularly. The adults were putted into 20 l volume plastic containers provided with well closing lids. Ventilation of containers was assured with holes bored into the lids. The lower third of containers were filled up with brown forest soil collected in the experimental field and moistened to 70% of the field capacity to creating ideal conditions for egg-laying. As a food source ears of maize with green pistils and immature corns were placed into the containers. After the preparations approximately 500 adults with 1:1 sex ratio were colonized into each container. The research materials were finally placed in the climate chamber of entomological laboratory of University of Pannonia Georgikon Faculty, Institute for Plant Protection. The mating and later egg-laying of WCR adults occurred by 26C°L/D 16/8 photoperiod. After 2-3 days fresh food was regularly placed into the containers till each adult died. From containers which did not contain any more living adults rest of food sources were removed and soil was loosen and mixed with planter shovel to provide homogenous egg distribution. The numbers of eggs in soil unit was determined in 5 containers with egg-washing method. On the basis of results it can be laid down as a fact that our method is capable for gaining soil containing large number of eggs for further infections of test containers. It can serve as a solid base for ecological studies as well, which have great importance in elaboration of a forecasting model.

VALIDATION OF THE BERLESE-FUNNEL TECHNIQUE FOR THRIPS EXTRACTION

H. CASTEELS, J. WITTERS, G. DE BONDT & J. DESAMBLANX
Institute for Agricultural and Fisheries Research (ILVO), Plant – Crop Protection
Burg. Van Gansberghelaan 96, BE-9820 Merelbeke, Belgium

Imported plant material is continuously examined at the Diagnostic Centre for Plants (ILVO) on the presence of quarantine insects and mites. Many of these organisms can be detected during direct observation of plant material using the dissecting microscope. This method is not always safe, because insects, in particular the winged adults, can move away during the manipulation of the examined plants. Results in the lab indicated that the Berlese-funnel method is more accurate. The validation of the Berlese-funnel technique for thrips extraction was necessary in order to be accredited to the standard EN ISO/IEC 17025. The apparatus used in the Diagnostic Centre for Plants is a 27-litre closed funnel system.
Thrips fuscipennis was collected from heavily infested *Rosa* plants a day before the experiments and used as target organism. All mobile stages of these thrips were counted and used for the experiments. Twenty leaves of *Acer pseudoplatanus* were contaminated artificially with 1, 5 and 10 thrips. Dataloggers were used for the registration of the temperature during the extraction period. The following parameters were validated: cleaning of the funnel, temperature during isolation, detection limit and isolation time.
Results showed that thrips can be present alive or dead in the funnel after removing the treated plants and can contaminate the next sample. The funnel must be cleaned with a vacuum cleaner and air compressor before running a new extraction. Contamination of the recipient is also possible from the environment. The optimal average temperature for the isolation of the thrips is between 35,74 °C and 39,38 °C . Based on the results we can conclude that an isolation time of 20 hours is necessary to obtain accurate data. Dependent on the number of thrips in the sample 87 to 95% is isolated after 20 hours. Results of the validation also showed that the detection limit is one thrips per sample with a probability of 95% for isolation after 20 hours.

ARTIFICIAL DIETS FOR LARVAE OF *ANARSIA LINEATELLA* ZELLER (LEPIDOPTERA: GELECHIIDAE)

P. DAMOS, Ch. SPANOUDIS & M. SAVOPOULOU-SOULTANI
Laboratory of Applied Zoology and Parasitology
Faculty of Agriculture Aristotle University of Thessaloniki
GR-54124 Thessaloniki, Greece
E-mail: matilda@agro.auth.gr, damos@agro.auth.gr

Maintenance of an insect colony under laboratory conditions is considered a pre-requisite for further study. However, numerous artificial diet formulas, such as dietary replacements or supplements, influence species growth and survivorship and display difficulties in utilization in laboratory settings. In this work, successful rearing in the laboratory is reported for the peach twig borer *A. lineatella* when developed on artificial diet. Furthermore, by using different nutritional media we made comparative studies in order to improve rearing conditions of *A. lineatella* in the laboratory. Thus, media based on alfa-alfa meal and Brewer's yeast as major components, significantly affected larval development and survivorship. Moreover, studies on artificial diets based on high concentrations of sucrose or containing additional casein also effected significantly development and survivorship, while in most cases a high susceptibility on microorganisms was also observed. A successful establishment of *A. lineatella* populations however, for successive generations in the laboratory was managed, when larvae developed on a nutritional medium based on lima beans. Such a diet was first proposed by Ashbys *et al*. (1956) for the development of the Tortricid *Cydia pomonela.* Except lima beans (380g) the diet contained 1360 ml distilled water, 31 g agar and preservatives. In addition, considerable improvement in growth and maintenance of successive generations of *A. lineatella* was observed on this diet in laboratory when compared to natural host.

THE EFFECT OF TEMPERATURE ON DEVELOPMENT OF *AGONOSCENA PISTACIAE* (HEM.: PSYLLIDAE)

Mohammad Reza HASSANI[1], GAdir NOURI-GANBALANI[2], Hamzeh IZADI[3] & Mehdi BASIRAT[4]

[1] Department of Plant Protection, Islamic Azad University, Rafsanjan Branch, Rafsanjan, Iran
[2] University of Mohaghegh Ardabili, Ardabil, Iran
[3] University of Vali-e-Asr, Rafsanjan, Iran
[4] Pistachio Research Institute, Rafsanjan, Iran

The pistachio is one of the most important agricultural products in Iran. The common pistachio psylla, *Agonoscena pistaciae* Burckhardt & Lauterer is a major pest of pistachio trees throughout pistachio-producing regions in Iran. The Developmental time for egg, nymph and the total period from egg to adult of *A. pistaciae* determined at different constant temperatures, i.e (15, 20, 25, 30 and 35^{o}C) at controlled conditions in growth chamber incubator. The Developmental times for different stages of *A. pistaciae* were used to calculate the developmental rates as r(T) = 1/t. The relationship between temperature and developmental rate was described by linear model. Temperature point 35 ^{o}C, above the linear portion of the developmental rate curve was not used to estimate the lower developmental threshold and thermal constant. Based on temperatures and developmental rates, lower developmental threshold (T_o) and thermal constant (K, degree-day) were estimated by hyperbole method as: Y = a + bT, for egg and nymph and egg to adult. The lower developmental threshold was estimated as c = -a/b and the thermal constant was estimated as k = 1/b. The lower temperature threshold for egg was obtained 8.18^{o}C and for nymph was obtained 10.39 ^{o}C. The lower temperature threshold form egg to adult was obtained 9.96^{o}C. Thermal constant of *A. pistaciae* for egg development was calculated 88 DD, and for nymph was calculated 241 DD. Thermal constant of *A. pistaciae* to complete development from egg to adult was calculated 326 DD. These information's could be incorporated in forecasting models of this pest.

INFLUENCE OF A MUTUALISTIC FUNGAL ENDOPHYTE ON POPULATION DEVELOPMENT OF *APHIS GOSSYPII* IN THE PHYLOSPHERE OF SQUASH

P.A. ALVARADO PRINCE, M.H. HAGEMANN*, J. KRANZ & R.A. SIKORA
Phytopathology and Nematology in Soil Ecosystems, Institute for Crop Science and Resource Conservation INRES, University of Bonn, Germany
University of Bonn, Nussallee 9, DE-53115 Bonn, Germany
*Corresponding author E-mail: michaelhelmut@googlemail.com

The role of mutualistic endophytic fungi in pest management systems for the biological of nematode and fungal pathogens is well studied. The fungal isolate *Fusarium oxysporum* strain 162 (FO162) has been repeatedly shown in the past to have induced resistance activity as well as repellency or lack-of-attraction effects on nematodes parasitizing different crops. In this study FO162 was tested for systemic bases biological control activity the aphid *A. gossypii.* The experiments tested the effect of seed dressing with FO162 on the number of leaf aphids in the shoots of squash (*Cucurbita pepo* L. var. *giromontiina*). Systemic activity toward *Aphis gossypii* was tested by placing 10 adults on the leaves of endophyte treated and control plants and then by recording population development after 8$\pm$1 days. The results showed a reduction in aphid population development in plants treated with the endophyte FO162 compared the untreated plants. The presence of the fungus in the root endorhiza was confirmed by re-isolating the endophyte from surface sterilized root samples taken at different soil depths. The results also showed clearly that the endophyte has a strong preference for the root elongation zone. The fungus was found in about 30% of all root samples. The fact that FO162 has never been detected in the shoots demonstrates that the negative effects of FO162 on aphid populations are of a systemic nature, moving acropetal into the shoot from the root system. It is important for biological management of pests that a mutualistic endophyte FO162 that reduces plant parasitic nematodes on a number of crops is also able to exert biological control activity toward aphids in the shoot tissue.

ULTRASTRUCTURE AND DESCRIPTION OF THE FIRST IMMATURE STAGE OF FOUR DIFFERENT SCALE INSECT SPECIES (HEMIPTERA: COCCOIDEA) IN EGYPT

A.K. MOURAD[2], Sahar M. BESHR[1], I.S. ABDEL-RAZAK[1] & K.S. MOURSI[1]

[1] Agricultural Research Centre, Plant Protection Research Institute
Scale Insects and Mealybugs Dept. Alexandria, Egypt
[2] Faculty of Agriculture, Saba-Basha, Plant Protection Dept.
Alexandria University, Alexandria, Egypt

First stage of four different species belonging to three families of the super family: Coccoidea are described and illustrated by using scanning electron microscope. These species are : *Mycetaspis personata* (Comstock) and *Fiorinia fioriniae* (Targioni Tozzetti), Family: Diaspididae; *Pulvinaria psidii* (Maskell), Family: Coccidae and *Icerya seychellarum seychellarum* (Westw.), Family: Margarodidae. The dimensions of the first stage (crawler), as well as some taxonomical aspects were examined and discussed. The obtained data showed that the first instar of the mealybug *I. seychellarum seychellarum* gave the largest diameter of 299.33 ± 0.94 µm in length and 183.44 ± 0.17 µm in width followed by the diaspidid species *F. fioriniae* recorded 208.1 ± 0.78 µm in length and 178.96 ± 2.34 µm in width, while the coccid species *P. psidii* measuring 119.17 ± 0.85 µm in length and 74.83 ± 1.03 µm in width and the smallest first stage crawler was the diaspidid *M. personata* measuring 85.50 ± 0.41 µm in length and 63.88 ± 0.27 µm in width. Also, the modes of insect protection are briefly discussed according to their means of egg laying.

SEASONAL TRENDS OF WHITEFLY POPULATIONS IN A MEDITERRANEAN TOMATO GROWING AREA

Mauro NANNINI, Francesco FODDI, Giovanni MURGIA, Riccardo PISCI, Francesco SANNA & Marco TESTA
Agris Sardegna - DIRVE, Cagliari, Italy

The whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum* and their associated viruses constitute a major threat to tomato crops in the Mediterranean region. Continuous host availability and mild climate are thought to be among the factors contributing to the outbreaks of whitefly-related problems in this area. We carried out a year-long survey to investigate the relative contribution of different plants, agricultural and not, and indoor/outdoor crops as hosts of the two whiteflies and the tomato yellow leaf curl disease (TYLCD) in a multi-crop system typical of tomato growing areas in southern Sardinia (S. Margherita di Pula, Cagliari, Italy). For this purpose, during 2005 we monitored whitefly population trends in different plots of a horticulture farm, evaluated seasonal changes in the infestation density of the two pests on the most represented host species, and assessed the incidence of TYLCD on tomato crops and susceptible weeds.
Whitefly catches on yellow sticky traps were found to be higher inside and along the external perimeter of greenhouses than in open field crops or uncultivated areas, suggesting significant adult movement between indoor and outdoor patches. In most plots flight activity increased between late spring and late summer, peaking in July. The number of immatures of the two whitefly species showed similar dynamics, but while *T. vaporariorum* reached the highest densities on greenhouse tomato crops in June, peak levels of *B. tabaci* were recorded between July and August on outdoor horticultural crops and weeds. The occurrence of TYLCD was detected almost all year round on weed hosts, but the highest number of infected plants was observed in June on long cycle tomato crops. The present survey has demonstrated the contribution of non-agricultural plants in the maintenance of tomato yellow leaf curl disease in the study site. However tomato crops established in summer as major reservoirs of TYLCD-associated viruses and presumably played a key role in the spread of the disease to autumn tomato crops. The implementation of a crop-free period between successive susceptible crops, or at least avoiding whitefly movement through greenhouse openings and completely destroying plants after final harvest, could probably reduce the severity of TYLCD epidemics in this area.

PERSISTENCE OF THE ENTOMOPATHOGENIC FUNGUS *LECANICILLIUM MUSCARIUM* ZARE AND GAMS UNDER OUTDOOR CONDITIONS

Sandra LERCHE, Helga SERMANN & Carmen BÜTTNER
Humboldt University of Berlin, Institute for Horticultural Sciences
Department of Phytomedicine,
Lentzeallee 55-57, DE-14195 Berlin, Germany
E-Mail: lerche74@hotmail.com

Positive results from laboratory trials, to proof the effectiveness of the entomopathogenic fungus *L. muscarium* against endophytic damaging larvae of the horse chestnut leafminer moth *Cameraria ohridella* DESCHKA & DIMIC, led to following outdoor trials. One aspect of the test was to determine the persistence of the fungus, which was used as commercial product Mycotal® (Koppert, NL) and as strain V24 from the department Phytomedicine. In different variants were tested several spore concentrations and the influence of an oil-containing addit (Koppert, NL). The trial took place on horse chestnut seedlings, 3 years old. The determination of persistence followed 1, 7, 14 days past application of the suspension (dpa) through the numbers of colony forming units (cfu) after impressing the leaves on agar plates.
Despite most unfavourable weather conditions, like above-average of temperature and hours with sunshine as well as low humidity and heavy rainfall, the fungus could be detected until 14 dpa, with differences between the variants. The application of the fungus led to moulding of larvae within the mines.
The sporulation of *L. muscarium* on the cadaver of the host under outdoor conditions proves the ability of the fungus to germinate, infect and kill the larvae followed by growing and sporulation at the cadaver. Furthermore the results show the persistence of *L. muscarium* on the plant-leaves during the time of trial.

Further investigations follow.

SOIL APPLICATION OF *BEAUVERIA BASSIANA* TO CONTROL *CERATITIS CAPITATA* IN SEMI FIELD CONDITIONS

Ali ALI, Helga SERMANN, Sandra LERCHE & Carmen BÜTTNER
Humboldt Universität zu Berlin, Institute for Horticultural Sciences
Department of Phytomedicine,
Lentzeallee 55-57, DE-14195 Berlin, Germany
E-mail: aligermany80@yahoo.de

The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) is a highly polyphagous pest of economic importance culture in Syria as in many other parts of the world and has up to 8 generations in each year.
The potential of the entomopathogenic fungus *B. bassiana* BALS (VUIL.) against adults of Mediterranean fruit fly *C. capitata* was evaluated in semi field conditions during the summer season 2008.
Soil (5-7 cm high) was filled into plastic container (27 cm × 32 cm). In one container 75 pupae, two days before emergency, were spread uniformly on the soil. Then the pupae, obtained from the laboratory breeding, were covered with soil (4–5 cm layer). After that, 30 ml suspension of fungal spores (4×10^8 Spores/ml) was applied to the soil surface using a dash bottle; this is in conformity with a spore density of 1.3×10^7 Spores/cm^2 on soil. Suspension was produced on biomalt agar in a laboratory. The germination of conidia was nearby 98%. After application the container was transferred to net-cages (45×45×63 cm) in outdoor. For the emerged flies water and food (1:4 yeast, sucrose) were placed in the cages. There were 3 replicates for the treatment and control.
One month after the soil application, the dead and living adults were collected. The dead flies were sterilized in 0.5% NaOCl for 2 sec. and than in 70% ethanol for 2-3 sec. supplementary. After washing them in sterile water, the flies were placed in humidity chambers and incubated at 20°C in darkness. Two days later the number of moulded flies was counted.
The semi-field evaluations of *B. bassiana* revealed a fly mortality of about 46% and in the control with 16%. In addition 72% of dead flies were moulded in the treatment.
These results indicated, that the entomopathogenic fungus *B. bassiana* was pathogen against the adults of *C. capitata* not only in the laboratory condition but also under field condition, because the most number of dead flies was infected. That means that *B. bassiana* could decrease the population of *C. capitata*. Therefore *B. bassiana* could be an effective factor to control *C. capitata* in combination with other control methods used in IPM.

EVALUATION OF POTENTIAL BIORATIONALS FOR THE EFFECTIVE MANAGEMENT OF COCONUT MITE, *ACERIA GUERRERONIS* KEIFER

Arulanandam VAKEESAN & Gunasingham MIKUNTHAN
Department of Agricultural Biology, Faculty of Agriculture,
University of Jaffna, Jaffna, Sri Lanka.

The coconut perianth mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae) inflicts severe damage on coconut and considered as a key pest of coconut fruits. To assess biological control as a management tool for *A. guerreronis*, the efficacy of different mycopathogens such as *Fusarium* sp. isolate GM 15, *Fusarium semitectum* and *Lecanicillium lecanii* each at $1x10^9$ cfu/ml, was evaluated both in laboratory and field keeping a botanical pesticide, Azadirachtin (0.3g/l) as a standard check and water alone as control. In laboratory assessment, young nuts exhibiting triangular patches of damage which indicated the presence of active colonies were injected with the biorationals in between the perianth and nut surface at the place of the symptom and the nuts were incubated for 72 hours. The results of the laboratory bioassay revealed that *F. semitectum* and *L. lecanii* exhibited their potential in suppressing the population of mites until 72 hours. At 72 hours post exposure, mites treated with the isolate GM 15 had an average of 89% mortality versus 80%, 79% and 66% from treatment with *L. lecanii*, *F. semitectum* and Azadirachtin, respectively. In the field, biorationals were sprayed to the crown region of the palms and the mites were counted by detaching nuts from the inflorescence prior to spraying as well as after spraying. Among the biorationals evaluated in the field, the isolate GM 15 (93.91) and *F. semitectum* (78.74) significantly reduced mite population on 23 days after spraying over control and were the best opted biorationals for the effective management of the mite. Azadirachtin (66.44) was the next best to reduce the population of mites, however *L. lecanii* was found less effective to control *A. guerreronis* in field. These findings indicated that the isolate GM15 and *F. semitectum* are promising options for controlling *A. guerreronis* both in laboratory and field.

NATURAL OCCURRENCE OF ENTOMOPATHOGENIC FUNGI IN POTATO FIELDS AND THEIR VIRULENCE AGAINST POTATO TUBER MOTH, *PHTHORIMAEA OPERCULELLA*

Ali DERAKHSHAN
Dept. of Plant protection, College of Agriculture
Shahrood University of Technology, Iran

Shahrood region is one of the important potato production regions in Iran. The potato tuber moth (PTM) causes serious damage to potato crop under stored and field condition. In this study, soil samples were collected from different potato fields and entomopathogenic fungi (EPF) were isolated by Galleria bait method. Two species, *Beauveria bassiana* and *Metarhizium anisopliae* were isolated. Out of 80 soil samples, 70% had EPF from which 39.28% had *M. anisopliae*, 32.15 % had *B. bassiana* and 27.57% had both species. Six isolates (3 from each species) were selected to test their virulence towards PTM. Bioassays were conducted using larval dip and tuber dip methods. In larval dip bioassay, there was no significant difference in larval mortality among isolates but MM8 isolate caused less larval penetration to tubers and maximum mycosis was observed in MM22 isolate. In tuber dip bioassay, maximum larval mortality and mycosis caused by MM22 and minimum larval penetration observed in BT11 isolate. Comparison between two bioassay methods showed that larval mortality and mycosis were significantly more in larval dip method whereas larval penetration to tubers was significantly higher in tuber dip method.

BIOLOGICAL AGENTS FOR WHITEFLY CONTROL IN SARDINIAN GREENHOUSE TOMATOES

Mauro NANNINI, Francesco FODDI, Lorena MANCA, Riccardo PISCI & Francesco SANNA
Agris Sardegna - DIRVE, Cagliari, Italy

To evaluate the effectiveness of alternative options for biocontrol of whiteflies in greenhouse tomatoes, an experiment was carried out during the cropping season 2005-2006 in one of Sardinia's major horticultural districts (S. Margherita di Pula, Cagliari, Italy). Twelve long-cycle and 17 short-cycle tomato crops (8 autumn and 9 spring crops) were surveyed. All of them were treated for insect pest control at the beginning of the growing season, but in 19 out of 29 cases whitefly natural enemies were also released (BCA greenhouses), at least four weeks after the last treatment. The following release programmes were tested: on autumn crops, 1 *Macrolophus caliginosus* and 12 *Eretmocerus mundus*/m^2; on long-cycle crops, 1 *M. caliginosus* (released in autumn or spring) and 24 *Encarsia formosa*/m^2 or 48 *E. formosa*/m^2; on spring crops, 1 *M. caliginosus* and 24 *E. formosa*/m^2 or 48 *E. formosa*/m^2. The cost of each option was fixed at approximately 0.25 euros/m^2. The remaining greenhouses were maintained as controls (no BCA greenhouses). While whitefly and mirid populations were monitored monthly, whitefly species composition and mortality of immature stages were estimated at least twice during the growing season.
On short-cycle autumn crops, the release of *M. caliginosus* and *E. mundus* produced negligible results in terms of *Bemisia tabaci* control. On long-cycle and spring crops, even if in June mortality rates in BCA greenhouses were found to be 2 to 3-fold higher than in no-BCA greenhouses, *Trialeurodes vaporariorum* population growth was not significantly affected by natural enemies. Among the beneficials tested, *E. formosa* proved to be the most effective; *E. mundus* and *M. caliginosus* did not establish well, probably owing to the persistence of insecticide residues, scarce prey availability and intense plant de-leafing. The presence of indigenous natural enemies of whiteflies was observed in most sites, but in general they contributed little to biological control. The present experiment showed that in Sardinian tomato greenhouses the use of beneficial insects may result in inadequate biocontrol of whiteflies. In particular the application of cultural practices which may disrupt the establishment and development of predator and parasitoid populations should be critically re-examined.

MONITORING OF THE ENTOMOLOGICAL DIVERSITY IN A PESTICIDE FREE ORCHARD: INVESTIGATION OF THE GEMBLOUX AGRICULTURAL UNIVERSITY CONSERVATORY

René Noël POLIGUI, Eric HAUBRUGE & Frédéric FRANCIS
Functional & Evolutionary Entomology, Gembloux Agricultural University
Passage des Déportés 2, BE-5030 Gembloux, Belgium

In orchards, the fruit production is currently oriented to integrated pest management. In order to develop efficient pest control strategy by using entomophagous beneficials and lowering the application of pesticides, sufficient knowledge of entomological populations is needed. Here, a systematic monitoring of insect diversity and abundance in a conservatory orchard of Gembloux was performed. Combining yellow traps and visual observations, 19 pest and 34 beneficials families were identified among the 10766 and 3985 insects caught in yellow traps and visual observations respectively. Calculation of diversity indices allowed us to demonstrate the variability according to fruit tree species and insect observation method. Highest insect diversity was registered in apple, then in cherry and finally in the apple-pear association and plum. Pests and beneficials represented 27% and 39% of the insect collections respectively. Aphids represented from 81% to 95 % of pests depending on fruit tree species and observation method. The predominant specie was *Brachycaudus helychrysi*. Aphidophagous guild was largely present and mainly constituted by Coccinellids and hoverflies. *Harmonia axyridis* Pallas and *Episyrphus balteatus* De Geer were the most abundant. Aphididae was found to be the most common orchard pests with a range of related aphidophagous beneficials. These results highlight the need of monitoring leading to potential reasoning strategies in integrated pest management.

DIAMONDBACK MOTH IN UKRAINE:CURRANT STATUS AND PROSPECTS FOR USE BIOCONTROL AGENTS

Yaroslav O. LICKAR & Tatyana R. STEFANOVSKA
Department of Crop protection/Entomology
National University of Bioresources and Environmental Science
13 Heroiv Onorony, UA-03014 Kiev, Ukraine

The diamondback moth (DBM), *Plutella macuiipennis* (L.) (Lepidoptera: Plutellidae) is is the most damaging insect pest of cabbage in Ukraine especially in the Southern part. The biology, damage, population dynamics of diamondback moth and effect of natural enemies on the level of infestation of this pest were studied in 2004-2007 in the laboratory and field conditions. Obtained results shows that generally pest has 2-3 generations although 5-6 can develop in the South .The most problems diamondback moth larvae causes in 2 or 2-3 generation. During outbraves which stared from 2004 68-100% of plants colonize by pest with level of infestation 15/ per one plant.

The fecundity and life longevity of diamondback were studied on white cabbage, red cabbage, broccoli, cauliflower and two basic weeds: shepherd's purse and cola. The host plant influenced fecundity and life longevity of the diamondback moth. Fecundity differs significantly and is highest for white cabbage.

The fauna of diamondback moth parasitoids is quite rich. All staged are affected by numerous parasitoids and predators. It was recoded about 22 parasitoid species during the study. Overall parasitism ranged from 18 to 60 % with big differences between areas. *Apanteles* (Cotesia) sp., *Diadegma* sp., *Trichogramma sp.*, were most common in all areas. Entomopathogenic nematodes *Steinernema* sp. is found as a natural enemies of diamondback moth.

Natural enemies complex contributes significantly to the control of Diamondback moth. Conservation and augmentation of natural enemies should be use in IPM systems to control diamondback moth on cabbage. Entomopathogenic nematodes is prominent biocontrol agents.

FUNCTIONAL RESPONSE OF *ORIUS LAEVIGATUS* AND *ORIUS NIGER* (HEM.: ANTHOCORIDAE) TO *FRANKLINIELLA OCCIDENTALIS* (THYS.: THRIPIDAE), *TETRANYCHUS CINNABARINUS* (ACAR.: TETRANYCHIDAE) AND *MYZUS PERSICAE* (HOM.: APHIDIDAE)

Mehmet KEÇECI[1] **& M. OKTAY GÜRKAN**[2]

[1] West Mediterranean Agricultural Research Institute, P.O.Box 35, Antalya, Turkey

[2] Department of Plant Protection, Ankara University Agricultural Faculty, Ankara, Turkey

Two anthocorid predator, *Orius laevigatus* (Fieber) and *Orius niger* (Wolff) were evaluated as a biocontrol agent for main greenhouse pests, *Frankliniella occidentalis* Pergande, *Tetranychus cinnabarinus* Boisd. and *Myzus persicae* (Sulzer) in Turkey. The functional response of both *Orius* species to immature greenhouse pests were measured separately in small cages (4 x 1.5 cm) at 25°C. The predators were starved for 24 h. Predation was assessed by placing single adult *Orius* together 5, 10, 20, 30, 50 individual of *F. occidentalis*, *T. cinnabarinus* and *M. persicae.* After 24 h, predators were removed and remaining individual were counted.
O. laevigatus had a maximum attack rate of 31.0 thrips, 26.8 spider mites 24.3 aphid while *O. niger* consumed 30.1 thrips, 25.3 spider mites 23.8 aphid in 24 h. Both predators showed a Type II functional response to the pests.

FUNCTIONAL RESPONSE *HIPPODAMIA VARIEGATA* (GOEZE) (COLEOPTERA: COCINELIDAE) TO PISTACHIO PSYLLA *AGONOSCENA PISTACIAE* (HEMIPTERA: PSYLLIDAE)

Fatemeh ASGHARI[1], Kamran MAHDIAN[1], Mehdi BASIRAT[2], Mohammad AMIN SAMIA[1] & Hamzeh IZADI[1]

[1]Department of Crop Protection, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan Rafsanjan, Iran. [2]Pistachio Research Institute, Rafsanjan, Iran

Agonoscena pistaciae Burckhardt and Lauterer (Hemiptera: Psyllidae) is economically the most important species among the psyllid pests of pistachio in Iran. The spotted amber ladybird *Hippodamia variegata* (Goeze) (Coleoptera: Cocinelidae) is one of the most prominent Coccinelid predators in pistachio orchards. It is a highly polyphagous coccinelid that feeds mainly on aphids and psyllids of pistachio plants. This coccinellid has received some attention in the literature, but information regarding the predation capacity of this predator is still limited. The functional response of *H. variegata* to densities of adult *A. pistaciae* was assessed in laboratory. Female adults of *H. variegata* exhibited a type II functional response. Theoretical maximum predation rate was estimated 240 prey nymph per day. The estimates of handling time (T_h) and attack rate (a) were 0.10 h and 0.20 h^{-1}, respectively. The mean number of prey killed per day by *H. variegata* females ranged from 10 at a density of 10 prey to 365 at a density of 400 prey. The data indicated that the *H. variegata* may be a useful candidate for the biological control of *A. pistaciae*.

SOME BIOLOGICAL CHARACTERISTICS OF *CLOSTEROCERUS FORMOSUS* (HYM., EULOPHIDAE) PARASITOID OF *LIRIOMYZA TRIFOLII* (DIPT., AGROMYZIDAE)

Shahram HESAMI[1], Hadi OSTOVAN[2] & Ebrahim EBRAHIMI[3]
[1] Department of Plant Protection, College of Agriculture, Islamic Azad University
Shiraz branch, Shiraz, Iran
[2] Department of Entomology, Islamic Azad University
Fars Science and Research branch, Marvdasht, Iran
[3] Insect Taxonomy Research Department
Iranian Research Institute of Plant Protection, Tehran, Iran

Biology of *Closterocerus formosus* Westwood was studied in laboratory conditions (25 ± 1 ^{o}C, 60 ± 5 % RH, 16 L: 8 D) on the vegetable leafminer, *Liriomyza trifolii* (Burges) on bean. This parasitoid is a solitary endoparasitoid that attacks larvae of leafminer. The female usually laid one egg in a host and did not use the same host for both host feeding and oviposition. The mean developmental time (from egg to adult emergence) was 15.4 ± 0.2 days. When 30 old larvae of leafminer provided daily, the female killed 349.5 ± 52.6 hosts in her life span, 216.4 ± 25.2 with parasitizing and 143.1 ± 34.7 with host feeding. Host stage preference was investigated using equal number of young larva (2 days old) and old larva (4-5 days old) in choice and no-choice preference tests in 24 hours period. The results indicated that in choice experiment the mean number of young larvae and old larvae parasitized were 0 and 12.4 ± 3.7, and in no-choice experiment were 0.5 ± 0.2 and 14.2 ± 2.8, respectively.

ARTIFICIAL SUBSTRATES FOR THE REARING OF PREDATORY BUGS

Maarten BONTE, Veronic DE PUYSSELEYR, Bjorn VANDEKERKHOVE & Patrick DE CLERCQ
Laboratory of Agrozoology, Ghent University, Gent, Belgium

The mirid bug *Macrolophus pygmaeus* (Rambur) and the anthocorid *Orius laevigatus* (Fieber) are both biological control agents of numerous greenhouse pests such as thrips, whiteflies and aphids. In this study the effect of several substrate types on nymphal survival and development for both predatory bugs was evaluated.
Data on nymphal survival showed that lipophilic substrates such as wax paper, bean pods and plastic were more suitable for rearing *O. laevigatus* than household paper. *Orius laevigatus* could not benefit from a plant substrate such as a green bean pod.
In general, the presence of plant material had a positive effect on the development of *M. pygmaeus*. No mortality was observed on plant material. The mortality on the artificial substrates was slightly higher (varying from 4 – 12 %). The developmental time of *M. pygmaeus* on plants did not differ from that on household paper with a bees wax layer but was shorter than that on the other artificial substrates.
Both predators can thus be reared without plant material as a substrate but *O. laevigatus* appeared to be less dependent on plant material than *M. pygmaeus*, even though both predators are facultative phytophagous. Replacing plant material with artificial substrates in commercial rearing systems may allow a reduction in production cost for both heteropteran predators. In order to obtain a fully artificial rearing system, an artificial oviposition substrate needs to be developed.

USE OF PLANT MATERIAL AND *ARTEMIA* CYSTS FOR THE PRODUCTION OF *MACROLOPHUS PYGMAEUS*

Bjorn VANDEKERKHOVE[1], Laurian PARMENTIER[1], Gilbert VAN STAPPEN[2] & PATRICK DE CLERCQ[1]

[1] Laboratory of Agrozoology, Department of Crop Protection, Ghent University, Belgium
[2] Laboratory of Aquaculture & Artemia Reference Center, Department of Animal Production, Ghent University, Belgium

Macrolophus pygmaeus Rambur is a polyphagous mirid predator that is a successful biological control agent against several crop pests in European greenhouses. The suitability of cysts of the brine shrimp *Artemia* sp. as a factitious food for this predator in the presence or absence of plant material was investigated. Cysts of the brine shrimp were offered to the predator: these were either non decapsulated & non hydrated (NDNH), non decapsulated & hydrated (NDH), decapsulated & non hydrated (DNH) or decapsulated & hydrated (DH). In a control treatment *E. kuehniella* eggs were offered. The foods were either offered on a tobacco leaf disc on agar or on wax paper substrates supplemented with a parafilm capsule filled with tap water. When plant material was available survival was very high (>90%) in all treatments. No differences in developmental rates, adult weights and female oocyte counts were found between the different *Artemia* cysts forms and *E. kuehniella* eggs, except for the treatment NDNH, which resulted in lower body weights and longer development. Hydration of the cysts did not have a significant effect on any of the tested biological parameters. When no plant material was available there were large differences in survival on the different foods: survival was lower than 10% for nymphs fed NDNH cysts and varied between 50-75% for the other food sources. When predators were offered decapsulated cysts, no differences in development, adult weight and oocyte counts were found between hydrated cysts and non hydrated cysts. The effect of plant material on the predator's performance can probably be explained by hydration of the *Artemia* cysts when they are placed on leaf discs. Hydration profiles showed that these cysts with an initial water content of ca. 4% absorbed water and reached a water content of ca. 20% within 12h. When non decapsulated cysts are hydrated, the cyst shell becomes weaker and is probably easier to penetrate by the predator's stylets, which could explain the importance of hydrating the cysts in the absence of plant material. When cysts are decapsulated, the cyst shell is removed and hydration is of a lesser importance regarding accessibility of the cysts to the predator than for non decapsulated cysts. These findings can be of practical relevance when considering *Artemia* cysts as a food source for the production of *M. pygmaeus* with or without plant material.

MASS REARING METHODS AND BIOLOGY OF *MICROPLITIS MEDIATOR* HALIDAY (HYMENOPTERA: BRACONIDAE) IN CHINA, A CANDIDATE FOR BIOLOGICAL CONTROL OF *HELICOVERPA ARMIGERA* (LEPIDOPTERA: NOCTUIDAE)

Li JIANCHENG , Lu ZIYUN , Guo JINXIA & Pan WENLIANG
Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences
Baoding, China

The mass rearing methods and biology of *Microplitis mediator* (Haliday), a solitary endoparasite of larvae of the cotton bollworm, *Helicoverpa armigera* (Hübner) and armyworm, *Mythimna separate* (Walker)was studied in the laboratory. *M. separate* were used as an alternative host to mass rear *M. mediator* because *H. armigera* are cannibalistic and difficult to mass-rear. M. mediator can parasite 1st to 4th instars larvae, but prefer 2nd and 3rd to 1st and 4th instars larvae. Parasitoid egression and pupation were dependent on the host instar parasitized and occurred from the 1st through the 4th instar. The mean developmental time from egg to prepupae of *M. mediator* within 1st to 4th instars of the host was 8.27, 8.30, 8.30 and 9.20 days, respectively. The adult longevity had significantly difference under different temperature conditions. Honey water can effectively enhance longevity of wasps. The wasps have the longest longevity of 15 d for female and 9 d for male in 18 with 10% honey water supply and the shortest longevity of 1 d in 38 with no food supply. The longevity of field released female and male parasitoids was 7.6 d and 3.9 d, respectively. Diapause in M. mediator is manifested during the pupal stage and normally occurs during the winter season. No diapause was observed at temperatures above 20 °C regardless of the photoperiod. In contrast, when larvae were exposed to 16, 18 and 20 °C combined with a photoperiod of LD 10:14, the percentages of parasitoids that entered pupal diapause was 97.9%, 87.8% and 26.2%, respectively. Critical photoperiods were determined to be LD 7.03:16.97 and 12.21:11.79 at 16 °C and LD 6.75:17.24 and 12.03:11.96 at 18 °C. The 2nd instar of the parasitoid was the most sensitive to diapause induction for the photoperiods and temperatures tested. Field experiments and surveys were conducted to evaluate the efficiency of using the parasitoid M. mediator to control populations of *H. armigera* in cotton fields in Northwestern China's Xinjiang Uyghur Autonomous Region. The results showed that the number of parasitized *H. armigera* increased with an increasing number of M. mediator cocoons released in the field. Large-area releases of M. mediator in cotton fields resulted in more than 60% parasitism and an 80% decrease in cotton boll and bud damage. The above results indicated that augmentative release of M. mediator has a good potential for the biological control of *H. armigera*.

BEHAVIOURAL RESPONSE OF PHYTOSEIULUS PERSIMILIS IN INERT MATERIALS FOR TECHNICAL APPLICATION

Dennis WENDORF, Helga SERMANN, Peter KATZ[1], Sandra LERCHE & Carmen BÜTTNER

Humboldt-Universität zu Berlin, Landwirtschaftlich-Gärtnerische Fakultät, FG Phytomedizin, Lentzeallee 55-57, DE-14195 Berlin, Germany
E-mail: helga.sermann@agrar.hu-berlin.de
[1] Katz-Biotech AG An der Birkenpfuhlheide 10, DE-15837 Baruth, Germanry

A large scale application of the predatory mite *Phytoseiulus persimilis* Athias-Henriot for use in the biological control of spider mites, for instance on cucumber fields, involves specific problems. Due to the need of mechanisation of this customary manual method, material used for transport and distribution of the predatory mites must be provided. This material has to hold the mites for the duration of the application and must be suitable for use in a mechanical procedure.
Therefore the behaviour of *Phytoseiulus persimilis* was tested in chosen materials. Special interest was given to the distribution of the animals in the material, the time of remaining in the material and certain factors that might have an impact on the time of remaining in the material.
The materials used in the laboratory studies were Millets pelts and Vermiculite (1-3 mm). To examine the effect of abiotic influences on the migratory behaviour and the time of remaining in the material, both the dampness of the materials was varied (0%, 5% and 10%) and the temperature (6°, 8° and 10°C) as well as the duration of exposure to the different temperatures (2, 4 and 16 hours) was modified. Moreover the influence of altitude of materials was tested. The time of emigration from the material was noted for each individual.
Emigration from all dry materials was completed 15 minutes at the latest after the beginning of the test. The increase of dampness had an obvious effect on the time of remaining in the material. In this respect the material Millets pelts showed the most favourable effect with 10% dampness.
Decreasing temperatures and increasing times of exposure prolonged the time of remaining in the material. The strongest effect on delaying the resumption of movement was recorded after cooling for 16 h with a temperature of 6°C. Increasing altitude of material the mobility of predatory mites will be influenced negatively above 75 cm. Up to 50 cm mites have not a problem to move in the material and the time of remaining can be prolonged considerably.
The effect of slowing down the mobility of the mites in the material has to be judged positively in respect of a mechanised application. It depends on the chosen form of application technology whether this effect is sufficient regarding the mechanised application process.

PHYSIOLOGICAL COSTS OF COLD STORAGE OF MUMMIES OF *APHIDIUS ERVI* (HYMENOPTERA: APHIDIIDAE): LONGEVITY IS AFFECTED

Ismail MOHANNAD[1], Vernon PHILIPPE[1], Hance THIERRY[2] & Joan VAN BAAREN[1]

[1] UMR CNRS 6553 ECOBIO, University of Rennes, France

[2] University of Catholic of Louvain, Belgium

E-mail: Mohannad.ismail@univ-rennes1.fr

Cold storage of natural enemies can be used to obtain large number of individuals . However, several studies pointed out negative effects on survival of parasitoids and life history traits (longevity, fertility,...). These effects increase with decreasing of the temperature and with the duration of cold exposure.

We tested the physiological costs during a short period of storage, which did not induce a high mortality in the parasitoide *Aphidius ervi*. This parasitoids is used to control different species of aphids.

We compared 5 treatments at 7°C on mummies 1-day-old (control at 20°C, 1 and 2 wk at 7°C constant, 1 and 2 wk at 7°C fluctuate). This temperature is above the development threshold of *A. evri* (6.6°C).

Our results showed that fecundity at the emergence and life time fecundity were not affected at all treatments. In contrast, we found that the longevity of females in the treatment constant 2 wk declined significantly with the duration of the storage. Hence, we suggested that males were more tolerant to the cold storage than females. The progeny sex ratio was male biased in the 2 wk treatment.

Water content decreased significantly in all treatments, whereas fat content decreased significantly only in the constant treatments. We suggested that the fluctuating treatments had un important role in economizing fat reserve.

We suggested that the decreased longevity, is probably linked to the diminution in fat content and water content.

A. ervi can therefore be stored successfully for 2 wk at 7°C in the fluctuating treatments without any detrimental effects.

ROLE OF BIOAGENTS IN PROTECTION OF WHEAT GENETIC RESOURCES

Tea ABRAMISHVILI & Nona CHKHAIDZE
Department of AgroEcology, Georgian State Agricultural University
Tbilisi, Georgia

From 27 species of world wheat genetic resources 14 species are Georgian. Georgian wheat species Zanduri - *Triticum timopheevi* Zhuk characterize comparative low productivity and high immunity to harmful organisms, but some of them Makha -– *T. macha Dek. et Men.* with high productivity and low immunity.

There are presented first record morpho-anatomical study of this two species. Ear pedicel of Zanduri - have rich trichome on all the surface. Trichome lengths are 4-36 µm. It has six quantity conductive fascicles. Hair root is rich. Root diameter is 90 µm, quantity of wood vessels -7. Rich trichoms of ear rod and deep disposition of conductive system leave out feeding of some of insects, damaging this species. Ear pedicel of Makha - has single trichoms with lengths 34-140 µm. Quantity of conductive fascicles have 16. Hair root is rich. Root diameter is 120 µm, quantity of wood vessels - 8. Such structure gives a chance to insect for feeding.

The greenbug - *Shizaphis gramina* Rond. is on a large scale pest insect demiging wheat culture in Georgia. The average injuriousness from its archive.

For protection Georgian wheat species from the *Shizaphis gramina* Rond. were conducted ecological safety methods, especially use effective bioagents - entomophagous Ladybird (7-spot) - *Coccinella 7-punctata*, which feeds very gluttony in ages of as a larva as adults.

There are firste recored a new aphidophagous specie - *Trombidiidae* sp. (Acariformes: Trombidiformes) of *S. gramina.* The mite has red and metamerized body with size 3-5 mm and bristle. Three pair of climberly foot covered with bristle. *Trombidiidae* sp. were attached on different body sites of *S. gramina:* ventral abdomen, ventral and dorsal thorax, on the head. Number of abundant are 1-6 mites on an aphid.

Also, *Trombidiidae* sp. were observed on the other wheat damaging bugs species: wheat great aphid -*Sitobion avenae* and maize grain aphid- *Aphis avenae*, which is feeding them.

The full study of new specie - *Trombidiidae* sp. is very important, which will have a grate practical use in future as a biological control against.

COMPATIBILITY OF BOTRYTICIDES WITH THE BENEFICIAL INSECT *BOMBUS TERRESTRIS*

Linde BESARD[1], Veerle MOMMAERTS[1], Guido STERK[2] & Guy SMAGGHE[1,3]
[1] Laboratory of Cellular Genetics, Department of Biology, Faculty of Sciences
Free University of Brussels, Brussel, Belgium
[2] Biobest NV, Westerlo, Belgium
[3] Laboratory of Agrozoology, Department of Crop Protection
Faculty of Bioscience Engineering, Ghent University, Gent, Belgium

Bombus terrestris bumblebees are important pollinators of wild flowers, and in modern agriculture they are used to guarantee pollination of vegetables and fruits such as tomatoes, sweet peppers, strawberries, apples and pears. In the field it is likely that worker bees are exposed to pesticides during foraging. The present research project was conducted to examine the lethal and sublethal effects on the reproduction and on the behaviour of the pollinator *B. terrestris* by botryticides. Botryticides are important to agriculture in the control of the plant pathogenic fungi, *Botrytis cinerea*. Six commercial botryticides were tested: Frupica, Rovral, Signum, Sumico, Switch and Teldor. In a first experiment bumblebee workers were exposed in the laboratory to the different compounds via three different routes: dermal contact, and orally via the drinking of treated sugar water and treated pollen. The compounds were tested at their respective maximum field recommended concentrations (MFRC). In another series of experiments the impact of sublethal concentrations of these fungicides was tested via sugar water on the foraging behaviour of bumblebee workers. In brief, the experimental setup consists of two artificial nests that we connected with a walking through tube of about 20 cm. In one nest the workers constructed brood, and in the other food (sugar and pollen) was provided. Before exposure, the workers were allowed a training to forage for untreated food; afterwards this was replaced by treated food. In general, the experiments showed that concentrations that "look" safe for bumblebees can have a negative influence on their foraging behaviour. Therefore it is recommendable that behaviour tests should be included in risk assessment tests for pesticides because impairment of the foraging behaviour can result in a decreased pollination, lower reproduction and finally in colony mortality due to a lack of food.

COMPATIBILITY OF COMMERCIAL BT INSECTICIDES ON MICRO-COLONIES OF *BOMBUS TERRESTRIS*

Veerle MOMMAERTS[1], Guido STERK[2] & Guy SMAGGHE[1,3]
[1] Laboratory of Cellular Genetics, Department of Biology, Faculty of Sciences
Free University of Brussels, Brussels, Belgium
[2] Biobest NV, Westerlo, Belgium
[3] Laboratory of Agrozoology, Department of Crop Protection
Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Bacillus thuringiensis (Bt) is a natural soil bacterium that is used worldwide for the control of pest insects as its protein crystals possess insecticidal activity. Moreover due to the intensive use of Bt in different crops like vegetables, ornamentals, flowers and fruiting plants, the question has raised whether Bt is safe for non-target organisms. Nowadays cultivators are using beside honeybees also bumblebees for the pollination of their crops such as tomatoes.
In this study the risk of two different strains of commercial Bt insecticides, *B. thuringiensis kurstaki* (Dipel® WG) and *B. thuringiensis aizawai* (Xentari® WG) on the biology of the bumblebee *Bombus terrestris* was assessed. In order to evaluate potential lethal and sublethal effects on the reproduction micro-colonies of worker bumblebees were exposed to 0.1% of each compound, representing the maximum field recommended concentration (MFRC), and this via three different routes of exposure: dermal contact and orally via treated sugar water and treated pollen. For both Bt compounds no loss of survival was scored after dermal contact treatment. But via treated sugar water, Xentari® at 0.1% killed all worker bumblebees, but with a lower dose of 0.01% (1/10 of the MFRC) mortality was zero. With Dipel® at 0.1% in the sugar water and in the pollen, no mortality was scored. Next to lethal effects, sublethal effects were evaluated. In the nests exposed to Xentari® at 0.1% via the pollen a significantly lower number of drones was produced ($P<0.05$); however, no detrimental effects were seen with a lower dose of 0.01% ($P>0.05$). For the treatments with Dipel®, the reproduction in the micro-colonies was normal (37.6 ± 5.5 drones per nest) as in the controls (39.5 ± 6.7 drones per nest). Then in a next step in our risk assessment study on side effects we evaluated with a new experimental setup in the laboratory the impact of sublethal concentrations of Xentari® (0.01% via the sugar water and the pollen) on the foraging behavior of bumblebees. Here no change in the behaviour of the workers was seen. Overall the results showed that the tested Bt insecticides cause an effect on the biology of *B. terrestris*. However, more information about environmental relevant concentrations is necessary before making final conclusions about the compatibility of these compounds with *B. terrestris*.

"TEPPEKI, SELECTIVE INSECTICIDE ABOUT *BOMBUS TERRESTRIS*"

Angela FANIGLIULO[1,2], Vittoria FILI[1], Rosa PACELLA[1], Soccosa COMES[1] & Aniello CRESCENZI[2]

[1]Bioagritest Test Facility, Bioagritest Srl - Centro Interregionale di Diagnosi Vegetale
Zona PIP lotto E2. IT-85010 Pignola (PZ, Italy)
E-mail: info@bioagritest.it; www.bioagritest.it

[2]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata. Viale dell'Ateneo Lucano Campus Macchia Romana 3A310
IT-85100 Potenza (PZ) Italy
E-mail: aniello.crescenzi@unibas.it

At a time when a highly controversial debate about the causes of the widespread deaths of bees is taking place all over Europe, which accused the agriculture and its practices with particular reference to the harmful effects of some insecticides, it seems important to point out as another insecticide, the Teppeki, can be selective about bumble and have a good compatibility with the activity of the apiaries. This insecticide has the active ingredient flonicamid (500 g/kg) belonging to a new chemical class, called pyridinecarboxamides: the product works systemic and is known as having a long lasting efficacy against all important aphid species. Bioagritest test facility of Pignola (PZ, Italy) has conducted in two successive production cycles an experimental trial on a tomato hydroponic cultivation within the Agricola Bonsai farm in Sibari (CS, Italy), whose objective was to measure the selectivity of flonicamid on *Bombus terrestris*, insects playing an important role in the pollination of certain species grown in greenhouse such as Tomato, Eggplant, Pepper and Cucumber. On the pollinated flower *B. terrestris* leaves some trace of its visit, a typical dark trademark: on the detection of the marking of flowers was based the testing program conducted by Bioagritest.

Two thesis were compared: *A, standard)* treatment with a foliar insecticide, the neonicotinoide acetamiprid, normally used for control of aphids and whiteflies (unlike other neonicotinoides - imidacloprid and thiametoxam - quite selective about *B. terrestris)* and *B. Teppeki)* foliar treatment with Teppeki, to the maximum dose indicated on the label. The experimental design included the use of randomized blocks with 4 repetitions (4 plots/thesis with 100 plants each).

In every thesis 6 *B. terrestris* hives were placed 2 days before treatment: the respective holes remained closed during the treatment and the 12 following hours.

In order to verify the pollination, by the detection of the flower marking, 2 flowers per plant were observed, for a total of 200 flowers per plot. The measurements were made on the 3rd (I relief) and 8th day (II relief) after treatment. Statistical analysis was performed by the use of *XLSTAT* data analysis and statistical software.

The analysis of collected data shows that flonicamid has a minor effect of interference with the activity of pollination by *B. terrestris*, compared to the standard used. 14 days after treatment, 3 hives per thesis were inspected in order to verify the status of the colonies (adults, larvae, eggs, pollen). The colonies appeared generally homogeneous as concerning the number of alive adults - 100 for each - all at the end of the development cycle. There was no dead adult. Two colonies, one for thesis, presented evidence of eggs. All colonies had low stocks of pollen.

Ultimately, treatment with Teppeki has not given any acute effect on *B. terrestris*, nor any effect of interference in respect of its pollination activity.

SIDE EFFECTS OF PLANT PROTECTION PRODUCTS AND ENVIRONMENTAL INTERACTIONS ON THE EUROPEAN EARWIG *FORFICULA AURICULARIA* L.

G. PEUSENS[1], R. MOERKENS[1], T. BELIËN[1] & B. GOBIN[1]

[1] Zoology Department, pcfruit
Fruittuinweg 1, BE-3800 Sint-Truiden, Belgium
[2] Evolutionary Ecology Group, University of Antwerp
Groenenborgerlaan 171, BE-2020 Antwerp, Belgium

Plant protection products (PPP's) are designed to control pests but can have negative side effects on non target arthropods thus disturbing the important population of natural enemies required for biological control. Although the European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae) is not considered to be a key beneficial in pit fruit, it is an important predator of several pests, e.g. woolly apple aphid. The impact of non selective compounds at crucial moments in their (univoltine) life cycle can be of significant relevance compared to insects with numerous generations. Foliar applications in spring when nymphs are migrating into the trees could reduce the number of adults in summer and subsequently affect the population size next year. Repeated treatments during summer on adults may have a cumulative effect resulting in less over wintering females which possibly exhibit poor reproductive performance. To obtain data on the side effects of formulated products, residual contact bioassays on bean leaves were carried out in the lab.

Although the majority of compounds proved to be harmless (< 30% mortality), others were harmful, e.g. thiacloprid (slightly), and even toxic, e.g. flufenoxuron, spinosad and indoxacarb. Depending on the mode of action of the PPP's, effects increased, declined or remained unchanged during the observation period. Insects showing sub lethal symptoms recovered partially or died eventually. When multiple rates of 4 compounds (1/27-th, 1/9-th, 1/3-th and 3-fold of the registered dose rate) were compared, a clear dose-response pattern was noticed. For instance, a 1/3-dilution of the registered dose rate of spinosad and indoxacarb still had a side effect of >50% and >70%, respectively. In field circumstances mortality may be higher as these earwigs are more easily predated or parasitized.

Understanding the earwig's population dynamics is essential for efficient practical biocontrol. It proves difficult to increase population levels to sufficient high numbers for optimal pest control. Local environmental factors might be limiting. Therefore, we tested two hypotheses that pertain to population limitation: 1. Bird predation during summer, 2. Small mammal nest predation during winter. Enclosure experiments showed no negative bird effect on earwig densities unless large bird flocks inhabited the area. Small mammals did not actively predate the overwintering nests, although other predatory arthropods may be important.

SIDE EFFECTS OF TEFLUBENZURON AND CHLORFENAPYR IN *ERIOPIS CONNEXA* EGGS (COLEOPTERA: COCCINELLIDAE)

Marilina FOGEL[1], Federico RIMOLDI[1], Samuel PINEDA[3], Marcela SCHNEIDER[2] & Alicia RONCO[1]

[1] CIMA, Facultades de Ciencias Exactas, UNLP, CONICET, La Plata, Bs. As., Argentina
[2] CEPAVE (CCT-La Plata- CONICET- UNLP), La Plata, Bs. As., Argentina
[3] Instituto de Investigaciones Agropecuarias y Forestales
Universidad Michoacana de San Nicolás de Hidalgo
Km. 9.5 Carr. Morelia-Zinapécuaro. MX-58880 Tarímbaro, Michoacán, Mexico

The conservation of the natural enemies in an agroecosystem is a goal for the IPM's promoters. However the pesticides commonly used for the control pest could reduce the natural enemies' populations due to their toxicity. *Eriopis connexa* is a generalist predator from the Neotropical Region, feeding on soft-bodied prey such as aphids, whiteflies, lepidopteran eggs, etc. Also, it is considered as a potential biological control agent in this region. The objective of this work was to evaluate the effects of teflubenzuron and chlorfenapyr on the egg stage of *E. connexa*. Commercial compounds and the maximum registered concentrations for their field use were employed: Nomolt® (15% teflubenzurón, 45 mg/L a.i., BASF Argentina S.A.) and Sunfire® (24% chlorfenapyr, 72 mg/L a.i., BASF Argentina S.A.). The experiments were carried out under laboratory conditions at 25±1°C of temperature, 75±5% RH and 16:8 L: D photoperiod. Mass of 25-30 eggs ≤ 48 h-old were treated by dipping in insecticides solutions during 25 seconds. The experiment was replicated 3-4 times. The controls were treated with the dissolvent (water) + 0.01% Tween® 80. The mortality and the development time for each instar stage were evaluated daily and during all the predator life span. No statistical differences were found between treatments with respect to survival rate for each development stage and in the accumulated survivorship. Although any effects were observed in the development rate from the neonate larvae to pupa but significant differences were found for each development stage. The role of the chorion as a protected barrier to pesticides is discussed.

COMPATIBILITY OF METHOXYFENOZIDE WITH *ERIOPIS CONNEXA*, *CHRYSOPERLA EXTERNA*, *TRISSOLCUS BASALIS* AND *TRICHOPODA GIACOMELLII*

Marcela Inés SCHNEIDER[1], Samuel PINEDA[2], Natalia FRANCESENA[1], Adriana TREUQUE[1] & Ana Mabel MARTÍNEZ[2]

[1] CEPAVE (CCT-La Plata CONICET, UNLP), La Plata-Bs. As., Argentina

[2] Instituto de Investigaciones Agropecuarias y Forestales
Universidad Michoacana de San Nicolás de Hidalgo
Km. 9.5 Carr. Morelia-Zinapécuaro. MX-58880 Tarímbaro, Michoacán, Mexico.

In Argentina, transgenic soybean resistant to glyphosate (RR) has expanded in recent decades. This resulted in a significant increase in the use of broad-spectrum insecticides together with the herbicide glyphosate. However, the Integrated Pest Management (IPM) paradigm claims for integrating natural enemies of pests, selective pesticides, and other cultural techniques. Broad-spectrum pesticides are toxic for most natural enemies.

Methoxyfenozide is a Moulting Accelerating Compound (MAC) belonging to the Insect Growth Regulator insecticides (IGR's). Commercial Inteprid® has been registered in Argentina for the control of soybean pests and represents one alternative to broad-spectrum insecticides.

The objective of this work was to evaluate de compatibility of this biorational insecticide with four relevant natural enemies associated to soybean pests (*Eriopis connexa, Chrysoperla externa, Trissolcus basalis and Trichopoda giacomellii*). Commercial compound and the maximum registered concentrations for their field use were employed: Interprid® (24% methoxyfenozide, 144 mg/L a.i., Dow Agrosciences Argentina S.A.). The bioassays were carried out in the laboratory under controlled conditions: 25±0.5 °C of temperature, 75±5% RH and 16:8 L:D of photoperiod. Harmful effects of methoxyfenozide on life parameters of the four natural enemies were evaluated by ingestion, dipping and residual exposure ways. The endpoints chosen for the evaluation were: mortality, development rate, longevity, parasitism, predation rate, fecundity and fertility.

Methoxyfenozide results harmless for the four natural enemies studied because their life parameters were similar to those observed in the controls.

This work confirms once again the compatibility of methoxyfenozide with natural enemies of the agricultural pests.

EFFECTS OF IMIDACLOPRID, INDOXACARB AND ENDOSULFAN ON EGGS, THE THIRD INSTAR AND PUPA OF GREEN LACE WING *CHRYSOPERLA CARNEA* IN LABORATORY

Gholam Reza GOLMOHAMMADI[1], Mir Jalil HEJAZI[1], Shahzad IRANPOUR[1], Seid Aboulghasem MOHAMMADI[2] & Mohammad Reza SAFARNEJAD[3]

[1]Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
[2]Department of Plant Breeding, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
[3] Department of Microbial Biotechnology
Agricultural Biotechnology Research Institute, Karaj, Iran

The common green lacewing is a powerful agent in biological control programs because of a wide geographical distribution, high compatibility with different agricultural systems, high searching ability and ease of rearing. Adults and larvae were reared on artificial diet and eggs of *Anagasta kuehniella* (Zell.), respectively. The toxicity of insecticides was assessed on eggs (dipping method), the 3rd instars and (contact method) and pupa (topical application) of *C. carnea*. In order to study the sub-lethal effects, the 3rd instars were treated with recommended field rate of each insecticide. The effects of the insecticides were assessed using fertility life table and IOBC methods. Rearing conditions were 26 ± 2 ºC, 60 ± 10% relative humidity and a photoperiod of 16: 8 h (L: D). The results revealed that the insecticides did not affect eggs even at doses higher than recommended field rates; therefore, LC_{50} was not estimated for this stage. The LD_{50} values, of endosulfan, imidacloprid and indoxacarb for pupal stage were estimated to be 144, 33, and 21 µg ai/insect, respectively. The insecticides did not affect 3rd instar larvae at the recommended field rate considerably. Therefore, LC_{50} was not estimated for this stage. The results showed that only net reproduction rate (R_0) was significantly affected by the insecticidal treatments. But no significant differences were observed among the insecticides tested. The highest and the lowest amounts of r_m were 0.178 and 0.169 belonging to control and indoxacarb treatment, respectively. Based on the IOBC classification method, imidacloprid, endosulfan and indoxacarb were slightly harmful (%30 < Total Effect Index < %79) to the 3rd instars *C. carnea*. In conclusion, based on both demographic and IOBC methods, the toxicity of the insecticides tested was a follows: indoxacarb > endosulfan > imidacloprid. Overall, if similar results are obtained in field conditions, these insecticides might be suitable candidates for use in integrated pest management programs in place where *C. carnea* plays an important role in controlling the arthropod pests.

EFFICACY OF FIPROLE INSECTICIDES ON BROWN PLANTHOPPERS, *NILAPARVATA LUGENS* (STÅL) ON RICE

Tina KRAMER[1] & Ralf NAUEN[2]
[1] Department of Plant Physiology, University of Jena, Germany
[2] Research Insecticides, Bayer CropScience AG, Monheim, Germany

Particularly in SE Asia brown plant hopper, *Nilaparvata lugens*, evolves in the last decades as one of the most important pests in intensive rice growing areas, including Thailand, Vietnam and China. Most notably changes in agricultural practices with greater reliance on synthetic insecticides as control measures facilitated the development of insecticide resistance, for example against carbamates and organophosphates.
Insecticide resistance in *N. lugens* can spread quite fast due to its migratory nature with travel distances up to 3000 km per season.
Since middle of 90´s neonicotinoids – a class of insecticides acting on the nicotinic acetylcholine receptor - are used to control this pest, and particularly in SE Asian countries such as Vietnam and Thailand field resistance developed very recently. Other chemical options for brown plant hopper control are the phenylpyrazoles acting as GABA-gated chloride channel antagonists, and known to show no cross-resistance to neonicotinoids.
This work was particularly designed to test the efficacy of the major neonicotinoid insecticide imidacloprid, as well as the phenylpyrazoles ethiprole and fipronil against an insecticide-susceptible reference strain of *N. lugens*. Special reference was on the biological mode of action of these compounds regarding egg development, hatching performance and acute contact activity.
Additionally environmental scanning electron microscopy offered a close view to a kind of egg protecting cover, excreted by the females of *N. lugens* after depositing the eggs in plant tissue.

BASELINE STUDIES AND INSECTICIDE RESISTANCE MONITORING IN POPULATIONS OF THE DAMSON HOP APHID, *PHORODON HUMULI* SCHRANK

Mirjam PATTEN[1] & Ralf NAUEN[2]
[1] Institute of Phytomedicine, University of Hohenheim, Germany
[2] Research Insecticides, Bayer CropScience AG, Monheim, Germany

The development of insecticide resistance is an important aspect in modern applied entomology. Particularly some hemipteran pests such as aphids and whiteflies are considered at high risk for resistance development due to frequent applications of insecticides. In some crops only a few insecticides are available for pest control and resistance development is often a question of time.
Damson hop aphids, *Phorodon humuli* Schrank are a major sucking pest in hop cultivations worldwide. The hop yield in Germany represents more than 20 % of the total global production. Damage to hop plants by *P. humuli* results from direct feeding, the excretion of honeydew and as a vector of viruses.
In the early sixties, populations of damson hop aphids developed resistance to organophosphates due to continuous insecticide applications of the same chemical class. The same happened between 1970 and 1990 when carbamates and pyrethroids were introduced and applied continuously. In 1993 the neonicotinoid insecticide imidacloprid was introduced and applied by stem and foliar application in almost every German hop garden due to failure of pyrethroids. Today imidacloprid is still used by hop growers along with two structurally different feeding inhibitors, pymetrozine and flonicamid.
The objective of this work was to check the efficacy of these insecticides against laboratory and field strains of *P. humuli*, in order to monitor for possible resistance and cross-resistance. Both field strains were collected in hop gardens in the Hallertau region (Bavaria, Germany) in 2007, because of anecdotal reports of lower efficacy of imidacloprid treatments. Leaf-dip bioassays were performed with the currently registered insecticides imidacloprid, pymetrozine and flonicamid. Additionally deltamethrin was checked for its efficacy since pyrethroids haven't been used for many years. Furthermore spirotetramat, a not yet registered tetramic acid derivative with a completely new mode of action was included to generate baseline susceptibility data.
Bioassay data indicated moderate resistance to imidacloprid which decreased while strains were maintained under laboratory conditions without selection pressure. The action of imidacloprid could be synergized by piperonylbutoxide, indicating the possible involvement of microsomal monooxygenases. Preliminary data suggest a certain level of cross-resistance between imidacloprid and pymetrozine. All populations were completely susceptible to deltamethrin, and baseline data for spirotetramat turned out to be very homogenous. Implications of the presented data for hop aphid resistance are discussed.

EFFECTS OF LARVAL EXPOSURE TO SUBLETHAL CONCENTRATIONS OF METHOXYFENOZIDE IN *SPODOPTERA FRUGIPERDA* (J.E. SMITH)

Samuel PINEDA[1], Nereyda ZARATE[1], Ovidio DÍAZ[2], Ana Mabel MARTÍNEZ[1], Marcela Inés SCHNEIDER[3], José Isaac FIGUEROA[1] & Guy SMAGGHE[4]

[1] Instituto de Investigaciones Agropecuarias y Forestales
Universidad Michoacana de San Nicolás de Hidalgo
Km. 9.5 Carr. Morelia-Zinapécuaro. MX-58880 Tarímbaro, Michoacán, Mexico
[2] Facultad de Agronomía, Universidad Autónoma de San Luis Potosí
Álvaro Obregón 64 Centro, MX-78000 San Luis Potosí, Mexico
[3] CEPAVE (CCT-La Plata- CONICET- UNLP), La Plata, Bs. As., Argentina
[4] Laboratory of Agrozoology, Faculty of Bioscience Engineering, Ghent University, Belgium

Methoxyfenozide belongs to a novel class of insect growth regulators (IGRs), the molting accelerating compounds or nonsteroidal ecdysteroid agonists, discovered by the company Rohm and Haas (Spring House, PA). This compound acts more slowly than neurotoxin insecticides because it disrupts the physiological development of insects rather than kill through direct toxic action. Although these effects could be important in the field, they have been relatively poorly investigated compared to immediate lethality. In this study, we investigated the lethal and sublethal effects of methoxyfenozide on *S. frugiperda*, the most serious maize pest throughout Latin America. Fifth instars were continuously fed, until pupation, with artificial diet containing 0.24 and 0.35 mg of active ingredient/kg diet, which correspond to LC_{10} and LC_{25}, respectively. Larval mortality was 8 and 26% in each concentration at seventh day after start the experiment. A progressive larval mortality of 12 and 60% for the LC_{10} and LC_{25}, respectively, also was observed until just before pupation. Treated larvae exhibited a significant lower pupal weights, and presence of deformed individuals (pupae and adults). Methoxyfenozide also caused a significant effect on the larval time development. Both male and female treated larvae lived around seven days more than those of the controls in both concentrations tested. In contrast, the compound did not affect the pupae and adults longevity. Finally, *S. frugiperda* adults that resulted from fifth instars treated with methoxyfenozide were not affected in the sex ratio. Our results suggest that the combination of lethal and sublethal effects of methoxyfenozide might have important implications on the population dynamics of fall armyworm.

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EFFECTS OF TEBUFENOZIDE IN REPRODUCTION OF THE FEMALE GERMAN COCKROACH, *BLATTELLA GERMANICA* (DICTYOPTERA, BLATTELLIDAE)

Samira KILANI-MORAKCHI[1], Nadia ARIBI[1], Jean-Pierre FARINE[2], Guy SMAGGHE[3] & Noureddine SOLTANI[1]

[1] Laboratoire de Biologie Animale Appliquée, Département de Biologie
Faculté des Sciences, Université d'Annaba, DZ-23000 Annaba, Algérie
[2] Université de Bourgogne, CNRS-UMR 5548, FR-21000 Dijon, France
Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, BE-9000 Ghent, Belgium

Tebufenozide, a dibenzoylhydrazine insect growth regulator, was applied topically (1 µg) on the most prevalent female German cockroach *Blattella germanica* and its effects on ovarian measurement and proteins contents were investigated during the first gonadotrophic cycle (0, 2, 4 and 6 days). Dissection of treated females showed a clear reduction of oocyte numbers and volume of basal oocytes. The ovarian protein content was also significantly reduced. In the second series of experiments, behavioural tests revealed that tebufenozide treatment of females (6 days old) caused a significant perturbation of the sexual receptivity of the untreated non-specific males with an increase in the number of contact antennae. Thus, in 20% of untreated males, the response to a calling treated females was altered with absence of contact antennae and wings raising probably caused by reduced production of female sex pheromone and in turn this provoked an obvious lower reproduction.

EFFECTS OF ROTENONE FROM DERRIS CRUDE EXTRACT ON ESTERASE ENZYME MECHANISM IN THE BEET ARMYWORM, *SPODOPTERA EXIGUA* (HUBNER)

Ajin RATTANAPAN & Suriyan PHAIRIRON
Department of Biology, Faculty of Science, Maha Sarakham University, Thailand
University, Khamreang District, Maha Sarakham 44150, Thailand
E-mail: ajinrat@hotmail.com

This research was elucidated the toxicity and detoxification enzyme mechanism of derris crude extract (*Derris eliptica* Benth) in the beet armyworm (*Spodoptera exigua* Hubner) by larvae dipping method. Derris crude extract was extraction by Soxhlet's apparatus with ethanol solvent. The ethanolic derris crude extract was diluted into 5 concentrations to test the toxicity value. The result showed that the toxicity dramatically increased when the concentration of substance and the time exposure were increased. Toxicity in terms of LC_{50} value against 2^{nd} instar larvae indicated that *ca.* 69.15 (r^2 = 0.98), 51.32 (r^2 = 0.99) and 46.60 (r2 = 0.98) ppm at 24, 48, and 72 hours, respectively. The *in vivo* study with Yang *et al.* (2004); Visetson and Milne (2001) modified method, revealed the derris crude extract that inhibited of esterase enzyme activities *ca.* 0.75-1.01 fold with not the protein concentration changed. This result indicated that, derris crude extract had efficacy for insecticide, therefore it had a possibility to be another insecticide alternative in *S. exiqua* control. Moreover, some separate experiments indicated that this extract was safe to human and environment.

ACUTE INSECTICIDAL EFFICIENCY OF SOME THAI BOTANICAL INSECTICIDES ON *SPODOPTERA EXIGUA, MYTHIMNA SEPARATE, ADOXOPHYES HONMAI* AND SOME INSECT PARASITOIDS

Vasakorn BULLANGPOTI[1*], Kanitsara CHAWNA[1] & Yooiichi KAINOH[2]

[1] Department of Zoology, Faculty of Science, Kastsart University
Phahonyothin Rd., TH-10900 Thailand

[2] Institute Agriculture and Forestry, University of Tsukuba, Ibaraki, Japan

Botanical insecticides have long been counted as attractive alternatives to synthetic chemical insecticides for pest management. The use of natural products readily available in the tropics and subtropics could help to reduce the need for imported pesticides, environmental fate problems and, thereby, would gradually increase the developing countries self-sufficiency. In this research, we are interested in insecticidal activity of four Thai botanical insecticides (*Eucalyptus cinerea, Jatropha grossypifolia, piper nigrum and amaranthus viridis)* on some insect-type pests and some parasitoids using dipping and impregnated filter paper methods. We observed the underlying effect of the extracts on the acute toxicity and behavior of treated second instar larvae. The toxicity for both tested methods showed that the best result for control *Mythimna separata and Adoxophyes honmai* was *piper nigrum* (*Mythimna separate*: LC_{50} = 579.83 ± 51.07 ppm for dipping method, 964.30 ± 191.42 ppm for impregnated filter paper method) (*Adoxophyes honmai*: LC50 = 887.82 ± 108.28 ppm for dipping method, 4464.35 ± 401.68 ppm for impregnated filter paper method). in contrast with the control of *S. exigua*, the best extract was *Jatropha grossypifolia* (LC_{50} = 996.87 ± 373.38 ppm for dipping method, 5195.98 ± 891.35 ppm for impregnated filter paper method). After the treatment, treated insects showed behavioral changes such as apparent inertia and paralysis. Moreover, the treated worms reduced in appetite, and some ignored eating artificial food. For parasitoids toxicity test, *Meteorus pulchricornis and Ascogaster reticulatus* were tested using impregnated filter paper method. The toxicity result show all extracts are no toxicity result to *Meteorus pulchricornis* although using the concentration up to 20,000 ppm under contract method with adult parasitoids. However, *Ascogaster reticulatus* was affected to this three extracts which show the toxicity up to 80% when treat at 10,000 ppm. After the treatment, treated insects showed behavioral changes such as avoid to runaway from extract. Thus, it may be concluded that the extracts may affect insect in changing their motion and dietary behaviours.

EFFICACY OF FIVE VOLATILE OILS AND THEIR MIXTURES AGAINST THE MEALYBUG, *ICERYA SEYCHELLARUM SEYCHELLARUM* (WESTW.) INFESTING SAGO PALM, *CYCAS REVOLUTA* IN ALEXANDRIA, EGYPT.

H.A. MESBAH[1], A. EL-KADY MAGDA[1], A.K. MOURAD[1], S.I. ABDEL-RAZAK[2], O.A. ZAGHOUL[1], M.L. BOTHINA WEHEDA[3] & S.E. ABD EL-RAHMAN[3]

[1] Faculty of Agriculture, Saba-Basha, Dept. of Plant Protection
Alexandria University, Alexandria, Egypt

[2] Plant Protection Research Institute, Dept. of Scale Insects and Mealybugs
Agricultural Research Centre, Alexandria, Egypt

[3] Horticultural Research Institute, Dept. of Landscape Gardening & Ornamental plants
Agricultural Research Centre, Alexandria, Egypt

Five tested plant volatile oils and their mixtures were evaluated to control the Margarodid, *Icerya seychellarum seychellarum* (Westw.) on growing Sago palms in Antoniades public garden, Alexandria, Egypt. The tested volatile oils at concentration rates of 0.5, 1 and 1.5 % (v/v) were Kamphor 20%; Dill 20%; Rose 30%; Peppermint 20% and Clove 30% (v/v). Their mixtures were Kamphor/Peppermint; Kamphor/Rose; at rate of 1:1 Kamphor/Rose/ Peppermint at 1:1:2 and Kamphor/Rose/Dill at 2:1:1. The calculated results as general mean of residual reduction percent for the whole inspection periods of the test indicated that the superior volatile oils in reducing mealybug were both Kamphor and Rose, followed by Dill, Peppermint and the least efficient Clove volatile oil.

The evaluated volatile oils mixtures showed that each of Kamphor/Rose/Peppermint, Kamphor/Rose and Kamphor/Peppermint mixtures occupied a higher rank of efficiency against the treated mealybug.

USE OF *ALLIUM SATIVUM* IN PULSE PROTECTION AGAINST *CALLOSOBRUCHUS MACULATUS*

Ahmed BOUGHDAD[1] & Fatima DOUIRI[2]

[1] Département de Protection des Plantes. Ecole Nationale d'Agriculture de Meknès
B.P. 40 ENA Meknès Maroc
E-mail : boughdad@enameknes.ac.ma

[2] Département de Biologie. Université Moulay Ismail, Facultés des Sciences
B.P. 4010 Béni M'hamed Meknès Maroc

Callosobruchus maculatus (Fab) (Coleoptera, Bruchidae) is the major pest of the strored seed's chickpea. In the perspective of developing alternative methods to control insect pests of agricultural stored commodities, powders, extracts and essential oils from *Allium sativum* L. were tested against *C. maculatus* reared on *Cicer arietinum* L. seeds at a temperature of 20-30°C and 65±5% relative humidity. The chickpea seeds were powdered, sprayed or fumigated by half, double or normal concentration occurred naturally in clove of garlic tested. On each plot treated or control, 10 newly emerged pairs were released. The number of dead bruchids and eggs, hatched and unhatched laid on the seeds were counted. At the end of their development, adults emerged from seeds treated or not, have been counted. Fertility and the success rate were calculated. Three replicates were performed for each plot. The essential oils of garlic were extracted by hydrodistillation and analysed by GC-MS. The majority of compounds were trisulfide di-2-propenyl(46.52%), disulfide di-2-propenyl(14.30%), trisulfide methyl 2-dipropenyl (10.88%) and diallyl disulfide (7.15%).The substances tested have repellent and toxic effects against *C. maculatus*. Fecundity raised from treated plots was lower than those obtained on control, according to formulation and concentration; it varied, e.g., from 17 to 59 eggs/10females in the fumigated plot with garlic essential oils vs 218 -310 in control; the fecundity decreased when the concentration increased. Longevity (in treated plot =1-3 days < in control = 13 days), the egg fertility (in plot treated = 0-25% < in control = 87-90%) and the success rate (treated plot = 0 < control = 80-90%) were also significantly affected in comparison with control; they decreased gradually with increasing concentration and no adult offspring has been obtained in fumigated plot with essential oils of the garlic; these compounds were showed more effective, the LC_{99}(24h) were 46.07 and 21.55 µl/l of air for males and females, respectively. The fumigation of stored products with the essential oil of garlic against insect pests could be integrated in pest management under storage conditions without risk to consumers and environment.

VAPOR ACTIVITY OF ESSENTIAL OILS EXTRACTED FROM FRUIT PEELS OF TWO CITRUS SPECIES AGAINST ADULTS AND EGGS OF *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE)

Salehe ABBAR[1], Gholamhosein MORAVVEJ[1], Saaiid HATEFI[1], Mohammad HASSANZADEH-KHAYYAT[2] & Majid AZIZI ARANI[1]

[1] Faculty of Agriculture, Ferdowsi University of Mashad, Mashad, Iran

[2] Faculty of Pharmacy, Mashhad University of Medical Sciences, Iran

Many investigations have been conducted to replace synthetic chemicals with natural and economical compounds to control stored-product pests in the last decades. In the present study, biological activity of essential oils of the fruit peels of two *Citrus* species including Lemon (*Citrus limonium* Risso.) and Tangerine (*Citrus reticulata* Blancko.) were examined on the adults and eggs of cowpea seed beetle (*Callosobruchus maculatus* Fab.). The oils were extracted from fresh peels by hydro-distillation method using a Clevenger apparatus. The bioassays were carried out on zero- to 2-day old adults and zero- to 2-day old eggs in the laboratory conditions. The adults were exposed to different oil concentrations for 3-24 hour periods in enclosed vials. The essential oils of *C. reticulata* and *C. limonium* caused respectively 100and 91% mortality in adults after 24 h by the presence of the highest concentration (285 $\mu l L^{-1}$). In all bioassays, adult mortality was associated positively with oil concentration and exposure time. Based on fumigant 24 h LC_{50} estimates on adults, the essential oil of *C. reticulata* proved to be more toxic (LC_{50}= 33 $\mu l L^{-1}$) and that of *C. limonium* lower toxic (LC_{50}= 235 $\mu l L^{-1}$). The eggs were exposed to different oil concentrations for 6 days periods in enclosed vials. The bioassays on eggs indicated that the lower LC_{50} value was attained by *C. reticulata* oil (LC_{50} = 38 $\mu L l^{-1}$) and the higher LC_{50} value by *C. limonium* oil (LC_{50} = 82 $\mu L l^{-1}$). In summary, the essential oil of *C. reticulata* exhibited higher lethal activity against adults and eggs.

INVESTIGATION ON PENETRATION OF THREE CONVENTIONAL FOODSTUFFS PACKAGING POLYMERS WITH TWO DIFFERENT THICKNESSES BY LARVAE AND ADULTS OF MAJOR SPECIES OF STORED-PRODUCT PEST INSECTS

Somayeh ALLAHVAISI[1], Ali ASGHAR PURMIRZA[1], Mohamad Hasan SAFARALIZADE[1], Guy SMAGGHE[2] & Amin SADEGHI[3]

[1] Laboratory of Entomology, Department of Plant Protection, College of Agriculture, Urmia University, Iran

[2] Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Belgium

[3] Laboratory of Entomology, Department of Crop Protection, Faculty of Agriculture, University of Kurdistan, Iran

Despite modern methods of packaging, stored agricultural products are still under attack by stored-insect pests. Therefore determination of the best polymer and appropriate thickness inhibiting the penetration of the insects must be considered. In this study, we investigated the ability of penetration and the rates of contamination by nine important stored product pest insects for three conventional flexible polymers (polyethylene, cellophane and polypropylene) at two thicknesses (16.5 and 29 µm), which are used as pouches for packing of agricultural products. We used adults of *Tribolium castaneum* (Coleoptera), *Sitophilus granarius* (Coleoptera), *Rhyzopertha dominica* (Coleoptera), *Callosobruchus maculates* (Coleoptera), *Oryzaephilus surinamensis* (Coleoptera), and larvae of *Plodia interpunctella* (Lepidoptera), *Ephestia kuehniella* (Lepidoptera), *Sitotroga cerealella* (Lepidoptera) and *Trogoderma granarium* (Coleoptera).

Results showed that for most of the species penetration occurred between 4 days and 2 weeks, but there were significant differences ($p \leq 0.05$) in the penetration of three polymers (cellophane, polyethylene and polypropylene) by the insects. Among the polymers, polyethylene with a thickness of 16.5 µm showed the highest degree of penetration and was the most unsuitable polymer for packaging of foodstuffs. Application of this polymer led to a complete infestation of the product and a lot of punctures were created by the insects. In contrast, no penetration was observed in polypropylene polymer with a thickness of 29 µm. Further on, adults and larvae of all species showed a much lower penetration when there was no food present in the pouches and this was the case for all polymers tested.

GROWING OF SCORPION WEED (*PHACELIA TANACETIFOLIA*) IN CONTROL OF SOIL-BORNE PESTS

András KOVÁCS, Miklós NÁDASY & Csaba BUDAI
University of Pannonia, Georgikon Faculty of Agriculture, Hungary, Keszthely

The *Phacelia tanacetifolia* is a very useful plant as a fore crop of all kinds of cultivated plant. It has no hosts or pests. Its growth is very quickly, after coming up it is in flower in one month. Autumnal plough under this plant is very useful, because it contains a lot of organic substances; decomposition products of Phacelia stall off soil-borne pests.

In laboratory raiser-pot research was made against the *Melonta* spp. (cockchafer grub). Into these kinds of pots soil and larvae in L_1-L_2 stadium were put. Our test plant was the *Phacelia tanacetifolia*, control plant was the autumnal wheat (*Triticum aestivum*). The efficiency percentage (H %) was determined from the mortal percentage of larvae. Schneider - Orelli formula was used for the calculation. Phacelia reduced the number of larvae by 66 %.

Determining the number of soil-borne nemathodes Baermann method was used in field. After Phacelia green crop, with 48 hour research, the mean number of nemathodes was 520 N/100 g soil, while in control field it was 844 N/100 g soil. So the Phacelia growing reduced the number of soil-borne nemathodes with 40 %.

In laboratory Petri-cup co-germination method was used for investigating the allelopathic effect of Phacelia. The germination percentage of Phacelia on the average was 93,5 %. The control plant was the Ambrosia artemisiifolia, it's germination percentage was 67,7 %. Test and control plants were germinated together which mean that hundred seeds of test and hundred seeds of control plant were put on humid filter paper in Petri-cup, in four repeat. With our laboratory data statistical-test was made with variation-analysis. In laboratory co-germination bioassays *P. tanacetifolia* reduced the germination of Ambrosia artemisiifolia by 53,8 %.

Because of fair results of our researches to our mind using Phacelia as green crops (in field or in greenhouse) would be instrumental in nutrient replacement, soil-borne reduction and weeds suppression.

Posters
Nematology

USING PSEUDOMONAS FLUORESCENCE CHAO FOR RESISTANCE INDUCTION AGAINST NEMATODE IN TOMATO

Sahar SARAFRAZ NICOO, Navazallah SAHEBANI & Heshmatallah AMINIAN
Department of Plant Protection, College of Aburaihan , University of Tehran, Iran

Plants show various mechanisms in reaction with different stresses and interferences where ROS could be addressed as one of those, particularly when a severe defensive action is addressed. ROS s including Hydrogen peroxide (H2O2), Hydroxyl radical (OH•) and related enzymes such as Super Oxide Dismutase (SOD), Peroxidase (POX) and Catalase (CAT) were studied in root of Tomato plant during seven days treatments. In fallowing included root pre-inoculated with CHAO and inoculated with nematode, root inoculated with nematode, root inoculated with *P. fluorescence* CHAO and control root (treated with distilled water). *P. fluorescence* CHAO is a bacteria categorized as PGPR showing high antagonist property in biocontrol of soil-born diseases .The test was aimed at resistance induction in Tomato plant against Root – knot nematode (*M. javanica*) by the aides of CHAO bacteria. This nematode is known as one of the three or five significant disease causes in plants, which has ubiquitous hospitality and extremely severe economic causality. Tests resultant declared that H2O2 and OH• amount has tow peaks, where during the first and second days it faces a considerable increment in comparison with the witness plant, then it is receded by decrement up to 5th where it experiences a peak before depreciation. Deviation in SOD amount and catalase activity reached its extreme at the third day where POX enzyme activity had its peak at 4th day and then depreciated. These alterations and depreciations were by far different from whatever observed in either after treatment or the witness plant. According to significant deviations and changes in over-treated plant by bacteria and treated with nematode, resultant proceedings suggest resistance induction in tomato plant. Methods utilized through this series of tests were benefited spectrophotometry on various pertinent wavelengths. (Changes and deviation in free radicals and associated enzymes were strangely correlated to each other where for instant increment in H2O2 amount was followed by reduction in SOD. Results suggest reduced number of gall, egg mass and eggs in the egg mass and finally reduced egg hatch on inoculated plant by elicitor in comparison with witness plants.

OCCURANCE OF ROOT-KNOT NEMATODE AND ROOT LESION NEMATODES IN POTATO FIELDS OF NIĞDE PROVINCE IN TURKEY

Mehmet ALI SÖĞÜT[1] & Zübeyir DEVRAN[2]
[1] Süleyman D. University, Agriculture Faculty, Plant Protection Department, Isparta, Turkey
[2] West Mediterranean Agricultural Research Institute (BATEM), Antalya, Turkey

Niğde province is one of the most important potato production regions of Turkey. Root-knot nematodes cause seriously yield losses in potato production in this region. A survey was carried out different locations of Niğde. Samples were collected from soil and galled potato tubers in the harvest time, 2008. Nematodes were extracted from soil using modified Baerman funnel techniques and examined under light microscope. Nematode species extracted from soil were identified using morphological characters. Egg masses and root-knot nematode female were collected from infected potato tubers using a small needle. Twenty perineal patterns of each sample were prepared and examined on a light microscopy for morpholocical identification. DNA was extracted from egg masses and second stage juveniles with DNAeasy Tissue and Blood Kit according to the manufacturer's protocol. Species-specific primers were used molecular identification of the root-knot nematode and root lesion nematodes. According to morphological and molecular characters, *Meloidogyne chitwoodi* and *Pratylenchus sp.* were determined as important plant parasitic nematodes in potato fields. *Meloidogyne chitwoodi* and *Pratylenchus sp.* were widespread and heavily infected about 76 % and 65 % of sampled areas, respectively. These nematode species were mixed 47 % of the survey area. Additionally, *Meloidogyne chitwoodi* and *Pratylenchus neglectus* was alone found 29 % and 17 % of sampled area, respectively.

AGRICULTURAL FEATURES OF BORG EL-ARAB REGION WITH SPECIAL REFERENCE TO NEMATODES, SOIL AND WATER CHARACTERISTICS AND GROWING PLANTS

M.M.M. ABD-ELGAWAD[1], S.S. KABEIL[2] & M.I.A. EL-TAWEEL[3]
[1] Phytopathology Department, National Research Center (NRC)
El-Behooth St., Dokki 12622, Giza, Egytp
[2] Mubarak City for Scientific Research and Technology,Borg-Elarab, Alexandria, Egypt
[3] Soil & Water Use Department, NRC, Egypt

A new entomopathogenic nematode, *Heterorhabditis* sp., was isolated in the present study from Borg El-Arab region, Egypt. SDS-PAGE for soluble proteins and quantitative determination of catalase, protease and peroxidase could be used to characterize and compare the new isolate with four others: *Heterorhabditis indica*, *Heterorhabditis* sp. El-Khatatba, *H. bacteriophora* D, and *H. bacteriophora* Hb88. In addition, two to nine of the following plant-parasitic nematode genera were present on each of ten sampled crops at Borg El-Arab; in a descending order of their population densities: *Tylenchorhynchus*, *Helicotylenchus*, *Pratylenchus, Criconemella, Xiphinema*, *Heterodera*, *Tylenchus*, *Trichodorus*, *Paratylenchus* and *Meloidogyne*. Other pathological features of plant pests and diseases common in Borg El-Arab were recorded. They complied with those disseminated in Egypt. Borg El-Arab soil and irrigation water were analysed to accurately study their features and determine the best crops for plantation. Consequently, as an important finding of this study, these crops are olives, sugar cane, groundnuts, sorghum, millets, wheat, fig and pear.

A NEW STEINERNEMATID NEMATODE SPECIES FROM "*MONTICOLUM*"-GROUP FOUND IN EUROPE

Sergei E. SPIRIDONOV[1], Lieven WAEYENBERGE[2], Dieter STURHAN[3] & Maurice MOENS[2,4]

[1] Center of Parasitology, Institute of Ecology and Evolution
Leninskii pr. 33, RU-119071 Moscow, Russia
[2] ILVO, Plant, Crop Protection
Burg. Van Gansberghelaan 96, BE-9820 Merelbeke, Belgium
[3] c/o Julius Kuehn-Institut, Münster, Germany
[4] Laboratory for Agrozoology, Ghent University
Coupure links 653, BE-9000 Gent, Belgium

The geographical distribution of species of entomopathogenic steinernematid nematodes often does not show clear patterns. Some cosmopolite species (like *Steinernema feltiae*) can be found nearly in every habitat, and the 'endemism' of certain steinernematids might be explained by inadequate sampling in other areas. *Steinernema glaseri*, e.g., was described as a characteristic component of North American soil ecosystems but was reported since then also from Europe and Asia. The species *S. monticolum* was described from Korea. Recently two close species - *S. robustispiculum* and *S. ashiunense* - were described from Vietnam and Japan, respectively. These three species are forming a separate phylogenetic *Steinernema* sub-clade ('*monticolum*" group). A steinernematid strain closely resembling *S. ashiunense* was found in the south of the Russian Far East (Vladivostok area), and a strain similar to *S. monticolum* was reported on Kamchatka peninsula. Thus, until now all species and strains of the '*monticolum'* group were only reported from Eastern Asia. In 2003 a beetle larva of the genus *Osmoderma* naturally infected with EPNs, was found on the soil surface in the north-eastern part of Germany (Mecklenburg). The cadaver with nematodes was transferred to the BBA Institute of Nematology in Münster, where a living culture on *Galleria mellonella* caterpillars was established. The morphology of these nematodes was studied under light and scanning electron microscope, and sequences of ITS rDNA and LSU rDNA were obtained after cloning of corresponding PCR-products. Because of contradicting literature data, the same sequences were obtained for the type culture of *S. monticolum* (Korea). This latter species was based on sequence-comparison the closest one to the nematodes found in Germany with 31 bp difference in the ITS region (about 5%) and 19 bp difference in LSU region (about 4%). Such differences correspond to the level of interspecific differences in the '*monticolum'* group. The nucleotide ITS sequence of the *S. ashiunense* strain from the Russian Far East was about 5 bp different from the Japanese type culture (less than 1%) and most probably represents intraspecific variability. The Kamchatka population demonstrated 3-4% ITS difference from *S. monticolum* and 4-8% difference from other members of this group. It can be concluded that the steinernematids of the '*monticolum'* group are widespread in East Asia but their diversity is still not described. Representatives of this group can be found also outside East Asia (Mecklenburg population). The European representative of this group seemingly constitutes a new *Steinernema* species. Morphological and molecular features of this steinernematid are described.

NEMATODE COMMUNITY AS POTENTIAL INDICATOR OF COMPOST MATURITY AND QUALITY

H. STEEL[1], E. DE LA PEÑA[1], P. FONDERIE[1], K. WILLKENS[2], G. BORGONIE[1] & W. BERT[1]

[1] Department of Biology, Ghent University, Belgium

[2] Institute for Agricultural and Fisheries Research, Plant Sciences, Merelbeke, Belgium

Composting is the heat-producing, aerobe disintegration of organic materials by several organisms which form a complex and fast changing community. Up to now only the dynamics of the bacterial community have been thoroughly investigated in relation to composting processes. This research focuses on the taxonomical composition and the succession of the nematode community during the composting process. Nematodes show several characteristics which make them ideal bio-indicators to analyze the ecosystem quality. Furthermore, the nematode diversity and density in mature compost are considered crucial to construct a solid soil food web. To characterize the nematode community, a compost heap prepared according to the CMC-method (Controlled Microbial Composting) was surveyed at different time intervals and at the same time several variables (temperature, pH, conductivity and moisture) were determined. The quantitative and taxonomical analyses of the compost samples clearly showed an abundant presence of nematodes in compost (except during the heat peak at more than 71 °C). However, major shifts in the nematode community occurred during the process. Three clear phases could be distinguished after the heat-peak. At the beginning of the process, directly after the heat peak, the nematode population is primarily built by bacterial feeding enrichment opportunists (cp-1) (Rhabditidae, Panagrolaimidae, Diplogasteridae) immediately followed by bacterial feeding general opportunists (cp-2) (Cephalobidae) and the fungal feeding general opportunists (Aphelenchiodidae). Thereafter, the bacterial feeding-predator opportunistic nematodes (*Mononchoides* sp.) became dominantly present. Finally, at the most mature stage, the fungal feeding Anguinidae is the most prominent group. These changes in nematode community and the associated indices illustrate the potential of nematodes to assess the compost maturity and quality. The biology of compost is an important criterion of mature compost especially when compost is used to inoculate the soil. Especially the remarkable high number of predatory nematodes, provided by certain stages of the composting process, can be an important factor of the suppressive capacity of compost.

SHIFTS IN BANANA ROOT EXUDATE PROLIFES AFTER COLONIZATION WITH THE NON-PATHOGENIC *FUSARIUM OXYSPORUM* ENDOPHYTE STRAIN FO162

Andreas KURTZ & ALexander SCHOUTEN
Soil Ecosystem Phytopathology and Nematology, INRES-Plant Health Department
University of Bonn, Nussallee 9, DE-53115 Bonn, Germany

Banana roots are becoming less infected by the burrowing nematode *Radopholus similis* when colonized by the non-pathogenic *Fusarium oxysporum* strain Fo162. It is assumed that a systemic reaction is triggered by the fungus, which is affecting the biochemical root exudate composition and thus causes reduced colonization by *R. similis*. To characterize these shifts, a continuous flow experiment was set up to collect root metabolites on a matrix (XAD-4). HPLC analysis did not show differences in the composition of the root exudates between plants colonized by the endophyte and the controls, but the accumulation of several compounds differed significantly. When these extracts were used in a bioassay with *Radopholus similis* none of the sample-treatment combinations had a significant attractive or repellent effect on the nematodes. This experiment shows that non-pathogenic *Fusarium oxysporum* strain Fo162 is able to upregulate the synthesis of at least some, so far unidentified compounds released by banana roots under hydroponic conditions. Further studies and optimization of the experimental setup are required to determine whether or not increase in metabolite concentration can affect nematode responses *in vitro* and ultimately *in vivo*.

A STUDY OF THE CHANGES IN QUANTITATIVE ACTIVITY OF SOME ENZYMES IN OLIVE CULTIVARS DURING THE INTERACTION BETWEEN OF VERTICILLIUM WILT AND ROOT-KNOT NEMATODE

Ayatollah SAEEDIZADEH[1], Ahmad KHEIRI[2], Javad ZAD[2], Hasan REZA ETEBARIAN[3] & Ali REZA BANDANI[2]

[1] Dept. of Plant Protection, Shahed University, Tehran, Iran
[2] Dept. of Plant Protection, University of Tehran, Karaj, , Iran
[3] Dept. of Plant Protection, Aboureyhan Campus, University of Tehran, Iran

Second stage juvenile (J_2) of root-knot nematode, *Meloidogyne javanica*, and microsclerotia of verticilliosis, *Verticillium dahliae*, were used as the source of inoculum for nematode and fungus respectively. One-year-old seedlings of olive cultivar, Zard, Roghani, Koroneiki and Manzanilla, were transplanted to pots containing 2000g of sterilized sandy loam soil. Experiment was conducted in completely randomized design with 32 Treatments and five replications. Treatments were as fallows: control, nematode alone, fungus alone and fungus+nematode. Pots were inoculated with (0, 2000, 3000, 4000) J_2 of nematode and/or (10 no/g soil) microsclerotia of fungus according to the treatments. Quantitative activity of polyphenol oxidase, catalase, proxidase and glucanase were determined by the pirocatechol, hydrogen proxide, guaiacol and laminarin-dinitrosalicylilate methods, respectively, on 1,10,20 and 30 days after inoculation. Results showed that these enzymes in leaves and roots of seedlings inoculated with pathogens increased ($p \leq 0/05$). In this study changes of these enzymes as compared with nematode alone and fungus alone treatments were increased in fungus+nematode treatments in seedlings ($p \leq 0/05$). Maximum of quantitative changes of these enzymes were observed in fungus+nematode ($4000J_2$) treatment on cv. Koroneiki. Based on the results obtained in this study, quantitative activity of these enzymes in olive roots and leaves were be more on cvs. Koroneiki, Roghani, Zard and Manzanilla, respectively ($p \leq 0/05$).

SPECIES OF *HETERODERA* CYSTS IN CEREAL FIELDS IN FLANDERS

Zeliha Çolak YILMAZ[1], Anne-Marie DEEREN[2], Nancy DE SUTTER[2] & Nicole VIAENE[2]

[1]Giresun University, Faculty of Arts and Sciences, Department of Biology
TUR28049 Giresun, Turkey
[2] ILVO, Plant, Crop Protection
Burg. Van Gansberghelaan 96, BE-9820 Merelbeke, Belgium
University of Ghent, Department of Biology, PINC
Ledeganckstraat 35, BE-9000 Gent, Belgium

Heterodera is a genus of cyst-forming nematodes that contains about 60 species. Some can cause serious yield reduction in crops, e.g. the beet cyst nematode *H. schachtii* and several species of cereal cyst nematodes. Lately, Northern European countries reported increasing damage in cereal crops due to *H. avenae*, as well as the presence of *H. filipjevi*, a species formerly associated with warmer climates. As few data are available on the occurrence of cereal cyst nematodes in Flanders, a survey was organized for the *Heterodera* species present in fields where cereals are grown. At 22 locations spread over Flanders, soil samples were taken during summer from 51 fields where cereals are rotated with mainly beet, vegetables and potato. A total of 112 samples were collected, at a rate of 1 sample per ha, each consisting of 60 soil cores taken 5 to 30 cm deep. Cysts were extracted from 1500 g soil per sample using the Seinhorst apparatus. Species identification was based on morphometrical and morphological observations on maximum 10 juveniles and 10 vulval cones per sample. No cereal cysts were detected, but *Heterodera schachtii* was found in 30 fields (59%) and was clearly the dominant species. Other *Heterodera* species found were *H. trifolii* (6 fields) and *H. mani* (3 fields), while cysts of the *H. goettingiana* group and of *H. carotae* were suspected in 1 field each, but their identification was not conclusive. *H. trifolii* and *H. mani* were present in the same field as *H. schachtii*, indicating that mixtures of cysts do occur, but perhaps are not discovered as identification was limited. This study emphasized once again the need for accurate molecular tools to make identification of *Heterodera* species less time consuming and dependent on specialized skills. The survey indicates that cereals cyst nematodes are not a problem in Flanders, but beet cyst nematodes might be responsible for yield reductions in several fields.

CHALANGES TO GROW OILSEAD RAPE *BRASSIKA NAPUS* IN SUGAR BEET ROTAIONS

Tatyana R. STEFANOVSKA
Department of Plant Protection/Entomology
National University of Bioresources and Environmental Science
13 Heroiv Oborony, UA-03041 Kiev, Ukraine

Across the Ukraine, the increase in planting area of oilseed rape *Brassicf napus* has been rapid for the last three years because of growing demand for bio fuel. Sharp increasing of commercial production of this crop permits colonization of insect herbivores and plant parasitic nematodes into these host plants.
Oilseed rape is attacked by 50 pest species, including sugar beet cyst nematode *Heterodera schachtii*. Planting of oilseed rape in traditional 5-6 field sugar beet-rotation especially where this nematode cause the problems can alter the herbivore pest complex associated with this and lead to significant reduce yield in both crops. The problem is aggravated by the absence of resistant to *H. schachtii* and withdrawing of nematicides from the agrochemical market. The objective of this study carried out in 1989-1991 and repeated in 2003-2006 is to compare life cycle and dynamic of *H. schactii* on sugar beet, oil rape, fodder radish, mustard and develop recommendation how to decrease the risk of yield reduction while it grows in sugar- beet rotations.
Researched was carried out in plot experiment in natural conditions. Study shows that all tested brassica crops are susceptible to *H. schachtii*. However there is a significant difference in population dynamics. The highest total number of brown cysts, eggs and all ages juveniles were observed in winter rape. *H. schasctii* developed two generations on sugar beet and one generation on mustard. The voluntary seed germination after harvest contributes to increasing *H. schachtii* population. Therefore it is necessary to destroy voluntary oilseed rape using chemical or physical way. This operation should be done in about 2-4 weeks. The exact time can be calculated using on temperature- based model. Growing "catch" crops of resistant fodder radish *Raphanus sativus*
(Cultivars Pegletta, Colonell, Picobello) or resistant mustard Accent can reduce population of *H. schachtii* about 80-90%. Growing regular fodder radish as a "catch" crop is also possible. The time of sowing is not early then August 20th. Calculation time of destroying of fodder radish should be taken in consideration that *H. schachtii* can complete life cycle without foliage.

A STUDY OF INTERACTION BETWEEN OF VERTICILLIUM WILT *VERTICILLIUM DAHLIAE* AND ROOT-KNOT NEMATODE *MELOIDOGYNE JAVANICA* IN OLIVE CULTIVARS

Ayatollah SAEEDIZADEH[1], Ahmad KHEIRI[2], Javad ZAD[2], Hasan REZA ETEBARIAN[3], Ali REZA BANDANI[2] & Fahimeh NIYASTI[1]

[1] Dept. of Plant Protection, Shahed University, Tehran, Iran
[2] Dept. of Plant Protection, University of Tehran, Karaj, Iran
[3] Dept. of Plant Protection, Aboureyhan Campus, University of Tehran, Iran

Second stage juvenile (J_2) of root-knot nematode, *Meloidogyne javanica*, and microsclerotia of verticillium wilt, *Verticillium dahliae*, were used as the source of inoculum for nematode and fungus respectively. One-year-old seedlings of olive cultivar, Zard, Roghani, Koroneiki and Manzanilla, were transplanted to pots containing 2000g of sterilized sandy loam soil. Experiment was conducted in completely randomized design with 32 Treatments and five replications. Treatments were as fallows: control, nematode alone, fungus alone and fungus+nematode. Pots were inoculated with (0, 2000, 3000, 4000) J_2 of nematode and/or (10 no/g soil) microsclerotia of fungus according to the treatments. Experiment was terminated after 10 months and fallowing parameters were determined i.e., fresh weight of root and stem, number of galls and egg masses per root system, and percentage of incidence of symptom on aerial parts, browning of vascular tissue, decrease of seedling height and stem/root tissue colonization by fungus. Results showed that presence of nematode caused reduction on colonization of the fungus in the root and stem and vice versa i.e. presence of fungus caused reduction on number of galls and egg masses produced by the nematode. Severe fungus wilt on aerial parts of Zard cultivare was observed when both pathogens were inoculated and mild fungus wilt was observed in fungus alone treatments of Koroneiki cultivare. Galling and egg mass production in root system were reduced in cvs. Manzanilla, Zard, Roghani and Koroneiki, respectively ($p \leq 0/05$). Based on the results obtained in this study, verticilliosis symptoms and galling of nematode in olive seedlings were be less on cvs. Koroneiki, Roghani, Zard and Manzanilla, respectively ($p \leq 0/05$).

CHANGES IN PROTEIN CONTENT AND ENZYMATIC ACTIVITY OF TOMATO ROOTS IN RESPONSE TO NEMATODE INFECTION

Mahfouz M. ABD-ELGAWAD[1] & Sanaa S. KABEIL[2]

[1] Plant Pathology Department, National Research Centre, Dokki 12622, Giza, Egypt

[2] Mubarak City for Scientific Research and Technology, Borg-Elarab, Alexandria, Egypt

The tomato cultivars, Super Marmand and GS-12 were susceptible to the root-knot nematode *Meloidogyne incognita* while the cultivar Castle Rock was moderately resistant as demonstrated by the common root gall index and plant growth parameters. Protein content and patterns as well as endo- and exo-glucanase activities were estimated one and two weeks after *M. incognita* inoculation and/or addition of garlic extract (600 g ground garlic cloves/l water) as bio-control agent. The effects of such treatments on the tested protein and enzymes were presented and discussed.

ROS(S) ROLES IN INDUCED RESISTANCE PLANTS BY SALICYLIC ACID AGAINST *MELOIDOGYNE JAVANICA*

Sahar SARAFRAZ NICOO, Navazallah SAHEBANI & Heshmatallah AMINIAN
Departement of Plant Protection
College of Aburaihan, University of Tehran, Iran

This nematode is known as one of the three or five significant disease causes in plants, which has ubiquitous hospitality and extremely severe economic causality. Plants show various mechanisms in reaction for different stresses and interferences where ROS could be addressed as one of those, particularly when a severe defensive action is addressed. SA is a fenolic compound which has important role in physiological activities in plants including induction of Anti-pathogenic defence reactions. ROS's including Hydrogen peroxide (H_2O_2), Hydroxyl radical (OH•) and related enzymes such as Super Oxide Dismotase (SOD), Peroxidase (POX) and Catalase (CAT) were studied on root of Tomato plant during seven days with different treatments. Treatments included root pre-treatment with SA and inoculated with nematode, root inoculated with nematode, root treatment with SA and control root (treated with distilled water).The test was aimed at resistance induction in Tomato plant against Root–knot nematode (*M. javanica*) by the aides of SA. Tests resultant declared that H2O2 and OH• amount has tow peaks, where during the first and second days it faces a considerable increment in comparison with the witness plant, and then it is receded by decrement up to 5th day where it experiences a peak before depreciation. Deviation in catalase activity reached its extreme at the third day where SOD amount and POX enzyme activity had its peak at 4th day and then depreciated. These alterations and depreciations were by far different from whatever observed in either after treatment or the witness plant. According to significant deviations and changes in over-treated plant by bacteria and treated with nematode, resultant proceedings suggest resistance induction in tomato plant. Methods utilized through this series of tests were benefited spectrophotometry on various pertinent wavelengths. (Changes and deviation in free radicals and associated enzymes were strangely correlated to each other where for instant increment in H2O2 amount was followed by reduction in SOD. Results suggest reduced number of gall, egg mass and eggs in the egg mass and finally reduced egg hatch on inoculated plant by elicitor in comparison with witness plants.

VIRULENCE OF THE LOCAL ENTOMOPATHOGENIC NEMATODES ISOLATES AGAINST *AGELASTICA ALNI* L. (COLEOPTERA: CHRYSOMELIDAE)

Huseyin YILMAZ[1], Beran FIRIDIN[1], IsmaiL DEMIR[2], Maurice MOENS[3,4] & Zihni DEMIRBAG[2]

[1] Department of Biology, Faculty of Arts and Sciences, Giresun University, Turkey
[2] Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, Turkey
[3] Institute for Agricultural and Fisheries Research (ILVO), Merelbeke, Belgium
[4] Laboratory of Agrozoology, Department of Crop Protection, Ghent University, Belgium

The alder leaf beetle, *Agelastica alni* L. (Coleoptera: Chrysomelidae) is one of the most serious pests of hazel and alder trees. The insect outbreaks occur almost every year in the Middle and Eastern Black Sea Region of Turkey where hazel plantations cover huge areas. We screened the virulence of four local isolates of entomopathogenic nematodes (*Steinernema carpocapsae* B122, *S. feltiae* B1, *Heterorhabditis bacteriphora* M3 and *H. megidis* P69) against last instar larvae of *A. alni*. In bioassays using 24-well plates with sterile sand, insect larvae were exposed to a concentration of 100 infective juveniles per individual. Larval mortality ranged from 42.7 to 84.5%. *Heterorhabditis megidis* P69 caused infection and mortality of larvae more quickly than the other isolates and has a potential as a biological control agent in IPM programs. Future work will focus on the determination of the efficiency of the isolates against the pest in field conditions.

DISTRIBUTION OF ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.) IN THE WEST MEDITERRANEAN REGION OF TURKEY

Zübeyir DEVRAN[1], Mehmet ALI SÖĞÜT[2], Adem ÖZARSLANDAN[3], Münevver GÖÇMEN[1] & Hatice İKTEN[1]

[1] West Mediterranean Agricultural Research Institute (BATEM), Antalya, Turkey
[2] Süleyman D. University, Agriculture Faculty, Plant Protection Department, Isparta, Turkey
[3] Plant Protection Research Institute, Adana, Turkey

Root-knot nematodes (*Meloidogyne* spp.) cause serious yield losses in protected vegetables grown in the West Mediterranean region of Turkey. Ninety- four populations of root-knot nematodes (*Meloidogyne* spp.) were collected from different vegetable growing locations in this region. Egg masses were picked up from the plant roots using a small needle in the laboratory. Tomato seeds were germinated in steam-sterilized sandy soil in seed tray, and 2- week-old seedlings were transplanted singly into 250 ml plastic pot. Plants were inoculated at the fourth true leaf stage with single egg mass. Plants were grown at 25 ^{o}C growing chamber and harvested 8 weeks after inoculation. Root systems were carefully washed under tap water. Population was identified using morphological characteristics and molecular techniques. To identify morphological characteristics, perineal patterns were examined on a light microscopy. For molecular characterization of root-knot nematodes, firstly, DNA was extracted from egg masses with *DNAeasy Tissue* and *Blood Kit* according to the manufacturer's protocol. Secondly, the five different species-specific primers were used for DNA analysis of the root-knot nematode samples. According to morphological and molecular methods, *Meloidogyne incognita*, *M. javanica* and *M. arenaria* were identified in the West Mediterranean region of Turkey. *M. incognita* was found the most common root-knot nematode species in the region.

HATCH TEST OF POTATO CYST NEMATODES FROM SERBIA WITH SELECTED STIMULANTS

Milan RADIVOJEVIĆ & Tijana STANČIĆ
Institute for Phytomedicine, Faculty of Agriculture, Beograd, Serbia

European Union coordinates measures to control or eradicate potato cyst nematodes (PCN) as quarantine pests all over the continent. In Serbia, these nematodes were detected in several restricted localities during recent years, and now are subject to various activities aiming eradication. Practical value of convenient hatching agents is e.g. to reliably demonstrate actual viability of cysts for statutory or research purposes; to evaluate ability of particular agents, especially those originating from locally relevant plants, to decrease or support PCN populations, etc. Selected substances known or suspected to act as stimulants for PCN hatching are being tested with local populations of both PCN species, but only the data for *Globodera rostochiensis* Ro 1 are ready to provide herein. Nine agents, stored in the fridge, were tested: Inorganic substances (NaO_3V 0.6 mM, $KMnO_4$ 0.5 %); Leaching from infested soil; Root diffusates of potato (cvs. Desireé and Cleopatra), tomato (cvs. Pokep FV and NS Jabucar), egg plant (cv. Jajcavec) and *Solanum nigrum*. Root difusates were extracted in 200 ml water by three-fold rinse from single plants grown in1 lit mixture of potting substrate, sand and terra-cotta granules 1:1:1. Tap water was control. For each agent, batches of 50 cysts carrying c. 5000 encysted larvae (L2), five-fold replicated, were kept in 3 ml vials at 20 ^{0} C for minimum12 weeks. Solutions in vials were replaced and counts obtained weekly. Cysts originate from infested field under potato crop cv. Desireé, locality Ponikve, June 2008. Initial cyst content of vivid larvae was estimated from 50 random cysts, individually dissected. Inorganic agents had significantly better effect than root diffusates and soil leaching. NaO_3V and $KMnO_4$ had similar high effect (c. 3000 L2 total hatch), but with an early peak of vanadate in 3rd and 4th weeks *vs*. very late peak of $KMnO_4$ in the 11th week. Soil leaching had a median effect (c. 1900 L2) and peak in 6th - 8th weeks. Amongst the plant root diffusates, the best effect was that of potato cv. Desireé, c.1500 L2 (peak in the 5th week), twice as much as cv. Cleopatra (c. 750 L2). Diffusate of tomato cv. Pokep VF hatched c. 1000 vs. 600 L2 of tomato cv. NS jabucar. Effect of egg plant was similar, even slightly worse than spontaneous hatch in water (c. 200 vs. 300 hatched L2, respectively). High stimulating effect of both inorganic agents demonstrates that, in the cysts examined, at least 60 % of the vivid inital content are hatchable and certainly alive L2. Between the two, sodium meta-vanadate has advantage of its early action for routine laboratory practice. Root diffusates and soil leaching(s) need further work.

RELATIONSHIP BETWEEN SOIL NEMATODE COMMUNITIES AND MULCHING IN VEGETABLE GROWING GREENHOUSES

Sevilhan MENNAN[1], Ayşe EKDIOĞLU[2] & Sezgin UZUN[2]
[1] Ondokuz Mayıs University, Agricultural Faculty, Plant Protection Department
TR-55139 Samsun, Turkey
[2] Ondokuz Mayıs University, Agricultural Faculty, Horticultural Department
TR-55139 Samsun, Turkey

Because nematodes live in the soil such a complex agroecosystem, lots of things especially related with soil, have a great influence on the communities of soil nematodes. The aim of the research was to determine the effects of mulching on soil nematode communities in tomato (*Lycopersicon esculentum* L. vr "Töre F1") planted soil in greenhouse. Soil samples were collected monthly and 3 times at August, September and October. Black coloured plastic material were use to mulching. Soil temperature was observed during the experiment. Extraction was done by using Modified Baermann Funnel method, nematodes were killed and fixed in heated TAF. Soil nematode communities were identified as a trophic level (bacterivours, fungivours, plant parasites, predators and omnivores). Ecological parameters were applied to the numbers and Maturity Index, Plant Parasitic Index, Channel Index, Fertility Index, Enrichment and Structure Index were found compared with parcels with muching or not. Mulching caused to increase of total soil nematodes community especially bacterivours and fungivours. Each month, soil nematode numbers were risen up including plant parasitic species in harmony with soil temperature.

TEMPERATURE EFFECT ON NOVEL ENTOMOPATHGENIC NEMATODE *STERNERNEMA SIAMKAYAI* STOCK, SOMSOOK ANS REID (N.SP.) ANS IT'S EFFICACY AGAINST *SPODOPTER LITURA* FABRICIUS (LEPIDOPTERA: NOCTUIDAE)

Wilaiwan WETCHAYUNT[1], Ajin RATTANAPAN[2] & Suriyan PHAIRIRON[2]
[1] Biological Control Research Group, Department of Agriculture
Ministry of Agricultural and Cooperative, Thailand
[2] Department of Biology, Faculty of Science Maha Sarakham University, Thailand
* Correspondence to: Ajin Rattanapan, Dept. of Biology, Faculty of Science, Mahasarakham University, Khamreang District, Mahasarakham 44150, Thailand
E-mail: ajinrat@gmail.com

The new entomopathogenic nematode species, *Steinernema siamkayai* is a new category from Thai strain of Thailand. The efficacy of the this new species and the well known previous species are *Sterinernema carpocapsae* and *Steinernema riobrave* against third instars larvae of common cutworm, *Spodoptera litura*, and greater wax moth, *Galleria mellonella* was evaluated under laboratory condition. The optimal temperature for entomopathogenic nematode *S. siamkayai* at 50 and 100 IJs per larva to penetrate into *S. litura* and *G. mellonella* larvae were highly suitable during 25 to 30°C. The mortality rates of *S. litura* and *G. mellonella* against *S. siamkayai* upto 100% when using more times and higher concentration than did *S. carpocapsae* and *S. riobrave*. When compare between the *S. riobrave* and *S. siamkayai,* they were used the same time but more concentration with 200 IJ per larvae to cause 100% mortality to these Lepidopteran larvae. The toxicity value in term of LC_{50} of the new entomopathogenic nematode, *S. siamkayai* to *S. litura* larvae at age of 10 and 12 days were indicated that 37.75 and 61.16 IJs per larva, respectively. Moreover, According to the results of this study, the investigation should be further conducted in order to develop increasing efficacy of *S. siamkayai* and symbiotic bacteria. In addition, especially, the specificity of *S. siamkayai* to the insects should also be investigates.

EFFICIENCY OF PROPOLIS EXTRACT ON FABA BEAN PLANTS AND ITS ROLE AGAINST NEMATODE INFECTION

E.M.A. NOWEER[1] & Mona D. DAWOOD[2]
[1] Plant pathology Department
[2] Botany Department, National Research Center

Propolis is a resinous substances collected by honey bees, It can not be used as a raw material, and it must be purified by extraction with solvent. Propolis samples were extracted by using three different solvents distilled water; 70% ethanol and acetone.
The propolis extract contains sterols and/or triteroenes, flavonoids and phenolic compounds. Few numbers of phenolic acids (coumaric, ferulic, salicylic and benzoic acid) were also detected on TLC.
Total chlorophyll increased with all treatments, but the magnitude of increase was more pronounced by applying the higher concentration (1000 mg/L). On the other hand, the ratio between chlorophyll a + chlorophyll b/carotenoid decreased with all treatments (Soil drench and foliar).
Addition of propolis extract with all treatments as soil drench to infected soil with nematode caused significant increase in some of the parameters of faba bean plants in most cases except the number of pods/plant which showed non significant effect, while, significant decrease observed in root length and shoot dry weight/plant as compared to control
Applying propolis extract as soil drench to infected soil with nematode caused an increase in carbohydrate content of the yielded seeds accompanied by a decrease in protein phenolic compounds; phytic acid and vicine content.
The data revealed that the propolis extract as soil drench reduced the juvenile-*Meloidogyne* sp.-population density per one kg soil and number of root-galls per one gm roots specially at the higher concentration (1000 mg/L).

EFFECT OF SOME ORGANIC AMENDMENTS AND BIOSYNTHETIC PRODUCTS ON ROOT-KNOT NEMATODES *MELOIDOGYNE JAVANICA* INFECTING BANANA IN MOROCCO

Z. FERJI[1], H. MAYAD[2], M. ALFALAH[1] & D. DE WAELE[3]

[1] Laboratoire de Nématologie, Institut Agronomique et vétérinaire Hassan II
Complexe Horticole d'Agadir, B.P 18/S, Agadir. Maroc.
E-mail : ferji@iavcha.ac.m ,

[2] Laboratoire de Symbiotes Racinaires et Biochimie végétale, Faculté des Sciences
B.P. 28/S, Agadir, Maroc

[3] Laboratory of Tropical crop Improvement, Catholic University of Leuven (KUL)
Kasteelpark Arenberg 13, BE-3001 Leuven, Belgium
E-mail: Dirk.DeWaele@biw.kuleuven.be

A new biological product Dazitol, and two organic amendments one composed of leaves, stem and seeds of the *Ricinus communis* the other consists a locally produced compost (Biocompost) were used to assess their effect on the management of the root-knot nematode *Meloidogyne javanica* on banana.

The best results of second stage larvae reduction (100%) were obtained by Dazitol. Whereas, nematode reductions obtained by *Ricinus* and *Ricinus* combined to Biocompost were 88.3%, 84 % respectively. In terms of root damage *Ricinus* treated plants showed the lowest root gall index (1.8) versus 3,5 recorded on the untreated banana plants.

Either alone or combined to Biocompost, *Ricinus* increases significantly the growth of the aerial plant part of banana (weight, height and circumference of the pseudo stem). The weight of Ricinus treated banana plants was 111 g compared to the control (42.5 g).

INTEGRATED ROOT-KNOT NEMATODE MANAGEMENT IN CARROTS

Nordalyn B. PEDROCHE[1], Luciana M. VILLANUEVA[1] & Dirk DE WAELE[2]
[1] Semi-Temperate Vegetable Research and Development Center
Benguet State University, La Trinidad, Benguet, Philippines
[2] Laboratory of Tropical Crop Improvement, Department of Biosystems
Faculty of Bioscience Engineering
Catholic University of Leuven, Leuven, Belgium

Root-knot nematode, *Meloidogyne incognita*, remains to be one of the most important economic constraints in agricultural production worldwide. However, root-knot nematode (RKN) population can be suppressed by addition of organic amendments. A greenhouse microplot experiment was conducted to determine if locally available organic amendments will reduce RKN population and improve the growth and yield of resistant and susceptible carrot cultivars.

Residues of broccoli, chicken manure and *Trichoderma* inoculant were incorporated into the soil artificially infested with root-knot nematodes. Untreated soil was provided to serve as control. Three months after transplanting, nematodes were recovered from the soil using the modified Baermann-tray technique and from the roots using staining technique. The number of root-knot nematodes was counted under the stereoscopic microscope.

In the susceptible cultivar New Kuroda, the lowest number of second stage juveniles (J2's) was recovered from the soil incorporated with broccoli left-over materials and *Trichoderma* inoculant while chicken manure-amended soil had the highest number of J2's. Galls and egg masses in secondary roots were highest in unamended-inoculated soil which is greatly different from broccoli-amended soil with solarization and *Trichoderma* inoculant.

On the other hand, chicken manure-amended soil had the lowest number of J2's in the resistant cultivar Chunhong, while broccoli-amended soil with *Trichoderma* inoculant gave the lowest number of galls and egg masses, although this number was exceedingly lower compared to the susceptible cultivar New Kuroda. Yield was highest in broccoli-amended soil with solarization and *Trichoderma* inoculant in both cultivars. Similarly, both treatments were able to decrease root-knot nematode population relative to untreated soil.

NEW RECORD OF *XIPHINEMA INDEX* THORNE & ALLEN, 1950 (NEMATODA: LONGIDORIDAE) FROM BULGARIA WITH DESCRIPTION OF THE MALE

Tatyana BILEVA[1] & Boryana CHOLEVA[2]
[1] Department of Agroecology, Agricultural University of Plovdiv, Bulgaria
[2] Faculty of Biology, Sofia University "St. Kliment Ohridski, Bulgaria

During a study of plant-parasitic nematodes from grapevine areas of South Bulgaria a population of *Xiphinema index* Thorne & Allen, 1950 was found in a new locality. Females and a male of this species are briefly described.

Posters
Herbology

DETECTION OF *CIRSIUM ARVENSE* L. IN CEREALS USING A MULTISPECTRAL IMAGING AND VEGETATION INDICES

Pavel HAMOUZ, Kateřina HAMOUZOVÁ & Josef SOUKUP
Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources Czech University of Life Sciences, Czech Republic

A patchy spatial distribution of weed populations provides a large potential for herbicide savings. However, new automatic methods are required, that will allow fast and reliable mapping of weed populations. The objective of this study is to find algorithms for detection of *Cirsium arvense* in cereals using airborne high resolution multispectral imaging. The image of three winter wheat stands was taken from helicopter using 3-band multispectral imaging system in 2007. Green (500–580 nm), red (630–710 nm), and NIR (735–865 nm) spectral bands were used. Spatial resolution of analysed images was 0.2 m per pixel. Reference areas of 2 x 2 m were marked in the field using white targets. Ground truth data were collected immediately after the flight. High resolution ground images of the sample areas were taken by digital camera. The shapes of *C. arvense* plants were manually extracted from these images and the aerial and ground information were compared by the overlay of the images. Various vegetation indices including NDVI and DVI were calculated and the correlation with ground data was tested. The correlation coefficients r = 0.781 – 0.851 were calculated for NDVI and r = 0.779 – 0.870 for DVI. The use of NDVI index provided lower correlation if other weeds were present in the crop.

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APERA SPICA-VENTI – THE MOST IMPORTANT WEEDY GRASS IN THE CZECH REPUBLIC IS RESISTANT TO ALS-INHIBITING HERBICIDES

Kateřina HAMOUZOVÁ, Pavlína TŮMOVÁ & Josef SOUKUP
Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Czech Republic

Whilst emergence of graminicide resistance in weeds has been a relatively recent phenomenon world-wide, it has occurred to varying degrees amongst grass weed species and is represented currently to different extents in the area of cropped land affected. The long-term monitoring and testing on resistance in *A. spica-venti* (APESV) have been done in the Czech Republic since 2000. *A. spica-venti* occurs regularly in 80 % of all observed localities mainly in the west and central part of the country. The increasing harmfulness was declared by 32 % of the inquired farmers. For no one weed that has evolved resistance have all the parameters determining resistance been known in advance, although they can explain the speed of evolution and propose practical measures to prevent its spread. Resistance in APESV to sulfonylurea was reported for the first time in 2004 after investigations of control failures in winter wheat and has been associated in the main with largely continuous winter cereal cropping with recurrent use of herbicides with the same mode of action. High degree of resistance to ALS-inhibiting herbicides was confirmed in 2 % of screened populations. In resistant populations the ED_{50} value in whole-plant bioassays for chlorsulfuron is over 50 times that of a standard susceptible population. Cross resistance, as a serious and substantial phenomenon in relation to graminicides, was confirmed to chlorsulfuron, sulfosulfuron and iodosulfuron. There is the evidence that the pattern of cross resistance is not consistent between populations, in terms of individual pattern of cross resistance or degree of resistance (RF=6.7-303). Now, we are investigating the genetic basis of resistance in *A. spica-venti,* which is an annual outcrossing species, with a considerable reproductive potential (mean value 22.746 ± 20.302 seeds per plant) and transient seed bank (up to 3 years). A substitution of cytosine by thymine (susceptible: CCC, resistant: TCC) at the position 76 (197) has occurred, that confers an exchange of the amino acid proline in the susceptible to serin in the resistant biotype.

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INFLUENCE OF LONG TERM USED HERBICIDES ON RESISTANCE DEVELOPMENT IN *APERA SPICA-VENTI* L. TO SULFONYLUREAS

K. ADAMCZEWSKI, R. KIERZEK & K. MATYSIAK
Institute of Plant Protection – National Research Institute
St. Wladyslawa Wegorka 20, Poznan, Poland

Silky bent grass (*Apera spica-venti* L.) is one of the most important weeds in winter cereals and winter rape infesting arable fields in Poland. This grass weed occurrences in winter cereals is noted at 60 percentage, that is about four million hectares. Silky bent grass is very competitive grass weed for winter cereals, in particular cultivars which grow not so high (short straw cultivars). Sulfonylurea herbicides are widely used for grass and broadleaf weed control in winter cereals in Poland developed resistance, especially in Silky bent grass (*Apera spica-venti).* In Poland sulfonylurea and isoproturon herbicides are commonly used for control of *A. spica-venti*. The sulfonylurea herbicides in practice are most popularly used, because they are cheap and efficient and play currently a role crucial in the chemical weed control in winter cereals. During last few years control of this important weed species with the sulfonylurea was not frequently applied.
The aim of the study was to evaluate the possibility of resistance increase after six years (2003-2008) used of some herbicide for control of *A. spica-venti* in winter cereals monoculture. The field experiments were conducted in Agricultural Experimental Station at Winna Gora. During six years the herbicides: chlorsulfuron, sulfosulfuron, iodosulfuron and isoproturon were applied. In fourth, fifth and sixth years *A. spica-venti* seed from the experiment was collected and used in greenhouse study. The obtained results indicated that after six years usage of the herbicides resistance of *A. spica-venti* to sulfonylurea herbicides were found. Results obtained in field condition were confirmed in greenhouse experiment. Resistance process was found also on untreated plots. It was indicated that resistance is transferred also by pollen. This results confirm date obtained by Adamczewski and Matysiak (2008), and by Busi *et al*. (2007). Tranel and Wright (2002) contribution is also indication that resistance of ALS-inhibiting herbicides can be transfers by seeds and pollen.

DETECTION OF ACCASE TARGET-SITE RESISTANT *ALOPECURUS MYOSUROIDES* (BLACK-GRASS) IN BELGIAN POPULATIONS

P-Y. MARECHAL[1], F.HENRIET[2] & B. BODSON[1]
[1] Gembloux Agricultural University (FUSAGx), Unité de Phytotechnie des Régions Tempérées, Passage des Déportés 2, BE-5030 Gembloux, Belgium
[2] Walloon Agricultural Research Centre (CRA-W), Pesticide Research Department Rue du Bordia 11, BE-5030 Gembloux, Belgium
Corresponding author: marechal.py@fsagx.ac.be

Black-grass is a common grass weed, widely spread in Northern Europe and also in Belgium. For ages, it has been an increasing problem in industrial crops, especially winter cereals. The first case of resistance in Belgium was reported in 1996 by Robert Bulcke. Yet the resistance mechanism was not specified. Since then, no more information was published about Belgium, while research continued in the United Kingdom and in France. Moreover, during the last decade, progress in molecular biology allowed to highlight the mechanism of target-site resistance. A simple PCR method allows to detect the mutation conferring resistance to herbicide.

After two years of resistance monitoring in Belgium, mostly in the Walloon part, some populations have been clearly identified as highly resistant to ACCase inhibitor. These populations have been tested by molecular biology so as to detect the single nucleotide polymorphism (SNP) involved in this case. The method employed was the Polymerase Chain Reaction Allele Specific Assays (PASA: Délye, 2002) for the mutation Ile-1781-Leu that confers a target-site resistance to ACCase inhibitors. Those analyses were performed on plant material issued from bioassays, either in glasshouses or in Petri dishes. Leaves have been collected from plants which survived a fenoxaprop-P treatment applied in a glasshouse single dose assay. Seedlings from resistant populations grown in Petri dishes containing either fenoxaprop-P or cycloxydim provided the second type of sample. Ile1781 mutants were discovered within three populations. Each mutant plant was heterozygote. Five of those samples have been sequenced to confirm PASA results and everyone was matching. Moreover, they were all issued from Petri dishes containing cycloxydim, known to be unaffected by enhanced metabolism, confirming that theses populations are indeed target-site resistant.

TREATMENT INFLUENCE ON HERBICIDE RESISTANCE LEVEL OF BELGIAN *ALOPECURUS MYOSUROIDES* POPULATIONS (BLACK-GRASS)

P-Y. MARECHAL[1], F.HENRIET[2] & B. BODSON[1]
[1] Gembloux Agricultural University (FUSAGx)
Unité de Phytotechnie des Régions Tempérées
Passage des Déportés 2, BE-5030 Gembloux, Belgium
[2] Walloon Agricultural Research Centre (CRA-W), Pesticide Research Department
Rue du Bordia 11, BE-5030 Gembloux, Belgium
Corresponding author: marechal.py@fsagx.ac.be

Black-grass is a common grass weed, widely spread in Northern Europe and also in Belgium. For ages, it has been an increasing problem in industrial crops, especially winter cereals. Therefore, farmers started to spray herbicide intensively and soon cases of failure occurred for different molecules and different modes of action. Black-grass populations have been tested in greenhouses to assess the influence of an herbicide treatment as to the resistance level regarding three different herbicides: chlortoluron, fenoxaprop-P and mesosulfuron+iodosulfuron.
Black-grass seeds were collected in field trials in six locations in Belgium, on individuals which have survived the herbicide treatment. Each population comes from trial plots, measuring 2 meters wide by 5 meters long and characterized by a single or a combination of products. Herbicides sprayed were isoproturon, flufenacet+diflufenican, ACCase inhibitors and ALS inhibitors. Seeds were also collected in the untreated plots. The population present in these last ones corresponds to the former population, before the herbicide selection pressure was applied. In the glasshouse assay, this population was used as the standard population to compare with other populations issued from the same field. The 'R' rating system was set up with this population to assess the evolution of resistance level, year in, year out. Rothamsted and Peldon populations were also included as cross-reference.
Each field population presented different behaviours towards herbicide applied in greenhouses and some cases of resistance can be highlighted. Generally, a reduction of treatment efficiency between field and greenhouse results was clearly visible for the whole of studied active ingredients. Indeed, a distribution shift of the populations towards higher resistance classes could be observed. This is particularly remarkable for active ingredients sharing the same mode of action. For example, it has been found that populations already sprayed with fenoxaprop-P on the field showed a higher resistance level to fenoxaprop-P than to mesosulfuron in the greenhouse test.

ARE PORTUGUESE *ECHINOCHLOA* SPP. POPULATIONS STILL SUSCEPTIBLE TO PROPANIL?

IsabeL M. CALHA & FATIMA ROCHA
Instituto Nacional de Recursos Biológicos I.P. (INRB I.P.), Unidade de Protecção de Plantas
Quinta do Marquês, PT-2780-155 Oeiras, Portugal
E-mail: isamc@dgpc.min-agricultura.pt

Propanil is the most important herbicide for rice weed management both at world and national level. Rice growers complain of poor control of *Echinochloa* spp.was monitored in Mondego and Sorraia river valleys, Portugal. Seed samples were collected from the affected area and tested. After the first screening of 37 populations, the sensitivity of six *Echinochloa* spp. populations to propanil was assessed in a growth chamber dose response study (with seven rates: 0- 7200 g a.i. ha^{-1}). Fresh weight was assessed 21 days after treatment and data was analysed using non-linear regression analysis and sensitivity indices ($SI=ED_{80}$, less sensitive/ ED_{80}, most sensitive) calculated for the two regions surveyed. The rates of 50% of plant growth inhibition (ED_{50}) were calculated from the fitted equations. Four populations where confirmed susceptible with ED_{50} values ranging from 89 to 1866 g a.i. ha^{-1}. Two other populations presented ED_{50} values of 6538 and 9536 g a.i. ha^{-1}. Mondego and Sorraia SI were 2.35 and 21.77 respectively. The pattern of propanil use in the two regions could explain the higher sensitivity of Mondego populations compared to Sorraia populations. The response to a single dose Petri dish bioassay (360 g a.i. L^{-1}) was similar among the six populations, denoting that this method was not so sensitive as the whole plant assay to discriminate between *Echinochloa* spp. populations. Further studies are needed with more doses and populations since this method allows for an answer within six days, compared with 41 days with the whole plant bioassay.

ALLELOPATHIC POTENTIAL OF RICE LEAVES, STRAW, AND HULL EXTRACTS ON BARNYARDGRASS (*ECHINOCHLOA CRUS-GALLI*)

Husrev MENNAN[1], Emine KAYA[1], Mevlut SAHIN[2] & Dogan ISIK[2]
[1] Ondokuz Mayıs University, Agriculture Faculty, Department of Plant Protection
TR-55139 Samsun, Turkey
[2] Black Sea Research Institute Samun, Turkey

Weeds are the most important constraint in rice production, causing considerable yield losses depending on weed species and rice cultivars. The use of herbicides were intensive control methods since past three decades in Turkey, but herbicides resistance in weed and their adverse effects on the environment has been main concern in last years. For this reason, it is important to find alternative and sustainable weed management methods. One of the ecological methods is the use of allelopathic potential of rice cultivars. Thirty rice cultivars (*Oryza sativa*) were grown at the Black Sea Agricultural Research Institute in Samsun, Turkey in 2007. The flag leaves of cultivars were harvested at the end of vegetative period. The hulls and straw obtained during harvest. The harvested materials (leaves, straw and hull) were dried at 24 ^{o}C and ground through 40 mesh screen. Aqueous extracts were prepared and centrifuged at 3000 rpm for 4 h. To evaluate the allelopathic effect of these extracts, 25 seeds of *Echinochloa crus-galli* were placed on filter paper in a Petri dish, and ten millilitres of each extract solution added to petri dishes. Distillated water was applied to *Echinochloa crus-galli* seeds as a control. The number of germinating seeds was recorded, and length of roots measured. All extracts showed an inhibitory effect on *Echinochloa crus-galli* germination, and straw extract inhibited these seeds than the other extracts. The average inhibition rate in *Echinochloa crus-galli* seeds from leaf extracts was highest for 'Martelli' (75.3%) and lowest for 'Gonen' (0%). Only 13 rice cultivars demonstrated over 50% inhibition. When straw extract were used, significant differences existed among the rice cultivars in their ability to suppress *Echinochloa crus-galli* germination. Extract of 'Negis' 'Koral' and Kizilirmak' were the most inhibitory of *Echinochloa crus-galli* seeds. While fifty cultivars inhibited germination more than 50%, six cultivars did not show any effect. The hull extract of 'Kiziltan' had 96.6% inhibitory effect on *Echinochloa crus-galli* seeds. The other cultivars did not exhibited more than 40%.

SPECIES DIVERSITY OF WEEDS IN WINTER WHEAT AND WINTER BARLEY FIELDS IN BULGARIA

Dina ATANASOVA
Institute of Agriculture – Karnobat, Bulgaria
E-mail: dinadadar@abv.bg

In 2008 was made monitoring to determine the degree of weed infestation on the field crops of winter wheat and winter barley. It was defined species diversity, degree of spreading of the important species and were determined regions which differ in level of weed infestation. In all crops was registered presence of perennial weed with deep taproot *Convolvulus arvensis* L. and *Cirsium arvense* (L.) Scop. In rarefaction crops with high density are met *Polygonum convolvulus* L., *Viola tricolor* L. and from ephemerals *Veronica hederifolia* L, *Fumaria officinalis* L., which sprouted ever since autumn.
Monitoring components of biodiversity can by itself provide a basis for evaluating weed management strategies.

CARFENTRAZONE-ETHYL: A NEW MOLECULE FOR THE CONTROL OF HAZELNUT SHOOTS

Angela FANIGLIULO[1,2], Vittorio FILÌ[1], Carmine Giuseppe MASSA[1] & Aniello CRESCENZI[2]

[1] Bioagritest Test Facility, Bioagritest Srl - Centro Interregionale di Diagnosi Vegetale
Zona PIP lotto E2. 85010 Pignola (PZ, Italy)
E-mail: info@bioagritest.it; www.bioagritest.it.
[2] Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali
Università degli Studi della Basilicata
Viale dell'Ateneo Lucano Campus Macchia Romana 3A310, 85100 Potenza (PZ) Italy
E-mail: aniello.crescenzi@unibas.it

Control of the shoots growth is an important practice for the management of hazelnut crop, in order to improve its qualitative and quantitative yield.
Bioagritest test facility has set up a trial in order to evaluate the efficacy of the contact herbicide SHARK® (carfentrazone 60g/l) for the control of hazelnut shoots. Experimentation was performed, according to EPPO guidelines and Principles of Good Experimental Practice (GEP), in a family-run hazelnut orchard in Nocera Inferiore (Salerno), southern Italy. Experimental design consisted in random blocks, in 4 repetitions. Two different dosages of SHARK 60EC – 0.35 and 0.40 lt/hl - were compared with a commercial formulate, Glufosinate-ammonium 2 lt/hl (Basta 11.33 SC, Bayer CropSciences), a non-selective herbicide for use in tree crops, vegetables, non-crop areas and as desiccant of potatoes and other crops.
Two applications were realised with an interval of 7 days. 4 scoutings were performed respectively a week, 2 weeks, a month and 2 months after the first application. They consisted in a visual and subjective evaluation on the whole plot of the desiccation of the shoots expressed as a percentage (intensity of necrosis as opposed to any green parts present), according to a scale from 0 to 100. The intensity of desiccation was evaluated together with eventual phytotoxic effects. Statistical analysis was performed by the use of *XLSTAT* data analysis and statistical software.
The chance of an unexpected rain in the hours after the first application produced an interesting result because the two doses of Carfentrazone-ethyl showed a rapid uptake in the tissues of shoots, much higher than that of the standard Glufosinate-ammonium. It is believed that this rapid uptake and, consequently, a fast-desiccation capacity, may represent a value for the use of Carfentrazone-ethyl on hazelnut shoots.
Carfentrazone-ethyl provided a good control of hazelnut shoots and, at the highest dose, it induced on all replicates a total dissecation. No effect of phytotoxicity was noticed.

WEED MANAGEMENT IN POME FRUIT ORCHARDS IN PORTUGAL

M. JÚLIA AFONSO
Direcção-Geral de Agricultura e Desenvolvimento Rural (DGADR)
Quinta do Marquês, PT-2780-155 Oeiras, Portugal
E-mail: juliaafonso@dgadr.pt

Portugal has good climatic-edafic conditions for pome fruit orchards and produces apples (*Malus domestica* Borkh.), pears (*Pyrus communis* L.), quinces (*Cydonia oblonga* Miller) and loquats (*Eriobotrya japonica* (Thunb.) Lindl).
To achieve yield rentability in these orchards, weed management is essential.
This paper presents the state of art, in Portugal, regarding to some cultural and social-economical aspects of the mentioned fruit orchards (e.g., cultivated areas, productions, main producer regions), the main weeds, the weed control methods currently used and, amongst them, the registered herbicides, with indication of their usage conditions (doses, application timings, spectrum of weeds controlled and some restrictions/precautions), according to the principles of Good Plant Protection Practice, Integrated Weed Management and Integrated Production programmes.

PHYTOTOXICITY OF DRIMYS WINTERI BARK FOR WEED SUPPRESSION

Nelson ZAPATA & Pamela MEDINA
Facultad de Agronomía, Universidad de Concepción, Chillán, Chile

Drimys winteri J.R. et G. Forster is a native tree of Chile, its bark contains numerous secondary metabolites, which have shown bioactivity like insecticide, fungicide, bactericide and herbicide. The aim of this study was to evaluate its phytotoxic properties for weed suppression, in order to use it in crop protection. A Dose-response experiment was established with *D. winteri* chopped bark mixed with soil (0.1, 0.2, 0.4, 0.8%), and with their extracts obtained with n-hexane (0.05, 0.1, 0.2, 0.4%). The plant growth substrate was put into pots, and four different species of weeds were sown (*Convolvulus arvensis* L., *Daucus carota* L., *Setaria pumila* Poir. and *Cichorium intybus* L.). We evaluated the emergency percentage, germination speed, plant height and accumulated biomass. *D. winteri* bark and the n-hexane extracts reduced significantly the emergency and growth of all weeds, but *C. intybus* and *S. pumila* were the most sensitive species to the bark and extracts tested, on the contrary *C. arvesisis* showed the greatest tolerance.

THE EFFECT OF WEED CONTROL METHODS AND SOYBEAN CULTIVARS ON THE GRAIN YIELD OF SOYBEAN

Kayvan ZAMAN[1], Amin SADEGHI[2], Shina ZAMAN[3], Bakhtiar MORADI[3], Asaad ROKHZADY[4] & Shahab JALALI[5]

[1]Young Researchers Club Sanandaj unit of Islamic Azad University, Sanandaj, Iran
[2] Laboratory of Entomology, Department of Crop Protection, Faculty of Agriculture, University of Kurdistan, Iran
[3]Tehran University (Abouraihan), Tehran, Iran
[4] Sanandaj unit of Islamic Azad University, Sanandaj, Iran
[5]Ardabil unit of Islamic Azad University, Ardabil, Iran

Successful weed control without using herbicides is one of the most applications for sustainable agriculture. Losses due to weeds have been reported one of the major limiting factors in soybean production. Weeds compete with soybean for light، moisture، and nutrients which becomes critical, in the early season. In this study, the effect of weed control methods (weed out، land cover with mulch straw and none weed out) and soybean cultivars (Clark, M7, M9, T.M.S) on the grain yield of soybean were investigated. The result showed that weed control methods significantly affect on plant height, pod number, first pod distance from soil level, hundred grain weight, grain yield, biological yield and harvest index compared none weed out. In addition, the highest level of first pod distance from soil and harvest index have been observed in mulch straw treatment. Also the results showed that yield and yield component were influenced by soybean cultivars by which the maximum grain yields and thousand grain weights recorded for the M9 cultivar. In conclude, our results showed that link of mulch straw and M9 soybean cultivar are good strategies for weed management and yield increment in soybean fields, concerning to sustainable agriculture.

THE EFFECT OF SEVERAL ADJUVANTS ON GLUFOSINATE EFFECTIVENESS IN *CONYZA* SPECIES

M.C. FERNANDEZ-CEREJIDO, F. BASTIDA & J. MENENDEZ,
Departamento de Ciencias Agroforestales, Escuela Politécnica Superior,
Campus Universitario de La Rábida. Universidad de Huelva, Spain

Ammonium glufosinate is a non-selective herbicide worldwide used for controlling a wide spectrum of broadleaved and grass weeds. However, its interaction with adjuvants has not been studied in deep. In these terms, better weed control would be achieved using the proper glufosinate tank-mixtures. The aim of this study has been to determine under laboratory controlled conditions the effect of several adjuvants on the effectiveness of glufosinate on the broadleaved weeds *Conyza albida* and *Conyza bonariensis*. It has been also determined the herbicide mixture adherence and the contact angle for the different treatments. The adjuvants used on the experiment were a mixture of methyl oleate and palmitate (MOMP), a dodecylbenzene ammonium sulphonate (DBAS), a paraffinic oil (PO), a poly-1-p-menthene (POLY), an alkylglycol ester (AGE), and a lecithin + propionic acid + non ionic surfactant based product (LI-700). The herbicide was applied at a 200 L ha^{-1} carrier volume using a precision lab sprayer. Half effective doses (ED_{50}) were recorded 28 days after treatment. On *C. albida*, the mixtures of the herbicide with poly-1-p-menthene or the paraffinic oil were the most effectives compared to glufosinate applied alone. On *C. bonariensis*, the alkylglycol ester and the mixture of methyl oleate and palmitate showed the best results. In adherence assays, the best results for *C. albida* were observed with the poly-1-p-menthene and the dodecylbenzene ammonium sulphonate mixtures. On *C. bonariensis*, the treatment with the paraffinic oil and the alkylglycol ester showed a better adherence than the glufosinate applied alone. In contact angle assays, data surprisingly showed no differences among treatments in both species. Although the increase on the effectiveness of glufosinate observed on both species when the herbicide is applied with these adjuvants may be explained in terms of adherence, contact angle values do not support the hypothesis, so adherence results may depend on more variables than just surface tension.

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RACES OF BACTERIAL BLIGHT, *XANTHOMONAS CITRI* SUBSP*. MALVACEARUM*, PRESENT IN IRANIAN COTTON CROPS

Azadeh SAEEDI MADANI[1], Abolghasem GHASEMI[3], Alireza MAREFAT[2] & Keyvan BEHBOUDI[1]

[1] Plant Protection Department, University of Tehran, Iran
[2] Plant Protection Department, University of Zanjan, Iran
[3] Plant Disease Department, Plant Protection Research Institute of Tehran, Iran

Bacterial Blight caused by *Xanthomonas citri* subsp. *malvacearum* is an important and potentially destructive cotton disease on susceptible cultivars. The disease was first reported in 1967 in Iran, and has been observed during 2000-2003 in central, and Eastern North cotton fields. Affected leaves, stems and bolls were collected from local cultivars in 2000 and from Sahel and Sayokra cultivars in 2003.
To race determination, bacteria were grown on NA for 24 hr, suspended in deionised water and adjust to 1×10^6 colony forming units (CFU)mL^{-1}. The abaxial side of the differential cultivars leaves were inoculated. There were two replicates per treatment. One using a strile syringe to inject the leaves, and the other spraying with pressure. They were then monitored for symptoms during 2 weeks. In incompatible interactions, characterized by hypersensitive reaction necrosis appeared while in compatible interactions, water-soaked lesions were evident. Following the system of Verma and Singh (1974) observations were compared with 32 bacterial blight races. Using NTSYS software, strains were devided into 4 groups. Of the 37 strains that were randomly selected and tested, 16% were race 1, 8% race 2, 57% race 6 and 19% race 18. Race 6 was prevalent, representing 57% of the whole strains. In the first isolation in 2000, race 18 was not found on any local cultivars, but was detected on Sahel and Sayokra in 2003. It suggests that race 18 can be a new form of former races which has a wider host range.

EXPRESSION OF RUST RESISTANCE COMPONENTS TO *UROMYCES VICIAE-FABAE* IN *VICIA FABA* COLLECTION

Mohsen KHALIFA[1], Mohammed BÉCHIR ALLAGUI[2] & Mourad SAADOUN[3]
[1]Plant Protection laboratory, National Institute for Agronomic Research of Tunisia (INRAT), Hedi Karray street, Ariana 2080, Tunisia

Faba bean rust caused by *Uromyces viciae-fabae* is a major fungal disease of cultivated faba bean in Tunisia. Eleven accessions were used in this study. These accessions were evaluated during two cropping seasons in glasshouse under rust artificial inoculation and in field under heavy natural rust infection. Assessment criteria to the faba bean rust reaction were latent period (LP), uredinia number (UN), telia number (TN), infection type (IT), hypersensitive reaction (HR), spore production (SP) and area under the disease progress curve (AUDPC). Resistance was identified in three accessions V-1272, VF-176 and ILB 3025 which expressed a long LP, a reduced UN, low IT and low value of AUDPC. High susceptibility to rust was also found in Fiord, ILB 4357, ILB 1820 and 987/255/95 characterized by short LP, susceptible IT and high value of AUDPC. Principal component analysis showed that the variables were grouped in two principal components which explained 80.97% of the total variance. The projection of the point-cloud representing the populations on the plan formed by the principal components 'Fact 1' (74.18%), in abscissa, and 'Fact 2' (6.80%) in ordinate, permitted to distribute the accessions in several groups. Three faba bean accessions (V-1272, VF-176 and ILB 3025) showed the lowest values of the major components used. In consequence, these accessions may be used as sources of durable resistance to faba bean rust. AUDPC, LP and SP could be used as powerful criteria for selecting accessions with higher level of resistance.

HOST-PATHOGEN-BIOCONTROL AGENT INTERACTION AS AFFECTED BY SEQUENTIAL APPLICATION OF Na_2CO_3 AND $CaCl_2$

G.M. MOLINU, G. ARRAS, A. DORE. , T. VENDITTI, A. PETRETTO & G. D'HALLEWIN

Consiglio Nazionale delle Ricerche, Istituto di Scienze delle Produzioni Alimentari
via dei Mille 48, IT-07100 Sassari, Italy
E-mail of presenting author: mariagiovanna.molinu@ispa.cnr.it

Following the endured use of a restricted number of fungicides, the citrus industry is facing the outbreak of *Penicillium digitatum* (citrus green mould) and *Penicillium italicum* (citrus blue mould) resistant biotypes in the packing houses. Among the alternatives to synthetic postharvest fungicides, encouraging, but insufficient results have been reported for biocontrol agents. The efficacy was significantly improved when these agents were combined with GRAS compounds or physical means, but the mechanisms involved are still not clear. The understanding of the host-pathogen-biocontrol agent interactions as affected by the combined treatments is fundamental to further improve the treatment efficacy. With this in mind we studied the interaction among oranges (*Citrus sinensis cv* Washington navel) inoculated with *P. digitatum* and treated in sequence with a 3% solution of Na_2CO_3 (SC) and one containing 1% of $CaCl_2$ with or without the antagonistic yeast (*Pichia guilliermondii* isolate 5A). Along with the direct effect of SC on *P. digitatum* pathogenesis, the induction of phytoalexins by SC and by the yeast played a key role in controlling pathogen growth. Also the application of $CaCl_2$ following SC resulted very important indeed, the application of this salt improved significantly the growth rate of the antagonist and improved the mechanical barrier to pathogen penetration. The results will be discussed trying to shed light on the different interactions and make conclusions strengthened by some scanning electron microscopy observations.

PLANT TISSUE COLONIZATION BY THE FUNGUS RACE 1.2 OF *FUSARIUM OXYSPORUM* F.SP. *MELONIS* IN RESISTANT MELON GENOTYPES

Hela CHIKH-ROUHOU, José MARIA ÁLVAREZ & Rafael GONZÁLEZ-TORRES
Centro de Investigación y Tecnología Agroalimentaria de Aragón. Unidad de Tecnología en Producción vegetal. Unidad de Sanidad Vegetal
P.o. Box 727, ES-50080 Zaragoza. Spain

Fusarium Wilt of muskmelon caused by *Fusarium oxysporum* f.sp. *melonis* (*Fom*) is one of the most threatening diseases of melon crops in Spain and elsewhere. Since 1976 four physiological races of *Fom* have been described, namely races 0, 1, 2 and 1.2. Race 1.2 was further divided into two patotypes: 1.2Y, which causes leaf yellowing before wilting, and 1.2W nonyellowing strains where wilting occurs without prior yellowing symptoms.

Once introduced into a field, *Fom* can persist even after rotation to non host crops because the fungus survives in the soil as chlamydospores, and is able to colonize crop residues and roots of most crops. Because of the persistence of the pathogen in the soil, Fusarium wilt of melon can only be properly controlled by the use of resistant cultivars or hybrids. No genes have been identified in melons that confer high levels of resistance to either 1.2Y or 1.2W. However, it have been found resistance to race 1.2 in Piboule genotypes, this potential resistance is under polygenic recessive control. This type of resistance is difficult to introduce into commercial cultivars, and only a few ones have been developed incorporating resistance to *Fom*, most of them are only used as rootstocks.

Nowadays many sources of resistance to *Fom* races 0, 1 and 2 are known, but it does not occur the same for race 1.2. For this reason during 2003-2006, it was done a screening of 110 melon accessions in CITA (Zaragoza) and a relatively high resistance to race 1.2 was found in four accessions, 3 of them are from Japan and the fourth one is a Portuguese accession (Chikh-Rouhou *et al*., 2007).

The objective of this research was to determine whether the resistant accession plants were able to stop fungal invasion of their root or stem.

The plant material used was the susceptible accession 'Piel de Sapo' and the resistant ones 'Shiroubi Okayoma', 'C-211', 'K.N.M' and 'BG-5384'. The *Fom* isolates used to prepare the inoculum were 37mls and Fom0125 belonging to 1.2W y 1.2Y respectively.

To test which plant regions were invaded by *Fom* race 1.2, and to examine the relationship between resistance and presence of the pathogen in the plant tissue, seedlings of the susceptible genotype and the resistant ones were inoculated with the two pathotypes of *Fom* race 1.2, and after 20 days, three plants of each accession were collected, surface-sterilized in sodium hypochlorite for 2min, followed by rinsing during 2min in sterile water and then dried on sterile filter paper.

Three slices were cut from the lower, middle and upper parts of the hypocotyl respectively from each plant of the above genotypes, and then were plated on Petri dishes containing sterile V8 medium for 7 days at 25ºC, to determine which parts of the plants were colonized. The diameter of the fungus mycelium developed from each section of the hypocotyls was measured. These data were ANOVA analyzed and the means were separated using the LSD test.

The results showed, that seven days after plating on sterile V8 medium, a massive growth of *Fom* developed from all segments of the susceptible genotype ('Piel de

Sapo'). All the slices of the 'Piel de Sapo' hypocotyls were colonized by the fungus, and the mycelium that emerged was dense and intensely colored. Most of the hypocotyl slices of the resistant genotypes were also colonized, but the diameter and the density of the mycelial mass was significantly smaller than those of the mycelium that emerged from the susceptible one. It appears that the extent of the colonization by the fungus in the upper segment was somewhat smaller than that in the lower and middle hypocotyl.

In our study, the susceptible genotype and the resistant ones differed in colonization of the hypocotyl by the pathogen *Fom* race 1.2, being the diameter of the mycelium produced from the hypocotyl slices of 'Piel de Sapo' significantly greater than that produced from the resistant ones. Indeed, we appreciated a decrease of the mycelium diameter in the upper part of the hypocotyl, for the resistant genotypes, which can indicate a restriction of the fungus to the lower parts of the plant. So it appears that resistant plants were able to restrict, to some extension, on their hypocotyls, the fungal invasion.

HOST INVOLVEMENT IN THE IMPROVED DECAY CONTROL FOLLOWING $NaHCO_3$ CO-APPLICATION WITH IMAZALIL

A. DORE, G.M. MOLINU, T. VENDITTI, A. PETRETTO & G. D'HALLEWIN
Consiglio Nazionale delle Ricerche, ISPA - Unità di Sassari
Traversa La Crucca 3, IT-07040 Sassari, Italy
E-mail of presenting author: antonio.dore@ispa.cnr.it

The growing concerns on health and environmental issues regarding the use of postharvest fungicides have implemented researches on alternatives. Until now, economically valid results with alternative approaches (physical means, biocontrol agents, GRAS compounds, induced natural resistance) have been attained only when two or more treatments were combined. Within this framework we may consider also approaches aimed at lowering the concentration of the synthetic fungicide employed. With this in mind, we investigated the possibility to reduce imazalil (IMZ) concentration by performing 2 min co-application dip treatments with $NaHCO_3$ (SBC). Experiments were carried out using lemon fruit (*Citrus limon* (L.) Burm .f. *cv* Verna), either inoculated or not with *Penicillium digitatum* before the co-application of 3% SBC with 0, 50, 100 and 250 µg/L IMZ. Lemons treated with 0, 50, 100, 250 or 1000 µg/L IMZ were used as controls. Inoculation was performed by injecting into each wound 20 µL of a suspension containing 10^5 conidia mL^{-1}. Then, fruit was kept 24 h at 25 °C and high relative humidity (RH) before treatment and subsequently storage at 20 °C and 90% RH for 5 days when decay was monitored. Wound-un-inoculated lemons were used to study host-fungicide interactions as affected by the co-application with SBC. The occurrence of natural decay was monitored after a 30 day storage at 8 °C and after 6 days of simulated marketing conditions at 20 °C and 75% RH. Comparable efficacy of 1000 µg/L IMZ, in controlling natural decay development during storage and SMC, was obtained with ten time less IMZ when co-applied with SBC. The same trend was observed with artificially inoculated fruit. Studies performed to shed light on this synergistic interaction evidenced, significant increases of IMZ residue along with phytoalexin induction and structural changes within the potential infection sites.

MOLECULAR DETECTION OF *CHALARA FRAXINEA* IN ASH TREE (*FRAXINUS EXCELSIOR* L.) USING REAL TIME PCR

Anne CHANDELIER, Frédéric ANDRÉ & Frédéric LAURENT
Walloon Agricultural Research Centre, Department Biocontrol and Plant Genetic Resources.
Rue de Liroux 4, BE-5030 Gembloux, Belgium
E-mail: chandelier@cra.wallonie.be

A few years ago, a new fungus was reported as the cause of *Fraxinus excelsior* decline in Europe. It was described as a new species *Chalara fraxinea* T. Kowalski sp. nov. A real time PCR assay was developed for the detection of this pathogen in common ash tree. PCR primers and Taqman probe, based on the internal transcribed spacer region of the multi-copy gene rDNA, were tested for specificity and sensitivity. The primers amplified a 81 bp fragment for *Chalara fraxinea* but did not amplify DNA from other *Chalara* species, nor from other fungi isolated from ash tree (pathogenic or saprophytic). The limit of detection reached 5 pg of genomic DNA per PCR. Moreover, naturally-infected samples were correctly detected. A procedure for the DNA extraction from wood material using an electric drill yielded appropriate DNA quality for real time PCR. This molecular method could be useful for routine analysis of this emergent pathogen and for epidemiological studies.

BIODIVERSITY OF *FUSARIUM* ISOLATED FROM MAIZE KERNELS IN IRAN

Vahid RAHJOO[1], Javad ZAD[2], Mohammad JAVAN-NIKKHAH[2], Sayyed Mahmood OKHOVVAT[2], Mohammad REZA BIHAMTA[3], Amir Mirzadi GOHARI[2], Jafar RAZZAGHIAN[4] & Sonja SLETNER KLEMSDAL[4]

[1] Seed and Plant Improvement Institute (SPII), Iran

[2] Plant Protection Dept., Faculty of Agriculture, University of Tehran, Iran

[3]Biotechnology Dept., Faculty of Agriculture, University of Tehran, Iran

[4] Plant Health and Plant Protection Division, Bioforsk

Norwegian Institute for Agricultural and Environmental Research, Norway

A total of 191 *Fusarium* isolates were recovered from maize ear samples collected from 11 different geographic regions in Iran during 2005. *Fusarium* isolates were identified based on morphological characters and using species-specific PCR assays. DNA sequence based identifications were also carried out using DNA sequences of the translation elongation factor 1-α (*tef-1*) gene region for some unknown isolates. Based on the morphological and molecular identifications, *Fusarium verticillioides* was the most prevalent species representing a frequency of 69.6% of the isolates. *F. proliferatum* was the second most common *Fusarium* species with a frequency of 26.7%. PCR analysis using the species-specific primers VER1/2 and PRO1/2 confirmed 95% and 89.4% of the morphological identifications of *F. verticillioides* and *F. proliferatum* respectively. Based on DNA sequence analysis, five isolates were identified as either *F. oxysporum*, *Fusarium* cf. *bullatum* or *F. thapsinum*. Two isolates based on a Blast search of GenBank appear to represent a new unnamed *Fusarium* species. In addition this is the first report of *Fusarium* cf. *bullatum* and *F. thapsinum* on maize kernels in Iran.

TREATMENTS WITH ACETIC ACID FOLLOWED BY CURING REDUCE POSTHARVEST DECAY ON *CITRUS* FRUIT

T. VENDITTI, G. D'HALLEWIN, A. DORE, M.G. MOLINU & A. PETRETTO
Consiglio Nazionale delle Ricerche, ISPA - Unità di Sassari
Traversa La Crucca 3, IR-07040 Sassari, Italy
E-mail of presenting author: tullio.venditti@ispa.cnr.it

Citrus fruit are susceptible to many postharvest diseases and disorders, but *Penicillium digitatum* and *Penicillium italicum* are the most common and serious pathogens during storage and marketing.
The continuous employ in packing houses of synthetic fungicides such as imazalil (IMZ) or thiabendazole for the control of these pathogens is promoting the selection of resistant biotypes. These considerations together with an increased attention for human health and the environment have multiplied the studies on new ecological technologies.
In recent years, researcher's studies focused on alternatives to the chemical control of postharvest decay, such as the utilization of GRAS compounds as well as physical methods. In the present study the sequential use of acetic acid (AAC) followed by curing is reported.
The lemon (*Citrus limon*) variety "Verna" and the orange (*Citrus sinensis*) variety "Jaffa", naturally inoculated, were treated with vapours of AAC performed at three different concentrations (15, 25 and 50 µL/L) for 15 minutes, after an incubation period of 24 hours at 27 °C and 90% relative humidity (RH).
After treatments, fruits were cured at 36 °C for 36 hours with 90% RH and subsequently stored at 8 °C and 90% of RH for eight weeks. Both *Citrus* varieties were also treated with IMZ at a concentration of 200 µL/L. At the end of the experiment decay and weight loss were evaluated.
After 8 weeks of storage, in the lemon variety, the lowest percentage of infected wounds was 1.5% for both: the fruit treated with IMZ or with AAC at 25 µL/L. The control and the treatment performed at 15 µL/L showed similar results with 16.6 and 13.6% of rotted fruit, respectively.
Different results were obtained with the orange variety; in this case the synthetic fungicide was the most effective with 18.0% of decay at the end of the storage period. AAC treatments were not as successful as on lemons, the best results were achieved even in this case, with AAC performed at 25 µL/L, but with 39.9% of decay. In both species the weight loss was not affected by the treatments.
These results show that a good control of postharvest decay could be achieved, on lemon fruit, by combining the effect of a GRAS compound such as AAC with curing. Conversely, the results obtained by applying this control method to the orange variety were not so promising. Further researches are needed to shed light on the different behaviour between the two species.

TEMPERATURE-DEPENDENT GROWTH OF *BOTRYTIS CINEREA* ISOLATES FROM POTTED PLANTS

Juan A. MARTÍNEZ[1], María J. GÓMEZ-BELLOT[1] & Sebastián BAÑÓN[1,2]
[1] Departamento de Producción Vegetal, Universidad Politécnica de Cartagena, Spain
[2] Horticultura Sostenible en Zonas Áridas, Unidad Asociada al CSIC-CEBAS, Murcia, Spain

Botrytis cinerea is a common aggressive saprophyte fungus which also invades injured plant tissues causing Botrytis blight (Grey mould) in most ornamental plants, including potted flowering plants. Several *B. cinerea* isolates from potted plants (*Pelargonium* x *hortorum*, *Lantana camara*, *Lonicera japonica*, *Hydrangea macrophylla*, and *Cyclamen persicum*) affected by Botrytis blight in the south of Spain were studied and identified by PCR. The isolates showed phenotypic differences between them, as previously reported by the authors. In this work we demonstrate that these isolates show different temperature-dependent growth phenomena, expressed as mycelial growth rates, conidiation (measured as the number of conidia per colony and time of appearance), mass of both aerial and submerged mycelia, and sclerotia production. Growth rates were assessed from differences in colony diameter, area, perimeter and mass of both aerial and submerged mycelium growing in potato dextrose agar culture medium (PDA). Three temperatures were used to measure these variables: 6, 16, and 26ºC and to establish the relationship by modelling the effects of temperature on the growth variables. *B. cinerea* showed a high degree of phenotypical variability and differences in its growth kinetics depending on the temperature and isolate in question. Conidiation did not occur at 6ºC and was highest at 26ºC, except in the *H. macrophylla* isolate, where this process was also insignificant at 26ºC. The growth rate of the isolates from *L. camara* was the highest only at 26ºC. At this temperature the growth rate of this isolate increased 5-fold faster than at 16ºC after 3 days growing on PDA, compared with the 2-fold increase observed in the other isolates. Growth rates were determined with time in all the isolates and the growth kinetics could be fitted to a typical equation on solid culture medium. After 14 days the overall appearance of isolates from *P.* x *hortorum* and *C. persicum* was quite different (depending on the temperature), while the other three isolates had a similar appearance (regardless of temperature). In general, the higher the temperature, the thicker the aerial mycelium. A temperature of 26ºC accelerated sclerotia production, except in isolates from *P.* x *hortorum* and *C. persicum* where this process slowed down as the temperature increased. Such phenotypical variability and growth rates differences may result in a differential response in plant-pathogen interactions when different isolates attack the hosts at different temperatures, so different plant protection strategies could be considered when *B. cinerea* attacks these potted plants.

SELECTION OF BACTERIAL ANTAGONISTS FOR THE BIOLOGICAL CONTROL OF *BOTRYTIS CINEREA* IN APPLE (*MALUS DOMESTICA*) AND IN COMPARISON WITH APPLICATION OF THIABENDAZOLE

S. PEIGHAMY-ASHNAEI, A. SHARIFI-TEHRANI, M. AHMADZADEH & K. BEHBOUDI

Department of Plant Protection, Faculty of Horticultural Science & Plant Protection
University of Tehran, Karaj, Iran

Fungal pathogens are the main cause of postharvest losses of apples. Grey mould, caused by *Botrytis cinerea* Pers.:Fr., is a severe disease worldwide on pome fruits. The aim of this paper was to determine if the attacks of grey rot on apple could be reduced by the biological agents *Pseudomonas fluorescens* and *Bacillus subtilis*, isolated from rhizosphere of different plants such as onion, cucumber and pistachio. In this study fifteen strains of identified *P. fluorescens* and *B. subtilis* were investigated. Biocontrol efficacy of strains were studied in dual culture assay, *In vitro*. The isolates were grown in nutrient broth (pH=6.9), at an initial inoculation of 1×10^5 CFU ml^{-1}. Then, Cells from over night cultures were inoculated by dipping a stainless steel rod with a probe tip 1 mm wide and 2 mm long into a suspension of 2×10^8 CFU ml^{-1}. A suspension of 10^6 spores ml^{-1} was then applied to each dry wound. Antagonistic efficacy of strains against *B. cinerea* on apple was studied *in vivo* condition, after ten and twenty days. Also, apple fruits were inoculated with Thiabendazole at 500 and 1,500 mg/liter. *P. fluorescens* P-35 and *B. subtilis* B-16 showed the most inhibitory zone in dual culture assay against *B. cinerea*, *In vitro*. After ten days, *B. subtilis* B-3 and B-16 showed the considerable results against *B. cinerea* on apple fruits and could reduce the grey mould from 100% to less than 36%. After twenty days, B-16 *(B. subtilis*) decreased the disease from 100% to less than 53%. Also, application of thiabendazol at 1,500 mg/litre was more effective and could reduce the disease from 100% to 25% and 60%, after 10 and 20 days respectively. So, results indicated that there is no significant difference among the treatments (thiabendazol and bacterial strains). So, the bacterial strains could not only control the disease but also be a reliable replacement instead of Thiabendazol.

SCREENING OF *PSEUDOMONAS* AND *BACILLUS* ISOLATES FOR POTENTIAL BIOCONTROL OF THE DAMPING-OFF OF BEAN (*PHASEOLUS COCCINEUS*)

S. PEIGHAMY-ASHNAEI, A. SHARIFI-TEHRANI, M. AHMADZADEH & K. BEHBOUDI

Department of Plant Protection, Faculty of Horticultural Science & Plant Protection
University of Tehran, Karaj, Iran

Damping- off caused by *Rhizoctonia solani* Kühn is one of the most widespread diseases of bean (*Phaseolus coccineus*) and cause serious yield losses under favourable environmental conditions. The objectives of the present paper, isolation of *Pseudomonas* and *Bacillus* isolates from rhizosphere of different plants such as pea, wheat, soybean and rape that could control *R. solani in vitro* and *in vivo* conditions. In this study fifteen isolates of identified *Pseudomonas fluorescens* and *Bacillus subtilis* were investigated for control of bean damping-off disease caused by *R. solani*. *In vitro*, biocontrol efficacy in dual culture and also final growth of isolates in NB were studied. The strains were grown in nutrient broth (pH=6.9), at an initial inoculation of 1×10^5 CFU ml^{-1}. Then, Cells from over night cultures used to inoculate soil at 1×10^9 CFU cm^{-3} soil. At the same time, fungal inoculum (infected millet seed with *R.. solani*) was added to soil at the rate of 2 g kg^{-1} soil. Antagonistic efficacy of strains against *R. solani* on bean was studied *in vivo* condition, after 14 days. *In vitro*, *P. fluorescens* P-6 and *B. subtilis* B-3 showed the most inhibitory zone in dual culture assay against *R. solani*. Also, the growth of P-6 (4.5×10^8 CFU) was significantly higher than in the other treatments. In greenhouse condition, *P. fluorescens* P-5 and P-6 showed the considerable results against *R. solani* and could reduce the damping-off from 100% to less than 30%. P-5, P-6 *(P. fluorescens*) and B-16 (*B. subtilis*) strains had the highest effect on fresh weight of bean. All of the isolates effectively controlled damping-off on bean. Root exudates are believed to determined which microorganisms colonized roots in the rhizosphere. The ability to colonize bean roots is variable between rhizobacteria, being this characteristic a reflection for their ability to compete for ecological niches in the rhizosphere.

EFFICACITY OF FUNGICIDES AGAINST *PHYTOPHTHORA CACTORUM* ON VIOLA

Liesbet BLINDEMAN[1] & Kurt HEUNGENS[2]
[1] PCS - Research Centre for Ornamental Plants
Schaessestraat 18, BE-9070 Destelbergen, Belgium
[2] Institute for Agricultural and Fisheries Research (ILVO) – Plant Unit - Crop Protection
Burgemeester van Gansberghelaan 96, BE-9820 Merelbeke, Belgium

Phytophthora cactorum caused significant losses to pansies during the heat wave at the end of summer 2006. Infected plants showed foliage that appeared meagre and chlorotic, with wilting occurring even when soil moisture is adequate. When uprooted, symptomatic plants possess a surprisingly healthy appearing and well-developed root system, but stem and root tissue at the ground line will be discoloured (purple to dark brown) and mushy. Older leaves turn yellow and when the stem base is attacked, the plant dies. *Phytophthora cactorum* was identified from stem and root tissue with both morphological and molecular techniques.
To evaluate the efficacy of different fungicides, healthy plants were infected with zoospores of a *Phytophthora cactorum* isolate collected from commercial plants. Eleven fungicides were evaluated and compared to an untreated control. Two fungicides were applied via root drenching 7 days before inoculation with zoospores of *P. cactorum*. The other fungicides were applied by spraying 24h after inoculation with *P. cactorum*.
Preventive drenching with the combined formulation of fenomidone + fosethyl offered the best protection against *P. cactorum,* while drenching with dimethomorf also resulted in an obvious reduction of infected plants. In foliar spraying, only a combined formulation of Mancozeb + metalaxyl-M gave sufficient protection.
In conclusion, preventive drenching appears to be the best solution to prevent infection with *P. cactorum* during heatwaves.

INVOLVEMENT OF BIOSURFACTANTS AND PHENAZINES PRODUCED BY *PSEUDOMONAS* CMR5C, CMR12C AND CMR12A-MUTANTS DURING BIOLOGICAL CONTROL OF RHIZOCTONIA ROOT ROT IN BEAN

Hoang Gia KHUONG HUA, Joke PANNECOUCQUE, Katrien DE MAEYER, Jolien D'AES & Monica HÖFTE
Laboratory of Phytopathology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, BE-9000 Gent, Belgium
E-mail: Monica.Hofte@UGent.be

Rhizoctonia root rot caused by *Rhizoctonia solani* is a major constraint in tropical bean production. As an environmental friendly alternative to chemical control, biological control of *R. solani* AG 2-2 WB and AG 4 HGI using antagonistic *Pseudomonas* bacteria was studied. The antagonistic potential of two *Pseudomonas* strains, CMR12a and CMR5c, isolated from cocoyam plants in Cameroon, was investigated. Both strains were selected based on their production of phenazine antibiotics and biosurfactants; two antifungal compounds which have earlier been reported to play an important role in the biocontrol potential of these strains.
Importance of phenazines and biosurfactants in biocontrol of *R. solani* in bean was evaluated during plant experiments with one phenazine deletion mutant (CMR12a-Δphz), two biosurfactant mutants (CMR12a-7.97 and CMR12a-CLP1), and one regulatory mutant knocked out in many secondary metabolites (CMR12a-GacA). Compared to the wild type strain, biosurfactant and phenazine mutants were reduced in their biocontrol capacity against *R. solani* AG 2-2 WB and AG 4 HGI. However, symptom development was still less severe compared to the diseased control. When the soil was treated with the phenazine and biosurfactant deficient CMR12a-GacA strain, biocontrol activity against *R. solani* AG2-2 and AG 4 was completely lost and seven days after inoculation with *R. solani*, the same level of elongate, sunken, red-brown lesions on the hypocotyls and lower stems was observed. These results indicate that both phenazines and biosurfactants are important compounds for the biological control of *R. solani* in bean.

UTILISATION OF INORGANIC SALTS IN FUNGAL CROP DISEASE MANAGEMENT IN THE UK

Thomas DELIOPOULOS[1], Peter S. KETTLEWELL & Martin C. HARE
[1] Crop and Environment Research Centre, Harper Adams University College
Newport, Shropshire, GB-TF10 8NB, United Kingdom

The aim of the study described in this communication was to reduce the environmental and food chain burden of conventional crop fungicides by exploiting results of preliminary research which has shown that suppression of fungal diseases may be possible from spray applications of inorganic salts. A global survey of the available scientific and technical literature on the use of inorganic salts in fungal disease control was therefore undertaken in order to fully explore the possibility of partially substituting conventional fungicides used in UK crop protection by inorganic salts. The survey has shown potential for the suppression of 49 fungal diseases of 35 plant species by 34 inorganic salts (bicarbonates, phosphates, silicates, phosphites and chlorides). However, only a proportion of these substances have been introduced into the global crop protection market. In the USA, 25 commercial inorganic salt-containing products have been registered by the Environmental Protection Agency (EPA) as "biopesticides" with approved uses for fungal disease control (14 phosphites, seven bicarbonates, four phosphates), while four inorganic salts have been approved by the National Organic Program (NOP) of the US Department of Agriculture (USDA) for use in organic farming, specifically for fungal disease control. By contrast, in the UK, only one salt, that is potassium bicarbonate, has been granted a Commodity Substance Approval by the Pesticide Safety Directorate (PSD: UK Department for Environment, Food and Rural Affairs, DEFRA) and can be used on all crops (outdoor and protected) as a horticultural fungicide. Research on the topic of fungal disease management by inorganic salts is ongoing in a joint project between Harper Adams University College and the Agricultural Development and Advisory Service (ADAS), UK. This 2-year PSD-funded project is entitled "Novel strategies for optimising powdery mildew management on outdoor cucurbits and protected herbs" and commenced in January 2009. In addition to efficacy and validation experiments, an important component of the project will be the establishment of links with key industry players so as to identify pathways for approval of inorganic salts. It is anticipated that this strategy would also facilitate future uptake of these products by UK growers.

EVALUATION OF TWO PROTEIN EXTRATION PROTOCOLS FOR *PICHIA ANOMALA* PROTEOME ANALYSIS

Mohammed BAJJI[1], Pierre DELAPLACE[2], Sergio MAURO[3], Philippe LEPOIVRE[1] & M. Haïssam JIJAKLI[1]

[1] Plant Pathology Unit, Gembloux Agricultural University, Gembloux, Belgium
[2] Plant Biology Unit, Gembloux Agricultural University, Gembloux, Belgium
[3] Biotechnology Department, Walloon Agricultural Research Centre, Gembloux, Belgium

Pichia anomala (strain Kh6) was isolated from the surface of apple fruits and selected for its high and reliable biocontrol activity against *Botrytis cinerea* and *Penicillium expansum*. Its main modes of action have until now been studied using essentially microbiological and molecular approaches. The study continues now using the proteomic approach and considering the *in situ* P. *anomala*/*B. cinerea*/apple interaction. Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) is one of the most powerful tools used for proteomic analysis. It combines two sequential separation steps, the first dimension via isoelectric focusing (IEF) and the second one by SDS PAGE. Although recent advances in 2-D PAGE, the extraction of the whole proteome and the removal of interfering contaminants still limit its application. Sample preparation constitutes indeed a critical influential step for IEF which in turn affects 2-D gel quality. The objective of the present work was thus to develop an effective protein extraction protocol designed for 2-D PAGE analysis of the proteome of *P. anomala* strain Kh6. As a starting point, two contrasting protein extraction protocols were chosen to be evaluated in terms of protein yield and one-dimensional (1-D) SDS PAGE and 2-D PAGE gel patterns. The first protocol uses a urea/thiourea-based lysis buffer whereas the second protocol utilizes a hot SDS-based lysis buffer with an additional precipitation step. The comparison model used consisted of apples treated with strain Kh6 alone (K) and apples first treated with Kh6 and then inoculated with *B. cinerea* conidia (KB). Growth kinetics of strain Kh6 on wounded apples was determined and found to be not affected by the presence of *B. cinerea* conidia. Proteins were extracted from yeast pellets collected at both the exponential and stationary phases of strain Kh6 growth on apples. The evaluation of both extraction protocols indicates that more proteins were extracted with the SDS protocol and, according to 1-D assays, higher molecular weight proteins were obtained with the 'urea/thiourea' protocol and, regardless of the protocol used, more bands were obtained during the exponential phase. 2-D assays are currently underway and the corresponding results will be presented.

POTENTIAL OF BTH TO INDUCE RESISTANCE AGAINST *BOTRYTIS CINEREA* IN TOMATO, BEAN AND CUCUMBER

Zabihollah AZAMI SARDOOEI & Monica HÖFTE
Laboratory of Phytopathology, Faculty of Bioscience Engineering,
Ghent University, Coupure Links 653, BE-9000 Gent, Belgium

The production of greenhouse-cultivated tomato, bean and cucumber suffers great economic losses due to grey mould, caused by *Botrytis cinerea.* Benzathiadiazole (BTH or BION) is a chemical analogue of SA (salicylic acid) and can induce resistance in a variety of plants. In this study, we investigate whether foliar applications of BTH can induce resistance to *B. cinerea* in tomato, bean and cucumber using different concentrations. Moreover, the phytotoxic effects of BTH were examined.

Tomato, bean and cucumber plants were sprayed with BTH at 10, 100 or 250 mg a.i. L^{-1} four days before inoculation with *B. cinerea*. The inoculation suspension contained 10^5 spores mL^{-1} and plants were inoculated by putting 10 droplets of 10 µl of conidial suspension on each bean and cucumber leaf or 10 droplets of 5 µl of conidial suspension on each tomato leaf.

In order to examine the phytotoxic effect of BTH on foliar parts of the plants, we included higher concentrations (500 and 1000 mg a.i. L^{-1}). We measured the length of the plants from the soil level to the apical bud. Furthermore, the influence of phytotoxicity on the phenotype, flowering and productivity was studied.Leaf treatments with a low concentration of BTH (10 mg a.i. L^{-1}) resulted in a strong protection of tomato leaves against *B. cinerea.* This low concentration, however, was not effective on bean and cucumber. A higher concentration of 250 mg a.i. L^{-1} BTH was necessary to control *B. cinerea* on bean and cucumber.

On tomato, concentrations of 10 and 100 mg a.i. L^{-1}did not have any phytotoxicity effect or growth blockage; whereas with the concentrations of 250, 500 and 1000 mg a. i. L^{-1}, chlorosis symptoms were detected. On cucumber, concentrations of 100 and 250 mg a.i. L^{-1} showed chlorosis, while the higher concentrations of 500 and 1000 mg a.i. L^{-1} had a severe phytotoxic effect and blocked the plant growth. On bean, phytotoxicity and growth prevention were observed when concentrations higher than 10 mg a.i./l were sprayed (i.e. 100 and 250 mg a.i. L^{-1}). Furthermore, flowering was severely reduced and fruits and leaves were deformed. Higher concentrations (500 and 1000 mg a.i. L^{-1}) caused severe necrosis on the leaves, deformation of the apical bud and sterility of the flowers.

Regarding the significant effect of 10 mg a.i. L^{-1} of BTH on induced resistance in tomato and the low level of phytotoxicity among the other concentrations, 10 mg a.i. L^{-1} can be used to control *B. cinerea* on tomato. BTH, however is not suitable to control *B. cinerea* on cucumber and bean, since low concentrations (such as 10 mg a.i. L^{-1}) are not effective and higher, effective concentrations are phytotoxic and cause serious damage on the leaves.

INFECTION OF RASPBERRY LEAVES BY *BOTRYTIS CINEREA* IN RELATION TO LEAF AND CANE AGE

Xiangming XU[1], Erika WEDGWOOD[2], Angela BERRIE[1] & Tim O'NEILL[2]
[1] East Malling Research, New Road, East Malling, West Malling, ME19 6BJ, UK
[2] ADAS Boxworth, Battlegate Road, Boxworth, Cambs, CB23 4NN, UK

Botrytis cinerea Pers, ex Fr. can cause diseases on both cane and fruit on raspberry. Previous observations in field crops led to formulation of the hypothesis that infection of canes arose from invasion by mycelium from the petioles of infected leaves and that only mature and old leaves are susceptible. Inoculation studies in protected crops were conducted to investigate infection of raspberry leaves and canes by *B. cinerea* with a particular emphasis on determining the timing of cane infection in relation to leaf infection. The results obtained in protected crops indicated that cane age rather than the leaf age *per se* influences the susceptibility of leaves; leaves on young canes were less susceptible to botrytis infection than those on old canes. On older canes, leaves of all ages, ranging from young expanding to old senescent, were equally susceptible. The overall incidence of cane infection from inoculation of leaves was very low; most of cane infection was likely to have resulted from the direct infection of canes and not through invasion via the petioles of infected leaves.

THE USE OF ALIETTE WG FOR CONTROLLING DEAD BUDS ON PEAR CONFERENCE WITH ADDITIONAL EFFECT ON THE RETURN BLOOM AND ON FLOWER BUD QUALITY

L. DE MAEYER, C. SCHOEVAERTS, K. D'HAEMER & D. PEETERS
Bayer CropScience SA-NV, BE-1831 DIEGEM, Belgium
A. GOMAND, H. GOOSSENS, J. VERCAMMEN & T. DECKERS
Pcfruit vzw, Fruittuinweg 1, BE-3800 St-Truiden, Belgium

Aliette WG is a systemic fungicide with active ingredient fosetyl aluminium for oomycete control in various crops and with registered bactericidal properties in pears on *Pseudomonas syringae* and *Erwinia amylovora*. Applied after blossom at 10 days intervals, at 3 x 2 - 3,75 kg/ha, Aliette WG reduces significantly the symptoms of dead flower buds in the next season. This positive effect of Aliette WG on flower buds is generally more evident in the lower located parts of an orchard where overall growth conditions are less favourable with higher risks of humidity and frost damage. The period of application of Aliette WG in May-June coincides with the process of intensive cell division when flower buds for the next season are initiated and formed on the spurs on the 2-year-old wood. Aliette WG applications carried out on Conference pears in the period of May-June 2007 clearly resulted in less dead flower buds and in more flower clusters during blossom of 2008. Also the quality of the individual flower buds was improved. Flower clusters in the Aliette WG plots had more flowers per cluster and were surrounded by more rosette leaves than those in the untreated. Consequently this has lead to better fruit set with more remaining fruitlets per flower cluster after June drop

WHITE MOULD OF COMMON BEAN INCITED BY *SCLEROTINIA SCLEROTIORUM* LIB. DE BARY IN EGYPT

Mostafa M.A. AMER, Islam ABOU-EL-SEOUD, Mohamed R. RASMY & Manar M. KHATER

Faculty of Agriculture, Saba-Basha, Alexandria University
P.O. Box. EG-21531-Bolkely, Alexandria, Egypt

White mould, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is a destructive yield-limiting disease of common bean (*Phaseolus vulgaris* L.) in Egypt. Forty eight isolate of *S. sclerotiorum* were isolated from diseased bean tissues taken from six geographical regions (Al-Behara and Alexandria governorates) during winter season in 2008. The pathogenicity studies showed that the tested bean cultivars (Bronco, Contender and Nebraska) varied in disease incidence. Contender cultivar was more resistant than other cultivars. Histology investigation of seedlings bean hypocotyls inoculated with *S. sclerotiorum* after 24, 48, 72 and 96 hours after inoculation indicated that penetration of bean seedlings occurred, during the first 48 hours after inoculation, through the epidermis and the outer layer of the cortex. 72 hours after inoculation, damage extended deeper into the cortical cells. Infection took place inter-and interacellularly after 96 hours more damage occurred. In addition, the invasion of the fungal hyphae through the cortical cells occurred both inter-, and intracellularly. Moreover, the observed of electron microscope both transmission and scanning investigations concluded that penetrating hyphae progressed through bean seedlings tissues leading to complete destruction of epidermis, fully colonization and death of cortical cells, partial invasion of vascular tissues. However, presence of the fungal structures in pith cells was observed.

THE EFFECT OF MICROALGAE ON THE CAUSAL AGENT OF GRAPE DOWNY MILDEW (*PLASMOPARA VITICOLA*)

L. NÉMETH L. & V. ÖRDÖG[1]
Department of Plant Protection and [1]Department of Plant Physiology and Plant Biotechnology,
Faculty of Agricultural and Food Sciences, University of West Hungary,
HU-9200 Mosonmagyaróvár, Hungary

Downy mildew caused by *Plasmopara viticola* has been one of the most significant diseases of grape ever since its first appearance in 1880 in Hungary. Efficient control of the disease requires repeated use of various chemicals. Therefore it is desirable to find environmentally friendly means of control strategies.
Our studies aimed at the use of microalgae for this purpose. Microalgae strains, MACC-14, MACC-76 and MACC-302 from the Mosonmagyaróvár Algal Culture Collection (MACC) were selected to study their effect on the development of *Plasmopara viticola* and the disease severity.
Plasmopara viticola isolate 2000/3 was propagated on grapevine variety Blue Franc in the greenhouse.
Microalgae strains grown in liquid culture were centrifuged and the biomass pellet was freeze dried. Pellets were resuspended in distilled water then ultra-sonicated.
The abaxial surface of detached young leaves as well as leaf disks (13 mm in diameter) of grapevine variety Blue Franc were sprayed with cell-free suspensions of the microalgae preparation at the concentrations of 5 mg/ml and 10 mg/ml, respectively. After drying, treated leaves were inoculated with an aqueous suspension of 10^4 zoosporangia per millilitre then incubated in closed agar plates under laboratory conditions. Water-treated and inoculated leaves served as controls.
Seven days after the initiation of experiments, leaf infected areas were measured in comparison to untreated controls. Of the microalgae strains, MACC-76 proved to be the most effective one inhibiting downy mildew sporulation by 100% and 91.2% at the concentrations of 10 mg/ml and 5 mg/ml, respectively. However, it also expressed phytotoxicity at both concentrations. The same concentrations of MACC-14 or MACC-302 exhibited somewhat lower inhibitory effects, i.e. 91.7% and 73.1% or 89.6 % and 64.3%, respectively, with no phytotoxicity.
Strain MACC-14 at a concentration of 10 mg/ml was also applied to grapevine in a field experiment in Pannonhalma, in 2002. Plants were treated two times in July. Treatments with Dithane DG (mancoceb 75%) as a standard fungicide served as positive controls, and untreated plots were the negative controls. On the basis of infections on treated and untreated plants, efficiency quotient was calculated. The quotients obtained were 50% with algal treatment and 90% with the fungicide treatment. Consequently, two sprayings with algal preparation in field experiments are likely to be insufficient. An increase in the number of treatments, however, might improve the efficiency and thus reduce chemical pollution of the environment.

DETERMINATION OF DEOXYNIVALENOL IN CORN CROP AT ARDABIL PROVINCE IN IRAN AND REALATED FUSARIUM SPECIES IN 2007-2008

Z. ALIAKBARI[1], H. AMINIAN[1], M. MIRABOLFATHI[2] & R. KARAMI-OSBOO[2]
[1] Dep. of plant Protection College of agriculture Abo-reihan Campus Tehran University
[2] Mycotoxin research lab, Iranian Plant Protection Research Institute
P.O. Box 1454, Tehran 19395, Iran
E. mail: n.aliakbari@gmail.com

Corn ear rot disease can reduce yield and quality of corn. Some of the *Fusarium* species that infect corn ears may produce mycotoxins such as trichothecenes, that are harmful, and can be fatal. Deoxynivalenol contamination of forty maize samples of Ardabil province (Moghan) were collected at harvest time were estimated. All of the kernels of each sample were grounded with Romer II mill. 200 ml distilled water was added to 25 g of each grounded samples and shook in 150 rpm for 30 min. Extracted Deoxynivalenol was filtrated by Whatman filter paper NO 1. 1.4 ml of filtrated solution was cleaned up through (R-biopharm) immunoaffinity columns. deoxynivalenol was measured using HPLC at 222 nm, the mobile phase was Acetonitrile: Methanol: Water (8: 8: 84v/v). Standard curve was drown in range of 12.5-2000 ng/g (R^2 = 0/9995). Validation of the method was determined using 500 and 1000 ng/g spike samples, the recovery of the method for 500 and 1000 ng/g spike samples were 101/9%, 96% respectively. 55% samples are contaminate to DON. The range of contamination was 35.4- 542.55 ng/g. The mean of contamination was 165.7 ng/g that was less than maximum rate limit of deoxynivalenol which was recommended in the world (1ppm). In mycological studies all of the 40 kernel samples cultured on Nash-synder and Czapek media to isolate *Fusarium* species. For identification of Fusarium species PDA,CLA and SNA media and Leslie species description were used. Based on morphological and some of the phvsiological characteristics *F. verticioillides* (36%), *F. proliferatum* (21%), *F. noniliforme* (15%), *F. nygamaie* (10%) *and F. oxysporum* (18%) were collected. To identify the toxicogenic character of each species, rice matrix were contaminated artificially with representatives of each species and deoxynevalenol of each inoculated media estimated using IAC+ HPLC method. In finally, *F. proliferatum* was toxicogenic within isolates.

BIODIVERSITY AND NEW RECORDS OF MICROFUNGI IN THE RUHR AREA (NORTH RHINE WESTFALIA), GERMANY

Nosratollah ALE-AGHA, Manfred JENSEN & Markus BRASSMANN
University Duisburg-Essen, FB BioGeo
Allgemeine Botanik, Research Group Microfungi
Universitätsstr. 5, DE-45117 Essen, Germany

During our investigations of the microflora in NRW (Duisburg, Düsseldorf and Essen incl. the greenhouse of the Botanical Garden) in 2007 and 2008, we were able to collect and identify about 55 species on trees, bushes and ornamental plants as parasites and saprophytes. Some of these species are new for Germany or have been only rarely collected until now. Most of the species belong the Ascomycotina, Basidiomycotina and Deuteromycotina, for example: *Arthrocladiella mougeotii* (LÉV.) VASSILKOV. on *Lycium barbarum* L., *Colletotrichum coffeanum* F. NOAK on *Coffea arabica* L. (new for Germany), *Colletotrichum trichellum* (FR.) DUKE on *Hedera helix* L., *Erysiphe buhrii* U. BRAUN on *Lychnis cf. coronaria* (L.) DESR. (Anamorph. *Oidium dianthi* JACZ.), *Erysiphe flexuosa* (PECK) U. BRAUN & S. TAKAM. on *Aesculus spec.* (new for Europe), *Erysiphe necator* SCHWEIN. = *Uncinula necator* (SCHWEIN.) BURRILL on *Cissus cf. rhombifolia Vahl.* (new for NRW), *Golovinomyces cichoracearum* (DC.) V.P. GELYUTA (*Oidium spec*.) on *Argyranthemum pinnatifidum* (L.f.) R. T. Lowe (new host), *Lobatopedis foliicola* P.M. KIRK on *Quercus robur L.* (new for NRW), *Mamiania coryli* DE NOT. on *Corylus avellana* L., *Marssonina juglandis* (LIB.) MAGNUS on *Juglans regia* L., *Oidium spec*. on *Dahlia variabilis* (WILLD.) DESF. (new for Germany), *Oidium longipes* NOORDELOOS & LOERAK on *Petunia hybrida* WILM., *Oidium sesami* H.D.SHIN on *Ibicella lutea* (LINDL.) VAN ESELT., *Passalora pastinacae* (SACC.) U. BRAUN = *Pseudocercosporella pastinacae* (P. KARST.) U. BRAUN (new for Germany), *Podosphaera tridactyla* (WALLR.) DE BARY on *Prunus laurocerasus* L., *Septoria cornicola* DESM. on *Cornus sanguinea* L., *Stigmina tinea* (SACC.) M.B. ELLIS on *Viburnum opulus* L., *Torula herbarum* (PERS.) LINK on *Potentilla argentea* L., etc. All species are located in the herbarium Mycotheca parva collection G.B. Feige & N. Ale-Agha.

BASELINE SENSITIVITY OF *STEMPHYLIUM VESICARIUM*, THE CAUSAL AGENT OF PEAR BROWN SPOT TO BOSCALID

Giulia ALBERONI, Davide CAVALLINI, Marina COLLINA & Agostino BRUNELLI
Department of Agri-food Protection and Improvement, University of Bologna, Italy

The control of *Stemphylium vesicarium*, the causal agent of pear brown spot, is a true concern in Italy for pear growers because many fungicide treatments are required from petal fall to fruit ripening. The occurrence of resistance to dicarboximides in the early 1990's and to strobilurins in 2006 complicated even more the control of this pathogen. The introduction in the field of new fungicides with different mechanism of action is thus fundamental to enlarge the range of available fungicide against pear brown spot. At the end of 2006-season boscalid was registered in Italy on pear. It belongs to carboxamide-fungicide group and inhibits the respiratory chain acting on the enzyme succinate dehydrogenase in the complex II. This mode of action is, therefore, different from both dicarboximide and strobilurin fungicides and it could be used in the cases of field resistance to these compounds. Nevertheless, boscalid is considered by FRAC with a medium risk of resistance for its specific target. In order to evaluate the sensitivity of *Stemphylium vesicarium* to this fungicide a *in vitro* assay was then developed, comparing different methods. A baseline sensitivity was then determined in a mycelial growth test on YBA-medium for 57 isolates collected before the market introduction of boscalid. The EC_{50} values obtained ranged from 0.11 to 0.81 mg a.i./l and represent the basal sensitivity of *Stemphylium vesicarium*, which was similar to other pathogens. These values will also be the reference to evaluate possible shift in sensitivity caused by the use in the field of this fungicide during the years.

NATURAL VARIATION IN DEFENCE RESPONSIVEVENESS AMONG ARABIDOPSIS ACCESSIONS

Shakoor AHMAD[1,2], Marieke VAN HULTEN[2], Ruth GORDON-WEEKS[1], Corné M.J. PIETERSE[2] & Jurriaan TON [1]

[1] Rothamsted Research UK

[2] School of Experimental Plant Sciences, Utrecht University Netherlands

Plants can develop an enhanced defensive capacity that enables a faster and stronger defence response upon attack by pathogens or insects. This phenomenon is called "priming" and provides protection against a wide range of harmful organisms. Induction of priming has been shown to yields broad-spectrum resistance with minimal reductions in plant growth and seed set. Hence, priming represents an adaptive and cost-efficient resistance strategy that increases the plant's ability to resist environmental stress[1].

Accordingly, it can be predicted that selected Arabidopsis accessions have adapted to hostile environments by acquiring constitutively primed levels of defence. In support of this, we identified various Arabidopsis accessions that possess a relatively high defensive responsiveness to the plant defensive hormones jasmonic acid (JA) and/or salicylic acid (SA) in combination with enhanced levels of pathogen resistance. Accessions with primed responsiveness to SA were more resistant to a biotrophic pathogen and expressed higher levels defence-regulatory transcription factors. One accession, Bur-0, showed enhanced responsiveness to both JA and SA in combination with broad-spectrum resistance to pathogens and herbivores [2]. Although Bur-0 showed enhanced responsiveness of the JA-inducible *PDF1.2* gene, the JA-inducible *VSP2* gene was less responsive. These antagonistic induction profiles indicate a priming of the ERF1/ORA59-dependent JA response and a simultaneous repression of the MYC2-dependent JA response in accession Bur-0.

To explore the genetic basis underlying the genetically innate priming of accession Bur-0, we will perform eQTL analysis of a fully genotyped population of recombinant inbred lines from a cross between accessions Col-0 and Bur-0.

THE INFLUENCE OF FUNGICIDES FOLIAR TREATMENTS ON THE WHEAT YIELD AND QUALITY

Elena NAGY, Ioan HAS & Dan CRISTIAN NAGY
Agricultural Research – Development Station Turda, Romania
E-mail: nagyln@yahoo.com

Wheat crops are damaged by numerous diseases which caused quantitative and especially qualitative yield losses in Transylvania conditions.The complex of foliar diseases : powdery mildew (*Blumeria graminis* f. sp. *trtrici),* leaf and glume blotch (*Septoria trtrici* and *Stagonospora nodorum*), rusts (*Puccinia striiformis*, *Puccinia recondita and Puccinia graminis*) and tan spot (*Pyrenophora tritici – repentis*) as well as head blight (*Fusarium* spp.) and ears blackening (*Alternaria* and *Cladosporium*) are the most frequently in wheat crops. Yield losses reaching to 30% from yield value depend on climatic conditions and wheat cultivar. The effect of fungicide foliar treatments and winter wheat was studied at ARDS Turda during two years. It was organized byfactorial trials after block split type with 3 treatments variants: untreated (T_0), 1 Treatment (T_1) applied at through early flag leaf emergence (ZGS38) and 2 treatments (T_2) applied through early flag leaf emergence (ZGS38) and in the end of flowering (ZGS73). The fungicides used contain: spyroxamine 250 g/l+tebuconazole 167 g/l+triadimenole 43 g/l at dose 0.6 l/ha, for the first treatment, respectively prothioconazole 125 g/l+ tebuconazole 125 g/l at dose 0,9 l/ha for the second treatment. In the field, attack degree for main diseases (%) and yield (kg/ha) and in the laboratory, baking parameters protein and wet gluten content (%) were determined. It also evaluated, thousand kernels weight (TKW), volumetric weight and percentage of diseased kernels.

The weather conditions from April, May, June months of 2 years is characterized by high temperature associated with weather deficit, were not very favorable of the diseases occurrence, it know that is essentially weather-dependent. Foliar diseases: powdery mildew, tan spot, leaf blotch and brown rust and ears diseases: *Fusarium* head blight (FHB) were presented in wheat crops. By applying of one single fungicide treatment, attacked leaf area by foliar diseases was significantly reduced in average with 50% and quite more at Turda 2000 and Apullum cultivars. Applying of 2 treatments diminished substantially diseased leaf area (3.8%) and the FHB attack (2.6%), with positively effect on the yield capacity. Applying one foliar treatment increases yield with 5.4-13.8 %, average being 9.5% and for two treatments with 14.0-20.1%, average being 16.4 %. in the two ears. For Turda 95 and Dumbrava wheat cultivars, the highest yield by 6436 kg/ha respectively 6462 were registered.

Between spikes and diseased kernels a positive and significant correlation exists, defined by equation: $y=1.0447x+5.7327$; $R^2 = 0.6268$. Besides substantially significant yield gains were really improved the quality in term baking due to gluten content. Applying two treatments with fungicides determined an evident increase reach up to 30.7 % of the wet gluten and to 11.7% of the protein contain.

INTEGRATED APPROACH TO TYLCD MANAGEMENT IN SARDINIA (ITALY)

Antonella SIRIGU[1], Mauro NANNINI[1] & Aniello CRESCENZI[2]
[1] Agris Sardegna - DIRVE, Cagliari, Italy
[2] Università degli Studi della Basilicata
Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Potenza, Italy

In Sardinia (Italy) *Tomato yellow leaf curl disease* (TYLCD) re-emerged in 2003, after a period of decline, producing severe yield losses in protected tomato crops. This epidemic outbreak highlighted the inadequacy of the approach to TYLCD management based chiefly on chemical control of its vector, the whitefly *Bemisia tabaci*, and led growers to extend the use of 40-mesh nets for greenhouse screening.

To evaluate the reliability of alternative practices for implementation within IP&DM programmes, in 2007 and 2008 we carried out two field experiments on greenhouse tomato crops planted in summer. In both trials we tested the use of UV-reflective mulches (UVRM) and the application for three weeks after planting of non-woven row covers (NWRC), against an uncovered, transparent film mulched control. Furthermore, we assessed the effectiveness of five applications of acibenzolar-S-methyl, a SAR elicitor, as subplot factor in a split plot design.

In 2007 because of the low incidence of the disease it was not possible to assess differences among the treatments. However a significant increase in plant growth, and production (+ 40% compared to control) was observed in UVRM plots. By contrast, in 2008 the study crop was seriously affected by TYLCD, to such an extent that, 4 months after planting, 100% of the plants in all plots were infected.

In the plots not treated with acibenzolar-S-methyl, the spread of the disease was initially delayed in NWRC plots compared to UVRM and control plots but, 3 weeks after removal of the cover, the differences among treatments became statistically not significant. In the plots treated with acibenzolar-S-methyl the dynamics of TYLCD infection during the first five weeks exhibited the same trend as in the untreated plots. Thereafter, the combination of UVRM and, to a lesser extent, of NWRC with the SAR elicitor resulted in a reduction of disease incidence with respect to the control (-46% and -35%, respectively).

In conclusion, all the control measures tested in the present work showed the potential for TYLCD management in greenhouse tomatoes. However, the most promising results were achieved using UV-reflective mulch and non-woven row cover in combination with acibenzolar-S-methyl. In view of its positive impact on plant growth and yield as well as on TYLCD infection, the use UV-reflective mulch warrants further investigation.

DIFFERENTIATION OF *MAGNAPORTHE GRISEA* SPECIES COMPLEX BY REP-PCR GENOMIC FINGERPRINTING

Parastoo MOTALLEBI , Mohammad JAVAN-NIKKHAH , Sayyed MAHMOOD OKHOVVAT, Khalil Berdi FOTOUHIFAR & Gholam Hosein MOSAHEBI
Department of Plant Protection, University College of Agriculture & Natural Resources
University of Tehran, Karaj 3158 - 77871, Iran

Magnaporthe grisea is the causal agent of blast disease on rice and leaf spot on some Poaceae weeds in Iran and other regions of the world. Infected samples were collected from rice and weeds including *Digitaria sanguinalis* (crabgrass), *Setaria italica* (foxtail millet), *Echinochloa crus-galli* (barnyard millet), and some unknown weeds during 1997 - 2005 and were preserved in collection of Mycology at the University of Tehran, Iran. In this study, genetic diversity of *Magnaporthe grisea* species complex isolates was studied based on DNA fingerprinting by rep-PCR, using of two primers including ERIC and BOX. The total DNA of 75 isolates was extracted and DNA fragments were amplified in a thermal cycler program using mentioned primers. Therefore, DNA fragments from 400bp to 3000bp were amplified. Based on cluster analysis for two primers (ERIC and BOX), eight fingerprinting groups (clonal lineages) and sixty haplotypes were identified. "A" clonal lineage was containing the highest number of isolates and became dominant clonal lineages with 35 isolates from rice and 3 isolates from *S. italica*, whereas the highest number of isolates obtained from *D. sanguinalis* belonged to "E" clonal lineage and was the second largest clonal lineage. Approximately all of the *M. grisea* species complex isolates from crabgrass and some of unknown weeds were separated from other isolates in 42% similarity. As a result, asexual fertility causes low diversity in populations of *M. grisea* species complex and speciation could be one of the reasons of differentiation between isolates from *D. sanguinalis* with other isolates. Overall, these data indicated a low level of genetic diversity in the Iranian *M. grisea* species complex population similar to that reported in other countries.

ROLE OF PHOTORESPIRATION IN THE DEFENSE MECHANISM OF *SITIENS*, AN ABSCISIC ACID-DEFICIENT TOMATO MUTANT, AGAINST THE NECROTROPHIC PATHOGEN *BOTRYTIS CINEREA*

Hamed SEIFI[1], Katrien CURVERS[1,2] & Monica HÖFTE[1]
[1]Laboratory of Phytopathology, Faculty of Bioscience Engineering, Ghent University
Coupure Links 653, BE-9000 Gent, Belgium
[2]Department of Plant Systems Biology, Flanders Institute for Biotechnology
BE-9052 Gent, Belgium
Department of Plant Biotechnology and Genetics, Ghent University, Gent, Belgium

Botrytis cinerea, a necrotrophic pathogen with broad host range, causes significant losses in tomato greenhouse cultivation every year. We showed before that resistance to *B. cinerea* in *sitiens*, an abscisic acid deficient tomato mutant, involves timely production of hydrogen peroxide and hence cell wall fortification in the epidermis (Asselbergh *et al*. 2007).

Using transcript-profiling analysis, we found genes differentially expressed in wild type and *sitiens* 8 hours post inoculation with *B. cinerea*, of which a subset was categorized as genes coding for enzymes involved in amino acid metabolism including peroxisomal enzymes catalyzing transamination in photorespiration pathway such as alanine:glyoxylate aminotransferase (AGT). Glyoxylate, the main substrate of different aminotransferases, is formed in peroxisomes via the photorespiration pathway by glycolate oxidase activity and H_2O_2 is the byproduct of this reaction. It is believed that photorespiratory hydrogen peroxide is one of the main metabolic sources of H_2O_2 in C3-plants (Fahnenstich *et al*. 2008) and therefore, photorespiration is known to provide protection against abiotic and biotic stresses (Wingler et al., 2000). In this study, we tried to unravel the mechanism that lies behind the interaction between photorespiration and *sitiens* defence response.

We inhibited the movement of peroxisomes towards the fungus penetration site by blocking molecular actin-myosin based motors, using specific myosin inhibitors: 2,3-butanedione monoxime (BDM) and N-ethylmaleimide (NEM).This resulted in increased susceptibility in *sitiens*. Using DAB staining upon infection, we observed a clear difference in the pattern of H_2O_2 accumulation in *sitiens* and wild type.

Furthermore, we studied the activity of glycolate oxidase (GO), a peroxisomal enzyme involved in photorespiration. Using an Enzyme-Coupled Assay to monitor GO activity over a time course in wild-type and *sitiens* plants, we found the highest enzyme activity in infected *sitiens* leaves incubated in dark conditions, 8 hours post inoculation. Moreover, we investigated the impact of light on defence-related reactions in *sitiens* against *B. cinerea*, considering light-triggered H_2O_2 production and its localization. We found that very high level of H_2O_2 induced during light treatment leads to susceptibility in *sitiens*, indicating that a very balanced level of H_2O_2 is needed to induce resistance in this mutant.

THE PSYR_1712 GENE IS A HOMOLOGUE OF *PPGL* IN *PSEUDOMONAS AERUGINOSA* WHICH CAN MODULATE FITNESS AND QUORUM SENSING OF *PSEUDOMONAS SYRINGAE*

Saeed TARIGHI & Pierre CORNELIS
Laboratory of Microbial Interactions, Department of Molecular and Cellular Interactions
Flanders Interuniversity Institute of Biotechnology, Vrije Universiteit Brussel
Pleinlaan 2, BE-1050 Brussels, Belgium

The production of *Pseudomonas syringae pv. syringae*'s AHL signal, 3-oxo-hexanoyl-homoserine lactone (3O-C6-HSL), requires the expression of the AHL synthase gene, *ahlI,* and the AHL regulator gene, *ahlR*. This AhlI-AhlR quorum sensing system has been demonstrated to be key in controlling traits that contribute to epiphytic fitness, such as exopolysaccharide production, and motility, as well as contributing to disease development during plant infection. The AhlI-AhlR quorum sensing system also is subject to modulation by additional regulatory proteins. A novel modulator, Psyr_1712, was identified as a positive modulator of AHL production. Psyr_1712 mutant had a delay in the lag-phase of its growth. Both a Psyr_1712 mutant and an *ahlI* mutant were significantly increased in pyoverdin production and they were hyper motile compared with the wild-type strain. The tobacco HR reaction in the case of *ahlI* mutant was faster and stronger than Psyr_1712 mutant and wild type. The mutants caused water soaked and necrotic lesions on bean pods like the wild-type strain but lesions caused by *ahlI* mutant were larger than wild-type whereas those incited by a Psyr_1712 mutant were larger than WT and smaller than *ahlI* mutant. Complementation of the Psyr_1712 mutant in trans showed higher production of signal molecules. Interestingly, QRT-PCR showed expression of Psyr_1712 mutant is under control of quorum sensing suggesting this gene is part of quorum sensing circuitry in *P. syringae pv. syringae* B728a.
The above results suggest that the Psyr_1712 gene is important for *P. syringae* to modulate fitness and quorum sensing.

PYTHIUM SPP. IN THE TROPICAL AEROBIC RICE CULTIVATION : IDENTIFICATION, PATHOGENICITY, INTRASPECIFIC VARIABILITY AND BIOLOGICAL CONTROL

Evelien VAN BUYTEN, Arne STEELANDT & Monica HÖFTE
Laboratory of Phytopathology, Ghent University, Belgium

Rice (*Oryza sativa* L.) is the principal staple food for more than 3 billion people in the world, especially in Asia and Africa. Growing rice in paddy fields contributes for 75% to the total Asiatic rice production and covers 55% of the cultivated Asiatic rice area. Due to a decrease in water supply, the paddy field system is threatened and this brings about a huge problem with regard to the increasing population. Saving irrigation water without lowering productivity is possible by the cultivation of aerobic rice. Therefore, an aerobic rice growing system was constructed for temperate regions like China and Brazil. Unfortunately, the system fails under tropical conditions due to a so called 'soil sickness' of unknown etiology that can cause harvest losses up to 100% after the first year of cultivation.
The international Rice Research Institute (IRRI, Philippines) recently collected a great amount of *Pythium* isolates from infected aerobic rice fields in the Philippines. Preliminary pathogenicity tests demonstrated the majority of those isolates to be very pathogenic on aerobic rice. Consequently, we started species identification using sequencing of the ITS region. Up till now we could divide a selection of the isolates into 4 major groups: *Pythium arrhenomanes*, *Pythium graminicola*, *Pythium inflatum* and an unknown species closely related to *Pythium myriotylum*. Using an agar-based *in vitro* assay we showed that only *Pythium arrhenomanes* and *Pythium graminicola* were pathogenic on the aerobic rice variety 'apo', indicating that these two species may be responsible for the observed soil sickness. To confirm this hypothesis, the identification of each isolate will be finalized and their pathogenicity against 'apo' will be further investigated.

PROTEOMICS ANALYSIS OF *M. TRUNCATULA*/ *R. SOLANACEARUM* PATHOSYSTEM

Ahad YAMCHI[1,2], Aghafakhr MIRLOHI[2], Michel ROSSIGNOL[3], Cécile BEN[1], Martina RICKAUER[1], Reza ZAREIE[2] & Laurent GENTZBITTEL[1]

[1] INP-ENSAT, Symbiose et Pathologie des plantes
BP32607, FR-31326 Castanet-Tolosan cedex, France
[2] Department of Agricultural Biotechnology, Faculty of Agriculture,
Isfahan University of Technology, Isfahan, IRAN
[3] IFR40, Plateforme Protéomique Génopole Toulouse Midi-Pyrénées
Institut de Pharmacologie et de Biologie Structurale
CNRS UMR 5089, FR-31077 Toulouse, France

A *Medicago truncatula* RIL populations derived from cross between A17 (susceptible parent) and F83005.5 (resistant parent) lines is already available (Vailleau *et al*., 2007). Ten individual lines were selected from each of the resistant and susceptible population thresholds. Plants, grown for 10 days in hydroponic Fahraeus medium, were inoculated on the cut root with *Ralstonia solanacearum* strain GMI1000. Mock-inoculated controls were treated similarly but with no bacteria. Root and stem pulse leaves were harvested on days 1 and 3 post-inoculation. Samples from resistant and susceptible individuals were pooled and analyzed by 2D gel electrophoresis. Similar samples were pooled to reduce the number of 2D analysis and to mask genetic backgrounds of the RILs. To ensure reproducibility of results, plant inoculation, tissue harvest and 2D analysis were repeated in 3 independent replica. Comparing the 2D gels, proteins spots that were consistently over- or under-expressed in the resistant lines but not in the susceptible ones were identified. The most promising protein spots were identified by peptide mass fingerprinting. Results showed that two pathogen related proteins,PR5 and PR10, were increased in roots of resistant but not in susceptible lines after inoculation with bacteria. Also, 14.3.3 and HSP90 proteins were increased in leaves and roots respectively in both of lines after inoculation but in resistant lines were higher than susceptible lines. Previously, 14.3.3 protein has been known as a phosphoserine/phosphothreonine binding protein which may play an important role in a wide range of cellular processes such as cell cycling, transcriptional control and signal transduction against biotic and abiotic stresses. 14.3.3 protein was induced in leaves at dpi1 and dpi3 after inoculation of plant in roots. In Arabidopsis, HSP90 associates to SGT1 and has a co-chaperone function to regulate correct folding of R proteins before and after activation by Avr effector. Also, we showed that tissue specific proteins after inoculation with bacteria were more variable between resistant and susceptible lines than tissue unspecific proteins. This result agrees well with previously reported. Using the complete sequence for the proteins/genes found in the previous point, primers were designed for each gene. These primers were used in quantitative RT-PCR experiments on susceptible and resistant RILs lines to confirm the expression of candidate protein/genes at mRNA level.

CLASSIFICATION AND GENETIC VARIABILITY OF RHIZOCTONIA ISOLATES ASSOCIATED WITH SUGAR BEET IN IRAN

Parissa TAHERI, Mahrokh FALAHATI RASTEGAR, Behrouz JAFARPOUR, Hamid ROHANI & Esmat MAHDIKHANI MOGHADDAM
Department of Crop Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran

The soilborne necrotrtrophic fungus *Rhizoctonia solani* is of relevance for agriculture, causing several diseases on more than 27 plant families. Sugar beet is one of the most economically important crops that is suffering from Rhizoctonia diseases causing significant yield losses every year in sugar beet growing area in the world. The complexity associated to correct identification of Rhizoctonia species, anastomosis groups (AGs) and subgroups by using traditional methods justifies the efforts to use molecular markers for isolate characterization. In this study, molecular methods were used to classify different taxonomic groups of Rhizoctonia isolates causing sugar beet root and crown rot, also to investigate the genetic variability of the isolates. Application of species-specific primers designed for amplification of rDNA-ITS region revealed that all of the 45 isolates obtained from sugar beet crowns and roots were *R. solani*. Analysis of rDNA-ITS polymorphisms revealed that 38 isolates belonged to AG2-2 IIIB, 4 were AG2-2 IV, and 3 were AG5 but this method did not show any molecular variation within each subgroup. Genetic structure of the isolates was investigated using repetitive element-PCR. The similarity matrices were analysed using unweighted pair group method with arithmetic mean (UPGMA) clustering method in combination with Jaccard's similarity coefficient and the obtained dendrogram showed small genetic distances within the isolates. Correlation was observed between resulted grouping of the isolates and their taxonomic identification. Analysis of molecular variances revealed that geographic origin is not an important factor affecting genetic structure of *R. solani* populations obtained from sugar beet and new genotypes have been introduced into a region over time.

AN EPIDEMIOLOGICAL SURVEY OF TYLCD IN SOUTHERN SARDINIA (ITALY)

Mauro NANNINI[1], Francesco FODDI[1], Giovanni MURGIA[1], Riccardo PISCI[1], Francesco SANNA[1], Marco TESTA[1] & Gian PAOLO ACCOTTO[2]

[1] Agris Sardegna - DIRVE, Cagliari, Italy

[2] CNR – Istituto Virologia Vegetale, Torino, Italy

Tomato yellow leaf curl Sardinia virus (TYLCSV) and *Tomato yellow leaf curl virus* (TYLCV) are among the most virulent pathogens of greenhouse tomatoes in Sardinia (Italy). To investigate the relationship between seasonal population trends of the vector *Bemisia tabaci* and the dynamics of TYLCD spread to susceptible crops, between May and October 2007 we carried out a survey in a tomato growing area located in the south of the island. On three farms specialized in the production of fresh market tomatoes we monitored, outside commercial greenhouses, the following parameters related to TYLCD epidemiology: mean weekly catches of the whiteflies *B. tabaci* and *Trialeurodes vaporariorum* on yellow sticky traps, ratio between the two whitefly species in adult samples collected on hosts not susceptible to the disease, proportion of *B. tabaci* adults carrying TYLCSV and/or TYLCV, and proportion of tomato plants infected by TYLCD after a two-week exposure to open field conditions.

Generally speaking, the flight activity of whiteflies increased during spring, reached a peak in May or June to gradually decline in summer. At the beginning of the survey, *T. vaporariorum* was found to be the prevalent species, but after a shift in composition of whitefly populations during July, *B. tabaci* became predominant. While the percentage of vector adults carrying the viral agents of the disease was relatively high up to July, with maximum values ranging between 14 and 25%, during the following months it decreased to less than 5%. The incidence of TYLCD in the plants exposed outside the greenhouses showed a similar trend in the sites surveyed, with two peaks roughly coinciding with the beginning and end of summer. Therefore two distinct phases of TYLCD spread were observed: from spring to mid-summer when the disease was transmitted by low *B. tabaci* populations with relatively high proportions of virus carriers; from mid-summer to autumn, when the disease was spread by larger vector populations with low percentages of individuals carrying the viruses. Further studies are necessary to gain a better understanding of the interactions among *B. tabaci* biotypes, TYLCSV/TYLCV and their hosts, which presumably play a role in the spread of the disease to tomato crops.

CORRELATION BETWEEN THE COLONIZATION OF THE RHIZOSPHERE BY *PSEUDOMONAS FLUORESCENS* AND BIOLOGICAL CONTROL OF COMMON BUNT IN WHEAT

Abbas SHARIFI-TEHRANI[1,2], Stefan KELLENBERGER[1], Mohsen FARZANEH[2], Maria PECHY-TARR[3], Christoph KEEL[3] & Fabio MASCHER[1]

[1]Agroscope Changins-Wädenswil, CH-1260 Nyon, Switzerland
E-mail: fabio.mascher@acw.admin.ch
[2]Department of Plant Protection, University of Tehran, Karaj, Iran
E-mail: asharifi@chamran.ut.ac.ir
[3]Department of Fundamental Microbiology, University of Lausanne
CH-1015 Lausanne, Switzerland
E-mail: christoph.keel@unil.ch

Fluorescent *Pseudomonas* spp. are ubiquitous bacteria in agricultural soils and have many characteristics that make them well suited as biocontrol agents of plant pathogens. The widely recognized mechanisms of biocontrol mediated by *Pseudomonas* bacteria are competition for an ecological niche for a substrate, production of inhibitory allelochemicals, and induction of systemic resistance (ISR) in host plants. Common bunt, incited by *Tilletia caries* can be a serious disease, causing yield and quality losses in wheat. In this investigation, the capacity of different strains of *Pseudomonas fluorescens* (CHA0, CHA0gfp2, CHA0mcherry, PF153mcherry and Q2-87mcherry) was studied to reduce common bunt of the wheat variety Apogee. The bacteria were co-inoculated with the pathogen onto the seed prior to planting. Results showed that the presence of certain bacterial strains reduced the incidence of the disease. Particularly the strains CHA0 and CHA0gfp2 exhibited the strongest effect to reduce the number of infected spikes while PF153mcherry showed the least effect. This high disease reduction level was positively correlated with the ability of CHAOgfp2 to colonize the rhizosphere. Strain PF153mcherry showed the lowest colonization levels of bacterial root colonization. These results suggest that, besides the criteria mentioned before, a good biocontrol agent must also be a good root colonizer.

EFFECT OF ESSENTIAL OILS IN CONTROL OF PLANT DISEASES

S. PEIGHAMY-ASHNAEI, M. FARZANEH, A. SHARIFI-TEHRANI & K. BEHBOUDI
Department of Plant Protection, Faculty of Horticultural Science & Plant Protection
University of Tehran, Karaj, Iran

In this study, antifungal activity of some essential oils extracted from *Syzygium aromaticum, Foeniculum vulgare*, *Cuminum cyminum* and *Mentha piperita* were investigated against grey mold of apple. The essential oils of *S. aromaticum* and *F. vulgare* showed considerable antifungal activities on PDA medium against *Botrytis cinerea*. Results indicated that the increasing of dosage of the essential oils caused to the more antifungal activity against *B. cinerea in vitro* condition. Also, biocontrol potential of two essential oils of *S. aromaticum* and *F. vulgare* in two concentrations 750 and 1000 µL/L were investigated on infected fruits, at 20 ˚C and darkness condition. After 10 days, results showed that the essential oil of *F. vulgare* in both of the concentrations was more effective than the essential oil of *S. aromaticum* against grey mould of apple and decrease the disease up to 15.5% in comparison with the check treatment (100%). After 20 days, biocontrol potential of the essential oils of *S. aromaticum* and *F. vulgare* at 1000 µL/L were more effective than the other treatments and the percentage of disease was evaluated as 41.6% and 50.8% respectively, in comparison with the check treatment (100%). Thiabendazole was more effective against grey mould of apple as compared to the essential oils of *S. aromaticum* and *F. vulgare*.

IN VIVO AND *IN VITRO* EFFECTS OF N AND K ELEMENTS ON WHEAT RESISTANCE TO *SEPTORIA TRITICI* LEAF BLOTCH

Wahiba HARRAT & Zouaoui BOUZNAD
Institut National Agronomique, El Harrach, Alger

Four modalities of fertilization were used in order to test the effect of N and K supply on *Septoria tritici* leaf blotch development on durum wheat cultivar (Vitron), witch is susceptible to this disease.
Tow tests are realized, one under field conditions where seedlings were inoculated artificially with conidia suspension of *Septoria tritici* and another under *in vitro* conditions where detached leaves taken at beginning heading-flowering stage and from the flag leaf, were also inoculated.
The obtained results indicate that a potassic supply before sowing seems to decrease the intensity of disease. The nitrogen supplies split with tillering and beginning heading stages promote the extension of the disease to the last leaf what causes considerable losses of yield. A foliar supply of potassium at the heading stage can remedy to this situation. The *in vitro* results confirm those of field test concerning the effect of N and K. Leafs samples taken from beginning heading–flowering stage are more susceptible than those taken from the flag leaf. The obtained results will be used in future experiments including pesticides to optimize yields.

OCCURRENCE AND DISTRIBUTION OF POTATO VIRUS Y STRAIN N(PVYN) ON CELERY IN IRAN

Neda KHOSHKHATTI[1], Mina HABIBI KOOHI & Gh. MOSAHEBI
Department of Plant Pathology, Faculty of Horticulture Science and Plant Protection
University of Tehran, Karaj
[1] E-mail: Neda2p@Gmail.com

Celery (*Apium graveolens*) is an important crop grown in many countries. Different types of diseases present a major constraint to Celery production and can lead to significant reductions in yield
Potato Virus Y, The type member of the genus Potyvirus (family Potyviridae) is one of the causal agents of viral diseases in celery (*Apium graveolens*). The virus is naturally transmitted by aphids in a non-persistent manner. During growing seasons 2006-2007 celery fields were visited through Tehran Province and A total 332samples based on selection of plants expressing symptoms like mosaic, vein clearing and motteling were collected. By using serological methods (ACP ELISA and DAS-ELIZA) with specific antisera of Potyviruses (DSMZ-AS-0537.1) and PVY (DSMZ-AS-0137.403) 17.64% of the samples were infected with PVY. The reaction of PVY infected samples were positive in TAS-ELIZA with specific monoclonal antibodies (MAbs) of PVYNstrain (DSMZ-AS-403.1).
Some biological and molecular characterizations of the virus isolate were determined. PVY Nstrain isolated from celery was reported for the first time in Iran and in the world in this survey.

COMPARATIVE ANALYSIS OF ELISA, ONE STEP RT-PCR AND IC-RT-PCR FOR THE DETECTION OF BEAN YELLOW MOSAIC VIRUS IN GLADIOLUS

Duraisamy GANESHSELVARAJ[1], Pokorný RADOVÁN[2] & Ludmilla HOLKOVÁ[3]
Faculty of Agronomy, Department of Crop Science, Breeding and Plant Medicine,
Mendel University of Agriculture and Forestry, Czech Republic

Three methods were compared for the detection of *Bean yellow mosaic virus* (BYMV) in gladiolus both in leaves and corms: ELISA, one step-RT-PCR and IC-RT-PCR. The initial screening of samples was done by DAS-ELISA to eliminate a high percentage of virus-negative samples, considerably reducing the number of plants to analyze further by one step RT-PCR and IC-RT-PCR. ELISA was more reliable method to detect BYMV in leaves but in corms it fails to detect. RNA was extracted from leaves and corms were used for the detection of BYMV by one step RT-PCR method. The one step RT-PCR method was more sensitivity and it was able to detect BYMV at very low concentration in leaves but in corms it amplified very weak signals. In IC-RT-PCR using the PAbs and specific primers in the region of the coat protein (CP) gene, this assays detected their respective target virus (BYMV) at low concentrations.

WHEAT CROWN AND ROOT ROTTING FUNGI IN MOGHAN AREA, NORTHWEST OF IRAN

Behzad HAJIEGHRARI
Department of Plant Production, Moghan Junior College of Agriculture,
University of Mohaghegh – Ardabili, Ardabil, Iran

Root and crown rot of wheat (*Triticum aestivum* L.) is a disease problem in many wheat producing area as well as Moghan wheat growing area, Northwest of Iran. In this study; injured plants of wheat with blighting, stunting, and death of mature plants, and necrotic lesions on seminal crown roots, subcrown internodes and crown, and basal stem tissue symptoms were collected from localities of different region of Moghan wheat growing area. Fungi associated wheat root and crown were isolated and identified based on general colony morphology, the morphology of mycelia, reproductive structure and taxonomic description. Also pathogenicity of 28 selected isolates from various identified species was determined on wheat (*Triticum aestivum* L. CV. Atila 4; a common grown cultivar of wheat in Moghan area) seedlings. The results indicated that The predominant pathogens implicated in causing common root and crown rot in this study was *Bipolaris sorokiniana*. in addition to *B. sorokiniana*; *F. culmorum*, *F. graminearum* and Gaeumanomyces/Phiolophora complex are the most widely distributed species on wheat growing belt in Moghan area respectively *F. solani*, *F. crookwellence*, *F. clamidosporum*, *F. moniliform*, *F. poa*, *F. udum* and *F. babinda* were wheat root associated species of Fusarium that ascertained during our investigation. *Rhizoctonia solani* AG8 was not isolated from wheat in all of samples. Pathogenicity test revealed that *B. sorokiniana*; *F. culmorum*, *F. graminearum*, *F. crookwellence*, *F. udum* and Gaeumanomyces/Phiolophora complex are pathogen actively and others are saprophytes involved in root destruction tissue without being the cause of injury.

CARNATION MOTTLE VIRUS, AN IMPORTANT VIRAL AGENT INFECTING CARNATION CUT-FLOWER CROPS IN MAHALLAT OF IRAN

Maliheh SAFARI, Mina KOOHI HABIBI, G. MOSAHEBI & A. DIZADJI
Department of Plant Protection, College of Horticultural Science and Plant Protection,
Univerrsity College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
E-mail: malihe.safari@gmail.com, mkhabibi@ut.ac.ir

One of the most important cut-flower crops grown worldwide on commercial scale is Carnation (*Dianthus caryophyllus* L.). It's the main production of Mahallat where is one of the most important ornamental plants production centres of Iran. Infection of carnation with pathogens like viral agents cause economic losses in carnation cut-flower crop. One of the viral agents of this flower is *Carnation mottle virus* (CarMV) which is the type member of genus *Carmovirus* and belongs to the family *Tombusviridae*. It is naturally transmitted by grafting and contacting between plants. Although its infection lead to mild symptoms, it weakens the plant to infection by other pathogens.

The carnation greenhouses of Mahallat were visited during 2008 January to April and 100 samples with mild mosaic symptom were collected and tested by DAS-ELISA using CarMV specific polyclonal antibody (DSMZ As- 0081). The results showed that 75% of samples where infected with this virus. Mechanical inocubation of *Chenopodium quinoa*, *C. amaranticolor* and *Spinacea oleracea* with extracted crude sap of CarMV infected carnation leaves in phosphate buffer (pH=7) resulted in appearance of chlorotic and necrotic local lesions on inoculated leaves 4-7 days after incubation.

The virus was partially purified using *C. amaranticolor* locally symptomatic leaves. Total soluble proteins were extracted from healthy and CarMV infected *C. amaranticolor* plants and beside partially purified preparation electrophoresed through 15% poly acrylamide gel according to SDS-PAGE standard procedure. Protein bands were electroblotted onto nitrocelluse membrane and incubated with CarMV polyclonal during western immunoblot analysis according to standard method. The result revealed a distinc protein band with Mr of 35.5 kDa in total protein preparation of infected plant and viral partial pure preparation, without any reaction in those of healthy plant.

RT-PCR carried out using total RNA extracted from infected plant by Rnease Plant Mini Kit and a pair of primers, CPu, CPd, corresponding to the flanking region of the virus CP resulted in amplification of a DNA fragment in expected size around 1kbp.

REAL TIME PCR-MEDIATED MONITORING OF *PUCCINIA HORIANA* DEVELOPMENT IN CHRYSANTHEMUM

Hossein ALAEI[1,2,3], Martine MAES[1], Monica HÖFTE[2] & Kurt HEUNGENS[1]
[1] Institute for Agricultural and Fisheries Research, Unit Plant - Crop Protection, Belgium
[2] Ghent University, Faculty of Bioscience Engineering, Department of Crop Protection, Belgium
[3] Rafsanjan Vali-e-Asr University, Faculty of Agriculture, Department of Crop Protection, Iran

Real-time PCR was used to detect and quantify the development of *Puccinia horiana* Henn. in infected leaves of *Chrysanthemum x morifolium*. Specifically, we evaluated the effect of the incubation temperature on pathogen development during the latent period. Detection and quantification of *P. horiana* was possible from the start of the infection (<1 dpi). Under optimal temperature conditions, *P. horiana* DNA accumulated exponentially between 2 and 12 dpi, followed by a more moderate increase, and eventually, a decrease in the amount of DNA present. Visual symptoms appeared as early as 8 dpi and peaked at 14 to 18 dpi. The amount of pathogen DNA increased from approximately 2 to 1000 ng per 100 mg randomly inoculated leaf tissue, eventually representing approximately 10% of the total DNA (leaf+fungus) extracted. A continuous suboptimal incubation temperature (6°C) as well as a supra-optimal day-temperature (28°C day/20°C night) clearly resulted in a slower and reduced DNA accumulation. Under these temperature regimes, visual symptoms were delayed, but did appear after 4 and 2 weeks respectively. A continuous supra-optimal incubation temperature (28°C) resulted in a lack of *P. horiana* DNA accumulation and absence of disease symptoms after 90 dpi, even after returning the plants to the optimal temperature for pathogen development. These data do not support the hypothesis that the pathogen spans the Western European summer period as latently present mycelium inside the plant.

BACTERIA *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS* - PATHOGEN OF POTATO

K.P. KORNEV[1], A.N. IGNATOV[2], A.N. KARLOV, E.V[3]. MATVEEVA[1], E.SH. PEKHTEREVA[1] & N.W. SHAAD[4]

[1] Russian Research Institute of Phytopathology, Moscow region, 143050, Russia
[2] Center "Bioengineering" RAS, Moscow, 117312, Russia
[3] Russian Agrarian University "MSHA", 127550, Moscow, Russia
[4] USDA-ARS, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD 21702, USA
E-mail: konstantin.kornev@gmail.com

Since 2004 yellow-pigmented *clavibacteria* caused severe damage in some region of Russian Federation. Mostly non-pigmented *Clavibacter michiganensis* ssp. *sepedonicus* (Cms) is known to be a dangerous potato pathogen. First strains of yellow-pigmented bacteria of genus *Clavibacter* were isolated from eyehole of diseased potato tubers and visually healthy tubers, obtained from Kaliningrad region. During 2006-2007 the same bacterial strains were isolated repeatedly from green plant with wilt, spot- or blight-like leaf necrosis, and from diseased tubers in Moscow Kaliningrad, and Nizhniy Novgorod region. In field symptoms of disease appeared at early-middle July. Infected tubers were the major source of infection. Pathogen penetrates into host plant through hydothodes or mechanical lesions. Chlorosis appears on the leaves along edge and later necrosis appears along the leaf veins. Vessels of stem become dark. Under favorable for the pathogen condition the infected plants die in the beginning of August. Infection of tubers occurs on early stages of tuber formation. In contrast to the ring rot caused by Cms, the progress of the disease caused by yellow *clavibacteria* on tubers was slower, and the worst damage was caused for growing plants. The vascular ring of tubers had yellow or brown color, but secondary infection was not typical. Over 50 strains of the pathogen were isolated from different regions of Russia. All main morphological, biochemical, physiological characteristics were studied with comparison to typical strains of Cms and *Clavibacter michiganensis* ssp. *michiganensis* (Cmm). All strains were assayed for ability to produce HRP reaction on tobacco cv. 'Xanthy' and Mirabilis and for pathogenicity – on plants from families *Solanaceae, Brassicaceae, Poaceae* and *Fabaceae*. For identification, specific primers for all subspecies *Clavibacter michiganensis* were used including CMM5/CMM6, PCA1/PCAR and 11 other specific PCR markers. Additionally, several genomic regions were amplified and sequenced for representative strains. All the tested strains were identified by the molecular markers as Cmm. All strains were avirulent to plants from *Brassicaceae, Poaceae* and *Fabaceae* family, and highly virulent to potato and tomato plants. Biochemical analysis has supported the molecular data. The strains isolated from potato were homogeneous in morphological, biochemical, physiological characteristics and most similar to typical strains Cmm NCPPB2979^T. It was concluded that new strains from potato belong to separate physiological group of Cmm, virulent for both potato and tomato plants. We offer to name strains of this group as race P (Potato) of *Clavibacter michiganensis* ssp. *michiganensis.*

REMOVAL OF CAULIFLOWER DEBRIS MAY REDUCE THE NUMBER OF MICROSCLEROTIA OF *VERTICILLIUM LONGISPORUM* IN SOIL

S.C. FRANÇA[1], K. SPIESSENS[2], L. DE ROOSTER[2], S. POLLET[3], D. CALLENS[3] & M. HÖFTE[1]

[1] Laboratory of Phytopathology, Ghent University, Belgium
[2] Proefstation voor de Groenteteelt, Sint-Katelijne-Waver, Belgium
[3] Provinciaal Onderzoeks- en Voorlichtingscentrum voor Land- en Tuinbouw Rumbeke-Beitem, Belgium

Verticillium longisporum is a soil-borne pathogen causing vascular wilt of Brassicaceae. Verticillium wilt is an increasing problem in European cauliflower production. *V. longisporum* is a close relative of *Verticillium dahlia* and therefore similarities in their life cycles are expected to occur. Microsclerotia of *V. dahliae* are formed in aerial and subterranean parts on senescing tissues of host plants and are released in soil during the next year after incorporation of plant debris. The contribution from aerial parts is far the largest and the removal of aerial debris of host crops is important to reduce the soil population of this pathogen. Concerning the formation of microsclerotia of *V. longisporum* on cauliflower debris little information is available. In Californian fields, microsclerotia were observed on infected cauliflower roots 8 weeks after harvest. Recently, we have found in pot experiments that large amounts of microsclerotia can be formed on cauliflower stem residues several weeks after harvest. The effect of the removal of cauliflower debris on the density of microsclerotia of *V. longisporum* in soil was tested in field experiments on naturally infested soils in Oppuurs (Antwerpen) and Ardooie (West-Vlaanderen) during 2007 and 2008. The removal of cauliflower residues (roots and stem) from the field led to a reduction in the number of viable microsclerotia in the soil in Oppuurs. Although in Ardooie this effect was not detected, less increase of disease severity in 2008 was observed in the plots where crop residues were removed in 2007. The removal of cauliflower rests is probably not a practical alternative for managing *Verticillium* in field because it demands a lot of work and would increase costs for the growers. However, these results highlight the potential of developing sustainable control strategies for *Verticillium* wilt of cauliflower based on the prevention of formation and/or destruction of microsclerotia in crop residues, for example via application of antagonists or use of practices which promote natural microbial competition.

PHYTOSANITARY OVERVIEW OF THE GROWING SEASON 2008 AT THE PLANT DISEASE CLINIC OF THE WALLOON AGRICULTURAL RESEARCH CENTRE

Sophie SCHMITZ[1] & Anne CHANDELIER[1]
[1] Walloon Agricultural Research Centre, Department Biological Control and Plant Genetic Resources, Gembloux, Belgium

A wide diversity of pests and diseases affects plants. The accurate identification of the cause of plant symptoms is a necessary condition to apply appropriate control methods. Through its diagnostic activities, the plant disease clinic of the Walloon Agricultural Research Centre provides answers to growers facing various pests and diseases problems and it constitutes a connection between growers and agricultural research.

About 250 analyses were realized during the past year. Most of the encountered cases concerned recurring problems such as *Verticillium* wilt and *Fusarium* root rot of ornamental plants or attacks by the horse chestnut leaf miner *Cameraria ohridella*. *Armillaria* root rot was also detected on a wide range of woody plants. As in the previous years, *Sphaeropsis sapinea* was still predominant on pine trees but the pathogen tended to infect with a higher frequency *Araucaria* species. Stone fruits were mainly affected by *Pseudomonas* attacks and many cases of pear rust (*Gymnosporangium sabinae*) were once more observed in pear orchards. Main pathogens of strawberry cultures were *Glomerella acutata* and *Phytophthora cactorum*. Vegetable crops were not spared by phytosanitary problems as *Allium* leaf miner (*Phytomyza gymnostoma*) still caused important damages to leek cultures and many soil samples originating from pea fields were infected by the soil pathogen *Aphanomyces euteiches*.

A few pathogens were newly encountered at the plant disease clinic during the growing season 2008. *Mycosphaerella pini*, responsible for red band needle blight, was identified for the first time in the Walloon region in a forest stand of *Pinus nigra* ssp. *nigra* var. Koekelare. *Mycosphaerella microsora* was also found causing leaf spot on *Tilia* while *Phytophthora cryptogea* was isolated from diseased *Buxus*.

INCREASING THE RESISTANCE OF FIELD TOMATO TO *PEPINO MOSAIC VIRUS*

Beata KUŁEK
Research Centre for Agricultural and Forest Environment,
Polish Academy of Sciences, Bukowska 19, PL-60-809 Poznań, Poland
E-mail: B.Kulek@interia.eu

A resistance degree of three cultivars: 'Atol', 'Betalux' and 'Promyk' of *Lycopersicon esculentum* Mill. to the pathogen was examined. The most susceptible to *Pepino mosaic virus* (PepMV) turned out to be the 'Betalux' cultivar, therefore it was chosen for further studies. For this cultivar, under the electron scanning microscope, a few particles of the virus were observed in leaves, and many virions arranged in the form of the bundle – in fruits. An influence of two inducers was examined - benzothiadiazol (BTH) dissolved in the water and chitosan dissolved in the acetic acid, at different concentrations, as well as the influence of milk with the addition of different amounts of the acetic acid on the degree of the disease development.

Different testing methods were applied: measuring the percentage of leaf surface covered by necroses and yellow spots as well as the percentage of infected area on fruits, detecting the presence of 3.3'-diaminobenzidine peroxidase, hydrogen peroxide and lignin in leaves with colourful histochemical reactions. It was stated that the 0.5 mM solution of benzothiadiazol induced the biggest increase in the systemic acquired resistance (SAR) – mainly in fruits which were least infected by the virus. The upper leaves, growing above the BTH treatment zone, were also highly protected.

The milk with the acetic acid had no influence on improving the condition of younger leaves (growing above the level of sprayed leaves). However, it contributed in a high degree, though a little bit lower than in case of BTH, to the protection of the tomato fruits. Natural resistance of the 'Promyk' cultivar is correlated with the higher level of hydrogen peroxide, the higher activity of the peroxidase and the greater accumulation of the lignin than in case of the susceptible cultivar – 'Betalux'. Benzothiadiazol induced defensive reactions at susceptible cultivar of the tomato, which correlated with an increase in the level of the above mentioned indicators of plants resistance, especially of hydrogen peroxide.

PRODUCTION OF DESTOMYCIN-A ANTIBIOTIC BY *STREPTOMYCES* SP. USING RICE STRAW AS FERMENTED SUBSTRATE

Houssam M. ATTA, Ashraf T. ABUL-HAMD & Hassan G. RADWAN
Botany and Microbiology Department, Faculty of Science (Boys)
Al-Azhar University, Cairo, Egypt

Hundred and twenty microbial isolates could be isolated from different soil samples collected from different localities in Egypt. One of the actinomycete culture AZ-H-A5 from three cultures was found to produce a wide spectrum antimicrobial agent when cultivated on rice straw. The actinomycete AZ-H-A5 could be isolated from a soil sample collected from Helwan district, Egypt. The nucleotide sequence of the 16s RNA gene (1.5 Kb) of the most potent strain evidenced an 85% similarity with *Streptomyces pseudovenezue*, EU841712 and *Streptomyces galilaeus*. From the taxonomic features, the actinomycetes isolate AZ-H-A5 matches with *Streptomyces rimosus* in the morphological, physiological and biochemical characters. Thus, it was given the suggested name *Streptomyces rimosus*, AZ-H-A5. The parameters controlling the biosynthetic process of antimicrobial agent formation including: inoculum size, different pH values, different temperatures, different incubation period, and different carbon and nitrogen sources, potassium nitrate, K_2HPO_4, $MgSO_4.7H_2O$ and KCl concentrations were fully investigates. The active metabolite was extracted using ethyl acetate (1:1, v/v) at pH 7.0. The separation of the active ingredient and its purification was performed using both thin layer chromatography (TLC) and column chromatography (CC) techniques. The physico-chemical characteristics of the purified antibiotic viz. color, melting point, solubility, elemental analysis, spectroscopic characteristics and chemical reactions have been investigated. This analysis indicates a suggested empirical formula of $C_{20}H_{37}N_{13}O_{13}$. The minimum inhibition concentrations "MICs" of the purified antimicrobial agent were also determined. The purified antimicrobial agent was suggestive of being belonging to Destomycin-A antibiotic produced by *Streptomyces rimosus*, AZ-H-A5.

GENE EXPRESSION IN *SPORISORIUM REILIANUM* EXPOSED TO ROOT EXUDATES

S.K. SABBAGH[1,2] & C. ROUX[2]

[1] Department of Plant Protection, Faculty of Agriculture, University of Zabol, Zabol, Iran
[2] Laboratory of "Cell Surface and Signalling in Plants"
University Toulouse-CNRS Joint Unit 5546 - Plant Biotechnology Institute
24 Chemin de Borde Rouge, Auzeville, BP 42617, FR-31326 Castanet Tolosan, France

Sporisorium reilianum f.sp zeae, a basidiomycetous fungus belonging to Ustilaginaceae, is the causal agent of the maize head smut disease. This soilborne pathogen infects the host plant at the seedling stage by penetrating roots. The infection is systemic, and disease symptoms become apparent only after the onset of flower development when the fungal sori replace male or female inflorescences. In order to investigate the mechanism of infection, we analysed the transcriptome of the fungus in response to root exudates during the previous phase of infection. Asuppression subtractive hybridization (SSH) was used to generate cDNA libraries representing genes differentially expressed in haploid cell forms of the fungus exposed to root exudates leading to1440 ESTs. By using cDNA macroarray hybridization, we identified 150 ESTs which were differentially expressed in response to exudates application. In this first transcriptomic analysis realized on *S. reilianum*, we show that maize root exudates may affect gene expression of the fungus during the previous step of infection and could play an important role in pathogenicity.

THE EFFECT OF FUNGICIDES ON SEED YIELD AND DISEASE CONTROL IN ITALIAN RYEGRASS

Georges RIJCKAERT
ILVO - Institute for Agricultural and Fisheries Research, Unit Plant, Merelbeke, Belgium

Under Belgian climatic conditions, the incidence of fungal diseases like mildew, crown rust and stem rust is much lower in seed crops of Italian ryegrass than crops of perennial ryegrass, because of the cleaning effect of the preceding forage cut and the much quicker growing rate of Italian ryegrass. However, in some mild and warm seasons, above diseases can give detrimental effects on seed yield, so a preventive fungicide programme would be very recommended in order to obtain consistently high seed yields over the years.
Six different fungicides and an untreated control were tested on two tetraploid varieties of Italian ryegrass, namely cv. Meroa and cv. Salomé (more tolerant to crown rust); one fungicide application took place at early ear emergence for all three trials (2006-07-08).
Only in the 2007-trial with very severe rust pressure, the most efficient fungicides increased seed yield by 22-25 % against the control (100%) and the tebuconazol-treatment (106.6%). Yield differences could be attributed to a healthier seed crop i.e. less withered flag leaf and to a higher thousand seed weight.
As both years 2006 and 2008 had very little disease pressure throughout the season, the best treatments (Allegro and Opera) resulted in a moderate seed yield increase of 6-7% in 2006, while 2008 did not give any yield response at all between the fungicide treatments and the untreated check.
Seed yield, yield components and disease development are discussed and explained in relation to the seasonal meteorological conditions.

GENETIC CHARACTERISATION OF THE *RALSTONIA SOLANACEARUM* POPULATION IN FLANDERS BASED ON GENES INVOLVED IN HOST PLANT ASSOCIATION

Rachid TAHZIMA, Annemie HOEDEKIE, Brigitte DE PAEPE, Martine MAES & Johan VAN VAERENBERGH

Institute for Agricultural and Fisheries Research, Plant Unit – Crop Protection,
Burg. Van Gansberghelaan 96 bus 2, BE-9820 Merelbeke, Belgium
E-mail: rachid.tahzima@ilvo.vlaanderen.be

Ralstonia solanacearum (Rs) is a complex species showing a broad variability at the infraspecific level. Genetic flexibility is possibly a driving factor in adaptation of the pathogen to new and specific environments.
Several outbreaks of potato brown rot (PBR) in Flanders and other regions in Western Europe caused by race 3, biovar 2-A (Phylotype II), were associated with irrigation from water courses in which woody nightshade or bittersweet (*Solanum dulcamara*) served as reservoirs for persistence and distribution of the pathogen. The first outbreak of PBR in Flanders occurred 20 years ago and still the bacterium can be isolated from its weed host along water courses. The objective of this study is to assess the genetic constitution of this persistent Rs population. A collection of isolates from the early PBR period (1989-1994) were studied. Phylotyping and sequevar analysis confirmed that all isolates belong to Phylotype II and constitute a phylogenetically homogenous group. PCR-RFLP and sequence analyses of genes involved in virulence, chemotaxis and competitive fitness enabling root invasion and colonisation of the xylem showed that the isolates from Flanders are clonal, with only minor sequence differences. This observation was further supported by 16S rDNA sequence data. Recent isolates will be sampled from the same area and the same bittersweet host where the bacterium is still surviving or causing infections and genetic characterisation has to reveal their relatedness to the early population. Additional pathogenicity bioassays are in process.

SEVERE OUTBREAK OF TOMATO YELLOW LEAF CURL SARDINIA VIRUS ON PEPPER IN SOUTHERN ITALY

Soccorsa COMES[1], Fanigliulo ANGELA[1,2], Rosa PACELLA[1] & Aniello CRESCENZI[1]

[1]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali – Università degli Studi della Basilicata. Viale dell'Ateneo Lucano Campus Macchia Romana 3A310
IT-85100 Potenza (PZ) Italy
E-mail: aniello.crescenzi@unibas.it
[2]Bioagritest Test Facility, Bioagritest Srl - Centro Interregionale di Diagnosi Vegetale
Zona PIP lotto E2. 85010 Pignola (PZ), Italy
E-mail: info@bioagritest.it; www.bioagritest.it.

During summer and autumn 2008 a severe outbreak of *tomato yellow leaf curl Sardinia virus* (TYLCSV) infection was observed in pepper crops under plastic tunnels in the ionic coast of Basilicata region. The diseased plants showed stunting, flower abortion, light mosaic or mottling, leaf distorsion, interveinal and marginal leaf chlorosis, upward curling of leaves. The disease is referred to as *pepper leaf curl disease* (PLCD). Symptoms were more severe on yellow varieties than on red ones. PLCD incidence reached, in same cases, values close to 50%. In particular the disease was very strong along the borders of the greenhouse. Moreover, large populations of whiteflies, identificated as *Bemisia tabaci*, were observed on the crop.
Detection assays for TYLCSV and *tomato yellow leaf curl* (TYLCV) were performed. 190 symptomatic samples were collected from different fields and assayed in DAS-ELISA using a broad-spectrum reagent combination (distributed by Bioreba AG) detecting TYLCV, TYLCSV and other *Begamovirus*es: of these, 176 samples resulted positive.
In order to discriminate between TYLCSV, TYLCV or any other *Begamovirus,* 15 samples resulted positive in ELISA test were analyzed by PCR using a couple of synthetic oligonucleotides allowing the amplification of the whole coat protein (CP) gene. RFLP analysis performed on the PCR product, 1008 bp long, showed the presence of only TYLCSV in all assayed samples.
The molecular characterization performed by phylogenetic analysis of the sequenced coat protein gene revealed that the isolate shares a similarity of about 97% with the corresponding sequence of a tomato TYLCSV isolate from Sicily (Z28390) and is almost identical with the pepper isolate CAB-It recovered in the same area in 2007 (TYLCSV was first recorded on pepper in Italy in 2007 in Policoro - MT, Fanigliulo *et al.*, 2008. *Comm. Appl. Biol. Sci*, Ghent University, 73/2, 2008). Its further diffusion in the monitored area and its hazard has to be connected with the presence of hosts alternative to pepper (tomato, *Solanum nigrum*, *Sonchus* spp.) and with the strong presence of *Bemisia tabaci*.

CYAZOFAMIDE: A FUNGICIDE AGAINST *BREMIA LACTUCAE* ON LETTUCE

Angele FANIGLIULO[1,2], Vittorio FILÌ[1], Carmine GIUSEPPE MASSA[1] & Aniell0 CRESCENZI[2]

[1]Bioagritest Test Facility, Bioagritest Srl - Centro Interregionale di Diagnosi Vegetale
Zona PIP lotto E2. IT-85010 Pignola (PZ), Italy
E-mail: info@bioagritest.it; www.bioagritest.it
[2]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali
Università degli Studi della Basilicata
Viale dell'Ateneo Lucano Campus Macchia Romana 3A310, IT-85100 Potenza (PZ) Italy
E-mail: aniello.crescenzi@unibas.it

Cyazofamide is a molecule with anti-mildew action, the sole representative of the cyanoimidazole class, inhibiting all stages of oomycetes fungal development. Its mode of action is new and unique: it acts inhibiting the Qi (Quinone Inside Inhibitor) site of the cytochrome bc 1 site in complex III of the fungal mitochondrial membrane, blocking the transportation of electrons in the respiratory chain.
During 2007 and 2008 summers, experimentation was performed by the Bioagritest test facility, according to EPPO guidelines and Principles of Good Experimental Practice (GEP), aiming at establishing the biological efficacy of Cyazofamide 25SC (formulate MILDICUT, Belchim) and Cyazofamide 400SC + an organosilicone adjuvant containing polyalkyleneoxide modified heptamethyltrisiloxane (Ranman 400 SC, Belchim, a contact fungicide, classified as a protectant with anti-sporulation activity and a limited systemic activity) on lettuce against *Bremia lactucae*.
The study was performed in Nocera Inferiore (Salerno), southern Italy, in a greenhouse with polyethylene cover. Experimental design consisted in random blocks, in 3 repetitions. Two different dosages of the formulate Mildicut - 3,5 lt/ha and 4,5 lt/ha - were compared with the unique dosage 0,350 lt/ha of Ranman and a commercial formulate: 2,4 % metalaxyl-M + 40 % copper, 4 kg/ha (Ridomil Gold R 46 WP, Syngenta Crop Protection). Four foliage applications were applied every 7 days. The intensity and diffusion of the disease were evaluated on leaves together with eventual phytotoxic effects. The extreme climatic conditions occurred during the course of study, with rather low temperatures and high humidity in the greenhouse, accompanied by high rainfall outside, allowed for extremely serious attacks by *Bremia Lactucae*, as to make the 3 replications of the checks strongly compromised.
Statistical analysis was performed by the use of *XLSTAT* data analysis and statistical software. Experimental results show the excellent effectiveness of Cyazofamide: both the two doses of Mildicut and Ranman, such as the standard formulate used in comparison, showed to be effective in the control of the disease, highlighting a strong biological activity against *B. Lactuce*. No effect of phytotoxicity was noticed on leaves.

SUSTAINABILITY OF INDIAN AGRICULTURE SANS PESTICIDES

Y.P.S. RATHI
B-55 Pallavpuram, Phase I, Meerut
(U.P.) India

In this era of Liberalization, Privatization & Globalization there is heavy international competition within India as well as abroad on all fronts and agriculture in particular. Dr. Norman Borlaug, Noble Laureate described the phenomenon of the growing population of 3rd world countries as "Frightening situation". Hence, the priority is clear cut before us as our population surpassed one billion. We have not only to sustain the food production but to continue to achieve higher and higher production to ensure that every mouth gets sufficient food.

Introduction of high yielding semi dwarf varieties of wheat and rice with basket of agrochemicals and ensured irrigation resulted in GREEN REVOLUTION. During this era of green revolution many contact and systemic pesticides were developed and widely used. Indiscriminate use of agro chemicals caused health hazards, environmental pollution and damage to eco-system. Quite often we used to read in newspaper and magazines sensational headings such as "flow of banned pesticides to third world countries", "Pesticides induce cancer and sterility", "Pesticides: a guest that overstayed", "Pesticides, a death harvest", etc. After reading such sensational news, we are forced to think why should the pesticides not be banned. However, at the same time we are not ready to forget the history of mankind filled with miseries of pest epidemics.

In recent years organic movement and awareness of environmental hazards, some people of the developed countries started talking of "Organic farming". But the question arises can the farmers of 3rd world countries afford and sustain the organic farming ? Can this " Kitchen garden strategy" of organic produce fill up the belly of our bulging population ? The only way out is to minimize the gap between the genetical/potential yield through integrated crop management. In this approach Integrated Pest Management (IPM) is an integral part wherein eco-system disturbance is minimized. It will not be an exaggeration that the pesticides are indispensable for tropical and subtropical countries because of the climate of these countries is highly congenial to the pests. The adoption of monoculture is highly prone to outbreak of pathogens/insects. Moreover, the cash crops always need pesticide umbrella. However, these arguments do not justify at all that we should use the pesticides to maximum. In fact the pesticides should be used in targeted judicious manner and hence IPM strategies should be developed against more and more diseases and insect pests. Besides, the farmers should be educated about the pesticides awareness and safe & effective application technology.

EFFECT OF DIFFERENT SALINITY LEVELS AND NA/CA RATIOS ON DIE-BACK DISEASE OF PISTACHIO

Vahid MOZAFFARI[1] & Mohammad-Jafar MALAKOUTI[2]

[1] Deptartment of soil Science, Vali-e-Asr University of Rafsanjan, Iran

[2] Deptartment of soil Science, Tarbiat Modarres University, Tehran, Iran

In the course of recent years the incidence of pistachio *Pistacia vera* L. die-back disease has spread extensively in many regions of Iran, resulting in lower yields in the affected orchards. A randomized complete block factorial greenhouse experiments were carried out to evaluate the role of calcium at different salinity levels in controlling die-back disease of Badami-Zarand pistachio rootstock seedlings; and come to understand weather seedling inoculation with a known causative fungus *Paecilomyces variotii* would produce die-back disease under treatments. The experimental treatments were included A: soil with different salinities (3, 7.5 and 15 dS/m), B: different ratios of Na/Ca (0.2, 1.5, and 10.0), in the saturation extracts. In order to measure the length of cankers on the seedlings, the barks were removed from the affected stems and the length of the darkened spots was measured. To evaluate plant responses, concentration of proline, photosynthesis rate and plant yield (leaf, stem and root dry weight) were measured. Correlation between salinity levels and rate of canker development revealed a significant effect of salinity on the disease development; the shortest and the longest cankers were found with salinity levels of 3 and 15 dS/m, respectively. Decreasing the ratios of Na/Ca, especially at higher salinity levels, significantly decreased canker lengths (more than 100% in some cases). Furthermore, the chemical analysis of leaves showed that by increasing the salinity levels from 3 to 15 dS/m leaves proline content significantly increased from 0.153 to 0.193 mg/g. Fw. Moreover, increase of Na/Ca ratio from 0.2 to 10 leaded to a dramatic increase of leaves proline content (about 32%). The results of photosynthesis measurement disclosed that increasing the salinity level up to 7.5 dS/m has no significant effect but in 15 dS/m level, photosynthesis rate was significantly decreased (42%). Mean leaf, stem and root dry weight diminished to 34, 30 and 21%, respectively when salinity level increased from 3 to 15 dS/m.

EPIDEMIOLOGY AND ECO-FRIENDLY MANAGEMENT OF YELLOW MOSAIC VIRUS OF GRAIN LEGUMES IN INDIA

Y.P.S. RATHI
B-55 Pallavpuram-I, Meerut-250110
U.P., India

Mungbean yellow mosaic virus (MYMV) also called as a" yellow plague"of grain legumes is vectored by whitefly (*Bemisia tabaci* Genn.) in a persistent circulative manner. Currently, it is a number one problem causing substantial yield losses in urdbean (Vigna mungo), mungbean (*V. radiata*), mothbean (*V.aconotifolia*) and soybean (*Glycine max*) in India.Yield losses even may go up to 100% in case a susceptible genotype gets infected at an early stage of crop growth. The foliage of host crop exhibits bright yellowing and the disease field can be recognized from far distance .A better understanding of the epidemiology and application of plant and cow products may provide new approaches to develop eco-friendly management practices to manage the disease. Studies on epidemiology in Tarai region of Uttarakhand state revealed that besides the population of whiteflies, weather parameters like temperature, rainfall and humidity play important role in the development of the disease epidemic. Whitefly population increased with increase in temperature . High relative humidity, heavy shower and strong winds in rainy season were found detrimental to whitefly adults. Further studies on host range revealed that ratoon crop of pigeonpea and/or some other weeds might be serving as primary source of inoculum.

Experiments were conducted to contain this disease through agronomic practice (manipulation in planting dates and intercropping with barriers (non-host) crops, insecticides (foliar and soil application, seed treatment),spray of cow products (milk, buttermilk, urine), neem oil and host resistance. Low disease incidence was recorded in early (June) and late (August) planting as well as low plant spacing (5cm). Seed dressing with carbofuran 3-G or phorate 10-G with two foliar sprays of 1.0% neem oil+1.0% butter milk +2.0% cow urine + 1.0% detergent significantly reduced the disease incident. Inter-cropping of non host crops with grain legumes were not found very effective barriers. Some genotypes viz. Pant U 19, 30, 35 and PU-1 GD (urdbean), PM 1,2,3 & 4 (mungbean) and PK 416, PK 564,PK 1029, SL 142(Soybean) resistant to MYMV have been identified.

None of the individual management approach was found highly effective against the vector / virus due to high vector population and wide range of host plants serving as initial foci of the whitefly vector and the virus through out the year. Moreover, a single whitefly adult is sufficient to initiate the infection. Hence the development of integrated management strategy having tolerant varieties in core, is the only promising way to manage this "yellow plague" of grain legumes effectively.

EVALUATION OF BIOLOGICAL EFFICACY OF ISOLATED *TRICHODERMA HARZIANUM* RAFI AGAINST *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI* (SACC.) W.

Hesamedin RAMEZANI
Department of Agriculture, Payame Noor University (P.N.U.), Iran
E-mail: hesam18@yahoo.com

Trichoderma harzianum was isolated from five soil samples from different locations of Abadehetashk District in specific medium. It was noticed that the population in term of colony forming unit (cfu) per g of soil varied from sample to sample ranging from 27.3×10^2 cfu/g of soil in Abadeh to 1.2×10^2 cfu/g of soil in Tashk. Mycelia growth rate of *Trichoderma* isolates did not show significant variation. The pigmentation of the isolates were almost varying from light green to dark green. Out of these, only isolates T_1, T_2 and T_3 had some conidia of larger size of 2.3 to 3.1 µ diam. Isolates T_1, T_2 and T_3 were found effective antagonist against *Fusarium oxysporum* f.sp. *lycopersici*, the wilt pathogen of tomato, as there overgrowth the mycelia growth up to 62, 70 and 52%, respectively. It was found that the isolate T_4 has least antagonistic effect on *Fusarium oxysporum* f.sp. *lycopersici*.
Key words: Biological control, *Fusarium oxysporum* f.sp. *lycopersici*, Efficacy, *Trichoderma harzianum*.

DETECTION, DISTRIBUTION, AND PURIFICATION OF MAJOR POTYVIRUSES OF ZUCCHINI SQUASH IN IRAN

Parissa TAHERI, Behrooz JAFARPOUR & Mahrokh FALAHATI RASTEGAR
Department of Crop Protection
College of Agriculture, Ferdowsi University of Mashhad, Iran

Viral diseases of zucchini squash are among the most important and destructive diseases of this crop, causing severe damage every year. Viruses in the potyvirus genus (Potyviridae family), including zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus-1 (WMV-1), and watermelon mosaic virus-2 (WMV-2), are the major viruses infecting zucchini squash worldwide. In the present study, successive sampling was carried out from zucchini plants with symptoms such as mosaic, malformation and colour breaking of leaf and fruit, and fruit blistering at different growth stages in the main growing areas of zucchini squash located in Khorasan-razavi province of Iran. Detection of ZYMV, WMV-1, WMV-2 was based on double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA). The infected samples with ZYMV, WMV-1, and WMV-2 were 186, 98, and 134 out of 500 collected samples respectively. Some of the samples showed infection with both WMV-1 and WMV-2 viruses simultaneously, indicating that the investigated viruses can not cross-protect each other. Thermal inactivation point, longevity in vitro, and dilution end point were determined for each virus. The most destructive virus was ZYMV with the widest host range determined using differential indicator plants, and WMV-2 was more destructive than WMV-1. The viruses were purified using polyethylene glycol (PEG)-6000 and centrifugation in caesium chloride gradient. The purification was confirmed by spectrophotometry and coat protein investigation using Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis (SDS-PAGE), that showed a protein bands with molecular weight of 36.391 and 34.047 kDa which are specific for ZYMV and WMV-2, respectively.

FIRST REPORT OF A NEW *SOBEMOVIRUS* FROM LETTUCE IN IRAN

M. KOOHI HABIBI[1], F.S. ABTAHI[1], S. WINTER[2] & G.H. MOSAHEBI[1]
[1] Department of Plant Protection, Collage of Agriculture, University of Tehran, Karaj, Iran
[2] DSMZ Plant Virus Department, c/o JKI, 38104 Braunschweig, Germany

Lettuce (*Lactuca sativa*) is one of the most common vegetables in the world. Several viruses have been reported from this crop among which Lettuce mosaic virus is the most prevalent and widely distributed pathogen of lettuce. During studies to identify the most significant virus diseases of lettuce in the Tehran province, infectivity studies conducted by mechanical inoculation of lettuce homogenates onto indicator plants revealed symptoms of systemic chlorosis on *Chenopodium quinoa* indicating for virus infections. There was no reaction in ELISA with any of the known lettuce viruses and other antisera. Adsorption preparations of leaf homogenates subjected to electron microscopical examination revealed virus-like particles with distinct isometric particle morphology. A specific polyclonal antiserum was raised against virus purifications (DSMZ AS-0913) and used in ELISA for specific virus detection. The virus was transmitted in a non persistent manner by the green peach aphid (*Myzus persicae*) and by *Aphis faba* to healthy lettuce, *Vicia faba and Chenopodium murale,* with symptoms becoming visible 10 to 15 days after inoculation. To determine the potential for seed transmission, a *C. quinoa* seed lot obtained from infected plants was used for a seed health, growing-on test. 14 of 279 seedlings showed virus symptoms and were verified for virus infections by DAS-ELISA. Seed transmission was not tested for lettuce however, with this high infection rates, this virus may pose a serious threat to lettuce and also to faba bean production in Iran.

Cloning and sequence analysis of specific cDNA clones prepared from virion preparations revealed a high sequence identity with a sobemovirus recently described from *Rubus*. Thus, the virus from lettuce in Iran has to be considered as a strain of Rubus chlorotic mottle virus, a virus hitherto not described from lettuce and from Iran.