Plant health for sustainable agriculture

CONFERENCE, 11 – 12 May 2015
Ljubljana, Slovenia

Book of Abstracts

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ORAL SESSIONS

Session 1: Challenges in Plant Health

Prioritising Actions to Enhance Plant Biosecurity: The UK Plant Health Risk Register

R. Baker[1], H. Anderson[1], S. Bishop[1], A. Korycinska[1], A. MacLeod[1], N. Parkinson[1] and M. Tuffen[1]

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The UK Plant Health Risk Register (RR) has been developed to assist with the prioritisation of actions that mitigate the threats posed by pests of plant health importance (1). It assigns ratings to pests according to appropriate scenarios. Thus for pests that are currently absent from the UK, the scenario would be that the pest is introduced. The ratings for likelihood and impact are based on rules that are designed to summarise key information on the pests themselves and other factors such as their pathways, hosts, distribution, spread and damage caused. Where information is lacking, expert judgement is used. A "value at risk" is obtained from economic and environmental data to ensure that the risk ratings reflect the value of the host sector to the UK. Risk ratings in the absence of mitigations are compared with those that take existing mitigations into account in order to identify those species that are priorities for additional actions, e.g. to enhance existing regulations, to prepare a contingency plan or to conduct a pest risk analysis. The RR is now used routinely to support decision making. The content is regularly updated to reflect new information received and priority actions that have been undertaken. Pests are frequently added and, by January 2015, the RR contained approximately 750 species. Originally developed as an Excel spreadsheet it has been adapted for the Internet (2). A research programme has been carried out to investigate how best to enhance the RR, e.g. to reflect uncertainty and represent economic, environmental and social impacts.

Keywords: pest risk ratings, prioritising actions

References: (1) Baker, R.H.A. et al. 2014. The UK Plant Health Risk Register: a tool for prioritizing actions. EPPO Bulletin 44: 187-194. (2) The UK Plant Health Risk Register. https://secure.fera.defra.gov.uk/phiw/riskRegister/

Acknowledgement

Many people: policy makers, stakeholders, scientists, economists and web developers, assisted with the development of the RR. A detailed list of acknowledgments is given in (1).

Sustainable Agriculture and Food Security: the Need to Integrate Crop Breeding, Nutrition and Health Management

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Over the last 40 years the use of synthetic chemical crop protection products (SCCPP) has increased rapidly and, with the possible exception of modern greenhouse production, current yield levels in intensive agricultural systems are now dependent on such inputs.

However, due to consumer concern about potential negative impacts and environmental and human health impacts of pesticides, there has been pressure from the EU, national regulatory bodies and supermarket drive quality assurance schemes to greatly reduce pesticide use, with many supermarkets ultimately aim at providing pesticide residue free produce. As a result, a range of widely used and highly active compound groups (e.g., organophosphorus pesticides and compounds with endocrine disrupting activity may not be available to farmers in the future). Resistance development and the increasing cost of SCCPPs are additional challenges faced by farmers.

Future crop protection practices will therefore have to focus on alternative crop protection approaches (breeding for resistance, biological, cultural, physical and mechanical crop protection) where active compounds are lost. Using examples of recent research, results from the FP6 QualityLowInputFood and FP7 NUE-Crops projects in the Nafferton Ecological Farming Group (NEFG) and the need to focus on "whole systems" approaches for crop protection will be described.

Keywords: alternative crop protection approaches, consumer concern, EU regulations, human and environmental health, intensive agricultural systems, Nafferton Ecological Farming Group, organophosphorus pesticides, synthetic chemical crop protection products

Root-Knot Nematodes *Meloidogyne* spp. as Emerging Pests

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Root-knot nematodes (RKNs) *Meloidogyne* spp. represent one of the most polyphagous and damaging genera of plant-parasitic nematodes with approximately 100 nominal species described so far. They are obligate parasites, distributed worldwide and they parasitize almost every species of vascular plants. The most important species are *M. incognita*, *M. arenaria, M. javanica* and *M. hapla*, sometimes referred to as the four "major" species mainly because of their wide distribution. However, several other RKN species are emerging as major problems for agriculture, especially in the light of changes towards environmentally sound production, trade patterns and climate. Most of the emerging species belong to the tropical group of RKNs that typically reproduce by obligatory mitotic parthenogenesis, which may possess aneuploid genomes and have been suggested to be hybrid taxa (1). These characteristics enable them remarkable adaptive phenotypic diversity such as wide host range and also the ability to overcome plant resistance *R* genes. In addition to that, two RKN species *M. incognita* and *M. javanica* were recognised as globally the most rapidly spreading plant pests (2). It is therefore crucial to study these species more intensively in order to develop efficient multi-option management approaches. The research at the Agricultural Institute of Slovenia is focused on: a) intra-specific variation studies of the tropical RKN populations using molecular markers, b) physiological plant response due to RKN infestation, c) studies of the population reproduction potential (number of generations) of RKN during the growing season and development of prediction models for their reproduction extent, d) management approaches using biological control agents, e) screenings of resistant germplasm for new *R* gene identification and the durability of *Mi* gene resistance in tomato against RKNs. In addition, we have started to develop non-invasive methods for the detection of RKN infestation using hyperspectral imaging.

Keywords: tropical RKNs, emerging nematode species, management, resistance, biological control agents

References: (1) Lunt, D.H. et al. 2014. *PeerJ* 2:e356; DOI 10.7717/peerj.356. (2) Bebber, D.P. et al. 2014. Global Ecol Biogeogr, 23:1398-1407.

Acknowledgement

The results were obtained in the frame of research projects L4-1021 financed by Slovenian Research Agency and Ministry of Agriculture and Environment. Parts of this work were realized within the frame of FP7 Project CropSustaIn, grant agreement FP7-REGPOT-CT2012-316205.

Activation of the Plant Immune System as a Sustainable Strategy for Pest Management

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Plants have developed sophisticated molecular mechanisms to detect pathogens and parasites and to activate immune response. Immune response in plants is regulated by phytohormones that are low molecular weight molecules which interact in a complex network to regulate also many aspects of plant growth, photosynthesis, flowering, reproduction, seed production and response to environmental abiotic challenges. Innate immune system in plants should be considered as within plant growth processes; thus, expressing constitutive defence systems occurs only at the cost of plant growth and encounters the risk of allocating resources to defence in the absence of natural pathogens and pests. An effective alternative is to fine-tune immune responses by modulating the "immunological memory" of plants, as it occurs in animals. An aspect of this modulation may be represented by the so-called "priming" by which previously attacked plants respond more quickly or more strongly to a subsequent attack. The priming may be realized by Systemic Acquired Resistance (SAR) that is typically induced following effector-triggered immunity (ETI) or, in other words, *R* gene-mediated resistance. Salicylic acid (SA) is the key regulator of either *R* gene-mediated resistance to biotrophic pathogens or SAR. Priming is a relatively low-cost mechanism of advancing plant defence, as resources are not used until the threat returns. Another distinct pathway that is associated with induced immune response in plants is based on the plant hormones jasmonic acid (JA) and ethylene (ET) functioning as signaling molecules. JA and ET are involved in an induced systemic resistance (ISR) mediated by beneficial soil organisms such as arbuscular mycorrhiza forming fungi (AMF) and plant growth promoting rhizobacteria (PGPR). Hormone network interactions are as important for plant immune system expression that pathogens and parasites have developed sophisticated molecular mechanisms to deregulate the biosynthesis of hormones and/or to interfere with hormonal signalling pathways thus impairing plant defence response.

It should be clear that there will be no effective durable crop protection strategy if the health of the soil and the quality of the irrigation water of the monitored cropping systems will be not carefully taken into consideration in any integrated pest management. A natural stimulation of the immune system of plants should be pursued by enriching the soil of the fields, particularly those intensively cropped, with AMF and PGPR. Therefore, a holistic approach to durable crop protection strategies should be pursued in the consideration of the very complex relationship occurring between plants and changing natural environments, which may be characterized by a vast array of biotic attackers and abiotic challenges, also in view of new concerns as global warming.

Keywords: Systemic Acquired Resistance, effector-triggered immunity, *R* gene-mediated resistance, microorganisms, priming

Session 2: Plant Pests and Diseases Diagnostics

Viruses of Major European Crops - Challenges of Disease Prevention and Control in Light of an Increased International Movement of Plant Materials and Intensified Agricultural Production

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Plant viruses and viroids present serious threats to the production of crops around the world. For viruses present in major European agricultural and horticultural crops, mandatory or voluntary certification programs are in place that limit the extent of virus infections and reduce the impact from the diseases. To prevent introduction of non-European viruses and their spread within the Community, protective measures exist that are formulated in a European Council Directive (2000/29/EC) which is in line with the processes prescribed in the International Standards for Phytosanitary Measures (ISPM).

The regulatory status of an organism is evaluated by a pest risk analysis (PRA) which consists of a pest categorisation describing the pest and its severity, the potential and probability of entry, establishment and spread and, the pest effects/impact. Plant viruses are associated with their particular host and can occur wherever the plant/crop is grown. There are only few virus species reported elsewhere absent from European crops. More often, viruses not present in Europe (non-European viruses) are particular strains of isolates of species present and resemble in their biological features those that are already there. The evaluation of "additional" threats from such new strains/ isolates is difficult especially when biological features (e.g. symptoms, transmission) are to be extrapolated from observations made in other environments or, are simply not known. The assessment of "new" viruses is particularly challenging when the connection between virus genomes and disease phenotype remains vague. However, even when a virus/viroid can be unequivocally attributed to severe diseases in one host, the likelihood of it being transferred to from another host is very difficult to assess particularly when biological parameters are extrapolated.

Plant viruses move with plant materials and because ornamental plants present a most substantial segment of international trade, the origin and volume of a particular consignment are considered and introduced in the risk rating. Viruses in ornamentals however can largely be overseen due to the enormous size of the consignment and absence of virus symptoms in latent infections. This makes virus diagnosis - sampling, testing and monitoring - prior and during the introduction process complicated and increases the chances of inadvertent virus introduction. However, while world-wide ornamental trade was responsible for introduction of viruses across continents, several recent serious outbreaks of virus diseases in major European crops provide evidence that the introduction of the virus type (species), the particular pathway of its entry and subsequently virus establishment and spread cannot be foreseen and predictions are associated with a high level of uncertainty particularly when non quantifiable elements are to be considered. Recent epidemics of virus diseases in tomato, cucurbits, maize and citrus are discussed to highlight the challenges of virus disease prevention and control.

Keywords: virus epidemics, international trade, prevention and control

Unravelling Interactions in Disease Complexes and Syndromes: the Case of Acute Oak Decline

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Plant pathologists have concentrated primarily on the single disease concept, where a pathogen acts in isolation to cause disease. In reality, interactions between organisms and with the abiotic environment lead to a level of complexity which is not readily investigated using standard procedures such as the fulfilment of Koch's postulates. A further layer of complexity occurs when microbial pathogens interact with invertebrates. Many examples of these disease complexes or syndromes can be found, especially within the context of forestry; for example: diseases such as pine wilt disease (beetle x nematode x bacterium); citrus greening (psyllid x bacterium); and various forms of forest decline (1). The declines may take decades to develop, often with a shifting myriad of interacting factors, both biotic and abiotic. The complexity within these systems makes epidemiological investigations particularly difficult to undertake, often requiring novel methods of analysis in the interpretation of data. In recent year in the UK an atypical form of oak decline, now termed Acute Oak Decline (AOD), has become apparent (2). As its name suggests, AOD develops much faster with tree mortality than the long-known chronic form of decline. Evidence suggests that AOD is a consequence of the interaction between an *Agrilus* beetle (3) and several clades of bacteria (4, 5), although the nature of the interaction is still under investigation: e.g. whether there is a vector association or an additive/synergistic interaction. In this talk the temporal and spatial development of AOD at several sites in the UK and the possible relationships with the occurrence and behaviour of *Agrilus biguttatus* will be presented.

Keywords: forest declines, pathogenic bacteria, *Agrilus biguttatus,* epidemiology

References: (1) Denman, S. and Webber, J.F. 2009. Q. J. Forest.103, 285-290. (2) Denman, S.et al. 2014. Forestry, doi: 10.1093/forestry/cpu010. (3) Brown, N. et al. 2014. Forestry doi: 10.1093/forestry/cpu039. (4) Brady, C. et al. 2010. Syst. Appl. Microbiol. 33, 444-50. (5) Denman, S. et al. 2012. Int. J. Syst. Evol. Microbiol. 62, 2451-2456.

Acknowledgement

The study was supported by the Forestry Commission (UK) and DEFRA project TH0108.

The Association of Grapevine Pinot Gris Virus with Grapevine Leaf Deformation and Mottling

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*Grapevine Pinot gris vi*rus (GPGV) is a *recently* described trichovirus, discovered by next generation sequencing (NGS) in the cv Pinot gris, seemingly associated with symptoms of leaf deformation and mottling (1). A survey on different cultivars from the Trentino region, in Italy, showed that GPGV infected 82% of tested vines and was associated with symptomatic (79%) but also with symptomless (21%) vines. Symptomatic and GPGV-infected Pinot gris vines induced symptoms on healthy Pinot gris or Traminer grafted vines, whereas GPGV-infected but symptomless vines did not. Analysis of small RNA libraries from two GPGV-infected Pinot gris accessions, showing or not symptoms, consistently reproduced the already known virome with *Grapevine rupestris stem pitting virus* (GRSPaV), *Grapevine rupestris vein feathering virus* (GRVFV) and the two viroids *Hop stunt viroid* (HSVd) and *Grapevine yellow speckle viroid 1* (GYSVd-1), infecting both vines. Whole genome phylogenetic analysis on seven GPGV isolates, originating from Italy, Czech Republic and Slovakia (2), clearly clustered those infecting symptomatic vines. Sequence analysis of two genomic regions, one encompassing part of the movement protein and the coat protein genes, the other corresponding to the RNA dependent RNA polymerase domain of the replicase gene, was performed on a group of accessions from Trentino, free from relevant viruses involved in leafroll, infectious degenerations and rugose-wood associated grapevine diseases. Results showed a distinct clustering of isolates from symptomatic vines. Furthermore, an intriguing polymorphism, involving the MP stop codon distinguished latent or symptomatic GPGV isolates, although its unique association to each group was not verified (3). These findings showed that GPGV population from Trentino underwent, in the infected vines, a different evolutionary dynamic, which selected for latent or symptomatic isolates.

Keywords: grapevine, trichovirus, disease, mottling, deformation, GPGV

References: (1) Giampetruzzi, A., et al. 2012. Virus Res., 163:262-268. (2) Glasa, M. et al. 2014. Arch. Virol. 159:2103-2107. (3) Saldarelli, P. et al. 2014. Phytopathology http://dx.doi.org/10.1094/PHYTO-09-14-0241-R

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Phytoplasmas of Grapevine: Molecular Diversity of Slovenian Strains and New Diagnostic Challenges

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Phytoplasmas, as fastidious wall-less mollicutes, colonize phloem tissue of many plants. They are associated with a severe and worldwide complex of the grapevine diseases called grapevine yellows (GY). GY are caused by different taxonomically unrelated phytoplasmas. Among them '*Candidatus* Phytoplasma solani', the causal agent of Grapevine Bois noir (BN), is the most widespread both in Europe and in Slovenia. Flavescence dorée phytoplasma (FDp), which causes the most serious phytoplasmal disease of grapevine, Flavescence dorée and '*Ca.* P. asteris', have also been found in Slovenian vineyards during the official survey by the Slovenian phytosanitary administration. The analysis of phytoplasma strains revealed several different genetic strains of FDp (1) and Bois noir phytoplasma (BNp) in Slovenian vineyards, which are presumably linked to different epidemiology. Diagnostic procedures used in our laboratory for monitoring of GY phytoplasmas is composed of a simple and quick homogenization step of crude extracts with DNA extraction based on the binding of DNA to magnetic beads and a real-time PCR detection system (2). For the absolute quantification of FDp, without the need for calibration curves, droplet-digital-PCR was developed (3). This new method has also a potential to be used for characterization of phytoplasma reference material (i.e., for phytoplasma DNA calibrants). For the on-site detection as well as for the screening detection in laboratories, loop-mediated isothermal amplification (LAMP) that gives result in 30 minutes including hands-on work, was developed for FDp (4) and it is in the progress for BNp.

Keywords: phytoplasma, grapevine, real time PCR, LAMP, digital droplet PCR

References: (1) Mehle et al., 2011. Bulletion of insectology, 64:S29-S30. (2) Mehle et al., 2013. Springer Protocols: Methods in Molecular Biology, 938: Chapters 12 and 22. (3) Mehle et al., 2014. Phytopathogenic Mollicutes, 4:9-15. (4) Kogovšek et al., 2014. Plant Pathology, Doi: 10.1111/ppa.12266.

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Session 3: Nonchemical (Alternative) Control of Pests and Diseases

The Use of Baculoviruses in Biological Control of Insect Pests

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One of the driving principles of integrated pest management (IPM) is the use of highly selective pesticides for a specific control of pests and thereby minimizing any harmful effects on human health and the environment. Baculoviruses are a group of insect-specific dsDNA viruses, which infect larval stages of Lepidoptera, Hymenoptera and Diptera. They are highly virulent to target insects and have a narrow host range. Hence, they represent the currently most selective agents available for the control of insect caterpillars in agriculture, horticulture and forestry. Therefore, they are ideal tools within IPM strategies (1). More than 600 different baculoviruses have been isolated from different insect species. Many of them have a great potential of being used as biocontrol agents. However, maybe less than 20 baculoviruses are being registered and used as biocontrol products worldwide. This presentation will review the strength and weakness, the opportunities and threats of baculoviruses in biological control of insect pests.

Keywords: Integrated Pest Management, host range, virulence, registration, application

References: (1) Eberle, K. E. et al. 2012. Microbial control of crop pests using insect viruses. pp. 281-298. In: (Abrol, D. P., Shankar, U. eds.) Integrated Pest Management: Principles and Practice, CABI, Wallingford, UK.

Phylogeny and Chemical Ecology of Plant-Associated Fungi and their Potential as Biofertilisers and Biocontrol Agents

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Fungi continue to be a rich source of unique secondary metabolites with strong and selective biological activities. Recently, there has been a renewed interest in their exploitation, owing to the lack of efficient antibiotics to combat multi-resistant pathogens and because of innovative approaches based on conjugates of fungal toxins with peptide linkers and antibodies for the development of new anticancer drugs. Many studies on the discovery of novel bioactive agents are now targeting fungal endophytes. However, random isolation/screening campaigns resulted in re-discoveries of trichothecenes and other hazardous mycotoxins, or other compounds that were already known from soil fungi for several decades.

Endophytic and other plant-associated fungi are now also being exploited as biofumigants, biofertilisers and classical biocontrol agents. They often overproduce secondary metabolites that may have detrimental effects on the host plants or even constitute hazardous mycotoxins. Therefore, it pays off to study interesting new fungal strains simultaneously for the production of bioactive metabolites and their antagonistic potential against pathogens. Curiously, even many plant pathogenic fungi have only been studied for phytotoxins, but remain to be screened for beneficial bioactivities. Many new fungal metabolites are still out there to be discovered, but it is very important where and how to look for the yet unexploited sources and how to treat them in the laboratory.

The key to success of this work is taxonomy and phylogeny. This will be demonstrated based on recent findings of our studies of the Xylariaceae, which serve as “model family” to correlate biological and chemical diversity. They are among the most frequently encountered endophytes. Our strategy will be explained, and some recently obtained metabolites from Xylariaceae (1,2,3), as well as other poorly studied plant-associated fungi groups, such as the capnodialean sooty blotch and flyspeck fungi (4), and even the notorious Ash Dieback pathogen, *Hymenoscyphus fraxineus*, (5) will be presented.

Keywords: fungi, endophytes, antibiotics, taxonomy, phylogeny

References: (1) Bills, G.F. et al. 2012. PLoS ONE, 7(10): e46687. doi:10.1371/journal.pone.0046687. (2) Pažoutová, S. et al. 2013. Fungal Divers, 60:107–123. (3) Surup, F. et al. 2014. Mycol Int J Fungal Biol, 5:110–119. (4) Surup, F. et al. 2014. J Agric Food Chem, 62:3525–3530. (5) Halecker, S. et al. 2014. Phytochemistry, 100:86–91.

Metabolomic Changes in Grapevine Leaves of Resistant Varieties after Infection with *Plasmopora viticola*

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Cultivated grapevine is attacked by several fungi; the two most common are *Plasmopara viticola* (grapevine downy mildew) and *Erysiphe necator* (powdery mildew) that arrived in Europe in the 1800s. These fungi require several treatments and can reduce the production and crop quality significantly. Different forms of resistance to natural *Plasmopara viticola* in several wild species from different continents (North America and Asia) were shown. Up to now, the studies on the natural defense mechanisms of the different species have shown that the infection of grapevine results into the production of different metabolites, in particular in the group of polyphenols. In this study, different metabolites in the leaves of plants from different species resistant to downy mildew were defined with markers RPV 1: 3: 10; 12 and analyzed by using metabolomic approaches. The UHPLC/MS/MS methods were applied to study qualitative and quantitative changes of polyphenols (1) and lipids (2) and in grapevine leaves of four resistant varieties (Solaris, Bianca, Jasmine and BC4) induced by the infection with *Plasmopara viticola* pathogen inoculation after 0, 12, 48 and 96 hours. The primary aim of the work was to find early stage biomarkers and to explain differences in metabolomic changes due to different forms of resistance. The results show that the changes in metabolomic profiles after infection varies in the varieties included in the study. This means that resistance to *Plasmopara viticola* defined with different markers RPV 1: 3: 10; 12 results as a consequence of diverse changes of metabolism.

Keywords: *Vitis*, *Plasmopara viticola,* resistance, metabolomics, biomarkers

Reference: (1) Vrhovsek, U. et al. 2012. Journal of agricultural and food chemistry, 60:8831–8840. (2) Della Corte, A. et al, Talanta, http://dx.doi.org/10.1016/j.talanta.2015.03.003.

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Insecticidal Proteins from Mushrooms for controlling the Colorado Potato Beetle

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Colorado potato beetle (*Leptinotarsa decemlineata* Say, CPB) is a major potato pest that has become resistant to various insecticides. Since herbivorous insects depend on the digestive breakdown of ingested proteins for normal growth, development and fertility, several types of protease inhibitors have been investigated for their insecticidal potentials, but with limited success. Proteins from higher fungi have attracted interest because of their exceptional characteristics (1, 2). Recently, new families of the cysteine protease inhibitors mycocypins were identified in edible mushrooms, namely clitocypin from clouded agaric (*Clitocybe nebularis*) and macrocypins from parasol mushroom (*Macrolepiota procera*). They were evaluated for their adverse effects and their mode of action on growth and development of CPB larvae (3, 4). They were shown to reduce survival and slow larval growth when expressed in potato or when their recombinant variants, expressed in bacterial expression systems, were added to the diet. The inhibition of digestive cysteine proteases, intestains, was shown to be the underlying mode of action. Their exceptional trait is lack of effect on gene expression of known adaptation-related digestive enzymes in CPB guts. Mycocypins' advantages also include great resistance to proteolytic degradation, high temperature and extremes of pH. These protease inhibitors from mushrooms are the first fungal proteins shown to have a negative effect on growth and development of CPB larvae and represent important new candidates for effective and environmentally friendly plant protection.

Keywords: crop protection, protease inhibitor, adaptation, transgenic potato, cysteine protease

References: (1) Erjavec, J.et al. 2012. Trends Biotechnol., 30:259–273. (2) Pohleven, J. et al. 2011. Appl Microbiol Biotechnol., 91:1141–1148. (3) Šmid, I. et al., 2013. Agric Food Chem., 61:12499–12509. (4) Šmid, I.et al. 2015. Pestic Biochem Physiol. in press.

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Identification of Host Plant Volatiles from Four Varieties of Caraway (*Carum carvi*) Using Dynamic Headspace Collection

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In many crops, host plant chemistry is known to affect host plant location by pest insects. Among other cues (e.g. visual or acoustic), volatile organic compounds (VOCs) can be used by pest insects to detect a suitable host. In Finland and in the Czech Republic, caraway crops are often under attack by the caraway moth, *Depressaria daucella*. Chemical cues released by *Carum carvi* plants could have an important role to attract adult *D. daucella* in search for oviposition or mating sites. Identification of host plant volatiles from *C. carvi* is thus an important step to develop potential attractants or repellents to manipulate the behaviour of *D. daucella* and its natural enemies. In this study, we measured host plant volatiles produced by four different *C. carvi* varieties commonly grown in the Czech Republic (Prochan, Rekord and Kepron) and in Finland (Sylvia). Plant volatiles were sampled in the laboratory using a dynamic headspace system of odour collection. Most volatiles that we identified using GC-MS are terpenes that are known to be ubiquitous, occurring also in other plants but at different ratios (1). We identified new volatiles that have never been reported to occur in *C. carvi* (2). Germacrene D was shown for the first time to be part of caraway odours, and dominated the blend in all *C. carvi* varieties. (+)-epi-bicyclosesquiphellandrene was detected in most samples and was another sesquiterpene not reported before. The second most dominant volatile was the sesquiterpene β-caryophyllene. Other mono and sesquiterpenes that were previously identified in *C. carvi* (α-pinene, β-pinene, p-cymene, cis- and trans-ocimene, (+)-limonene, α-terpinene, and sabinene) were also found in most varieties, but in small amounts. Overall, the highest variability was observed for relative amounts of β-caryophyllene but were not due to the variety.

Keywords: host plant volatiles, *Carum carvi,* Germacrene D, *Depressaria daucella,* headspace collection

References: (1) Bruce, T. J. A. et al. 2005. Trends Plant Sci. 10: 269-274. (2) Borg-Karlson, A-K. et al. 1994. Phytochemistry, 35:111-119.

Acknowledgement

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Natural Substances Against Bird Damage - Development of a Biological Repellent

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Bird damage to seeds and seedlings of crops is widespread, especially in organic farming because there is no adequate protection. In Germany this especially applies for maize, which is damaged mainly by pheasants (*Phasianus colchicus*), corvids and pigeons (*Columba livia*). A biological repellent could be a measure to avoid such bird feeding similar to chemical seed treatments in the conventional farming. First steps for developing an alternative bird repellent consisted of tests of seed treatments with published substances likely to affect bird feeding behaviour (anthraquinone, pulegone and methyl anthranilate). However, the substances failed to repel feeding by pigeons and pheasants. A systematical screening of various plant extracts has started in 2012 used as treatment of corn seeds. By multiple- and two-choice experiments in aviaries, plant extracts with strong repellent effects on pigeons and pheasants could be identified. Further seedling and field experiments with best substances are still ongoing. Results of the screening of plant extractions will be presented and consequences will be discussed.

Keywords: bird repellent, plant extractions, aviary and field tests

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Session 4: Biotechnology and Plant Breeding for Plant Protection

Functional Analysis of the Roles of Nematode Effectors in the Susceptible Plant-Nematode Interaction

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The potato cyst nematode *Globodera pallida* has biotrophic interactions with its host. The nematode induces a feeding structure – the syncytium – which it keeps alive for the duration of the life cycle and on which it depends for all nutrients required to develop to the adult stage. Interactions of *G .pallida* with the host are mediated by effectors, which are produced in two sets of gland cells. These effectors suppress host defences, facilitate migration and induce the formation of the syncytium. The recent completion of the *G. pallida* genome sequence has allowed us to identify the effector complement from this species. We have examined the expression profiles of all effector candidates using RNAseq; this analysis shows that the majority of effectors fall into one of three clusters of sequences showing conserved expression characteristics (invasive stage nematode only, parasitic stage only or invasive stage and adult male only). In addition, we show that effectors target a diverse range of structures in plant cells, including the peroxisome and the nucleus. We have identified effectors that suppress host defences. Yeast 2 hybrid screens have allowed the identification of potato proteins that are targeted by nematode effectors. This analysis shows that nematodes manipulate host biochemistry to improve the nature of the sycnytium as a food source and that nematodes target similar classes of host proteins to other biotrophic pathogens.

Keywords: Potato cyst nematode, genomics, effectors, cell biology

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Genetic Resources and Plant Breeding

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According to (1) genetic enhancement of crop species has been the major cause of yield progress realised over the last 30 years whereas the contribution of agronomic factors is of minor importance in overall yield progress. Plant breeding is therefore considered a technology that plays a key role in achieving the increase in global food supply by 60% until the year 2050 required to cope with the demands of a growing world population (2, 3). Plant breeding is a complex process composed of three major activities: plant species protection and conservation of genetic resources as public service task, pre-competitive research and competitive breeding activities (4).

Genetic variation is the indispensible basis of breeding progress. Genetic resources are managed in breeding pools, conserved in gene banks or maintained through wild species protection programs. Although the wild relatives of domesticated species are mainly known as important source of resistances genes they are also used to complement the allelic variation in itself of breeding pools to promote breeding progress in yield and quality characters.

This paper will discuss how conservation of and access to plant genetic resources can be improved in Europe and how benefits arising out of the use of these resources can be shared in a way that will assist breeding progress and thereby the development of sustainable agricultural production systems.

Keywords: Plant breeding, genetic resources, germplasm management, Europe

References: (1) Laidig *et al.* 2014. Theo Appl Genet, 127:2599. (2) Hahlbrock 2011. Naturwissenschaftliche Rundschau 64(2):61. (3) Foley *et al.* 2011. Nature 478:337. (4) Neumann 2013. Journal für Kulturpflanzen 65:253.

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Resistance of Wild *Lactuca* Germplasm to Diseases and Pests, and their Exploitation in Lettuce Breeding

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The taxonomy, bio-geography and gene pools, biodiversity and resistance variation of wild *Lactuca* species are described (1, 2, 3, 4). The reported variation in reaction to pathogens and pests of wild *Lactuca* spp. is summarized, including viral pathogens (e.g. Lettuce mosaic virus - *LMV*, Mirafiori lettuce virus /Lettuce big vein virus – *LBV*, Beet western yellows virus - *BWYV*, Tomato spotted wilt virus – *TSWV*, Lettuce necrotic stunt virus – *LNSV*), bacterial pathogens (corky root - *Rhizomonas suberifaciens*, bacterial leaf spot - *Xanthomonas campestris* pv. *vitians*), oomycete and fungal pathogens (downy mildew - *Bremia lactucae*, powdery mildew - *Golovinomyces cichoracearum*, anthracnose - *Microdochium panattoniana*, stemphylium leaf spot - *Stemphylium* spp., sclerotinia drop - *Sclerotinia* spp., verticillium wilt - *Verticillium dahliae*, fusarium wilt - *Fusarium* spp., pythium wilt - *Pythium tracheiphylum*), nematodes (potato cyst nematode - *Globodera rostochiensis*, root-knot nematode - *Meloidogyne* spp., *incognita, hapla, javanica, enterolobii*), insects and mites (the green lettuce aphid - *Nasonovia ribisnigri*, the green peach aphid - *Myzus persicae*, the potato aphid - *Macrosiphum euphorbiae*, leafminer - *Liriomyza* spp.) (1, 2, 4). The approaches used to exploit wild *Lactuca* spp. in lettuce breeding are dicussed and known examples of lettuce cultivars with traits derived from wild *Lactuca* spp. are described (1, 3).

Keywords: Biodiversity, gene pools, *L. serriola*, *L. saligna*, *L. virosa*, breeding strategies, transfer of resistance

References: (1) Lebeda, A. et al. 2014. Eur. J. Plant Pathol. 138:597. (2) Lebeda, A. et al. 2009. Euphytica 170:15. (3) Lebeda, A. et al. 2007. Lettuce (*Asteraceae*; *Lactuca* spp.), p. 377. In: R. J. Singh (Ed.), Genetic Resources, Chromosome Engineering, and Crop Improvement, Vol. 3, Vegetable Crops. Boca Raton: CRC Press, Taylor and Francis Group. (4) Lebeda, A. and Mieslerová, B. 2011. Plant Pathol. 60:400.

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Potato breeding for resistance at the Agricultural Institute of Slovenia

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Potato breeding has a long tradition at the Agricultural Institute of Slovenia since 1947. 15 potato varieties were released until 1990, but after that, most of them were eliminated from the production due to their high susceptibility to $\mathrm{PVY^{NTN}}$. Breeding for extreme resistance to PVY was an obvious choice of the new breeding programme that started in 1993. The main focus of the programme is breeding of varieties adapted to Slovenian growing conditions and customer demands. The scale of the programme is between 10.000 to 20.000 seedlings per year. The crossings are made in screenhouse. Mass selection against potato virus Y is performed after artificial inoculation at the seedling stage, followed by selection for important qualitative and quantitative traits in subsequent 10 field generations. Most of susceptible genotypes are discarded within next two field generations. In the 4th field generation plant material is divided into two groups: first for further selection and screening process for different traits and second for keeping it healthy for possible further multiplication. Healthy plant material is transferred to *in vitro* conditions for multiplication before registration starts. Resistances against potato virus Y (R_{ysto}), potato leafroll virus, potato wart and against yellow and white potato cyst nematodes were introduced into new Slovenian varieties. For late blight major *R* genes were introduced form species *Solanum demissum, S. bulbocastanum* and *R* genes of Sarpo variety group. First new Slovenian late blight resistant variety KIS Kokra was released in 2010.

In the last 12 years varieties Pšata, Bistra, KIS Sora, KIS Mirna, KIS Sotla, KIS Kokra, KIS Mura KIS Krka and KIS Vipava have been bred. They have high yielding potential, excellent quality performance, high resistance level and are among best performing varieties on Slovenian national list.

Keywords: potato, resistance breeding, PVY, late blight, nematodes

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Potato Virus Y Infection Hinders Potato Defence Response and Renders Plants more Vulnerable to Colorado Potato Beetle Attack

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In the field, plants are challenged by more than one biotic stressor at the same time. In the present study, the molecular interactions between potato (*Solanum tuberosum* L.), Colorado potato beetle (*Leptinotarsa decemlineata* Say; CPB), and *Potato virus Y*^{NTN} (PVY^{NTN}) were investigated through analyses of gene expression in the potato leaves and the gut of the CPB larvae, and of the release of potato volatile compounds. CPB larval growth was enhanced when reared on secondary PVY^{NTN}-infected plants, which was associated with decreased accumulation of transcripts associated with the antinutritional properties of potato. In PVY^{NTN}-infected plants, the ethylene signalling pathway induction and induction of auxin response transcription factors was attenuated, while no differences were observed in JA signalling pathway. Similarly to rearing on virus-infected plants, CPB larvae gained more weight when reared on plants silenced in the JA receptor gene (*coi1*). Although herbivore induced defence mechanism is regulated predominately by JA, response in *coi1*-silenced plants only partly corresponds to the one observed in PVY^{NTN}-infected plants, confirming the role of other plant hormones in modulating response. The release of β-barbatene and benzyl alcohol was different in healthy and PVY^{NTN}-infected plants before CPB larvae infestation, implicating the importance of PVY^{NTN} infection in plant-to-plant communication. This was reflected in gene expression profiles of neighbouring plants showing different degree of defence response. The present study thus contributes to our understanding of plant responses in agro-ecosystems.

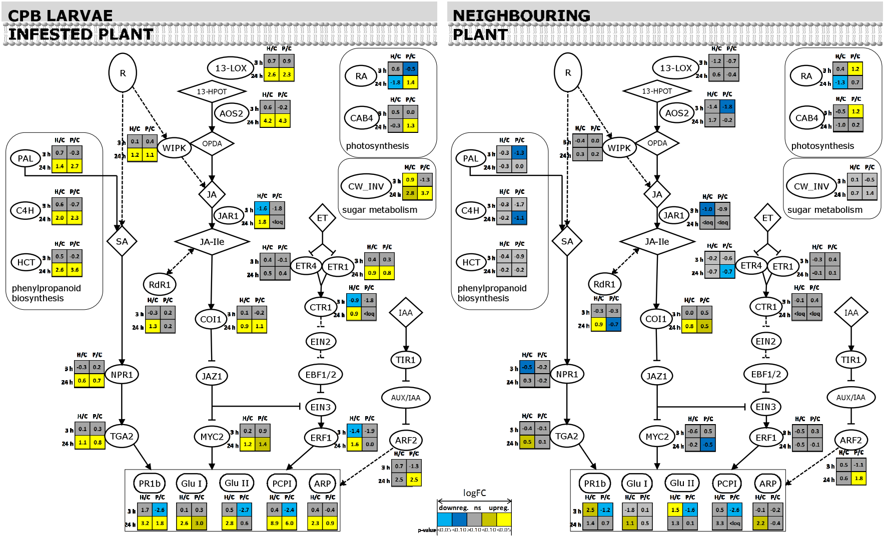

Figure 1: Differential expression of genes involved in plant defence responses in PVY^{NTN}-infected and healthy cv. 'Igor' potato plants after infestation with CPB larvae. H/C, comparison between CPB-larvae-infested healthy and non-infested healthy control plants; P/C, comparison between CPB-larvae-infested PVY^{NTN}-infected and non-infested PVY^{NTN}-infected control plants.

Keywords: Leptinotarsa decemlineata; *Solanum tuberosum* (potato); insect midgut adaptation; gene expression; plant defence

Use of Proteomics in Breeding for Drought Tolerance in Common Bean

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Drought is an abiotic stress that strongly influences growth, development and productivity of common bean. Sixty percent of the world's common bean grows under drought conditions and drought causes yield losses up to 80% in some regions. The mechanism of drought stress response in common bean has not been intensively studied therefore it is still not well characterized due to genetic complexity of drought response and tolerance. Understanding the response of common bean to drought is the first step in the breeding of tolerant cultivars. This study is focused on the improvement of drought tolerance in common bean with a proteomic approach. Research on plant proteomes has provided beneficial information for a comprehensive understanding of the protein networks in plants in response to external stimuli. The aim was to analyse drought-responsive proteins in leaves of two cultivars differing in their response to drought, Tiber and more sensitive Starozagorski čern. Differences in protein abundance between control and stressed plants were compared. Fifty-eight proteins whose abundance changed significantly were identified by mass spectrometry in Tiber and 64 in Starozagorski čern. The majority of identified proteins were classified into functional categories that include energy metabolism, photosynthesis, ATP interconversion, protein synthesis and proteolysis, stress and defence related proteins. Based on our results, certain identified proteins could be used as markers in the selection process for drought tolerance in common bean. The most suitable proteins would be those, exhibiting contrasting abundance patterns between cultivars, such as oxygen evolving enhancer proteins. To conclude, the application of proteomic technologies is useful in revealing the plant mechanisms to drought stress response, tolerance and identifying candidate biomarkers for use in molecular breeding strategies. The integration of proteomics into the field of crop science is helping to enrich genome annotation efforts and accelerate the improvement of crop productivity.

Keywords: proteomics, drought stress, *Phaseolus vulgaris*, drought tolerance

Acknowledgement

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Session 5: Prognosis and Decision Supporting Systems

Development and Delivery to Growers of Decision Support Systems for Sustainable Crop Protection

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Several Decision Support Systems (DSSs) have been developed for crop management but these DSSs have contributed little to practical agriculture because of the so-called 'problem of implementation' which is under-utilization due to both technical limitations of the DSSs and to farmer attitude towards new technologies. The implementation problem has to be specifically addressed in the development of DSSs. A recent DSS for sustainable management of vineyards, named vite.net®, is briefly described with focus on actions taken to increase its practical implementation. The DSS is intended for the vineyard manager, i.e, the grape-grower or the advisor suggesting to him the proper actions about the vineyard management; the information provided by the DSS is tailored to a vineyard, or part of a vineyard, or a number of vineyards that are uniformly managed throughout the season. The DSS has two main parts: (i) an integrated system for real-time monitoring of the vineyard components (air, soil, plants, pests, and diseases) and (ii) a web-based portal that analyses these data by using advanced modelling techniques and then provides up-to-date information for vineyard management in the form of alerts and decision supports. The DSS: (i) focuses on the important vineyard problems with a holistic approach (the DSS incorporates overall management solutions for viticulturists); (ii) uses automation and integration in data collection, and supports flexible input efforts by the user; (iii) contains fit-to-purpose, mechanistic, dynamic models; (iv) has a user-friendly interface and provides complete and easy-to-understand information; (v) is delivered through the Internet and thereby enables both continuous updating by the provider and flexible access by the user; (vi) assists the decision makers (by providing necessary information) rather than replacing them; vii) combines "push" and "pull" communication modes with the end-users. Potential end-users were involved during the DSS development and testing so as to obtain insight into how users make decisions. In addition, the benefits of the DSS were efficiently communicated to end-users via seminars and visits to demonstration vineyards.

Keywords: Grapevine, disease modelling, crop protection, decision making

References: (1) Rossi, V. et al. 2012. Phytopathologia Mediterranea, 51: 457-479. (2) Rossi, V. et al. 2010. In: Oerke, E.C. et al. (Eds.), Precision Crop Protection - The Challenge and Use of Heterogeneity. Springer, Dordrecht, The Netherlands, 241–258. (3) Rossi, V. et al. 2014. Computers and Electronics in Agriculture, 100: 88-99.

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A Generic Decision Support System for Integrated Weed Management

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Since 1987, Aarhus University (AU) in Denmark has designed a 'decision support system' named 'Crop Protection Online – weeds' (CPO-weeds). CPO-weeds is targeted mainly for farmers and crop advisors to promote integrated weed management (IWM). CPO-weeds now includes all herbicides in 32 crops and 105 weed species. More than 2,000 field validation trials have been executed to test different prototypes in different crops, and the presently released version has demonstrated robust weed management and 20-40% potential of reducing input of herbicides.

The user supports a field report, and CPO-weeds returns a list of treatment options, integrating legal restrictions. In Denmark, 350 Danish crop advisors (100%) and about 700 farmers (3%) subscribe to CPO-weeds, and educational institutions have integrated CPO-weeds in the examination criteria. Consequently, CPO-weeds may be perceived as a professional point of reference.

A professional version has been released also in Norway, and presently, prototypes are in field validation in Germany, Poland, Spain, Italy, Slovenia and China. Provisional results from validation trials indicate robust weed management and potentials for reduction of herbicide use. These results also indicate that CPO-weeds possess generic qualities, which may be suitable and potent for upscaling. These prototypes also integrate new designs for integration of mechanical weed control and measures to prevent/delay herbicide resistance. Including these developments, CPO-weeds specifically addresses 7 of 8 general principles as listed in Directive 2009/128/EC (IPM).

Decision Support Systems for German Vegetable Growers

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Decision support systems (DSS) play an important role in integrated pest management systems. With the help of DSS the use of pesticides can be avoided or reduced to a minimum. At the moment, DSS are offered only for a few pests and diseases to German vegetable growers and advisors (www.zepp.info). Simulation models are available for three major insect pests (*Delia radicum* L. - cabbage root fly, *Chamaepsila rosae* (Fabricius) – carrot fly and *Delia antiqua* (Meigen) – onion fly) and for two severe diseases (*Peronospora destructor* (Berk.) Caspary – downy mildew on onions and *Stemphylium vesicarium* (Wallr.) E.G. Simmons – purple spot on asparagus). The DSS for root flies simulate the population dynamics of these pests for the four different developmental stages. Thereby advisors and growers receive information on the time period and the intensity of the occurrence of the pests. This information can be used for sending forecasting reports, targeted insecticide application etc. For *Stemphylium vesicarium* on asparagus the probability of the disease severity value and for *Peronospora destructor* on summer onions the sporulation and infection risk are calculated. At present, further DSS systems are developed for white fly control in tomato crops (1, 2) and wire worms (3). Furthermore, investigations on the automatic detection of pests in cabbage crops (esp. *D. radicum* and different Lepidoptera species) are running in the framework of a federal research project (www.wega-online.org). Comparing the number of available DDS systems for German growers and advisors with the large number of pests and diseases which regularly threaten the crops and have to be controlled by pesticides this is only a very small proportion. Therefore, an urgent need exists to fasten the development of more DSS to promote the implementation of IPM-tools in practise.

Keywords: Decision support systems, *Delia radicum*, *Chamaepsila rosae, Delia antiqua, Peronospora destructor, Stemphylium vesicarium*

References: (1) Böckmann. E. et al. 2014. Julius-Kühn-Archiv, 447, 302-303. (2) Böckmann. E. et al. 2014. Journal of Pest Science DOI 10.1007/s10340-014-0601-7. (3) Jung, J. et al. 2014. Journal of Applied Entomology, 138, 183-194.

Application of remote sensing imagery in precision agriculture

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Remote sensing is a group of non-invasive data acquisition methods, where we collect data passively (e.g. by using sunlight) or actively (e.g. by using lasers) from up to several kilometers away. In imagery remote sensing we use imaging sensors, which divide the light spectrum in bands of a given size. In this regard we refer to broad- (e.g. multispectral) and narrow-band (e.g. hyperspectral) sensors. Information technologies enable us to integrate remote sensing imagery with data from other sources (e.g. GIS), thus allowing for precision agriculture. High spatial resolution remote sensing data is a cost effective and fast method of mapping spatial variations in crop productivity, disease presence and spread, and environmental and physiological conditions. This data then enables managers to improve management strategies and react to the presence of diseases and stressors in a timely manner. Farming practices can be changed throughout the season, maximizing profit while minimizing costs. To illustrate this point we provide three examples of remote sensing precision agriculture in vineyards. The first is a model using multispectral satellite imaging for identifying grapevines infected with grapevine yellows. Second is a model for determining the amount of sugar in grapes, and thirdly, first results of identifying rotting grapes without visible signs of decay. These last two models use hyperspectral imaging data.

Keywords: remote sensing, multispectral, hyperspectral, precision agriculture, grapevine yellows, chemometrics

Acknowledgment

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Session 6: **IPM and Good Agricultural Practice**

How Agro-Ecological Research Helps to Address Food Security Issues under new IPM and Pesticide Reduction Policies for Global Crop Production Systems

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Food security issues and associated drivers are discussed in relation to food losses caused by pests and crop protection issues. Key drivers include rapid human population increase, global climate change, loss of beneficial on-farm biodiversity, reduction in per capita cropped land, water shortages in some areas and EU pesticide withdrawals under policy 91/414 EEC, together with related follow-on Directives and Articles, including 2009/128/EC on sustainable use of pesticides. IPM (Integrated Pest Management) is compulsory for all EU agriculture from 2014. IPM offers ‘toolboxes’ of complementary crop- and region-specific solutions to address these pressures and aims for more sustainable solutions by using complementary technologies.

IPM is compatible with organic, conventional and GM cropping systems and aims to reduce pests below economic thresholds by utilising key ‘ecological services’ (ES), particularly biocontrol. A review for several major crops covering 26 countries indicates that IPM can reduce pesticide use and also increase yields in most cases studied. Landscape scale ‘ecological engineering’ research, together with public and private sector crop development is producing more durable pest-resistant cultivars (conventional and GM), novel semiochemicals, biopesticides, precision pest monitoring tools based on ‘biomimicry’, molecular diagnostics and predictive spatio-temporal models. These are being tested as components of novel ‘IPM toolboxes’, using multi-disciplinary research and modelling at a farm and landscape scales. Integrative and synergistic use of existing (pest-resistant cultivars, pheromones, biocontrol agents) and new crop protection technologies (e.g. biopesticides, GIS-based regional monitoring, stacked pest and disease resistance traits) will help meet future food production needs in developed and developing countries. Examples of IPM for soft fruit and vegetable brassicas will be provided.

Keywords: Ecological services, food security, Integrated Pest Management, landscape ecology, pesticides

References: (1) Birch, A.N.E. et al. 2011. J Exp Bot. 62: 3251-3261. (2) Bjorkman, M. et al. 2011. Phytochemistry, 72: 538-556. (3) Birch, A.N.E. et al. 2007. Pedobiologia, 51: 251-260.

Acknowledgement

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Non-targeted Side Effect of Microbial Fungicides on Grapevine: Fact or Fantasy

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Microbial biocontrol agents are commonly regarded as environmentally friendly plant protection products compared to synthetic chemicals, although this does not mean there are no potential hazards associated with them. Based on this concept, the EU Regulation 1107/2009 (1) requires eco-toxicological studies and the evaluation of the fate in the environment for all plant protection products, including microorganisms. When the active ingredient is a microorganism additional questions related to its potential ability to multiply and spread in the environment are often posed by the evaluators. In particular the impact of the application of a microbial fungicide on natural microbial communities, either in soil or phyllosphere, is often questioned. Natural microbial communities in the environment are highly variable and the most competitive species that have adapted to that niche commonly prevail. And so most of the studies report that the release of a microbial fungicide has negligible or only transient effect on resident microflora. In particular the effect of the introduction of a microbial fungicide is expected to be minimal on carbon and nitrogen cycles and never greater than the application of manure or compost. Assessment of the impact on birds and other terrestrial vertebrates, aquatic organisms, arthropods, non-target soil meso- and macrofauna, terrestrial non-target plants or any other plants would be warranted on a case-by-case basis, however, also thanks to the mechanism of action of most of the microbial biocontrol agents and their fate in the environment, almost no observation of impact were reported so far. In case of wine making, if the microbial biocontrol agent can grow in the must and exhibits antagonistic activity against *Saccharomyces cerevisiae,* then possible interference with the fermentation cannot be excluded, therefore specific tests could be necessary in this context. Although the precautionary principle shall always be applied in the registration of a new compound either synthetic or natural, based on the existing scientific literature and the mechanism of action the risk of unwanted side-effects of the application of several microbial fungicides seems to be more phantom than reality. Specific case studies on *Trichoderma atroviride* and *Lysobacter capsici* on grapevine will be discussed.

Keywords: fate in the environment, microbial populations, fermentation, ecotoxicology, *Lysobacter capsici, Trichoderma atroviride*

References: (1) Official Journal of the European Union, 24.11.2009, L 309/1

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Population Dynamics of *Globodera pallida* in Relation to Temperature

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Potato cyst nematodes (PCN) are persistent and economically important pests of potato with widespread distribution in potato growing areas worldwide. In the EU, the European Council Directive 2007/33/EC aims at preventing further spread of PCN and controlling existing populations. Resistance can be highly effective in suppressing PCN however many popular varieties are susceptible to either or both species and commercial pressures have a strong influence on cultivar selection. Management of PCN thus generally involves using a combination of strategies, and in the UK this often includes the use of nematicides.

An important factor in PCN multiplication is soil temperature but the relationship between PCN multiplication in the field and soil temperature is not well characterised. We have been undertaking experiments in controlled environments and in the field to examine this relationship with the aim of developing risk models for PCN, particularly with regard to 2 generations of PCN developing within one crop cycle.

Average soil temperatures in potato drills differ between geographic locations and growing seasons in the UK. Our results indicate that both species of PCN, *Globodera rostochiensis* and *G. pallida*, hatch more and develop more quickly as temperatures increase (1). Current average soil temperatures in potato drills in the UK are below the optima for multiplication of both species indicating that higher population multiplication could occur if the trend for increasing soil temperatures continues. The implications of this for different management strategies need to be considered.

Keywords: climate change, nematodes, risk model, management

References: (1) Kaczmarek, A. et al. 2014. *Phytopathologia Mediterranea* 53: 248-257.

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Sustainable Territorial Wines (STW): Field-Scale Application of DSS and Best Practices for Plant Disease Management in Friuli Venezia Giulia Region

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Environmental concern and awareness are nowadays particularly considered by the consumers and the general public, thus also the new EU Directive 2009/128/EC moved towards this direction. Precise monitoring of pest and diseases during the season allows growers to manage properly the pesticide strategies in the vineyard, and the application of modelling helps to choose the best timing for spraying. A three-year-work-project was carried out in collaboration with winegrowers of the main wine-production areas of Friuli Venezia Giulia, applying the most updated knowledge concerning integrated and organic pest management on 345, 611 and 741 hectares in 2012, 2013 and 2014, respectively. The method was based on the application of: i) the precise monitoring of pest/diseases with a geo-referenced database, ii) the Decision Support System vite.net® (www.horta-srl.com) for diseases epidemiology and management, iii) field check of sprayer efficacy, and iv) selection of active substances based on environmental risk and efficacy. During the three years of investigation, the application of the Sustainable Territorial Wines (STW) method allowed to decrease the number of sprayings by 3 and 2 in case of integrated and organic vineyard management, respectively, as compared with the baseline strategies usually applied by the winegrowers in the same area. The pesticides less impacting human health were used (lower toxicology class: harmful and toxic pesticides were excluded) and their rates optimised based on disease pressure and phenological stage. Sprayer efficacy was evaluated in 120 field checks using water sensitive paper, positioned in different parts of the canopy: in this way, the distribution of pesticides was improved for the different canopy situations by selecting proper nozzles and machine working conditions. In summary, STW allowed growers to reduce the cost for pesticide application of about 190 €/ha or 150 €/ha in case of integrated and organic pest management, respectively.

Keywords: integrated pest management, organic pest management, sprayer efficacy, DSS, pesticide rates

Acknowledgement

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Biodiversity & Runoff Best Management Practice for Protection and Productivity

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Agricultural Best Management Practices (BMP) like Operation Pollinator (OP) and MARGINS (Managing Agricultural Runoff Generation into Surface Water) projects are dedicated to achieve environmental goals; biodiversity enhancement, runoff and soil erosion management in agricultural landscape. These practices are met with increased farming productivity while generating public goods.

Syngenta initiated 700 ha OP fields between 2009-2014 on agricultural areas in Hungary, Croatia, Greece to demonstrate OP field effectiveness in biodiversity enhancement. Monitoring results demonstrated improvement of species diversity and abundance of Hymenoptera pollinating insects. A higher abundance of Apidae (6-12 fold), Bombidae (3-20), Megachiliadae (2-13), Andrenidae (5-20) was detected on OP fields compared to current agricultural ruderal margins. Flower rich plant mixtures of OP were developed based on local plant species which provided season long and multi-year food sources (nectar, pollen) and nesting sites for pollinators. Cutting regime was developed to sustain multi-year habitat for pollinators. Monitoring proved that on OP fields biodiversity increased to levels above the existing agricultural margins (3).

In the MARGINS project vegetative buffer zones (VBZ) were used to reduce runoffs, soil erosion and related agrochemical runoffs from agricultural fields to surface water. In a long term research in Hungary, the role of VBZ proved to reduce runoffs by 10-75%, soil erosion by 50% and pesticide runoff by 75%. (1, 2). MARGINS is a concept of multifunctionality for biodiversity and runoff management.

Operation Pollinator and MARGINS demonstrate that land use following BMP principles can integrate crop production needs with soil, water and biodiversity protection within fields, vineyards and orchards. Agri-environment funding will support the implementation of BMPs and achieve some key objectives of Common Agricultural Policy reform in EU (4).

Keywords: pollinators, Hymenoptera, runoff, erosion, margins

References: (1) Dyson, J. 2011. XIV. Symposium in Pesticide Chemistry, Piacenza. (2) Balázs, M. et al. 2011. Hungarian Geographical Bulletin 60 (2): 117-133. (3) Batary, P. et al. 2010. Agriculture, Ecosystems & Environment. 136(1-2): 35-39. (4) Kertesz, A. et al. 2014. International Soil and Water Conservation Research 2(1): 91-96.

Acknowledgement

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POSTERS

P1 RNAseq Analysis of the *H. schachtii* Transcriptome Reveals New Putative Effectors

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The beet cyst nematode (BCN) *Heterodera schachtii* depends on a set of effectors for the induction and maintenance of the syncytium inside the root. However, little is known about the type and role of such effectors in the process. The identification of new putative secretory proteins (PSPs) can lead to the discovery of new BCN effectors. We used RNAseq analysis to identify PSPs that have their transcripts up-regulated during the post infective stages, since such proteins are expected to play a role in the process of parasitism. By comparative analysis with other available plant-parasitic nematode sequences, we further selected for plant-parasitic nematode specific PSPs (PP-PSPs). The annotation of the identified PP-PSPs showed enrichment in particular gene ontologies, such as metabolic and catalytic protein activities in addition to those regulating cell growth and development. In order to validate PP-PSPs, we are using *in situ* hybridization to verify PP-PSP gene expression within the esophageal glands. The gene knock-down approach by RNAi is being used to determine the effect on infectivity of *H. schachtii*. Our results can lead to the identification of new types of effectors important for the parasitism strategies of BCN in particular and cyst nematodes in general.

Keywords: beet cyst nematode, putative secretory proteins, nematode gene expression

P2 Tracking Within-Plant Virus Population Diversity by Next Generation Sequencing

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RNA virus populations are one of the fastest evolving biological systems. They exist as a cloud of diverse sequences (quasispecies) (1). Using next generation sequencing (NGS), variability of viral populations has been investigated in-depth for few important human and animal viruses, but little research has been conducted on plant viruses. The focus of our research is *Potato virus Y* (PVY), a single stranded (ss)-RNA virus. Sequences corresponding to ss-RNA viruses infecting plants constitute at least three distinct, but interconnected pools: (I) ss-RNA molecules packed in viral particles, (II) double stranded (ds)-RNA molecules formed during replication and (III) virus derived small interfering RNAs (vsiRNAs) (2). We hypothesized that sequence diversity between these pools is similar but could slightly differ due to the errors or genetic bottlenecks introduced during viral cycle. Illumina deep sequencing of two different pools of viral sequences was employed: vsiRNAs (3) and ss-RNA isolated from purified viral particles (4). The data was analysed to search for variants present in each of the two pools. The results show highly similar mutational landscape of the within-host virus population between both pools, with no notable hotspots across the viral genome. Nevertheless, some differences were observed between the two sequence pools - small RNAs showing higher level of variation. Further experiments would be needed to elucidate their exact origin. Sequencing of viral particle pool enhanced the efficiency of consensus viral genome sequence reconstruction. Non-homologous recombinations were commonly detected in viral particle pool, with a hotspot in 3` untranslated and coat protein region of the genome. The methods tested in this study will serve as a baseline for the future research, which will be focused on dynamics of within-plant PVY population structure during adaptation to different potato cultivars.

Keywords: plant virus, quasispecies, NGS, small RNAs, viral particles, SNPs, recombinations

References: (1) Domingo et al., 2012. Microbiology and Molecular Biology Reviews, 7:159-216. (2) Mlotshwa et al. 2008. Trends in Plant Science, 13:375–382. (3) Kreuze et al. 20090. Virology, 388:1-7. (4) Rupar et al. 2012. Journal of Chromatography A, 1272:33-40.

Acknowledgement

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P3 PR-1 Gene is Up-Regulated in Tomato Roots During Antagonistic Interaction Between Pathogenic *Agrobacterium tumefaciens* and *Meloidogyne ethiopica* Root-Knot Nematode

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Crown gall-causing bacteria (*Agrobacterium* spp.) and root knot nematodes (*Meloidogyne* spp.) are economically important plant pests. They share a similar host range and similar parasitism/disease symptoms resulting from water and nutrient flow interference in host plants. According to many field and pot trial results, the synergism is most likely involved in the *Agrobacterium-Meloidogyne* interaction (1, 2), although one study describes it as antagonistic (3). However, the host defence responses to bacterial infection and nematode infestation are different. In a pot trial study we observed the interaction between a population of *M. ethiopica* (4) and a strain of pathogenic *A. tumefaciens* on roots of RKN susceptible tomato hybrid. In one observation, the reproduction of *M. ethiopica* was significantly lower after1st reproductive cycle (two-fold, $p<0.001$) when nematodes were added to the roots previously colonized by *A. tumefaciens*. To discern the plant defence pathway responsible for antagonistic effect we conducted a relative gene expression study (qPCR) using SYBR Green I chemistry. We selected six target genes involved in either systemic acquired resistance (SAR) or induced systemic resistance (ISR) to determine which pathway is altered in parts of tomato roots not in a direct contact with both studied organisms at 0, 4 and 12 dpi. We used a unified Wilcoxon-Mann-Whitney test for the Cq data analysis (5) and four reference genes for data normalization. The target gene PR-1 for pathogenesis-related protein 1 had the highest up-regulation at 12 dpi in the treatment where antagonism between *A. tumefaciens* and *M. ethiopica* was observed in the pot trial. As PR-1 is involved in salicylic acid defence response we propose that the observed low reproduction of *M. ethiopica* on *A. tumefaciens*-colonised roots could be the result of activated SAR pathway in tomato roots.

Keywords: *Agrobacterium, Meloidogyne,* tomato, interaction, PR-1, qPCR

References: (1) Süle, S. et al. 1995. J Phytopathol, 143: 169-171. (2) Elwakil M. A. et al. 2003. Pak J Plant Pathol, 2, 1: 1-9. (3) Fakhouri, W. D. et al. 1996. Pak J Nematol, 14, 1: 49-54. (4) Širca, S. et al. 2004. Plant Dis, 88: 680. (5) De Neve, J. et al. 2013. Stat Appl Genet Molec Biol, 12, 3: 333-346.

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P4 Gene Expression in Necrotic Lesions of PVY^{NTN}-infected Potato Tubers

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Tuber necrotic strain of *Potato virus Y* (PVY^{NTN}) causes potato tuber necrotic ringspot disease in the susceptible potato cultivars. Infected plants develop mild to severe mosaic on leaves and necrotic ringspots on tubers. The quality and quantity of the yield is decreased. Development of tuber necrosis was observed to be inhibited by storing the tubers at low temperature (4°C) in the first months after harvest (1). The aim of our study was to evaluate gene expression in PVY^{NTN} infected tubers stored at different conditions in order to aid better understanding of mechanisms underlying tuber necrosis development.

Study was performed on potato tubers of Slovenian cultivar Igor highly susceptible to PVY^{NTN}. Tubers of different health status, stored at different time intervals and temperature conditions, were sampled for microarray analysis. Potato genome 44K POCI microarrays were used to determine the differentially expressed (DE) genes between necrotic and non-necrotic tissue.

Overview of the DE genes was performed using MapMan Ontology and Wilcoxon Rank Sum Test (2). The most significant differential expression belonged to biotic stress and pathogenesis-related proteins (proteinase inhibitors), secondary metabolism of phenylpropanoids (lignin biosynthesis) and several signalling, regulatory and defensive (kinase activity regulation, cell wall degradation) functions.

High differential expression of five genes determined by microarrays was confirmed with reverse transcription quantitative real-time PCR (RT-qPCR). Selected genes were involved in biotic stress response (respiratory burst oxidase homolog), hormone metabolism (salicylic acid carboxyl methyltransferase), protein modifications (and Avr9/Cf-9 induced kinase 1), signalling (phosphate induced protein 1) and cell wall degradation (tumour-related protein).

Keywords: Potato Virus Y, PVY, potato, tuber necrosis, differential gene expression

References: (1) Dolničar, P. et al. 2011. Am. J. Potato Res., 88:318-323. (2) Ramšak, Ž. et al. 2014. Nucleic Acids Res., 42(D1):D1167:D1175.

Acknowledgement

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P5 The Biochemical Mechanisms of Defence of Maize Leaf on Herbivore Pest Insects Attacks

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Interaction between host plant and insects is a dynamic system and also co-evolutionary process. That system involves development of the defence mechanisms by plants, as well as, counter adaptations by the herbivore insects. Defence mechanisms involve either biological barriers or biosynthesis of phytochemicals, which act as phagodeterrents, repelents or even as toxins. Plant secondary compounds may be induced by herbivore attack and these may vary with the herbivore species and the amount of leaf area removed in each plant species. Different phenolic compounds are considered to play an important role in plant defence mechanisms (1). Maize is one of the most important crops in Europe. The most important arthropod pest of maize in Europe is the European corn borer, *Ostrinia nubilalis* (2). Pests of importance for central and south-east Europe included in this bioassay are western corn rootworm (*Diabrotica virgifera*), cotton bollworm (*Helicoverpa armigera*) and cereal leaf beetle (*Lema melanopus*). Objectives of this project were to determine whether these insect herbivores induce biosynthesis of different plant phenolics in maize leaves and then to compare it with antioxidant capacity of plant samples. Assays were conducted in year 2014 in the field condition near the town of Bečej, province of Vojvodina, Serbia. Maize cultivar used for this assay was NS 640. Plants respond to physical and chemical changes associated with insect feeding through accumulation of phenolic compounds (total phenolics, tannins and flavonoides). Punithavalli et al. (3) also observed that there was increase of concentrations of different phenolic compounds in rice plants after insects attack. Concentration of the total anthocyanins in leaves of maize was not affected by the biotic stress induced by herbivore insects. Phenolic compounds were reported as antioxidant factors involved in oxidative stress reactions caused by different biotic and abiotic factors. Antioxidant capacity of maize leaves extracts was assessed by 5 different methods: DPPH radical scavenging test, ABTS, FRAP, phosphomolybdenum method and NBT test. A positive relationship between antioxidant activities and total phenolic contents was observed. Our results demonstrate that attack of herbivore insects (*D. virgifera, H. armigera* and *L. melanopus*) induce biosynthesis in maize leaves and elevate antioxidant capacity.

Keywords: antioxidant capacity, herbivore insects, maize, phenolic compounds

References: (1) Shahida Perveen, S. et al. 2001. OnLine Journal of Biological Sciences, 1:496. (2) ENDURE, 2012. Final report on the Maize Case Study: Key pests and options to reduce pesticides in eleven European regions. (3) Punithavalli, M. et al. Rice Science, 20:363.

P6 The Impact of the Reduced Amount of Mesotrione on Photosynthetic Pigments Content According to the Irrigation Water Quality

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After application to plants or soil, pesticides used in agriculture for insects and weed control, can migrate into surface water and groundwater (1). The highest percentages of cultivated plants are irrigated using water from canals, and the presence of herbicides and their metabolites may cause damage of the plant population. The samples for water analyses were taken from the Danube Tisa Danube canal from the locality Vrbas (45°34′10″N/ 19°38′16″E). A multi-residue method was used to determine presence of twenty-one herbicides and their transformation products with isoproturon-d6 and atrazine-d5 as internal standards in surface water using liquid chromatography tandem-mass spectrometry (LC-MS/MS) with ESI (2, 4). Metamitron, terbuthylazine, atrazine, prometryn and metolachlor as well as terbuthylazine-desethyl (metabolite of terbutilazine) were detected. *Ambrosia artemisiifolia* L., invasive weed species, was grown in the laboratory in controlled conditions. For its irrigation canal water collected during the months of May, June and July was used. Control was irrigated with distilled water. Plants were treated with a reduced dose of mesotrione. The content of photosynthetic pigments (chlorophylls a, b and carotenoids) as a response to irrigation with polluted water, was determined (3). The detected residues of terbutilazine-desethyl and prometryn were above in all water samples. The quantities of photosynthetic pigments (chlorophyll a and carotenoides) were higher in the measured samples irrigated with water samples with detected herbicide residues (metolachlor, terbutilazine and atrazine) and their metabolites (terbuthylazine-desethyl) in comparison with control.

Keywords: mesotrione, *Ambrosia artemisiifolia,* photosynthetic pigments, herbicide residues, irrigation water.

References: (1) Ismail, B.S. et al. 2012. International Journal of Basic & Applied Scineces, 12, 6, p.85-90. (2) Milutinovic, V. et al. 2012. , Annual MGPR Meeting 2012 and International conference on food and health safety: Moving towards a sustainable agriculture, Belgrade, p.74. (3) Weltsttein, D., 1957. Exp.Cell Res. 12, p.437-440. (4) Official Journal of the European Union, 2008: Directive 2008/105/EC of the European parliament and of the council. Available at: http://faolex. fao.org/ docs/ pdf/eur84568.pdf

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P7 Influence of Herbicides on Chlorophyll Fluorescence in *Ambrosia trifida* L.

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Since the mid-20th century, *Ambrosia artemisiifolia* and *Ambrosia trifida* have migrated to farm fields and early successional sites in Europe, South America, and Asia. *A. artemisiifolia and A. trifida* are among the most competitive weed species for summer crops. *A. trifida* has caused corn and soybean grain yield losses ≥50%. The goal of our research was to test the influence of herbicides on chlorophyll fluorescence as a reaction of *Ambrosia trifida* L. to stress conditions. The trials were carried out under field conditions. The seed of *A. trifida* was collected in 2010. Plants were treated at 2 leaf growth stage with compounds containing active ingredients e.g. glyphosate, dicamba, clopyralid, 2.4-D, tembotrione + isoxadifen-ethyl and imazamox. Fluorescence parameters (basic fluorescence Fo, maximum fluorescence Fm, relationship between variable and maximum fluorescence Fv/Fm and effective yield of chlorophyll fluorescence Φ_{FSII}) were measured at 18, 42, 66 and 90 hours after application. Analysis of results showed that the strongest effect on *A. trifida* was caused by tembotrione + isoxadifen-ethyl. The most reliable evaluation parameter was variable fluorescence with value 3.5 times lower compared to the value measured in untreated plants 90 hours after application of tembotrione + isoxadifen-ethyl. Parameter Fv/Fm 90 hours after application was 0.279±0.277, which confirmed highest susceptibility of this weed species to tembotrione + isoxadifen-ethyl. Parameter Φ_{PSII} showed to be the least reliable one because values measured after all compound applications were not significantly different compared to the untreated values. That is the reason we classify this parameter as unreliable for stress measurement in *A. trifida* after herbicide applications. Chlorophyll fluorescence is fast, sensitive and reliable method, but what has to be taken in consideration is the fact that there are plant populations which get over the stress through different mechanisms. In those cases there are no differences in fluorescence yield and photosynthesis which will be the subject of our research.

Key words: chlorophyll fluorescence, photosynthesis, *Ambrosia trifida* L.

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P8 Healthy Plants as a Criterion for Entering Foreign Markets

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Under the auspices of the World Trade Organisation the flow of legitimate trade in plants and plant products is facilitated by several agreements. However, the Agreement on Sanitary and Phytosanitary Measures (SPS measures) enables that countries implement food safety, animal health and plant health regulations in benefit of agriculture, nature, economy, and people. Based on SPS measures harmful and non-compliant goods can be intercepted and deterred already at external state border, while compliant and secure trade is rapidly admitted to inspection and customs formalities and released to the market without disruption. Owing to the increased international trade through the past decades, the emerging food safety and phytosanitary risks have been more and more difficult to mitigate. Constant interceptions of new and regulated harmful organisms show increasing trends in vegetables, herbs and fruits. Despite these commodities had been officially certified in the country of origin to contain no quarantine pests, 1885 consignments from 41 countries were intercepted at the European Union Border Inspection Points (Anon., 2014):

- Tephritidae fruit flies (482) on *Mangifera* (234), *Momordica* (62), *Psidium* (32), *Annona* (17) and other fruits (*Luffa, Langenaria, Capsicum, Manilkara, Trichosanthes, Syzyngium, Trichosanthes*);
- Aleyrodidae white flies (290) on *Ocimum* (136), *Corchorus* (63) and other herbaceous plants (91);
- Agromyzidae leaf miners (105) on *Gypsophila* (42) and other herbaceous plants (*Apium, Artemisia, Coriandrum, Chrysanthemum, Solidago, Eryngium, Trachelium, Ocimum*);
- *Frankliniella*, or other Thripidae thrips (350) on *Momordica* (169), *Luffa* (140), *Solanum*, *Orchids*
- *Anoplophora*, *Monochamus* and other longhorn beetles on wood packing material (72) and on other wood and bark products (176);
- Other pests: *Thaumatotibia leucotreta* (150) on *Capsicum* and *Citrus*; *Spodoptera litoralis* (27) on *Rosa* cut flowers; *Anthonomus eugenii* (14) on *Capsicum*; Psyllidae (15) on *Murraya*, etc.
- Plant diseases: *Phyllosticta citricarpa* (56), *Xanthomonas axonopodis* pv. *citri* (35), *Xanthomonas campestris* pv. *vesicatoria* (6) and Plum Pox Virus (18) on planting material.

The Port of Koper is Slovenia's principal EU entry point with significant imports of fruits and vegetables. Development of the information and other tools together with training that improves risk analysis, targeting, and inspection are necessary to keep the safety and security of trade, and for enhancing the competitiveness of agriculture and food industry. Bilateral agreements as the Transatlantic Trade and Investment Partnership that is currently the subject of negotiations between the US and the EU shall facilitate the market access for plants and plant goods also with efficient procedures and regulatory cooperation of the partners. The equivalent rules and controls bilaterally agreed will simplify the approval and certification process and provide higher guarantees against the spread of plant pests (Anon, 2015).

Keywords: consignment, interception, international trade, phytosanitary measure, plant pest

References: (1) Anon. 2014. Non-EU trade alert list (1 Jan 2014- 31 Dec 2014).- Directorate General for Health and Food Safety, Unit E2, European Commission, B-1049 Brussels, Belgium http://ec.europa.eu/food/plant/plant_health_biosafety/alert_list_trade_non_eu/index_en.htm

(2) Anon. 2015. TTIP – Transatlantic Trade and Investment Partnership and Regulation: an overview.- The European Commission, 10 Feb 2015 http://trade.ec.europa.eu/doclib/docs/2015/february/tradoc_153121.1.2%20TTIP%20and%20regulation%20overview.pdf

P9 Root-Knot Nematodes (*Meloidogyne* spp.) from Vegetable Growing Greenhouses in the Middle Black Sea Region of Turkey

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Root-knot nematodes (*Meloidogyne* spp.) are one of the most important pests in vegetable growing areas of Turkey (1). In previous reports, root-knot nematodes from greenhouses in the Middle Black Sea Region (only Samsun and Tokat province) of Turkey were identified based on perineal patterns of females, and *Meloidogyne incognita* was the common species (2,3). Identification of *Meloidogyne* species based on perineal patterns morphology alone is not easy due to the high variability within the species and the overlap between species. In this study, we aim to overcome the difficulty in identifying root-knot nematode populations collected from different greenhouses in the Middle Black Sea Region (Samsun, Tokat, Amasya, Çorum, Ordu, Sinop province) using not only morphological (perineal pattern morphology), but also biochemical (esterase phenotype) and molecular (PCR with species-specific primers) techniques. Ninety populations were collected from infested roots in different locations. Each isolate was established from a single egg mass and maintained on susceptible tomato plants (cv. Falcon) in a glasshouse culture at 24±2 °C. All identification studies were made from tomato cultures. Morphological identification was based on a minimum of 10 perineal patterns (4). Esterase patterns were obtained from young egg-laying females electrophoretically (5). For molecular analysis, DNA was extracted from egg masses using DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's instructions. PCR amplification was done using previously described species-specific primers. All results of this study were evaluated together and of populations, 38 *M. arenaria* (42,22%), 37 *M. ethiopica* (41,11%), 11 *M. javanica* (12,22%) and 4 *M. incognita* (4,44%) were identified. This result differs from what has been reported in previous studies, because *M. incognita* was not the common species. This could be a result of the different identification methods. This study indicates the wide distribution of *M. ethiopica* in this region.

Keywords: *Meloidogyne*, greenhouse, identification, Middle Black Sea Region, Turkey

References: (1) Devran, Z. & Söğüt, M. A. 2010. Phytoparasitica, 38:245 (2) Katı, T. & Mennan, S. 2006. XXVIII Symposium of the European Society of Nematologists, Blagoevgrad, Bulgaria, 5-9 June 2006. p. 130. (3) Akyazı, F. & Ecevit, O. 2011. Anadolu J AgrSci, 26:1-9. (4) Taylor D. P. & Netscher, C. 1974.Nematologica, 20:268 (5) Pais, C. S. & Abrantes I. M. de O. 1989. Journal of Nematology, 21: 342.

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P10 DNA markers for species identification and phylogenetic inference in *Meloidogyne ethiopica* and other tropical root-knot nematodes

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Root-knot nematodes (RKNs) *Meloidogyne* spp. are the most damaging of all plant-parasitic nematodes in terms of yield loss due to their broad range of host plants. For effective agricultural management of RKNs accurate species identification is crucial. Species identification of RKNs is based on morphological, biochemical (isoenzymes) and molecular (species-specific DNA regions) characters, and determination of host range. Identification of RKNs is especially challenging due to highly similar morphology across species, poorly defined boundaries among species, intraspecific variability and hybrid origin. *Meloidogyne ethiopica* identified in Slovenia in 2003 represented a first finding of this species from the tropical group of RKNs in Europe (1). Since then, this species previously reported only from tropical regions has been reported from other Southern European countries (2, 3, 4). The species is a member of tropical RKNs group of which most species multiply by mitotic parthenogenesis. We have compared populations of *M. ethiopica* from different geographical regions (Slovenia, Greece, Turkey, South Africa and Brazil). The studied populations exhibited three different esterase izoenzyme profiles. Four DNA regions which were historically used in phylogenetic studies of nematodes including RKNs were evaluated as markers for species identification and studies of phylogenetic inference. rDNA regions (ITS, 18S and 28S) showed high intraspecific variability making these markers not suitable for *M. ethiopica* species identification and phylogenetic inference. However, the seqences of mDNA (containing 3' end of COII gene, tRNA-His gene and 5' end of 16S rRNA gene) region clustered *M. ethiopica* in a monophyletic group when compared to other species of RKNs. Recently described sister species *M. luci* formed a subgroup together with all the European populations of *M. ethiopica* mtDNA sequences. High intraspecific variability of rDNA region could be explained by the hybrid origin of this apomictic species. This study highlights the importance of selection of an appropriate DNA marker for *Meloidogyne* spp. diagnosis.

Keywords: root-knot nematode, *Meloidogyne ethiopica*, identification, phylogeny

References: (1) Širca, S. et al. 2004. Plant Dis. **88**: 680. (2) Maleita C.M. et al. 2012. Plant Dis. 96: 865-874. (3) Conceição, I.L. et al. 2012. Eur J Plant Pathol. **134:** 451-457. (4) Aydınlı, G. et al. 2013. Plant Dis. 97: 1262.

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P11 Evaluation of Silencing of Parasitism Genes in *Pratylenchus vulnus*

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Root-lesion nematodes (*Pratylenchus* spp.) are economically important migratory endoparasites of agricultural, horticultural and industrial crops (1). Control strategies for these and other nematodes involve a variety of approaches ranging from environmentally unfriendly chemicals to resistant crop varieties, cultural practices and biological control strategies, such as trap cropping or the use of natural enemies. The current strategy involves the use of molecular techniques to engineer nematode resistant plants. One of the most promising strategies involves the application of RNA interference (RNAi) in nematodes in order to understand the function of genes essential to development and parasitism. Several parasitism genes (*Pv-eng*-8, *Pv-Far*-1, *Pv-set*-1 and *Pv-msp*-1) have been isolated and fully characterized in *P. vulnus*. These genes were chosen because they are involved in migration, establishment and reproduction inside the roots of the host plant. Silencing of these genes has been carried out by soaking to introduce dsRNA. To investigate long-term effects of RNAi in *P. vulnus*, the carrot disc technique is used and 30 nematodes were added for each disc (2). Preliminary results of silencing of *Pv-eng*-8 show a significant lower reproduction (53%) compared to control nematodes. Silencing experiments of the other parasitism genes are still in progress aiming to search for novel essential target genes to control root-lesion nematodes.

Keywords: root-lesion nematode, endoglucanases, *Pratylenchus vulnus*, RNA silencing, carrot disc

References: (1) Smiley, R. W. et al., 2005. J. Nematol., 37:45. (2) Fanelli, E. et al., 2014. Plant Pathology 63:1436.

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P12 Longidorid Nematodes: Do We Really Know Them? Balkan Peninsula Issues

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Longidorids belong to one of the most diverse nematode family occurring in all continents and associated with a great number of higher plants. Their economic importance is determined by the role they play in the complex of plant diseases directly or as vectors of nepoviruses (1, 2, 3). The purpose of this presentation is to review the diversity (including distribution patterns) of longidorids on the Balkan Peninsula and to discuss the current approaches to identification and diagnostics of the species. Family Longidoridae consists of seven genera (ca. 520 species), three of which being the most diverse and species rich – *Xiphinema* Cobb, *Longidorus* Micoletzky and *Paralongidorus* Siddiqi, Hooper & Khan. Hitherto, 27 species belonging to the first genus have been recorded from the Balkans which represents 10% of the world and more than 1/3 (37 %) of the European fauna. Genus *Longidorus* is represented even better with 35 recorded species (22% of the world and about half (49.3%) of the European fauna). Only one *Paralongidorus* species has been recovered so far from the Balkans. Despite the long history of research, diversity of those genera is greatly underestimated, moreover that cryptic species have been revealed by using molecular methods (4). However, the knowledge about genetic diversity of the species in this family is far from complete, yet, which doesn't allow to reconstruct their phylogeny and elucidate the evolutionary relationships between species and genera. The application of holistic/integrative approach in their investigation (morphology, genetics, biology, host plant associations, interaction with other organisms, biogeography) will certainly fill the gaps in knowledge on longidorids although important results have been achieved so far. Rapidly increasing knowledge of nematodes and how to use this knowledge in training, research and development of new technologies and strategies for effective and sustainable control of plant parasitic nematodes are real challenges to fundamental and applied nematology.

Keywords: *Longidorus, Xiphinema, Paralongidorus/Siddiqia,* diagnostics

References: (1) Hunt, D. 1993. Aphelenchida, Longidoridae and Trichodoridae: their systematics and bionomics. CAB International, Wallingford, 352 pp. (2) Taylor, C.E. & Brown, D.J.F. 1997. Nematode vectors of plant viruses. CAB International, Wallingford, 286 pp. (3) Decraemer, W. & Robbins, R.T. 2007. Journal of Nematology, 39:295. (4) Gutiérrez-Gutiérrez, C. et al. 2010. Zool. Scripta, 39:483.

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P13 Hyperspectral Remote Sensing as a New Approach for Early Root-Knot Nematode Detection

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The root-knot nematodes (RKNs) of the genus *Meloidogyne* are the most polyphagous and damaging of all plant-parasitic nematodes. RKNs are sedentary endoparasites that invade and migrate through the root before initiating specialized feeding cells and cause the development of characteristic giant-cells and root galls. As a result, the uptake of water and nutrients by the roots become severely disrupted. Annual economic losses due to RKNs infection of crops have been estimated at several billion U.S. dollars (1), but this could be a significant underestimate, as many growers, particularly in developing countries, are unaware of plant-parasitic nematodes (2). Plants affected by RKNs show symptoms similar to drought and nutrient stress and therefore their symptoms are often misdiagnosed. Early and correct identification of biotic or abiotic stress is crucial for selection of effective control measures and prevention of yield losses. The conventional methods of detection rely on visual examination of plants and often result in detection of RKNs after the optimal time for control of the pest has already passed. Determination of specific hyperspectral signatures could be a better approach for early detection of the infestation by RKNs as hyperspectral sensors are able to detect changes in spectral signatures before these become visible to the naked eye. In our study, two hyperspectral cameras covering wavelengths from 400 to 2500 nm, with a spectral resolution of 3.7 or 5.5 nm and minimum spatial resolution of 0.06 mm were used. To establish a hyperspectral fingerprint database of biotic and abiotic stress symptoms we set up a pot experiment with tomato plants in a greenhouse. The plants were infested with three different levels of *M. incognita* (low - 1egg/cm^3, middle - 10 eggs/cm^3 and high - 50 eggs/cm^3), and two different water regimes were used. Hyperspectral images of the plants in the experiment were recorded every two weeks. In this study we evaluated the potential of hyperspectral image analysis as a new approach for early detection of RKNs.

Keywords: RKNs, spectral signatures, management, detection, abiotic stress, biotic stress

References: (1) Elling, A.A., 2013. Phytopathology, 103:1092-1102. (2) Nicol, J.M. et al. 2011. Current nematode threats to world agriculture. In: Genomics and Molecular Genetics of Plant-Nematode Interactions (Jones, J.T. et al. eds), pp. 21–44. Heidelberg: Springer.

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P14 Methods for Detection and Identification of Potato Cyst Nematodes *Globodera* spp. in Croatia

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Since the first detection of golden potato cyst nematode *Globodera rostochiensis* in 2001 in Croatia an overall intensive national survey is performed each year. Survey of *Globodera* spp. in Croatia is carried out by Institute for Plant Protection and Phytosanitary Inspection Service. The presence of potato cyst nematodes in the field is mainly controlled by visual inspections and taking soil samples prior and during vegetation period and processing them in a laboratory. The potato processing facilities are controlled too. For the routine cyst extraction from soil samples we are using the flotation technique (Fenwick can) and the extracts are checked under the binocular microscope. The isolated cysts and juveniles are subject to identification based on morphological and morphometrical characters under microscope with digital camera and software. In order to improve and complement morphometrical identification of potato cyst nematodes, molecular methods were implemented in our laboratory. There are different versions of PCR tests described in valid EPPO Protocols (2, 3) and available literature (1). In Croatia we have implemented the in-house modified version of the Dutch National Plant Protection Organization (NPPO-NL) protocol. The major difference between conventional PCR described by Bulman and Marshall (1) and Dutch modified version is in amplification program and primer sequences (4). Positive controls of *Globodera rostochiensis* and *G. pallida* obtained from NPPO-NL were used during the optimization process. Under our laboratory conditions this modified version showed high level of accuracy.

Keywords: *Globodera*, potato cyst nematodes, detection, identification, PCR

References: (1) Bulman, S. R. & Marshall, J.W., 1997. New Zeland Journal of Crop and Horticultural Science 25, 123-129. (2) EPPO, 2009. PM 7/40 (2): *Globodera rostochiensis* and *Globodera pallida*. *EPPO Bulletin* 39, 354-368. (3) EPPO, 2012. PM 7/40 (3): *Globodera rostochiensis* and *Globodera pallida*. *EPPO Bulletin* 43, 119-138. (4) van de Vossenberg, B. T. L. H. et al., 2014. *EPPO Bull*etin 44(1), 27-33.

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P15 Postharvest Decay of Onion Bulbs in Serbia

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Onion (*Allium cepa* L.) is an important vegetable crop grown widely for its value in the content of sugars, vitamins and minerals. Onion is highly resistant to pathogen invasion, and some diseases only develop after harvest causing losses ranging from a trace to 50% or more. Some of the well known species pathogenic to onion during storage include *Aspergillus niger*, various *Fusarium* spp., and *Botrytis* spp. which may invade in the field and later develop in storage (2). Economically important are a number of *Penicillium* spp. causal agents of onion blue mold (3). During 2013 onion bulb decay caused significant economic losses at a storage facility in Stara Pazova. Several types of symptoms were observed, and intensity ranged from mild to severe, resulting in complete rot. Both dry rot with dark to black decomposed tissue and soft rot with pale yellow to brown, soft and watery tissue occurred on the bulbs. Bluish green sporulation was abundant on the surface and between decayed scales, in some cases. Others had black discoloration on the outer scales, with deposits of black conidia between the outer dry scales, vertically, along the veins. Onion bulbs with white mycelium present over rotted areas and on the stem plates were also observed. Bulbs with rot symptoms were collected twice: two weeks and four months after harvest. Several isolates were obtained from decayed onion bulbs using standard procedure of spore or tissue transfer on potato dextrose agar medium. Pathogenicity was proven by wound inoculation of healthy surface sterilized onion bulbs cv. Meranto. Pathogenic isolates were identified based on morphological features (i.e. colony and reproductive structure morphology and size). *Penicillium* isolates were also grown on differential media, and production of cyclopiazonic acid was tested. Molecular identification was based on the β-tubulin locus (1) and internal transcribed spacer (ITS) region (4) via PCR and the amplified products were sequenced. *Aspergillus* spp. was identified as a causal agent of onion bulb rot two weeks after harvest, while *Aspergillus* spp., *Fusarium* sp., and *Penicillium* spp. caused onion bulb decay four months after storage, in single or mixed infection. The identification of these species is important not only because they cause onion losses during storage, but also because these fungi are the threat of mycotoxin contamination.

Keywords: onion bulb, postharvest, pathogens

References: (1) de Jong, S.N. et al. 2001. Mycol. Res, 105: 658. (2) Pitt, J. I. and Hocking, A. D. 2009. In: Fungi and Food Spoilage. Springer, New York. (3) Schwartz, H. F. and Mohan, K. S. 2008. In: Compendium of Onion and Garlic Diseases and Pests. Second Edition, APS Press, USA 47-55. (4) White et al. 1990. In: PCR Protocols: A Guide to Methods and Applications. Academic Press, USA.

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P16 Pathogenicity of *Fusarium* Species Isolated from Sugar Beet (*Beta vulgaris* L.) Plants in Pasinler District of Erzurum, Turkey

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The genus *Fusarium* is one of the most economically important genera of fungi and includes many pathogenic species which cause a wide range of plant diseases (1). This study was conducted in 2009 to determine the pathogenicity of *Fusarium* species isolated from sugar beet (*Beta vulgaris* L.) plants in Pasinler district of Erzurum. As a result of the isolation studies, 194 *Fusarium* isolates were obtained in total. Of these isolates, 37.63% were *F. equiseti*, 31,44% were *F. oxysporum*, 13,92% were *F. acuminatum*, 10,82% were *F. solani*, 4,12% were *F. heterosporum*, 1,55% were *F. avenaceum*, and 0,52% were *F. graminearum*. Pathogenicity tests on sugar beet isolates belonging to *F. acuminatum* (P2-8A1), *F. equiseti* (P1-6), *F. heterosporum* (P10-30), *F. oxysporum* (P8-24, P9-36) and *F. solani* (P8-2) were found to be more virulent than the other *Fusarium* isolates. Sugar beet was found to be a new host of *F. acuminatum* and *F. graminearum* in Turkey.

Keywords: Sugarbeet, *Fusarium* spp., pathogenicity

Reference: (1) Nelson, P.E. et al., 1981. *Fusarium*: Diseases, Biology and Taxonomy. The Pennsylvania State University Press, University Park and London.

Acknowledgement

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P17 The Most Important Fig Mycoses in Montenegro

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Fig has been cultivated in the southern and central part of Montenegro for a long time. Fig fruits are used in nutrition as fresh and dried. Until recently, fig was not grown on large areas, mainly near houses and in gardens, so the intensity of plant diseases was not of great importance. However, in recent years fig plantations were set up (from several hundreds to over 1000 trees), and certain diseases have been observed whose intensity threatens the cultivation of this crop and jeopardize the yield.

In last few years, agricultural producers had problems with two mycoses which were very economically significant. During the summer of 2012 in fig plantations in the region of Podgorica, massive fruit rot caused by the fungus *Alternaria alternata* was detected (1). Sunken, round and light brown spots were noticed that eventually led to the complete decay of fruits. Disease incidence in the fields ranged from 15 to 20%. Another very important mycosis in figs, fig rust, spread out in late summer and early fall of 2014, both in productive plantations and in nurseries. The leaves of diseased plants showed numerous yellow to brown colored spots on the upper side and reddish-brown pustules on the lower side. The leaves became twisted upwards with rusty and necrotic areas and then dried. Almost all leaves with those symptoms prematurely fell down, leaving bare branches and unripe fruits. Microscopic observations in laboratory revealed that the disease is caused by the phytopathogenic fungus *Cerotelium fici*. Extensive outbreaks of fig rust were recorded several times in last century in Montenegro (2). The disease is favored by humid and warm summer conditions as it was in 2014.

Keywords: fig fruit rot, *Alternaria alternata*, fig rust, *Cerotelium fici*

References: (1) Latinović, N. et al. 2014. Plant Disease, Vol. 98, No. 3: 424.3. (2) Mijušković, M. 1963. Naša poljoprivreda i šumarstvo 9, 4, 49-52.

Acknowledgement

The study was supported by Phytosanitary Directorate of Montenegro, project “Fruits protection with monitoring of pesticides residues”.

Fig rust symptoms on the lower side of the leaf

P18 Significant Incidence of Black Rot of the Lower Stem Portion of Potato ("Blackleg") in Montenegro in 2012

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Potato diseases caused by phytopathogenic bacteria in Montenegro are of less importance compared to those caused by fungi, fungus like organisms and viruses. Of all bacteria caused diseases, the greatest economic impact have wet rot of tubers and black rot of the lower stem portion of potato ("blackleg") which are caused by *Erwinia carotovora* ssp. *carotovora*, *Erwinia carotovora* ssp. *atroseptica* and *Erwinia chrysanthemi*. During the cold and wet period after planting and before sprouting of potatoes, as it was in 2012 (the period from February to April precipitation sum was 552.1 mm), "blackleg" disease often causes lot of damage in Montenegro. Damages usually occur in conditions of cold and rainy springs, especially in the parcels where used seed tubers cut just before planting are. The disease usually occurs in the period between planting and sprouting, and infected plants do not form tubers. Planting of cut tubers of the same parties and the same varieties of potatoes (Jaerla, Class B, imports from the Netherlands), in the surroundings of Podgorica, was carried out in the period from 15 February to 15 March 2012. All producers have cut tubers before planting, except that the four producers did it for 15-20 days before planting (healing of tuber cuttings), while the remaining 4 did this operation just before planting (without healing of tuber cuttings). High rainfall in the first part of the potato vegetation season, followed by slightly lower average air temperatures have caused slower sprouting of potato and huge occurrence of this disease in all plots. Symptoms of the disease were observed to a greater extent during April, and examination of all eight potato crops was done in June. Examination showed the presence of the disease in all examined crops, but significantly higher degree of infection was observed in crops with earlier planting times, especially those where potato tubers were cut just before planting. The percentage of infected plants in the plots where tubers were cut just before planting was very high and it was from 37.1 to 62.8%, while the parcels where tubers were cut 15-20 days before planting disease was present in much smaller scale. The percentage of infected plants in these plots ranged from 6.6 to 25%.

Key words: Potato, bacterial diseases of potato, potato blackleg

P19 The Rape Phyllody Disease: Is There a New Player in the Old Game?

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Oilseed rape or rapeseed (*Brassica napus* ssp. oleifera (DC.) Metzg.) is an important industrial plant and the rape phyllody disease, ascribed to phytoplasmas, can have devastating effects on its yield (1). The diseased plants sampled in 2009 in Croatia showed typical rape phyllody symptoms like green and deformed flowers, small and deformed siliques and seed reduction. Also, atypical stem necrosis, leaf chlorotic spots and necrotic changes more reminiscent of a *Turnip mosaic virus* (TuMV) infection (1) were recorded. The presence of phytoplasmas and viruses was investigated with the aim of characterizing pathogens involved in this rape phyllody outbreak. Expectedly, by PCR-RFLP and 16S rRNA gene sequencing, an aster yellows (AY, '*Candidatus* Phytoplasma asteris') strain (2) was identified with the closest relatedness to the 16SrI-B subgroup strains. Multi-gene sequence characterization of phytoplasma house-keeping *tufB*, *secY*, *groEL* and ribosomal protein genes plus a highly variable *amp* gene confirmed its AY affiliation. As opposed to the analyzed house-keeping genes, the AY specific *amp* gene encoding an immunodominant membrane protein, showed a significant variability suggesting possible influence on insect vector specificity and transmissibility. This way, the first detailed molecular characterization of '*Ca*. P. asteris' associated with the rape phyllody disease was obtained. Biological and serological assays revealed the co-infection with TuMV. The full TuMV genome sequence of the Croatian isolate CRO184A and its phylogenetic analysis classified it into the world-B phylogenetic lineage, whilst no evidence for interlineage recombination was obtained. This was the first finding of a '*Ca*. P. asteris' and TuMV co-infection in rapeseed, or any phytoplasma and virus simultaneously infecting a brassicaceous host (3). Due to the worldwide distribution and pathogenicity of the AY phytoplasma and TuMV as individual plant pathogens, their co-occurrence should be considered in future rape phyllody cases and this research lays foundation for investigating their possible synergistic effects.

Keywords: '*Candidatus* Phytoplasma asteris', multigene analysis, *Turnip mosaic virus*, genome

References: (1) Rimmer, S.R. et al. 2007. Compendium of Brassica Diseases. APS Press, St. Paul, MN, USA. (2) Lee, I.-M. et al. 2004. Int. J. Syst. Evol. Microbiol., 54:1037. (3) Šeruga Musić, M. et al. 2014. J. Appl. Microbiol., 117:774.

Acknowledgement

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P20 Optimising Droplet Digital PCR Analysis Approaches for Detection and Quantification of Bacteria: A Case Study of Fire Blight and Potato Brown Rot

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Digital PCR currently represents the most straightforward absolute quantification of the target nucleic acid copy numbers. Here we present the first assessment of droplet digital PCR (ddPCR) for detection and absolute quantification of two quarantine plant pathogenic bacteria: *Erwinia amylovora* and *Ralstonia solanacearum*.

The evaluation combined the determination of the ddPCR performance parameters on a defined set of samples with known health status, including: (i) NTCs; (ii) negative plant material; (iii) serial dilutions of target DNA; and (iv) artificially prepared samples with target concentrations relevant to routine testing (in this case, log 3 cells/mL plant extract). In the ddPCR format, the performance of the *E. amylovora* assay was comparable to its qPCR format, despite the reduced number of replicates (one in ddPCR against three in qPCR), which makes the ddPCR the first choice for characterisation of in-house reference materials, and for any application where quantification is also required. In contrast, for the *R. solanacearum* assay, where previous data and the data from this study indicate that its design might not be optimal, the ddPCR format significantly improved both its analytical and diagnostic sensitivity when analysed with a manual global threshold that was defined on a set of reference samples. In general, the proprietary QuantaSoft analysis required data of high quality, while manual threshold selection was more suitable for non-optimal assays. The data analysis and interpretation of the ddPCR was considerably simplified in comparison with qPCR.

In addition to being suitable for detection and quantification of target copies in plant samples after the DNA extraction and purification step, both of these ddPCR assays were successfully used for absolute quantification of in-house reference materials and control samples for interlaboratory studies. This is of particular importance in the field of plant pathogen diagnostics, where no reference materials are commercially available.

Keywords Bioanalytical methods, droplet digital PCR, molecular diagnostics, plant pathogenic bacteria

P21 Current impact and future directions of high throughput sequencing in plant virus diagnostics: the drivers of COST Action 1407

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The ability to provide a fast, inexpensive and reliable diagnostic for any given viral infection is a key parameter in efforts to fight and control these ubiquitous pathogens. The recent developments of high-throughput sequencing (also called Next Generation Sequencing - NGS) technologies and bioinformatics have drastically changed the research on viral pathogens. It is now raising a growing interest for virus diagnostics. This review provides a snapshot vision on the current use and impact of high throughput sequencing approaches in plant virus characterization. More specifically, this presentation highlights the potential of these new technologies and their interplay with current protocols in the future of molecular diagnostic of plant viruses. The current limitations that will need to be addressed for a wider adoption of high-throughput sequencing in plant virus diagnostics are thoroughly discussed. This paradigm change gave rise to the COST Action 1407 which is currently launched. This Action, its objectives and expected impacts will be presented.

Keywords: plant virus, diagnostic, high throughput sequencing

P22 Emaraviruses of Rubus Plants – From Symptoms to Detection

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Raspberry leaf blotch virus (RLBV) was recently identified associated with raspberry leaf blotch disorder in Scotland and England (1). It was also found in association with similar symptoms in Serbia, Finland and Bulgaria (1, 2, 3). Virus-like symptoms that indicated a possible infection with RLBV were observed on leaves of some wild and cultivated *Rubus* plants in Slovenia. The leaf symptoms differed between plants and included chlorotic mottling, yellow chlorotic and necrotic spots and patches, yellowing along the veins and leaf deformations. Attempts to amplify specific products with different RLBV specific primers were unsuccessful therefore next generation sequencing was used to identify the possible cause of the symptoms. Several assembled contigs with similarities to RLBV genomic sequences were identified. RT-PCR detection primers were developed and used to detect this virus in symptomatic plants.

Keywords: Emaravirus, RLBV, NGS, detection

References: (1) McGavin et al., 2012. J. Gen. Virol., 93: 430-437. (2) Bi et al. 2012. Plant Disease, 96: 1231. (3) Mavrič Pleško et al. 2014. J. Plant Pathol., 96: 437.

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P23 Presence, Diversity and Epidemiology of Viruses in Difficult Samples e.g. Environmental Water Systems

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Environmental water is an important route for waterborne plant viruses (1, 2). To study the presence, survival and between-host transmission of viruses in water, efficient concentration and sensitive diagnostic methods are needed, such as concentration using monolith chromatography and different techniques of amplification and detection of viral nucleic acids (PCR), including digital droplet PCR. Due to the specifics of viral genome, there is a lack of efficient generic diagnostic methods, with the exception of metagenome studies using next generation sequencing (NGS). NGS can be used for viral community studies in the samples and also for discovery of new pathogenic microbes in the environment. Many viruses cannot be easily propagated in host organisms and often high titre of virus is needed to infect a host. Thus, at low viral concentration, problems arise how to detect viral presence and measure their amount, infectivity or success of antiviral procedure. In such conditions, the distinction between assembled virions and viral nucleic acids could pose a problem. Possible solutions of indicated questions will be presented with the examples of plant viruses present in the water, using different diagnostic methods.

References: (1) Mehle, N. and Ravnikar, M. 2012. Water Research, 46(16)4902-4917. (2) Mehle, N. et al., 2014. Applied and environmental microbiology, 80(4)1455-1462.

P24 Importance of Virus-Vector Interaction Studies for Management of Virus Infections in Vineyards

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Scale insects are economically very important pests, infesting various fruit crops and ornamental plants all over the world. In addition, they are known vectors of different viruses. Grapevine leafroll associated viruses (GLRaVs) cause one of the most important and widespread viral diseases of grapevine and are transmitted by scale insects. During a survey of scale insects occurring in Slovenia many soft scales and mealybugs have been found (2). The soft scales *Neopulvinaria innumerabilis*, *Parthenolecanium corni, Pulvinaria vitis* and the mealybug *Planococcus ficus* were found on grapevine (2, 3). Moreover, they are all known vectors of GLRaVs. Due to climate changes and limited use of registered chemical controls of pests in vineyards, the presence of scale insects in vineyards is constantly increasing. Two local populations, one of *N. innumerabilis* and one of *P. ficus* were used in transmission studies of GLRaVs (1, 4). They were found to transmit two viruses, GLRaV-1 and GLRaV-3, but not GLRaV-4. GLRaV-1 and -3 can be regularly found in Slovenian vineyards and the presence of their vectors could lead to higher incidence of virus infections. The results of transmission studies can help us to better understand the role of vectors in the spread of these viruses in Slovenian vineyards and to understand the epidemiology of vector transmitted virus diseases. Finally, the studies can help us to develop the effective disease management practices to limit spread of viruses and their vectors in Slovenian vineyards.

Keywords: soft scales, mealybugs, grapevine leafroll-associated viruses

References: (1) Mavrič Pleško, I. et al. 2011. In: Maček J., Trdan S. (Ed). Abstract volume. Ljubljana: Plant Protection Society of Slovenia, 47-48. (2) Seljak, G. 2010. *Entomologica hellenica*, 19, 99-113. (3) Seljak G., Žežlina I. 2007. In: Maček, J. (Ed.). Lectures and papers presented at the 8th Slovenian Conference on Plant Protection, Radenci. Plant Protection Society of Slovenia, 233-237. (4) Štrukelj, M. et al. 2013. In: Trdan, S., Maček, J. (Ed.). Lectures and papers presented at the 11th Slovenian Conference on Plant Protection, Bled. Ljubljana: Plant Protection Society of Slovenia, 344-347.

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P25 Current Status of Economically Important Viruses Infecting Grapevine in Montenegro

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Presence of *Grapevine fanleaf virus* (GFLV), *Grapevine leaf roll-associated virus 1* (GLRaV-1), *Grapevine leaf roll-associated virus 2* (GLRaV-2) and *Grapevine leaf roll-associated virus 3* (GLRaV-3) have been reported in 2006 in the Skadar Lake basin, one of the two main grapevine-growing areas in Montenegro (1). During the 2012 and 2014 growing seasons, extensive monitoring have been undertaken encompassing 20 new locations in coastal and continental region. In total, 206 samples were collected from 14 grapevine cultivars. All samples were examined by DAS-ELISA using antisera against GFLV, GLRaV-1, GLRaV-2 and GLRaV-3. Positive results were obtained for GFLV, GLRaV-1 and GLRaV-3. The results showed that 63% of tested samples were infected with at least one of the detected viruses. The most prevalent virus in the continental region (Podgorica, Nikšić and Danilovgrad districts) was GLRaV-3 (36,8%), than GLRaV-1 (9,4%) and GFLV (8,5%), while in the coastal region (Kotor, Tivat, Bar and Ulcinj districts) GLRaV-3 and GLRaV-1 were detected in the lower percentage (19,6% and 7,8%, respectively). However, the incidence of GFLV (15,7%) was higher in the coastal (Mediterranean) region. The results showed difference in virus infection percentage among investigated grapevine cultivars ('Black Magic' (100%), 'Primitivo' (80%), 'Žilavka' (50%), 'Ribier' (50%), 'Vranac' (43,5%), 'Kratošija' (35,7%), ‘Radovača’ (25%) and 'Cardinal' (21,4%)), indicating high level of infection in the autochthonous varieties ('Vranac' and 'Kratošija') and urgent need for their systematic clonal selection. Further investigation is being undertaken to confirm and characterize these viruses and to determine the status of other viruses affecting grapevine.

Since the main nurseries and mother blocks are situated in the main grapevine-growing area of Montenegro, important step in the virus disease management would be their isolation from the commercial vineyards. Phytosanitary measures according to the EU certification standards need to be employed urgently.

Keywords: viruses, grapevine, cultivars, ELISA

References: (1) Zindović, J. et al. 2014. Bulletin OEPP/EPPO Bulletin 44(1):60-64.

P26 Entomopathogenic Fungi: Options for Production and Formulation to Get a Better Efficacy

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Often, positive results of laboratory experiments with entomopathogenic microorganisms are not confirmed under field conditions. Reasons are complex. For example, the mode of action is complicated, micro-organisms can not survive under harsh environmental conditions or they are not available at the right time and place. Therefore, it is essential to produce and formulate entomopathogenic fungi in an efficient way. Two examples will be given.

The application of the entomopathogenic fungus *Metarhizium brunneum* strain JKI-BI-1339 (= F52) against soil dwelling pests requires specific product formulations. Production of conidia on solid media, although possible, takes long time and is labor intensive. Additionally, problems with the application of grains, used in media as substrata, are reported. Therefore, we investigated the possibility of formulating mycelial fragments. Mycelium was produced in a liquid fermenter and was homogenized to get a flowable suspension. Experiments on fluid bed drying demonstrated that mycelial fragments can be coated on millet at temperatures of 50° C. After coating, the fungus was growing and sporulating on the surface of the millet under humid conditions. Further optimization steps and the practicability of mycelial fragments based formulations will be discussed.

Within a national funded project for control of the plum fruit moth *Cydia funebrana*, we treated artificial hideouts of the pest with *Isaria fumosorosea,* strain JKI-BI 1496. Spore production by the fungus was best in liquid culture, while the production of conidia on solid media was not sufficient. Submerged spores had a slightly better efficacy than aerial conidia. One constrain of applying living microorganisms is their environmental stability. Therefore, experiments on the persistence of JKI-BI 1496 on bark mulch were investigated over two months under semi-field conditions. Despite intensive UV radiation and heavy rainfall, a long persistence over the whole experimental time of both aerial conidia and submerged spores was investigated. Further laboratory experiments underlined that humidity of the mulches is important for the efficacy of JKI-BI 1496. Further results indicate that water-based formulations show an interesting alternative to former preferred oil-based formulations.

Keywords: biocontrol, entomopathogenic fungi, production, formulation

Acknowledgement

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P27 Laboratory Testing of Entomopathogenic Fungi against Wireworms (*Agriotes* spp.) and their Rhizosphere Competence

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Wireworms are soil-burrowing larval stages of click beetles (Coleoptera: Elateridae) and major pests of crops including wheat, maize, and numerous vegetables in many parts of the world. The aim of this study was to assess the virulence of 4 entomopathogenic fungal strains against field collected larvae of *Agriotes* spp. The fungi were also tested for their rhizosphere competence, plant tissue colonization (endophytism) and potential growth stimulating effects to maize and wheat. The tested strains represented *Metarhizium anisopliae*, *M. brunneum*, *M. robertsii,* and *Beauveria brongniartii*. All were isolated from various substrata in Slovenia. Additionally, the product 'Naturalis', based on the entomopathogenic fungus *Beauveria bassiana* ATCC 74040 and product 'Delfin WG', based on the bacterium *Bacillus thuringiensis* var. *kurstaki*, were used as reference biocontrol agents. The virulence to wireworms was assayed by dipping the wireworms for 10 seconds into conidial suspensions that contained 1 x 10^8 viable conidia ml^{-1}. The larval mortality was observed on a weekly basis for up to 56 days. To induce fungal growth and sporulation on dead or immobile larvae lacking a coat of sporulating mycelium, these larvae were removed from the test vessels and incubated separately in sterile 24-well plates. The rhizosphere competence and potential plant growth promoting effects were assayed by coating maize and wheat seeds with fungal conidia. The following parameters were evaluated: fungal rhizoplane colonization rate, plant root and shoot tissue colonization rate, seed germination rate, root and shoot length and root and shoot biomass. Abbott's corrected mortality at day 56 ranged from 30.0 to 60.0%. *Metarhizium brunneum* isolate 1868 and *M. anisopliae* 1154 caused higher wireworm mortality than the reference biocontrol agents. All strains colonized roots although the roots of maize and wheat were colonized to varying extent. The isolates tested were not isolated as endophytes. Some isolates stimulated growth, whereas others decreased root or shoot length or biomass. This effect was plant-fungus pair specific; on several occasions contrasting results were observed between maize and wheat and the same fungal isolate.

Keywords: biological control, endophyte, entomopathogenic fungi, rhizoplane, wireworms

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P28 Field Evaluation of Fungal Based Biological Control of Cabbage Root Fly

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The aim of this research was to assess a potential biological control strategy to protect cauliflower plants from cabbage root fly (CRF; *Delia radicum*) in a field experiment. The strategy was based on young plantlet inoculation with seven entomopathogenic or potentially plant growth promoting fungal species. The following fungal species were tested: *Metharhizium anisopliae*, *M. brunneum*, *Beauveria bassiana*, *Clonostachys solani*, *Trichoderma atroviride*, *T. koningiopsis* and *T. gamsii*. The fungi were isolated from various substrata in Slovenia. A commercial product based on *B. bassiana* (Naturalis) was used as a positive control. The field experiment mimicked semi-normal agronomic practice in cauliflower production. 2.3 x 10^7 conidia were applied to individual 4 weeks old cauliflower plantlets as a drench 24 hours before transplanting to the field. All fungal isolates were infective to one or more CRF life stages (eggs, larvae, pupae or imago), as assessed in laboratory in-vitro bioassays. *M. anisopliae* decreased the average plant weight at harvest. *T. atroviride* and *T. koningiopsis* treated plants had the lowest mortality. The lowest number of pupae and live larvae were counted in the Naturalis treatment followed by *M. brunneum* treatment. The highest number of pupae and live larvae were counted in the control treatment. The lowest root quality class indicating lowest damage to the roots was recorded in Naturalis treated plants, followed by *T. atroviride* treated plants. The results indicate that in addition of entomopathogenic fungi (e.g. *M. anisopliae* and *B. bassiana*) also soil fungi (e.g. *Trichoderma*) exhibit potential to control cauliflower plant form soil dwelling CRF maggots.

Keywords: Biological control, *Delia radicum*, entomopathogenic fungi, insect; soil pest

P29 Research and Evaluation of Novel Biopesticides: Antibacterial Proteins Isolated from Basidiomycete Fruiting Bodies and Bacteriophages

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Applying biopesticides represents an alternative management strategy against plant disease agents, complementary to conventional treatments. They are particularly important when pesticides are not available or harmful organisms are resistant to them. Our research focuses on the (i) discovery of novel antibacterials against bacteria causing wilt diseases, (ii) development of model systems for studying factors affecting the efficiency of bacteriophages against soft rot bacteria, and (iii) development of tracking methods for novel bacteriophages.

In the search for novel antibacterials against *Ralstonia solanacearum* we have screened 150 protein extracts from 94 mushroom-forming basidiomycete species. Growth inhibition assays identified 15 protein extracts showing inhibitory activities (10 %). Antibacterial activity correlated with the protein concentration in extracts. The extracts inhibited specifically the growth either of few or all 12 tested *R. solanacearum* phylotypes and also other bacteria, e.g. *Escherichia coli*. The activity of extracts of *Tricholoma saponaceum*, *Suillus variegatus* and *Clitocybe geotropa* extracts observed in *in vitro* tests was confirmed also *in planta*. Fungal protein extracts significantly delayed symptom development and/or symptom severity when inoculated together with plant pathogenic bacteria into plant stems. Size-exclusion chromatography led to the isolation of several active fractions that are now further studied.

A model system for bacteriophage research consists of *Dickeya* spp. causing soft rots of *Phalaenopsis* orchids and their respective bacteriophages from different Caudovirales families isolated from plant material and wastewater. Classical approaches have been complemented by de-novo genome sequencing of bacteria and bacteriophages allowing us to develop specific and quantitative real-time PCR and digital PCR assays suitable as tracking tools. Further characterization and development of the model system is underway, taking into account quantitative interactions among *Dickeya* spp., bacteriophages and their environment.

Keywords antibacterial proteins, phage therapy, detection methods

P30 Combined Application of *Isaria fumosorosea* and *Steinernema feltiae* Against the Colorado Potato Beetle Larvae

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The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), has become one of the most difficult insect pests to control due to its resistance to most registered pesticides (1,2). Development of new biological control method targeting this pest might solve the resistance problem and contribute to sustainable crop production. Laboratory experiments were conducted to assess the efficacy of *Isaria fumosorosea* (syn. *Paecilomyces fumosoroseus*) (WIZE) Brown & Smith (Hypocreales: Cordycipitaceae) strain CCM 8367 (3) against *L. decemlineata* when applied in combination with the entomopathogenic nematode *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) strain Ustinov. Last instar larvae were individually placed into plastic Petri dishes and 1 ml of the CCM 8367 blastospore suspension was applied topically to them. Then, 1 ml of water with 500 IJ of *S. feltiae* was added in the middle of each Petri dish.

The obtained results showed that the combination of *I. fumosorosea* and *S. feltiae* increased the mortality of *L. decemlineata* larvae compared to single biocontrol agent applications (Fig. 1). Similar synergistic effect of entomopathogenic fungus and nematodes was reported in another study (4). The size of first-generation *S. feltiae* adults recovered from fungus-treated host larvae was similar to the control, i.e., without fungus, indicating that nematodes developed normally and produced progeny.

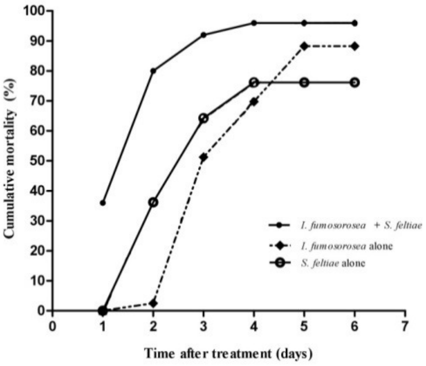

Figure 1: Cumulative mortality of *L. decemlineata* larvae treated by *I. fumosorosea* and *S. feltiae*.

We can conclude that simultaneous application of both biocontrol agents increases their efficacy against the Colorado potato beetle larvae. The research will continue to verify if this combination can be recommended for application in the field.

Keywords: entomopathogenic fungi, entomopathogenic nematodes, *Leptinotarsa decemlineata*, biological control, synergistic effect

References: (1) Zichová, T. et al. 2010. Pest Manag Sci, 66: 853-860. (2) Szendrei, Z. et al. 2012. Pest Manag Sci 68:941–946. (3) Prenerova, E. et al. 2013. Strain of entomopathogenic fungus *Isaria fumosorosea* CCM 8367 (CCEFO.011.PFR) and the method for controlling insect and mite pests. Patent No. US 08574566. (4) Barbercheck, M. and Kaya, H. 1991. Environ. Entomol. 20:707–712.

Acknowledgement

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P31 Control of Plum Sawflies (*Hoplocampa flava* and *Hoplocampa minuta*) by Three Entomopathogenic Nematodes

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Plum sawflies are among the most damaging pests of plum. The current control strategy implies insecticide application. No other measures are reported that could be used for plum sawflies management. Because entomopathogenic nematodes (EPN) are highly efficient against soil dwelling stages of some pests, these strategies were also evaluated against plum sawflies. Three species of EPN *Steinernema feltiae*, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* are tested under laboratory and field conditions to assess effectiveness against larval and adult stages of plum sawflies. Laboratory tests were performed in Petri dishes filled with sterile sandy soils. 500, 1000 and 2000 infective juveniles (IJ) were applied against 10 sawfly larvae per Petri dish. Age of larvae was considered by testing larvae 1 day after they exited last infested fruit, and 10, 20 and 40 days older larvae. In treatments with larvae 1 day after they exit last infested fruit, mortality was 92-100 %, whereas no mortality was observed for older larvae. To set up a field test against adult sawflies, plum trees were covered with an insect proof net. Above mentioned nematode species were applied to the soil surface at the dosage of 0.5 million IJ per square meter before the anticipated day of adult emergence. In 2013, the reduction of fruit infestations was 90-98%, in 2014, 30-90%. EPN are highly effective against sawfly larvae before they make the cocoon and against adult sawflies. Correct time of application is essential for high efficacy.

P32 Tiny Wasps - Big Success: the Success Story of *Trichogramma* Egg Parasitoids in Biological Control

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According to a recently published report on Biological Control in Germany, the tiny wasp *Trichogramma brassicae* (Hymenoptera, Trichogrammatidae) is the most widely used invertebrate biological control agent and released on more than 22,000 ha in the open field. It is used as a biological control agent of the European corn borer, *Ostrinia nubilalis*. *Trichogramma brassicae* is one out of nearly 200 species of this genus occurring worldwide and is commercially produced by several companies in Europe. The wasps are field-released by hand using various devices or by innovative application methods, e.g., mikrokopters. Quality of the mass-produced wasps is regularly investigated by the JKI since many years following IOBC-Guidelines and in general, the products do meet the expectations very well. Due to the fact that *T. brassicae* parasitizes eggs, the pest species is killed before hatching of larvae and any feeding damage is prevented. Pest management systems based or at least involving *Trichogramma* are therefore highly attractive, especially because suitable methods for their mass production and application are available. The JKI in Darmstadt, Germany, curates a »gene-bank« of several living strains and species of *Trichogramma* . These species originate from collections in several countries and they have different life histories and host preferences. We investigate their potential to control well-known old, but also new, invasive pest species like *Cydalima perspectalis* and *Tuta absoluta*. Furthermore, we explore interactions with other natural enemies and possibilities to enhance their efficiency in the field.

Keywords: inundative release, egg parasitism, maize

Acknowledgement

We want to thank the companies AMW Nützlinge GmbH, BIOCARE GmbH and UFA Samen Nützlinge for providing information material on application methods. We are highly grateful to Dr. Sherif A. Hassan for his inspiration to research and use of *Trichogramma* since several decades.

P33 Alternative Strategies to Manage Downy Mildew (*Plasmopara viticola*) for Reducing Copper applications through Bioagent or Biostimulant treatments

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Copper compounds have been used extensively against Downy Mildew worldwide. Nowadays it is well known that copper has negative effects on the soil environment, specifically on microorganisms, and therefore also on the activities of roots. Integrate pest management of vineyards can recruit many alternative substances that are effective against Downy mildew, while organic pest management strategies only allow copper applications (European regulation CE 473/2002 limits the use of copper at 6 kg/ha/year).

In the seasons 2013 and 2014, an experimental trial was set up for the comparison of different strategies against Downy Mildew with low copper rates and alternative bioagents or biostimulants, with the aim to reduce copper applications. Eight different treatments were compared in 2013 and eleven in 2014. In detail we wanted to compare the effects of the following treatments:

1. UNT, untreated;
2. Copper, label dosage rates;
3. Copper, Vite.net DSS suggested rates;
4. Frontiere and Oomisine (BioAtlantis Ltd., County Kerry, Ireland), algae extracts;
5. Previen (Fertenia srl, Bellizzi, Italy) weed / algae extracts and phosphites;
6. Multi-protek (Haifa-Italia, Bologna, Italy) phosphite + copper (2013 only);
7. Bma Xeda (Heda-Italia, Forlì, Italy) (2013 only);
8. Previen Bio, weed / algae extracts at 2 water volumes (2014 only)
9. Dinamico (Fertenia srl, Bellizzi, Italy) weed extracts (2014 only);
10. Chitoplant solution (Agritalia, Villa Saviola, Italy) shellfish extracts (2014 only);
11. Glutex 90 Cu (Agrisystem srl, Lamezia Terme, Italy) chelated low rate copper (2014 only);
12. Integrated strategy.

The results obtained showed good performances of copper at low rates in comparison with the integrated strategy. Good efficacy was ascertained only for treatments with Previen. All other tested bioagents and biostimulants showed low efficacies against Downy Mildew. Applying these substances need to be coupled with other active compounds in order to ensure protection against Downy Mildew.

Keywords: *Plasmopara viticola*, no copper, weed extracts, algae extracts, alternative strategies, phosphite

Acknowledgement

The study was supported by the Interreg IV A IT-SI project VISO Viticulture and sustainable development of local resources in the wine industry.

P34 Effects of Aqueous Plant Extracts on *Trialeurodes vaporariorum* (Insecta, Homoptera: Aleyrodidae) adults

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Results of some authors suggest that aqueous plant extracts could potentially be used for managing greenhouse whiteflies (1). Some plant extracts showed an insecticidal effect on adult insects by killing them or exhibited complete inhibition of feeding activity of the insect pests (2). Plant extracts are currently being studied as an ecologically friendly alternative to manage plant pests. The aim of this study was to evaluate effectiveness of aqueous extracts from *Satureja montana* L., *Salvia sclarea* L. and *Calamintha glandulosa* L. as contact toxicants against the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae). Adults of whitefly were collected in the greenhouse. The bioassays were carried out using groups of 20 *T. vaporariorum* on single pepper nursery plants that had been treated with aqueous plant extracts (0.1% and 0,2%). Aqueous extracts were applied together with an adjuvant (Trend) for a better adhesion to the leaf surface. Mortality was checked after 24, 48 and 96 h. Mortality rates were always above 50% when checked after 96 h. Extracts from *C. glandulosa* caused a 50% mortality of insects after 96 h when applied at a concentration of 0.1%. *Salvia sclarea* extractes resulted into a mortality of 57% (0.1% concentrated extract) and 55% (0.2% concentrated extract) after 96h. The most effective formulation was the 0.2% concentrated extract from *S. montana* showing a mortality of 68% of insects after 96h. The main extract constituent of *S. montana* was caffeic acid, which has a strong insecticidal activity (3,4). Caffeic acid is one of many phenolics that are part of defense mechanisms of plants against microbial infections, insects and other predators (4). Water extracts from different plant species and their active components are natural sources of biocides. Our results show that the use of natural substances could be an alternative method of insect control.

Keywords: aqueous extracts, mortality rate, *Trialeurodes vaporariorum*

References: (1) Dehghani, M. and Ahmadi K. 2013. Bulg. J. Agric. Sci. 19: 691. (2) Farooq, M. et al, 2011. Pest Manag. Sci. 67: 493. (3) Pavela, R. et al. 2009. J. Biopest. 2: 48. (4) Harrison, F. et al, 2003. J. Agric. Food Chem. 51: 2943.

P35 Preliminary Results Regarding the Potential Phytotoxic Activity of *Thymus* spp. Aqueous Extracts

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Phytotoxic natural compounds enable the development of alternative biocontrol products (1). Therefore an increasing number of species is screened for the identification of such substances (2). The current paper assesses the phytotoxic potential of aqueous extracts of three *Thymus* species: *T. comosus*, *T. dacicus* and *T. praecox* against *Raphanus sativus* and *Brassica oleracea* test species. The germination percentage was significantly inhibited (63–95%) in both test species especially when the aqueous macerates were tested. In *R. sativus*, the speed of germination (SG) was lowered by up to 68% and the accumulated speed of germination (AS) by 71% when aqueous macerates and hydrosols of the three *Thymus* species were tested; in *B. oleracea*, mainly macerates were active. The coefficient of the rate of germination was the least affected germination index. Plantlet initial growth was reduced by macerate treatments in a significant manner: in *R. sativus*, with up to 54% decreased root and up to 53% decreased stem lengths. Meanwhile, some of the hydrosols presented a stimulatory activity in radish stem elongation. The growth of *B. oleracea* plantlets was significantly reduced by only one macerate and by hydrosols prepared from plants in the flowering stage of the three *Thymus* species tested. The observed phytotoxic activity can be assigned to the presence of bioactive secondary compounds in tested extracts, rather than to physico-chemical factors, as indicated in spectrophotometric analyses and through pH and conductivity measurements. The differential response of the two test species may suggest that the tested extracts are to some extent selective.

Key words: aqueous extracts, germination, initial growth, phytotoxicity, spectrophotometry.

References: (1) Soltys, D. et al. 2013. In: Herbicides - Current Research and Case Studies in Use, Price A., Kelton J. A. (ed.), InTech, 517-42. (2) Dayan F.E., Stephen O. D. 2014. Plant Physiology Preview, American Society of Plant Biologists.

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P36 Some Experiences with Alternative Control of Pests in Hops with CORA Agrohomeopathie™ Products

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Agrohomeopathy is a practice where principles of homeopathy are used to strengthen and protect cultivated plants. In 2013, preliminary experiment with CORA Agrohomeopathie™ products was conducted in the Savinjska Valley. Four different preparations were used at three application times. Hop plants treated with systemic insecticides against damson-hop aphids and non-treated plants on the same field served as the control. Treatment was carried out with the usual spraying equipment and two protocols were tested.

Phenological development of hop plants, emergence of downy mildew and occurrence of pests were monitored. Treated hop plants developed well despite hot weather and drought conditions. Conditions were generally unfavorable for development of downy mildew. First appearance of the red spider mite (*Tetranychus urticae*) on treated plants was recorded on 8th August. The hop flea beetle (*Psylliodes attenuatus*) was present in both generations and caused some damage on leaves and cone stalks. Constant low pest populations caused no economically significant damage. The damson-hop aphid (*Phorodon humuli*) showed interesting population dynamics. Populations were assessed according to EPPO standards and were higher on treated plants than on control plants including non-treated plants. Population reached peak seven weeks before the harvest and later ceased. No damage was recorded on cones harvested on 6th September. Calculated yield was 1862 kg/ha and the level of 7.4% of alpha acids was reached.

The appearance of damson-hop aphids indicated that some other mechanisms of control are involved in the interaction between pests and treated plants resulting in potential win – win situation. Preliminary experiment with Cora Agrohomeopathie™ products showed that hop plants were in a good condition through entire growing season despite no irrigation. The yield was comparable to average yields of that season, the quality was better than average and the damage caused by pests in the cones was not apparent. Results encouraged us to continue with similar experiments also during next seasons.

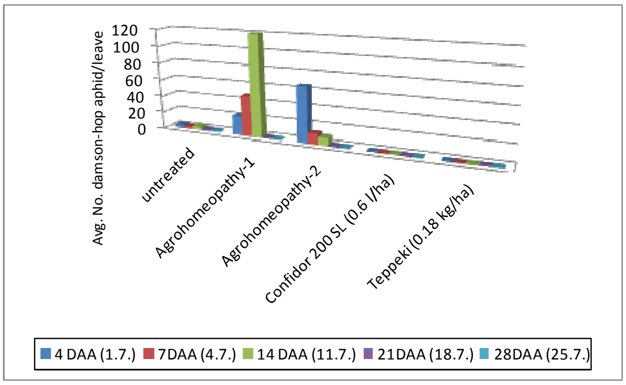

Figure 1: Average number of damson-hop aphid individuals per leaf during July 2013

Keywords: Agrohomeopathy, alternative pest control, preliminary experiment, damson-hop aphid, hop

P37 Impact of Biopesticide Formulation Type on the Efficiency

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Pesticides provide significant benefits to humans by controlling pests that affect agricultural crops. However, there are many risks associated with pesticide use because of their adverse effects on the environment. Consequently, there is an increasing public pressure for the development of safer alternatives. Biopesticides based on microorganisms may represent a desirable path in crop protection if they are highly target specific and without toxic residues and negative impact on the environment (1). Formulation of biopesticides has often been recognized as a critical point as it ensures economic and convenient use, prolongs shelf life of the product, and enhances field efficacy. Formulations are composed of an active ingredient (microorganism metabolites, cells or spores), carriers, adjuvants, compounds that promote optimum efficacy of the active ingredient, retain moisture or promote spreading of the biopesticide. Selection of an appropriate formulation type can improve product stability, enhance and extend activity, and may reduce inconsistency of field performance of many bioligically active agents (2, 3).

The objectives of our studes are: to investigate the possibility of developing different types of effective biopesticide formulations starting from two promising antagonistic strains of *Bacillus subtilis*, to screen adjuvants suitable for the application with developed biopesticide formulations, and to test bioactivity of the developed formulations using an isolate of *Monilinia fructicola* as a model pathogen.

Two different formulation types (two suspension concentrates /SC/ and one suspo-emulsion /SE/) and three plant-oil-based adjuvants were developed. The most effective was SE formulation, which was effective in both, *in vitro* and *in vivo* experiments. The experiment on wound-inoculated apple fruits showed that the formulation was as effective as a reference chemical fungicide and that its effectiveness remained stable for at least 24 hours after the application.

Keywords: biological control, *Bacillus subtilis*, *Monilinia fructicola*

References: (1) Woods, T.S. 2003. In: Encyclopedia of Agrochemicals, Wiley & Sons, New York, USA, (2) Chandler, D. et al. 2011. Philosophical Transaction of The Royal Society B, 386:2–13. (3) Fravel, D.R. 2005. Annual Review of Phytopathology, 43:337–359.

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P38 Metabolomic Approach for understanding Biochemical Mechanism of Grapevine Resistance to *Plasmopara viticola*

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Downy mildew is one of the most important diseases of grapevines (*Vitis vinifera* L.), especially for the European varieties caused by the oomycete pathogen *Plasmopara viticola* (Berk. et Curt.) Berl. et de Toni.

This pathogen was reported for the first time in Europe in 1878, where it was probably imported from North America. To reduce sprayings, *V. vinifera* cultivars were crossed in the past with resistant *Vitis* spp, to select resistant hybrids, but the biochemical mechanisms underlying the resistant phenotypes are poorly understood. Plants respond naturally to a multitude of stress conditions and the biosynthesis of protective chemicals is one of their major strategies (1). The metabolome, typically defined as the collection of small molecules produced by cells, offers a window for interrogating how mechanistic biochemistry relates to cellular phenotype (2). A metabolomic approach was applied, enabling the analysis of hundreds of biomarker compounds of different chemical classes allowing a better understanding of defense response. Particularly primary and secondary metabolites and lipids were analyzed. The aim was to cover all important classes of plant metabolites and to aim at identifying early stage biomarkers within the first 96 hours after pathogen inoculation.

Keywords: oomycete, resistance, metabolomics, *Vitis*

References: (1) Harborne J. B., 1999. Biochem. Syst. Ecol. 27:335–367. (2) Patti G.J., et al. 2012. Nat. Rev. Mol. Cell Biol. 13:263–269.

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P39 Exploring the Prospective Toward Sustainable and Cost Effective Vineyard Management

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Grapevine canopy microclimate, particularly temperature and relative humidity conditions around the clusters, may be of crucial importance for predisposing grapes to different incidences of microbial infections. Different timing of leaf removal leading to different microclimate scenarios, were therefore tested in a Pinot noir (*Vitis vinifera* L.) vineyard. For each leaf removal strategy, alternations of microbial counts and effectiveness of fungicide applications against *Botrytis cinerea* was measured. In parallel, grape quality and yield parameters were followed in two vintages to reveal experiment related alterations. Prospective for cost and energy savings was finally calculated based on all observations. The results revealed that pre-flowering leaf removal is more effective for controlling microbial infections than late (veraison) leaf removal. Also good quality parameters were retrieved with this strategy. Pre-flowering treatments also reduced bunch compactness (17% reduction) representing another critical factor influencing microbial diseases. Furthermore, reduced yields (30% in 2010 and 24 % in 2011) obtained by pre-flowering leaf removal strategies reduces the need for cluster thinning and consequently total energy consumption. We calculated a total of 27 % of reduced costs and 46 % reduced energy consumption. Accordingly, this novel technique presents a sustainable and cost-effective Pinot noir vineyard management strategy that could be applied in the Vipava valley and similar geo-climatic areas.

Key words: *Botrytis cinerea,* cost effective vineyard management, microbial ecology, 'Pinot noir' (*Vitis Vinifera* L.), polyphenols, pre-flowering leaf removal, sustainable viticulture

References: (1) Sternad Lemut, M. et al. 2013. Journal of Agricultural and Food Chemistry, 61: 8976-8986. (2) Sternad Lemut, M. et al. 2015. Australian Journal of Grape and Wine Research, In press.

Acknowledgement

The work was partially financed through European Union funds, the European Social Fund of Operational Programme for Human Resources Development for the period 2007–2013, first development priorities: Promoting entrepreneurship and adaptability, policy priorities 1.3: Scholarship Schemes. Part of research was performed within the Creative Core programme (AHA-MOMENT) with contract no. 3330-13-500031, co-supported by RS-MIZS and European Regional Development Fund Research supported by Slovenian Research Agency, project BI-BG/09-10-009 and by ADP 2014 Project founded by the Autonomous Province of Trento, Italy.

P40 Protection of fauna for the sustainable maintenance of protected areas and parks: Natural Resource Project "University City"

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Research on the diversity of trees and shrubs in parks located in protected areas was conducted within the resource management project "University City" in Banja Luka since 2011. 81 species of trees and shrubs (1,2) and 31 nesting bird species were recorded in a park area encompassing 28 ha (3). Also, other permanent residents of vertebrate groups have been observed in the territory of the park. At the same time, the health status of plant species was determined by recording registered plant diseases and harmful organisms such as insects, mites and weeds. Recently it has become evident that the increased use of chemicals for plant protection presents a threat for the environment, disrupts the stability of ecosystems, creates resistance in harmful organisms and has other adverse effects. Accordingly, pesticides are becoming increasingly unpopular. This paper presents a synthesis of previous research approaches and addresses the possibilities of developing biological control and biological protection measures. The goal is to improve the conditions for the survival of beneficial organisms. The presented research provides the basis for distinguishing important ecological key elements that were partly seen and their idioecological characteristics. They include various insect taxa from the orders Coleoptera, Neuroptera, Diptera, Hymenoptera, etc.; birds of the orders songbirds (Passeriformes) and owls (Strigiformes); and mammal insectivore species such as bats (Chiroptera). Bats are predators of species that are harmful for the encountered dendroflora of the studied protected areas. Protective measures include the establishment of new and maintaining of existing habitats and establishment of corridors and feeding places for beneficial species. Infrastructure must be planned in order to keep beneficial species in the area established as well as to attract others.

Keywords: fauna, biodiversity, biological protection, biological control, green areas

References: (1) Anonymus 2013. Program for management of protected area with sustainable use of natural resources "University city" for the period 2013 - 2015. Genetic Resources Institute of the University of Banja Luka. (2) Kadić et al. 2013. Proposal for the protection of the area for management of the resources complex "University city" in Banjaluka. The Republic Institute for Protection of Cultural, Historical and Natural Heritage of the Republic of Srpska. Banja Luka. (3) Sjeničić et al.2013. Breeding birds in urban parks of Banjaluka. "Skup" No. 5(1), 39-46. Faculty of Sciences, University of Banja Luka.

Acknowledgement

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P41 Involvement of Potato Ethylene Response Factor in Response to Potato Virus Y

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Potato *(Solanum tuberosum L.)* is the world's most widely grown tuber crop and potato virus Y (PVY) is one of the major potato pathogen causing severe crop loss in different areas worldwide. To better understand the potato defence response against PVY we studied the role of *ethylene response factor* (*ERF*) genes from group IX, since they have been related to the plant defence response and defined as important elements on hormone crosstalk.

Potato *ERF-IX* genes were identified and classified in this study. Among them, *StERF* was selected for further analyses based on previous transcriptomics experiments performed in our group (1). Expression patterns of the gene in hypersensitive resistance (HR) potato cultivar infected with PVY pointed to its importance as a signalling component in potato defence response. Using virus-induced gene silencing (VIGS) we demonstrated that PVY systemic spread is delayed in *StERF* silenced plants. We further examined the potential hormonal signalling involved in the expression of *StERF* and demonstrated that our gene integrates several signalling pathways. Getting more insights into the regulation of the gene, localisation studies showed that StERF strongly accumulated in cell nucleus after PVY infection.

Taken together our results suggested the importance of *StERF* in potato-PVY interaction. Therefore the data contributes to better understanding of the complex network of plant defence signalling pathways.

Keywords: *Solanum tuberosum*, PVY, ERF, plant-virus interaction, functional analysis

References: (1) Baebler, S. et al. 2014. Journal of Experimental Botany, 65(4):1095-1109.

Acknowledgement

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P42 The Role of microRNAs (miRNAs) in the Response of Potato (*Solanum tuberosum* L.) to Infection with Potato virus Y

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MicroRNAs (miRNAs) are a class of small ~21-24 nucleotides long non-coding RNAs capable of negatively regulating gene expression by targeting mRNAs for cleavage or translational repression and thus play important regulatory roles in plant development and responses to environmental stress (1). Therefore, it is likely that levels of some miRNAs might be an important factor of potato plant immunity against the Potato virus Y^{NTN} (PVY^{NTN}), which *causes* substantial losses in potato production by decreasing yields and affecting the quality of potato tubers.

Five miRNAs, miR168, miR172, miR390, miR482 and miR5300, were selected to compare their expression profiles in two potato genotypes, tolerant Désirée and sensitive NahG-Désirée, depleted in salicylic acid (SA), in the early response to infection with PVY^{NTN}. RNA was extracted using TRIzol and then miRNA quantification was performed using two step RT-qPCR with Taqman miRNA-specific stem-loop reverse transcription primer to synthesise cDNA and subsequent amplification using Taqman hydrolysis probe. Our results show that after infection with the PVY^{NTN} the amount of miR172 and miR390 in Désirée increased and levels of miR390, miR482 and miR5300 did not change, while in NahG-Désirée levels of all five miRNAs remained unchanged. The data obtained by RT-qPCR, were compared with results of next-generation sequencing technology. Comparison revealed some inconsistency in RT-qPCR and RNA-seq obtained data, since each method introduces certain bias, however increased quantity of miR172 and miR390 in Désirée after infection with PVY^{NTN} was shown. It can therefore be concluded that their increased quantity in addition to SA could contribute to the establishment of tolerance to infection against PVY^{NTN}.

Future challenges include better understanding miRNAs mechanisms of regulation, especially in resistant plants, and developing new antiviral strategies with either over-expression of natural miRNAs or inhibition of miRNAs activity. Thus, these plants should be able to resist plant stress without compromising their yields.

Keywords: potato, PVY, miRNA

References: (1) Debat H.J. and Ducasse D.A. 2014. Plant microRNAs: Recent Advances and Future Challenges. Plant Mol Biol Rep, 32:1257-1269.

Acknowledgement

National Institute of Biology, Department for Biotechnology and Systems Biology, Omics group (K. Gruden et al.).

P42 Transcriptional Regulatory Network in Potato – Potato Virus Y Interaction Signalling

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Plant hormones are crucial signalling molecules that coordinate all aspects of plant growth, development, reproduction and defence. Three hormones are especially important for plant immune response. The SA, JA and ET signalling pathways represent the backbone of the defence signalling network, with other hormonal signalling pathways feeding into it. The importance of salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) as dominant primary signals in local and systemic induced defence signalling has been well documented. However, the way these signal molecules function in a complex network of interacting pathways is less well understood and the majority of research has been done on model plant species and very little on potato. Our aim is to upgrade our current understanding of the roles of SA, JA and ET in the plant's immune system and crosstalk between defence hormone signalling pathways, with a focus on promoter analysis of chosen genes from defence signalling network.

In order to understand transcriptional network of signalling components in potato following PVY infection we decided to analyse the promoters of some genes that are crucial components in plant defence signalling pathways. One of them is transcription factor MYC2 from jasmonic acid pathway that regulates PCPI expression.

Promoters obtained from different potato cultivars were sequenced, compared to the available model genome sequence and analysed with TRANSFAC and PlantCARE. The results showed that promoter sequences of the same gene differ between cultivars. Furthermore, each gene can have different promoter sequences within the same cultivar. Using TRANSFAC and PlantCARE we managed to characterized transcription factor binding sites and established how transcription factor binding sites vary between promoters of the same gene within each cultivar.

Keywords: potato virus Y, plant immune response, jasmonic acid, promoter analysis, MYC2

P43 Dynamics of Primary Metabolism–Related Responses in Potato-Potato Virus Y (PVY^{NTN}) Interaction

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Potato virus Y (PVY) is a major pathogen that causes substantial economic losses in worldwide potato production. Different potato cultivars show different levels of resistance to PVY, from severe susceptibility, through tolerance, to complete resistance. The aim was to better define the mechanisms underlying tolerant responses of potato to infection by the particularly aggressive PVY^{NTN} strain.

Time series experiment was performed that included whole-transcriptome analysis, nontargeted proteomics, and photosynthetic activity measurements in tolerant potato cv. Désirée and its transgenic counterpart depleted for accumulation of salicylic acid (NahG). Faster multiplication of viruses can occur in the transgenic plants, with these plants developing strong disease symptoms. The results reveal the extensive dynamics of responses related to photosynthesis at the level of transcription in nontransgenicDésirée plants. In the first days after infection, genes related to photosystems were significantly up-regulated. With onset of viral multiplication, however, there was down-regulation of these genes. Specific and constant up-regulation of some RuBisCO transcripts which were shown to interact with viral proteins was detected in nontransgenic plants. Analysis of NahG plants revealed consistent repression of photosynthesis-related genes. A constant reduction in the photochemical efficiency in the NahG plants from the onset of viral multiplication was detected; in nontransgenic plants this decrease was only transient. The transient reduction in net photosynthetic rate occurred in both genotypes with the same timing, and coincided with changes in stomatal conductivity.

Down-regulation of photosynthesis-related gene expression and photosynthetic activity was shown. In addition induction of light-reaction components in the early stages of infection was detected. As some of these components have been shown to interact with viral proteins, their overproduction might contribute to the absence of symptoms in cv. Désirée. We show that detailed time-series analyses are necessary to improve our understanding of the plant defense mechanisms.

Keywords: plant–pathogen interactions, potato virus Y, Potyviridae, salicylic acid, *Solanum tuberosum*

Acknowledgement

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P44 Biological Diversity of Wheat and its Improvement for Organic Agriculture

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The project "BIOFARM" addresses characterization of wheat genetic resources variability from different regions of the world. Within the project we have estimated the genetic diversity of different species and subspecies of goatgrass. Using different selection methods, we created new varieties of emmer wheat and spelt for organic farming. The Slovak samples of goatgrass, coming from two different genetic pools, were analysed for DArT polymorphism, and identified as genetically different from the original accession DIM 132. The emmer sample has been submitted to the State Varietal Tests (DUS). The later results will be used along with molecular analyses to reveal phylogenetic relationships within wheat taxa and contribute to the evolutionary and taxonomic knowledge of the tribe Triticeae. In frame of the project we also performed research on presence and activity of selected defensive enzymes under abiotic (drought, heavy metal pollution, low temperature) and biotic stresses (pathogenic fungus *M. nivale*). In addition, molecular markers were used to screen a set of more than 60 wheat varieties for potential of accumulating metal (cadmium) ions, resulting in identification of varieties with low- eventually higher potential risk for production of contaminated food. The obtained results and information will be applied in climate change research, for breeding new wheat varieties, in conventional and organic agriculture, in the agri-environment studies and forecasts, for preparation of scientific articles and in the educational process.

Keywords: organic agriculture, wheat, goatgrass, emmer wheat, polymorphism, DArT

Acknowledgement

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0197-10, APVV-0661-10 and by the Operational Programme Research and Development for the project: "Implementation of the research of plant genetic resources and its maintaining in the sustainable management of Slovak republic" (ITMS: 26220220192), co-financed from the resources of the European Union Fund for Regional Development.

P45 Genetic Diversity of Croatian Winter Wheat Varieties

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Around 20% of human food production relays on wheat. Weather extremes are experienced most frequently in the Central European countries having the greatest influence on ecosystems and in addition, the appearance of new plant pathogens, pests and weeds can be expected in some regions, due to the global warming (1). Climate change evokes new demands, such as adaptability to various stresses as well as new approaches both in plant breeding and in the strategy and policies of biodiversity conservation. Sustainability and development of new genetic diversity in wheat germplasm is one of the main prerequisites for successful wheat breeding programs. Danger of genetic erosion especially exists in smaller breeding programs and in selection for limited production area with similar growing conditions (2). Croatian winter wheat breeding has a long tradition but it is relatively small in comparison with countries having large production. Use of SSR markers in evaluating genetic diversity of winter wheat varieties is limited in Croatian breeding programs (3). Therefore, aim of this study was to evaluate genetic diversity of Croatian winter wheat varieties using SSR markers as a powerful tool for assessing genetic diversity. Forty winter wheat varieties, from three Croatian breeding centres were included in the study. They were registered from 1931 until today and were or still are grown on the large areas. A set of 26 microsatellite markers were used, covering three wheat genomes and 42 chromosomes. A total of 108 alleles were detected with the average gene diversity (He=0.58). Microsatellites were very efficient in determination of population structure, grouping the varieties almost perfectly according to pedigree.

Keywords: winter wheat, diversity, SSR markers

References: (1) Juroszek, P., von Tiedeman A. 2012. Eur J Plant Pathol.136:21–33. (2) Fu, Y.B., Somers, D.J. 2009. Crop Sci. 49: 161-168. (3) Dvojković, K. 2009. Doktorska disertacija, Sveučilište u Zagrebu, Agronomski fakultet, Zagreb, Hrvatska

P46 Wild *Lactuca saligna* Richness for Lettuce Breeding

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Substantial progress in modern breeding of lettuce (*Lactuca sativa* L.) is supported by exploitation of wild related species, donors of valuable traits, e.g. resistance (4). Willow-leaf lettuce (*Lactuca saligna* L.), strategic species in lettuce breeding, is largely distributed over the world, but only a limited area of natural distribution is represented by accessions in the world's germplasm collections (3). Knowledge of its biodiversity is rather limited. Complex research activities were developed in the Czech Republic in cooperation with some other countries (e.g. Israel). Original data on ecology and distribution of *L. saligna* in Europe, North America and Israel were obtained, new seed samples were acquired for further studies. Large variation of morphological and phenological traits among samples originating from different eco-geographic conditions was recognized. New sources of resistance to economically important races of lettuce downy mildew (*Bremia lactucae*) (1,5) and powdery mildew (*Golovinomyces cichoracearum*) were identified. Resistance against *B. lactucae* is considered as a nonhost resistance. Samples originating from various eco-geographical conditions (Near East *vs.* Mediterranean Basin) differ significantly in their molecular polymorphism (AFLP) and they are genetically different (2). There were recognized natural interspecific hybrids between *L. saligna* and some other related *Lactuca* spp. (*L. serriola*). F1 hybrids raised from the controlled hybridization of *L. saligna* with *L. sativa* were released, F1 progeny were identified by morphological traits and proved by isozymes. Recently, *L. saligna* is broadly used in commercial lettuce breeding.

Keywords: willow-leaf lettuce, biodiversity, molecular polymorphism, disease resistance, interspecific hybridization

References: (1) Beharav, A. et al. 2008. Israel J. Plant Sci. 56:195. (2) Kitner, M. et al. 2008. Israel J. Plant Sci. 56:185. (3) Lebeda, A. et al. 2004. Bot. Rev. 70:328. (4) Lebeda, A. et al. 2009. Euphytica 170:15. (5) Lebeda, A. et al. 2014. Eur. J. Plant Pathol. 138:597.

Acknowledgements

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P47 Verification of PCR Method for Detection of the Most Commonly Used Structural Elements in the Genetic Modification of Plants

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The highly specific method of the Polymerase Chain Reaction (PCR) is the method of choice in the EU for the detection of GMOs. For the application of new validated PCR method, the laboratory must confirm that the same can be used for the intended purpose.

This process involves verifying the entire process of conducting laboratory methods on the performance basis. Many of the procedures used, are now standardized and available in the form of working protocols and recommendations of the European Commission Joint Research Centre (JRC) or the guidelines for Good Laboratory Practice (GLP). Their adoption is a prerequisite for application of molecular genetic methods for the analysis of the presence or absence of GMOs. The main objective of this study, conducted in the laboratory of Molecular Diagnostics in Federal Institute of Agriculture Sarajevo, was a verification of GeneScan protocol to assess the presence of two genetic sequences commonly used in the production of transgenic plants - Cauliflower mosaic virus (CaMV) CaMV 35S promoter and NOS terminator. CaMV 35S promoter is preferred over other potential promoters, due to its very high activity and transcription and it isn’t heavily influenced by environmental conditions. The second sequence, NOS terminator, was derived from gram-negative soil bacterium *Agrobacterium tumefaciens* and used as a transcriptional terminator. Skills and ability of our laboratory to perform PCR were confirmed through participation and successful performance of PCR analysis through a Proficiency Test. Establishing prescribed validated methods for the detection of GMOs in national laboratories enabled better control over the circulation of products that may contain or originate from GMOs, timely detection and therefore enabling the appropriate action of institutions including the possible prohibition of product placing on the market of Bosnia and Herzegovina.

Keywords: PCR verification method, GMOs, CaMV 35S, NOS

P48 Forage Crops Breeding for Improvement of Yield, Quality and Disease Resistance

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Alfalfa and red clover are the most researched perennial crops in major forage legumes breeding programs. Agronomical interests for these crops are based on its numerous advantageous characteristics such as high yield potential, suitable feeding value and favourable environmental impact. Perennial forage legumes breeding programs are usually based on mass selection, phenotypic recurrent selection and synthetic development using polycross method. The main objective of most breeding programs is the development of new varieties with high forage yield and quality and multiple disease resistances. Breeding program of perennial forage crops at the Agricultural Institute Osijek is existing and is continuously carried out for over than 50 years. In this period, the Institute has inscribed 19 varieties of alfalfa and two varieties of red clover and has as well a rich collection of genetic variability and diversity of domestic and introduced germplasm. Development of new varieties is a very long-lasting and difficult process where in the first place all the available germplasm needs to be evaluated, by means of large number of individual plants/cuttings in breeding nurseries over multiple years. In the each of several cycles of selection, the superior plants are selected and

intercrossed, to generate new populations with improved important agronomic traits. In the climatic conditions of Republic of Croatia the major alfalfa and red clover diseases are Sclerotinia crown and stem rot, Verticillium wilt, Fusarium wilt, Common leaf spot, Downy mildew and Powdery mildew. In our breeding program we are selecting alfalfa and red clover plants carrying natural resistance to the major pathogens occurring naturally in the field. These resistant plants are the basis for the development of new varieties. In sustainable plant production systems forage crops have an important place due to numerous agricultural and environmental benefits.

Keywords: alfalfa, red clover, breeding method, yield, diseases

P49 Trees of Old Slovenian Apricot Cultivars Exibit Tolerance Towards '*Candidatus* Phytoplasma prunorum'

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In Slovenia apricots were traditionally grown in Primorska and Posavje regions of Slovenia. Local cultivars were grafted on *P. insititia* root suckers. In the past several types of local cultivars 'Pišeška marelica' from Posavje and 'Debeli flokarji' and 'Catarji' from Primorska were selected and included in the list of cultivars recommended for growing in Slovenia. Today new foreign cultivars are grown in intensive orchards, but apricot cultivation is severely hampered due to damages caused by '*Candidatus* Phytoplasma prunorum' ('*Ca*. P. prunorum') and *Plum pox virus* (PPV). Both pathogens are widespread in Slovenian stone fruit growing areas. Aphids that transmit PPV and *Cacopsylla pruni*, the vector of '*Ca*. P. prunorum', are also abundantly present; therefore the infection pressure of both pathogens is very high. In the frame of two research projects local cultivars from various locations were sampled and tested for the presence of '*Ca*. P. prunorum' and PPV. Samples were collected from 8 trees of 'Pišeška marelica', 2 of 'Budanjska marelica', 2 of 'Bela pašta', 3 of 'Catarji', 10 of 'Debeli flokaji' and 2 of 'Drobni flokarji'. With exception of one tree of "Pišeška marelica" all other trees proved to be infected with '*Ca*. P. prunorum', but none of them showed symptoms of phytoplasma infection. Several hypotheses might be postulated to explain the absence of symptoms and will be discussed in the presentation. Some of them will be checked in the next years, since the cultivars are potentially valuable for growing in regions infected with '*Ca*. P. prunorum' and an interesting source for apricot breeding. Additionally, most of the cultivars might be resistant or less susceptible to PPV, since the virus was detected only in one sample from cultivar 'Bela pašta' and another one from cultivar 'Debeli flokarji' although PPV is highly spread in both regions.

Keywords: apricot, '*Candidatus* Phytoplasma prunorum', *Plum pox virus*, tolerance, local cultivars

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P50 Applicative Aspects of Marker-Assisted Selection (MAS) and *in vitro* Tissue Culture for Plant Protection and a Truly Sustainable Agriculture

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Considering the rapidly growing world population, the impacts of climate change and the detrimental impact of agricultural systems on the environment, sustainable forms of food production need to be developed (1). Biotechnological plant breeding techniques such as Marker-Assisted Selection (MAS) and *in vitro* tissue culture are part of an alternative and non invasive biotechnology that could be one part of the solution to the food crisis and impact of climatic changes on food production (2). We focused in particular on some applicative aspects of these biotechnology techniques that are suitable to be used in technology transfer projects with the aim to implement this technology in agro-food industry. Advance in agro-food sector of Emilia Romagna (Italy) and Slovenia were investigated to determine the main actors and collect data regarding (bio-) technologies that still need to be implemented by agro-food industry. Data was collected by interviews of different actors from most relevant institutions of research and industry and used for actor analyses and multi-level perspective (MLP) analyses (3). The analyzed actors' aspects regarding this challenge were: interest, attitude, power, proposed solutions, criteria of the solution, expectations. Analyzed aspects of MLP were niche-innovations, sociotechnical regimes and landscapes. Results enlightened different points of views of actors and gaps where technology transfer is recommended (controlled seeds production, selection of new varieties, protection of crops from new invasive pests, citizen science, varietal passports). Compared with Slovenia, Emilia Romagna presented a well developed smart specialization of agro-food sector, R&D oriented on GMO-free technology and a supportive landscape. Acquired knowledge is useful to propose innovative business ideas or collaborations for technology transfer.

References: (1) Kang, Y. et al. 2009, Progress in Natural Science 19:1665-1674. (2) Collard, B.C.Y. & Mackill, D.J. 2008. Phil. Trans. R. Soc. B, 363:557-572. (3) Geels, F.W. & Schot, J. 2007. Research Policy 36:399-417.

Keywords: biotechnology, food supply, plant protection, climate change

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P51 Development of genetic map and analysis of quantitative trait loci for drought tolerance in common bean (*Phaseolus vulgaris* L.)

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Because of the climate changes, higher temperatures and changed rainfall pattern, drought affects every year more than half of the common bean production worldwide (1). The development of varieties with a strong tolerance to abiotic stress is the primary goal of many common bean breeding programs in the world (2, 3). Common bean is susceptible to drought, but the mechanisms of its response are still not well characterized.

The population of 80 F8 recombinant inbred lines (RILs) based on the crossing of drought tolerant cultivar Tiber and susceptible cultivar Starozagorski čern was grown in a greenhouse under controlled conditions. At the stage of flowering, RILs were stressed by withholding irrigation. Morphological and physiological parameters that discern the two parental lines, e.g. seed and flower color, days to flowering, seed yield, one seed mass, water potential and photosynthetic fluorescence were scored at different stages of drought. Both parental lines and all RILs were checked for polymorphisms using DNA. Overall, 476 microsatellites and 256 AFLP markers were tested. One hundred twenty-seven polymorphic markers were used to construct the common bean genetic map. Markers were arranged into 11 linkage groups (LGs) based on common bean consensus map and two additional unclassified LGs. QTL analysis showed linkage between molecular markers and QTLs for days to flowering and to one seed mass on LG1 and LG9 consensus map and pointed to some other potential linkages which will be examined in our future work.

QTL analysis will be employed to identify informative markers that are closely linked to loci involved in the response of common bean to drought stress. These markers are expected to significantly improve our breeding programs aimed at development of drought tolerant bean varieties.

Keywords: common bean, drought stress, genetic markers, QTL mapping, breeding

References: (1) Ceullar-Ortz, S. M. et al. 2008. Plant cell environment, 31:1399-1409. (2) Miklas, N. P. et al. 2006. Euphytica, 147:105-131. (3) Beaver, J. S. and Osorno, J. M. 2009. Euphytica, 168:145-175.

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P52 GoMapman: Helping Translational Research in Agriculture

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Potato (*Solanum tuberosum* L.) is most widely grown tuber crop and the fourth most important food crop with a continuous progression of the world production of tubers that is challenged by changing environment and new emerging diseases. Understanding its biology by using systems biology tools can contribute to plant breeding and development of efficient agricultural practices. However, the exploitation of data obtained by high-throughput wet lab methods is hindered by dispersed data and unavailability of potato specific functional annotation and visualization tools. The genome of diploid potato was sequenced three years ago and enabled better integration of the existing data.

To combine existing information on potato genes and enable data integration from various datasets, we have merged information from two potato gene models and two sets of potato unigenes to 35609 gene paralogue groups. For easier data interpretation we have adapted plant-specific MapMan ontology and designed a centrally maintained database and a web interface GoMapMan (www.gompaman.org) (1) for browsing, searching and editing the ontology and gene annotations. Within the ontology, genes of the model species Arabidopsis and 4 crop species (potato, tomato, tobacco and rice) are connected through orthologues. The main features of GoMapMan include 1) dynamic and interactive gene product annotation through various curation options, 2) consolidation of gene annotations for different plant species through the integration of orthologue group information, 3) traceability of gene ontology changes and annotations, 4) integration of external knowledge about genes from different public resources, and 5) providing gathered information to high-throughput analysis tools via dynamically generated export files.

Keywords: potato, translational agriculture, gene ontology, orthologue genes

References: (1) Ramšak, Ž. et al. 2014. Nucleic Acids Res. 42:D1167.

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P53 Evaluation of Plant Cell Viability by the Use of Chlorophyll Fluorescence

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Plants living under natural conditions are exposed to many adverse factors that interfere with the photosynthetic process, leading to declines in growth, development and yield. The recent development of Chlorophyll *a* fluorescence (ChlF) represents a potentially valuable new approach to study the photochemical efficiency of leaves. Specifically, the analysis of fluorescence signals provides detailed information on the status and function of Photosystem II (PSII) reaction centres, light-harvesting antenna complexes, and both the donor and acceptor sides of PSII. The main goal of our study was to assess the sensitivity of testing procedure and usefulness of different parameters derived from the basic parameters of chlorophyll *a* fluorescence as well as from fluorescence kinetics analysis for their application in screening of wheat genotypes. We discuss the potential scientific and practical applications of this innovative methodology especially for the purposes of crop phenotyping and monitoring.

Keywords: Chlorophyll fluorescence, wheat, crop phenotyping, breeding

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P54 Weed Control with Foramsulfuron and Thiencarbazone-methyl in Sugar Beet Tolerant to ALS-Inhibiting Herbicides

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Sugar beet with tolerance to acetolactate synthase (ALS)-inhibiting herbicides, represents a new tool that offers unique solutions in weed control. Safety for the crop is guaranteed because in ALS-inhibitor tolerant sugar beets the ALS-inhibiting herbicides can't bind with target ALS enzyme.

The aim of this study was to evaluate the sugar beet response and efficacy in weed control of post-emergence single and split application of foramsulfuron (50 g·ha^{-1}) + thiencarbazone-methyl (30 g·ha^{-1}) compared with standard mixture of desmedipham + phenmedipham + ethofumesate + metamitron applied three times. Field trials were conducted in 2013 and 2014 in location Novi Sad (Serbia). The plots of 20 m^2 size were arranged in a complete randomized block design with four replications. The results obtained show that tolerance to ALS inhibitors and foramsulfuron + thiencarbazone-methyl are excellent new tools for the effective control of the most important annual weeds in sugar beet. Single and split application of ready to use mixture of foramsulfuron + thiencarbazone-methyl have achieved excellent efficacy (>95%) of hard-to-control weeds: *Abutilon theophrasti, Amaranthus retroflexus, Ambrosia artemisiifolia, A. trifida, Chenopodium album, Datura stramonium, Xanthium strumarium, Sinapis arvensis, Solanum nigrum* and *Sorghum halepense*. Consistent weed control was achieved regardless of whether foramsulfuron and thiencarbazone-methyl were applied once or twice, with complete selectivity to the sugar beet (including two times higher rates of herbicides than the recommended), and without negative influence on root yield and sugar content. Sugar beet tolerant to ALS-inhibitors will provide growers safer, very flexible and consistent season long weed control, with only 1-2 applications of foramsulfuron + thiencarbazone-methyl. This technology needs to be managed to make it sustainable in the future.

Keywords: sugar beet, foramsulfuron, thiencarbazone-methyl, weed control, crop tolerance

P55 Vineman.org: Integration of plant resistance, cropping practices, and biocontrol agents for enhancing disease management, yield efficiency, and biodiversity in organic European vineyards.

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The VineMan.org Project aimed at designing, developing, and testing innovative cropping systems for managing organic vineyards in Europe. The main focus was to improve the control of common vine plant diseases (downey and powdery mildew, grey mould), to enhance grape production efficiency in terms of sustainability, yield and berry composition. Furthermore, we aimed to increase the microbial biodiversity in the organic vineyards and to minimize the environmental impact of agriculture in the grape growing areas. To reach the goals 9 research groups performed different tasks in order to integrate plant resistance against fungal pathogens, cropping practices, and use of BCAs depending on environmental conditions. Methods for inducing the innate immunity of plants against fungi and oomycetes pathogenic to *Vitis vinifera* were evaluated and the effect of some viticulture management options on the development of the target diseases was investigated, with particular focus on canopy structure and cluster/berry morphology modifications. Study of the relationships between the target pathogens and the environmental conditions with emphasis to the development of weather-driven, mechanistic, dynamic models for predicting plant disease epidemics was carried out. Fitness, impact, and efficacy against the main grape diseases, was evaluated in four BCAs already registered in the EU as microbial biopesticides. Development of new strategies based on design-assessment-adjustment cycle was made and tested in field trials. Evaluation and monitoring of the microbial communities present on leaves and berries was done. Early leaf removal, resulting in modified microclimate and changed bunch compactness and berry skin structure, was effective in reducing grey mould incidence on grapes. The entomopathogenic fungus *Lecanicillium lecanii* was used against the leafhopper *Scaphoideus titanus* and proved to be virulent to its second instar nymphs. Web-based decision support system for organic viticulture tested in vineyards reduced the number of copper treatments against downy mildew by 36% on average.

Keywords: organic viticulture, biocontrol agents, microbial biodiversity

Acknowledgement

The study was supported by CORE Organic II Funding Bodies.

P56 VIPS – an Open Source Technology Platform for Prognosis and Decision Support

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VIPS is an Open Source technology platform developed for international collaboration on prognosis and decision support, where results from forecasting models can be easily distributed to users anywhere. The system is based on 14 years of experience with a web based forecasting and information service for integrated management of pests and diseases in cereals, vegetables, and fruit crops in Norway. The system allows for local adaptations, including language, incorporation of models and other services.

VIPS was tested in Sweden and Bosnia and Herzegovina in 2014. In 2015, testing will continue in Bosnia and Herzegovina, while the system will be put into production for several disease models in Sweden and Norway. This includes *Phytophthora infestans* in potato and *Fusarium* spp. in oats.

As a part of a project financed by the Norwegian ministry of Foreign affairs with focus on ICT development as a tool for improving IPM in Bosnia and Herzegovina, the system will include forecasting models relevant for apple orchards (*Venturia inaequalis, Cydia pomonella*) and vineyards (*Plasmopara viticola* and *Lobesia botrana*). Several weather stations have been installed in the fruit growing region of Tuzla, and one in the research vineyard of the University of Mostar. Combining data from these locations with weather prognosis, the models will be tested and validated in these regions. An important goal is to provide a flexible and sustainable tool for further improvement of IPM in Bosnia and Herzegovina.

Keywords: Prognosis, *Venturia inaequalis*, *Cydia pomonella*, *Plasmopara viticola*, *Lobesia botrana*

P57 Modified QuEChеRS for Ergosterol Determination by HPLC-DAD

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Ergosterol is a specific component of fungal membrane and other cellular constituents. It is good for the quantification of growth rate and fungal biomass in various matrices such as plant roots, stems or fungal cultural filtrate. Recently, solid phase extraction (SPE) has replaced liquid-liquid extraction (LLP) in ergosterol determination. But the growing concern over the environmental protection has led to the decrease in the application of toxic solvents and development of extraction method based on SPE in order to avoid LLP as a purification procedure. Further decrease in solvent application as well as in sample preparation time, results in the development of a number of alternative extraction procedures. Anastasiades et al. (2003) developed a quick, essential, cheap, efficient, robust and safe method (QuEChERS) to overcome the limitations of the existing preparation methods. In our study, the ergosterol was extracted from spiked sunflower stalk using an extraction procedure based on QuEChERS. The amount of 2 g of fine homogenised sample was mixed with 4 mL of MeCN, 2 ml of BHT and 4 ml mixture of KOH in MeCN. After extracting on vortex mixer, the sample was shaken for 1 hour on 80 °C. After shaking the anhydrous magnesium sulfate, sodium chloride, trisodium citrate dehydrate and disodium hydrogen citrate sesquihydrate were added. The mixture was shaken vigorously for 1 min and centrifuged for 5 minutes at 4000 rpm. After the centrifugation 5 mL of supernatant was transferred into a clean-up tube containing $MgSO_4$, PSA and C18. After the centrifugation for 5 minutes at 4000 rpm, the supernatant was analised by HPLC-DAD. Thus, an efficient, sensitive and specific method was developed for the determination of ergosterol. The linearity from 0.5–10.0 µg/mL, exhibit good R^2, over 0.999. LOD was 0.2 mg/kg with LOQ of 0.5 mg/kg. The average recovery for the concentration in ranges from 1.0 to 10 µg/mL was 97.9 ± 6.19%.

Keywords: ergosterol, QuEChERS, HPLC-DAD

References: (1) Anastassiades, M. et al. 2003. J AOAC Int, 86:412.

Acknowledgement

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P58 Sorbent Influence on Validation of Fungicide Residue Determination in Sour Cherries: Statistical Analysis

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Matrix effects are a major concern in pesticide residue food analysis. They can cause serious problems because they severely compromise qualitative and quantitative analysis of compounds at trace levels (1). Sour cherries are rather demanding as a matrix in which the pesticide residues are determined due to the present pigments. Therefore, the mixtures of $MgSO_4$/PSA/GBC; $MgSO_4$/PSA/AC and $MgSO_4$/PSA were added in the validation procedures as they have a strong affinity towards planar molecules, thus causing discolouration of the extract. The LC-MS/MS combined with electrospray ionization (ESI), was applied for the validation of triazine and strobilurine fungicides in sour cherries after using different sorbents for cleaning the extract. The one-way ANOVA applied for the validation of fungicide residue analysis in sour cherries comprised the sorbent influence in terms of recoveries and matrix effects. ANOVA did not show any statistical significances regarding the influence of pesticide groups and concentrations as an independent variable on the recovery as a dependent variable (for $p<0.05$), but proved to be of high statistical significance with the sorbent mixtures $MgSO_4$/PSA ($p_m=0.000$ for $p<0.01$). When applying Fisher's LSD test high statistical differences between all combined pairs (sorbents) were found (all values were $p=0.000$ for $p<0.01$ (for $MgSO_4$+PSA vs $MgSO_4$+PSA+GBC; $MgSO_4$+PSA vs $MgSO_4$+PSA+AC and $MgSO_4$+PSA+GCB vs $MgSO_4$+PSA+AC). The calculation of matrix effects (Fisher's LSD test) pointed at high statistical differences (for $p<0.01$) in the values of matrix effects on the pesticide determination between myclobutanil, triadimenol and penconazole in relation to tebuconazole, difenoconazole, azoxystrobine, krezoxym-methyl and pyraclostrobine (except penconazole and krezoxym-methyl where statistically significant differences were found, $p<0.05$) and high statistical significance between tebuconazole and krezoxym-methyl and statistically significant values in relation to tebuconazole-difenoconazole and tebuconazole-pyraclostrobin.

Keywords: statistics, fungicide residues, sour cherries, LC-MS/MS

References: (1) Fernandez-Alba, A. et al. 2014.10th EPRW, Dublin, Ireland, Book of abstracts, 54.

Acknowledgment

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P59 Nematode Dynamics Under Minimum Tillage

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Agriculture has transformed over the past centuries dramatically to become highly dependent on non-renewable fossil fuels, synthetic fertilizers and plant protection agents. This often occurs at the cost of soil degradation and soil food web disturbance. Future agriculture must not only be productive but also provide ecosystem services such as carbon sequestration, pest and disease suppression, nutrient cycling and water storage to become sustainable in the long-term. Sustainability is especially fostered by reduced tillage and the presence of a permanent layer of plant residues. Under such conditions, soil compaction is reduced, soil structure improved, water infiltration enhanced, soil organic content increased and microbial activity stimulated. However, continuous soil cover, especially associated with permanent growth of plants, might also enhance plant-parasitic nematode densities due to permanent food supply. The effect of minimum tillage on nematode dynamics was studied within the EU-funded project "Optimizing subsidiary crop applications in rotations". The rotation consisted of clover-grass, winter wheat, cover crop, and potato. The following treatments were studied: Plough versus minimum tillage, white clover versus subterranean clover as undersown crops in winter wheat, vetch versus a 1:4 mix of fodder radish and black oats as cover crop following winter wheat, and with and without 5t DM ha^{-1} compost application. Initially, the field was infested with *Helicotylenchus*, *Meloidogyne*, *Paratylenchus*, *Pratylenchus* and *Tylenchorhynchus*. *Pratylenchus* and *Helicotylenchus* increased under wheat and *Meloidogyne* was supported by white clover and subterranean clover as undersown crops. Population densities of all nematode taxa declined during the following catch crop and potato. There was no difference in nematode population dynamics between plough versus minimum tillage, nor between compost versus no compost. Results will be discussed considering the recent literature.

Keywords: sustainable agriculture, nematodes, crop rotation, subsidiary crops

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P60 Plant Protection Product Residues in Grapes and Wine from Kras Winegrowing Region

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Plant protection product (PPP) residues (215 active substances) were analysed with two multiresidual methods: gas chromatography coupled with mass spectrometry (GC/MS) and liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) in grapes “Refošk” (*Vitis vinifera* L.) and wine Teran PTP. In 2011, 2012 and 2013 73 samples of the “Refošk” grapes were collected from the vineyards at Kras winegrowing region at the time of harvest. 82 samples of the Teran PTP wine vintage 2011, 2012 and 2013 were collected from wineries 9 month after fermentation and analysed.

Active substances were found in 68 grape samples (93.2%). All the active substances found were allowed in the integrated pest management (IPM), and their content levels were below the MRLs determined by EU legislation. Most of the substances found were fungicides. Only 3 of the active substances found were insecticides. Although active substances were found in many grape samples, only 1 sample in 2011 contained residues at the level between 50-100% of the MRL value (1.4 % of all analyzed samples). Twenty-three samples (31.5%) contained residues at the level between 10-50% of the MRL value. All other grape samples with residues (58.9%) contained active substances at levels that were less than 10% of the MRL value. Results showed that farmers were using PPPs properly and in accordance with good agricultural practice and that the grapes were safe for consumers.

In the three-year period (2011 – 2013), active substances were found in 55 wine samples (67.1% of all analysed samples). All the active substances found were allowed in the IPM for grapes. Although active substances were found in many wine samples, their contents were low, therefore we concluded that the wine Teran PTP is safe for consumers.

Key words: Kras (Karst), grapes, wine, Teran PTP, plant protection product residues

Acknowledgement

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P61 Concentration of Cu, Fe and Zn in Cabbage Produced under Different Fertilization and Mulching Regimes

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Optimal use of fertilizers, cultivars with improved genetic resistance and cultural practices that may change agroecological factors that influence the accessibility of nutrients are very important for control of diseases and successful vegetable production. Cabbage is one of the widely cultivated crops around the world. The reasons are modest requirements for growing conditions, the possibility of cropping throughout the year, the high consumption per person and economic effects. The trial was conducted to determine the effect of different doses of mineral and organic fertilizers and mulching on quality and yield of cabbageheads. Experiment was done by split-plot method where the main plot consisted of the treatment with (using the black plastic sheeting) or without mulching. Sub-plot consisted of nine fertilizing treatments, with mineral and organic fertilizers (swine or beef well rotted manure and NPK, in different doses and combinations) in four replications. Mulching led to a significant increase in the concentration of the analyzed elements in comparison with the same fertilization treatment without mulching. Concentration of Cu increased significantly in variants fertilized using well rotted pig manure and $N_{33}P_{33}K_{63}$. Concentrations of Fe and Zn had the same tendencies depending on the fertilization regime. Addition of NPK, alone or in combinations with manure, stimulated uptake and accumulation of Cu, Fe and Zn in the cabbageheads. The highest concentration of all analysed elements, and the highest yield, was recorded in cabbageheads fertilized with 20 t ha^{-1} of well rotted pig manure together with $N_{55}P_{55}K_{105}$ and mulching. Fertilization practices, that can lead to increase in concentration of Cu, Fe and Zn in cabbage, on one hand, may help to alleviate the problem of their deficiency in human diet, and on the other hand may significantly increase cabbage resistance to various diseases.

Keywords: cabbage, fertilization, copper, iron, zinc

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P62 Targeted control of new pests in established systems using *Aleyrodes proletella* as an example

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Targeted chemical control is one element of integrated pest management (IPM). Action thresholds are a helping tool to decide whether and when a pesticide treatment becomes necessary. In Germany action thresholds are available for most pests infesting cabbage for instance. This system was questioned when several years ago the cabbage whitefly *Aleyrodes proletella* Linnaeus became an important pest affecting the production of Brussels sprouts, kale, savoy and kohlrabi. Until now, there is only little information available on the population dynamics of *A. proletella* and the reasons for its increasing relevance. Investigating the hibernation habitats and the migration behaviour we found that oilseed rape offers an exclusive hibernation habitat in northern Germany. In the western and south-western areas overwintering cabbage crops represent the most suitable hibernation hosts. Invading of spring planted cabbage crops takes place from May to July. Susceptible cabbage species can be infested with several thousand whiteflies leading to serious reductions in quality and yield.

Action thresholds should be easy to apply and not time consuming. Therefore, a binomial yes/no decision indicating the percentage of infested plants was tested using Brussels sprouts as example. Thresholds included were 25%, 50% and 80% infested plants as well as a higher one of 80% plants infested with 50 larvae minimum. A higher yield compared to the untreated control was achieved with all thresholds which did not differ significantly. Sprout quality was classified in nine classes from 1=clean to 9=completely polluted. Sprouts are marketable below class 3 which was only achieved with the low thresholds of 25% and 50% infested plants, resulting in a small potential for saving treatments under conditions with heavy infestation.

Precondition for applicable thresholds are effective pesticides. To date their number is limited. Regarding resistance management as well the active substance Cyantraniliprol seems a promising candidate.

Keywords: *Aleyrodes proletella*, hibernation, action threshold, yield, quality

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P63 Core Organic Plus Project: ReSolVe – Restoring Optimal Soil Functionality in Degraded Areas within Organic Vineyards

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Soil erosion and improper land preparation methods present a problem in organic vineyards, where compensating measures, for example with externally introduced fertilizers, are not permitted. As a consequence, vine health and grape production and quality is often negatively influenced, although problems typically occur only in parts but entire vineyards. The developing problems can be related to soil compaction and oxygenation, poor organic matter content, plant nutrient availability, and water deficiency.

The *ERA-NET Core-Organic plus* project *ReSolVe* (that started in March 2015) is a transnational and multidisciplinary research project aimed at testing the effects of selected agronomic strategies for restoring soil functionality in degraded areas within organic vineyard. Organic vineyards were selected in 5 countries (Italy: Chianti hills and Maremma plain, Tuscany; France: Bordeaux and Languedoc; Turkey: Adana and Mersin; Spain: La Rioja; and Slovenia) and degraded areas within these vineyards will be treated with *organic soil recovering methods* including treatments with compost, green manure with winter legumes, and dry mulching with cover crops. The strategies will be evaluated according to their efficiency to improve plant health and root growth, grape yield and quality, and effect on soil ecosystem services.

The project involves 9 research groups in 6 EU countries (France, Italy, Slovenia, Spain, Sweden, and Turkey) and experts from different disciplines such as soil science, ecology, microbiology, grapevine physiology, viticulture, and biometry. We will disseminate restoration techniques and the monitoring methodologies developed and tested during the *ReSolVe* project in guidelines for vine-growers. The research group in Slovenia will focus on the characterization of ecological service providers, fungi and bacteria, from rhizospheres before and after restoration techniques were performed.

Keywords: viticulture, soil functionality, biodiversity, soil management, ecological service providers

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P64 Herbicide Resistant Weeds: a Major Threat to Weed Management in Orchards

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Tree fruit crops are important part of Turkish agriculture, economy and trade. IPM programs in orchards have been used in Turkey for more than two decades. Weed IPM had mainly focused on prevention, good cultural practices, and mechanical and chemical control. Herbicide used in orchards, however, has been increasing due to simplicity, effectiveness, and cost. For example, glyphosate application became the main weed management practice in orchards replacing other IPM tools. The use of glyphosate has increased 10 times in the last decade, which can lead to development of herbicide resistance and shift to more tolerant weed species as it happened in other countries. Glyphosate resistance is difficult to manage and may result in the use of other herbicides that are more damaging to environment and health. Our research is currently focused to expand the use of cover crops and mulch as part of weed IPM programs. In addition, we have developed robust outreach programs to educate farmers to prevent the development and manage herbicide resistant weeds including farmer to farmer education, workshops, publications, online training, and timely blogging.

Keywords: IPM, herbicide resistance, weed management

P65 Suboptimal doses of nitrogen impact wheat growth and accumulation of defence molecules

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This work is focused on investigating the effects of (sub) optimal concentrations of nitrogen on wheat plants (*Triticum aestivum* L. cv. Genoveva) grown hydroponically. Nitrogen is a significant factor limiting plant growth, and plants frequently encounter with different nutrition conditions in their natural habitats. Deficiency (0 mM) as well as excess of nitrogen (35 mM) in the environment seriously interfere to the natural metabolic balance of plants and have many negative effects on the physiology and growth of plants. Data show that N availability correlates well with biomass growth and content of photosynthetic pigments. Altered levels of free proline were detected in plants pointing to different defense strategy of shoots and roots under different nitrogen conditions. Most importantly, the profile and activities of typical plant defense enzymes – glucanases and chitinases – were affected by doses of N in media suggesting a possible impact on defense potential of plants to additional environmental stress. The obtained data could be applicable for optimization of fertilization strategy for sustainable agriculture.

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CropSustaIn
Kmetijstvo. Učinkovitost. Raziskave. Znanje.

CropSustaIn aims at improving scientific research for the development of sustainable crop production and environmentally friendly plant protection strategies in Slovenia at changing climatic conditions. A key objective of the project is the creation of a new network integrating the Agricultural Institute of Slovenia (KIS), through the collaboration of national and international research institutions, agricultural holdings and companies. Newly established links of KIS with leading European agricultural research institutions will provide access to foreign knowledge flanked with technological modernizations through CropSustaIn. It enforces close collaboration between researchers at KIS and the industry and allows optimized result exploitations.

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