



***XXI Convegno Nazionale
Società Italiana di Patologia Vegetale
(SIPaV)***

***Difesa delle piante per l'alimentazione
e l'energia***

BOOK OF ABSTRACTS

Edited by: Giordano L., Spadaro D., Gonthier P.

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Book of abstracts

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*XXI Convegno Nazionale
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Difesa delle piante per l'alimentazione e l'energia

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**XXI CONVEGNO NAZIONALE
SOCIETÀ ITALIANA PATOLOGIA VEGETALE
CENTRO CONGRESSI TORINO INCONTRA
TORINO, 21-22-23 SETTEMBRE 2015**

PROGRAMMA

Lunedì 21 Settembre 2015

12.00 Registrazione dei partecipanti e affissione Posters (n. da 1 a 68)

14.30 Apertura dei lavori e saluti delle Autorità

15.00 - 15.30 *Relazione su invito*. EUROPE ON ITS WAY INTO A BIOBASED ECONOMY – PERSPECTIVES FOR PLANT AND AGRO-RESEACH. C. Patermann

Prima sessione – Eziologia ed epidemiologia. Moderatori: P. Capretti e N. Luchi

15.30 - 15.50 DETECTION, HOST PREFERENCE AND ROLE ON TREE STABILITY OF WOOD DECAY FUNGI IN URBAN ENVIRONMENT. L. Giordano, F. Sillo, P. Gonthier

15.50 - 16.10 PATHOGENICITY OF ETIOLOGICAL AGENTS OF CROWN ROT DISEASE ON ORGANIC BANANA IN DOMINICAN REPUBLIC. M.A.M. Kamel, P. Cortesi, M. Saracchi

16.10 - 16.30 RACE TYPING AND MOLECULAR CHARACTERIZATION OF *XANTHOMONAS CAMPESTRIS* pv. *CAMPESTRIS* STRAINS OCCURRING IN ITALY. P. Bella, C. Moretti, C.P. Strano, M. Zaccardelli, F. Branca, R. Buonauro, J.G. Vicente, V. Catara

16.30 - 16.50 ‘*CANDIDATUS* PHYTOPLASMA PHOENICIUM’ ASSOCIATED WITH ALMOND WITCHES’-BROOM DISEASE: INSIGHTS INTO THE STRAIN POPULATION STRUCTURE AND THE INTERACTIONS WITH HOSTS. F. Quaglino, M. Kube, M. Jawhari, Y. Abou-Jawdah, C. Siewert, E. Choueiri, H. Sobh, P. Casati, R. Tedeschi, M. Molino Lova, A. Alma, P.A. Bianco

16.50 - 17.10 THE NEW DEAL IN VIRUS DISCOVERY: A MAJOR CONCERN FOR “MINOR” CROPS. M. Morelli, M. Chiumenti, P. Saldarelli, A. Giampetruzzi, A. Minafra

17.10 - 17.30 SYNTHETIC CLONES OF GRAPEVINE ALGERIAN LATENT VIRUS (GALV) DEVELOP A TYPICAL *TOMBUSVIRUS* INFECTION IN *NICOTIANA BENTHAMIANA* INFECTED CELLS. A. Lovato, A. Polverari, D. Maffi, F. Faoro

17.30 - 18.00 Conferimento del Premio “G. Scaramuzzi” 2015

18.00 - 20.00 “*Apericena*” di benvenuto

Sessione serale *Opificio delle idee*. Moderatori: I. Baccelli e B. Scanu

20.00 - 20.15 *DIPLODIA SAPINEA* AND CLIMATE CHANGE: SPECIES DISTRIBUTION MODELS OF THE MOST IMPORTANT PINE SHOOT PATHOGEN IN ITALY. L. Bosso, H. Rebelo, N. Luchi, G. Maresi, D. Russo, G. Cristinzio

20.15 - 20.30 RAPID SPREAD AND GENETIC DIVERSITY OF *PEPINO MOSAIC VIRUS* IN TOMATO CROP IN SICILY. S. Panno, S. Davino, G. Iacono, M. Davino

20.30 - 20.45 COMPARATIVE GENOMICS BETWEEN THE INVASIVE FOREST PATHOGEN *HETEROBASIDION IRREGULARE* AND THE NATIVE SIBLING SPECIES *H. ANNOSUM* PROVIDE A GLIMPSE INTO THEIR DIVERGENT ADAPTIVE EVOLUTION. F. Sillo, M. Garbelotto, P. Gonthier

20.45 - 21.00 LASER MICRODISSECTION OF GRAPEVINE LEAVES HIGHLIGHTS SITE-SPECIFIC TRANSCRIPTIONAL CHANGES AT THE EARLY STAGES OF DOWNY MILDEW INFECTION. L. Lenzi, C. Caruso, P.L. Bianchedi, I. Pertot, M. Perazzoli

21.00 - 21.15 GENOME PERTURBATION CONSEQUENT TO GENE DELETION BY HOMOLOGOUS RECOMBINATION. A. Grottoli, M. Beccaccioli, W. Sanseverino, C. Fanelli, M. Reverberi, V. Scala

21.15 - 21.30 UNRAVELLING PLANT-MICROBE INTERACTIONS: SUCCESSFUL COLONIZATION OF LETTUCE BY TAGGED BIOCONTROL *STREPTOMYCES*. X. Chen, M. Bonaldi, C. Pizzatti, A. Kunova, M. Saracchi, A. Erlacher, G. Berg, P. Cortesi

- 21.30 - 21.45 *HOPA1'* A NEW PUTATIVE T3SS EFFECTOR OF *PSEUDOMONAS SAVASTANOI* pv. *SAVASTANOI* STRAIN DAPP-PG722, THE CASUAL AGENT OF OLIVE KNOT DISEASE. C. Cortese, M.P. Castañeda-Ojeda, C. Ramos, R. Buonaurio, C. Moretti
- 21.45 - 22.00 DOWNY MILDEW CONTROL AND VEGETATIVE GROWTH OF GRAPEVINES UNDER ALTERNATIVE TREATMENTS TO SYNTHETIC FUNGICIDES. V. Mancini, E. Feliziani, A. Servili, G. Romanazzi

Martedì 22 Settembre 2015

9.00 - 9.30 *Relazione su invito*. NETWORK EPIDEMIOLOGY AND PLANT TRADE. M.J. Jeger, G. Stancanelli, M. Pautasso

Seconda sessione – Genomica e interazione pianta-patogeno. Moderatori: M. Lorito e R. Marcato

- 9.30 - 9.50 GENOME PLASTICITY MEDIATED BY TRANSPOSABLE ELEMENTS DRIVES THE EVOLUTION OF VIRULENCE IN THE VASCULAR WILT PATHOGEN *VERTICILLIUM DAHLIAE*. L. Faino, M.F. Seidl, B.P.H.J. Thomma
- 9.50 - 10.10 FUNGAL β -GLUCANS PROTECT THE FUNGAL CELL FROM PLANT THAUMATIN-LIKE PROTEINS AND CHITINASE. R. Marcato, L. Sella, S. Vincenzi, M. Sturlese, S. Moro, F. Favaron
- 10.10 - 10.30 ANALYSIS OF *PHYTOPHTHORA* DIVERSITY IN ORNAMENTAL NURSERIES USING METABARCODING APPROACHES BASED ON CLONING/SANGER SEQUENCING AND 454 PYROSEQUENCING. M.I. Prigigallo, A. Abdelfattah, A. Biasi, S. Mosca, M.G. Li Destri Nicosia, S.O. Cacciola, G. Magnano di San Lio, L. Schena
- 10.30 - 10.50 CA^{2+} TRANSPORT IN *PSEUDOMONAS SAVASTANOI* pv. *SAVASTANOI*-OLIVE PATHOSYSTEM. C. Moretti, L. Granieri, C. Cortese, M. Mazzoni, A.M. Del Pino, R. Buonaurio, C.A. Palmerini
- 10.50 - 11.10 THE MAPK CASCADE IN PLANT INNATE IMMUNITY IS REGULATED BY PEROXYNITRITE-MEDIATED TYROSINE NITRATION. B. Sottocornola, D. Bellin, L. Tengfang, A. Amoresano, M. Delledonne, E. Vandelle
- 11.10 - 11.30 NUCLEOLAR LOCALIZATION, PATHOGENIC EFFECTS AND SILENCING SUPPRESSOR ACTIVITY OF THE P15 PROTEIN FROM GRAPEVINE VIRUS B. S. Davino, S. Ruiz-Ruiz, P. Serra, R. Flores
- 11.30 - 11.45 *Pausa caffè*
- 11.45 - 13.00 Sessione poster – Ezioologia ed epidemiologia/Genomica e interazione pianta-patogeno. Moderatori: M. Ruocco e L. Schena
- 13.00 - 14.00 *Pranzo di lavoro*
- 14.00 - 14.30 *Relazione su invito*. THE GOOD AND BAD SCIENTIFIC COMMUNICATION. P. Bianucci

Terza sessione – Malattie post-raccolta e micotossine. Moderatori: M. Reverberi e S.M. Sanzani

- 14.30 - 14.50 BIOLOGICAL CONTROL OF POST-HARVEST FUNGAL PATHOGENS BY *AUREOBASIDIUM PULLULANS*: COMPETITION ASPECTS. A. Di Francesco, L. Righetti, S. D'Aquino, M. Mari
- 14.50 - 15.10 *FUSARIUM INCARNATUM-EQUISETI* SPECIES COMPLEX FROM CEREALS: PHYLOGENY AND VARIABILITY OF TRICHOHECENE BIOSYNTHETIC GENE CLUSTER. A. Villani, R.H. Proctor, D.W. Brown, T.J. Ward, A.F. Logrieco, A. Moretti, A. Susca
- 15.10 - 15.30 EFFICACY OF COLD PLASMA IN THE REDUCTION OF AFLATOXINS ON HAZELNUTS. D. Spadaro, A. Prella, I. Siciliano, M.C. Cavallero, D. Vallauri, A. Garibaldi, M.L. Gullino
- 15.30 - 15.50 CHARACTERIZATION OF *MONILINIA* spp. POPULATIONS IN SOUTH ITALY AND DETERMINATION OF THEIR SEXUAL BEHAVIOR. D. Abate, C. Pastore, S. Pollastro, D. Gerin, R.M. De Miccolis Angelini, C. Rotolo, A. Spadoni, F. Faretra
- 15.50 - 16.00 *Pausa caffè*
- 16.00 - 16.15 Affissione Posters (n. da 69 a 116)
- 16.15 - 18.00 Assemblea sociale
- 20.30 *Cena sociale*

Mercoledì 23 Settembre 2015

9.00 - 9.30 *Relazione su invito*. FUNGICIDE DEVELOPMENT FOR THE 21ST CENTURY. G.M. Kemmitt

Quarta sessione – Difesa. Moderatori: M.L. Gullino e S. Sarrocco

9.30 - 9.50 COMPETITIVE MULTITROPHIC INTERACTIONS IN THE BIOCONTROL OF FUSARIUM HEAD BLIGHT. S. Sarrocco, F. Valenti, R. Baroncelli, G. Piaggieschi, G. Vannacci

9.50 - 10.10 CONTROL OF SOIL-BORNE PATHOGENS ON POTTED VEGETABLES WITH MICROORGANISMS ISOLATED FROM SUPPRESSIVE COMPOST. M. Pugliese, G. Castella, L. Battisti, M.L. Gullino, A. Garibaldi

10.10 - 10.30 INFLUENCE OF THINNING TREATMENT ON THE OCCURRENCE OF TREE DECLINE IN DECIDUOUS OAK FORESTS. N. Anselmi, A. Saraceni

10.30 - 10.50 CONTROL OF GRAPEVINE DOWNY MILDEW BY PROTEIN HYDROLYSATES. N. Lachhab, S.M. Sanzani, M. Adrian, A. Chiltz, S. Balacey, M. Boselli, A. Ippolito, B. Poinssot

10.50 - 11.10 NEW FUNGAL HYBRIDS THAT COMBINE IN A SINGLE STRAIN THE PRODUCTION OF DIFFERENT BIOACTIVE SECONDARY METABOLITES AND ENHANCED BENEFICIAL EFFECTS ON PLANTS. G. Manganiello, S. Lanzuise, M. Ruocco, F. Vinale, R. Marra, N. Lombardi, A. Pascale, F. Lacatena, A. Djella, L. De Vitto, S.L. Woo, M. Lorito

11.10 - 11.30 ROLE OF SINGLE SITE-SPECIFIC ALLELE REPLACEMENT INTO SVHK1 LOCUS IN THE STUDY OF *STEMPHYLIUM VESICARIUM* DICARBOXIMIDE AND PHENYLPYRROLE FUNGICIDES RESISTANCE. K. Gazzetti, A. Ciriani, A. Brunelli, M. Collina

11.30 - 11.45 *Pausa caffè*

11.45 - 13.00 Sessione poster – Malattie post-raccolta e micotossine/Difesa. Moderatori: P. Cortesi e C. Fanelli

13.00 - 13.15 Chiusura lavori



PREMIO SCARAMUZZI



PREMIO SCARAMUZZI

UNRAVELING THE TRITROPHIC INTERACTIONS BETWEEN
FRUIT HOST-PATHOGEN-ANTAGONIST IN THE POST-HARVEST ENVIRONMENT**H. Banani^{1,2}**

¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: bouda.banani@unito.it

Fruits and vegetables post-harvest losses due to the attack of several fungal pathogens can reach levels of up to 50% depending on the commodity. Besides the economic losses, some of these fungi may represent a potential health risk because of mycotoxin production. To control post-harvest diseases of fruits, synthetic fungicides, when permitted, are the primary means used. However, the emergence of fungicide-resistant strains and the willingness to use safer and eco-friendly treatments, have generated interest in the development of alternative non-chemical methods to reduce post-harvest losses. Biological control using microbial antagonists has emerged as one of the most promising alternatives to fungicides, either alone or as part of an integrated pest management. The antagonistic activity of biocontrol agents has been widely demonstrated, however the mechanisms of action of most biocontrol agents of post-harvest diseases has not yet been fully understood which is important to know for a successful implementation of post-harvest biocontrol technology. Therefore a part of my PhD focused on this aspect; we have successfully cloned, expressed in *P. pastoris* and confirmed the involvement of a novel endochitinase gene *MfChi* and the protease gene *ALP5* in the biocontrol activity of the antagonistic yeasts *Metschnikowia fructicola* AP47 and *Aureobasidium pullulans* PL5 successively. Our work proved that the alkaline serine protease ALP5 and the endochitinase MfChi could be developed as post-harvest treatments with antimicrobial activity for fruits undergoing a short shelf life to control *Botrytis cinerea*, *Monilinia fructicola* and *Monilinia laxa* on pome and stone fruits. Moreover, besides developing biocontrol agents as alternative approaches to chemicals to control the pathogens, we should also be familiar with the decaying agents, such as their nature and the molecular basis of the infection. Due to recent technological advances, sequencing has revolutionized biological research and has become the forefront of biological experimentation in the last decade. Therefore, we have sequenced for the first time *Penicillium griseofulvum* PG3 which is associated with blue mould decay, the most important post-harvest disease of pome fruit worldwide. The fungal pathogen has the ability to simultaneously produce both detrimental and beneficial Secondary Metabolites (SM). Our work aimed to analyse some important SM clusters present in the studied strain in order to gain insight into SM synthesis in *P. griseofulvum*. Genome-wide analysis of PG3 genes revealed a complete putative gene cluster for patulin biosynthesis, and partial griseofulvin and roquefortine C clusters. Besides the bioinformatics analysis of these gene clusters, we quantified the SM production *in vitro* and during disease development on apple. Furthermore, the SM detected in infected apple were examined by studying the expression kinetics of their key genes under controlled conditions. In addition, we found additional SM clusters in PG3, including those potentially responsible for the synthesis of penicillin and cyclopiazonic acid. These findings provide relevant information to understand the molecular basis of SM biosynthesis in *P. griseofulvum* and this resource will allow further research directed to the overexpression or blocking of specific SM synthesis, to assess its potential in terms of biotechnological applications for beneficial SM, such as griseofulvin.



INVITED PRESENTATIONS



EUROPE ON ITS WAY INTO A BIOBASED ECONOMY – PERSPECTIVES FOR PLANT AND AGRO-RESEACH

C. Patermann

Director (ret.), European Commission

New knowledge about plants, animals, microorganisms and insects during the last decades has prompted deliberations, primarily from Europe and here by the European Commission, to use systematically and in a systemic way this new knowledge as the basis of a new economic concept, the Bioeconomy. Having originally been a RTD concept, the so-called Knowledge-Based Bioeconomy or KBBE, the Bioeconomy is now regarded to be an economy as such using biological resources from the land and the sea, including waste (biomass) as inputs to food, feed, industrial and energy production. Its aims are twofold: produce sustainably new renewable raw materials in agriculture, forestry, fisheries and aquaculture, and/or process such feedstock into new value-added products in the Food, Feed and Industrial Biobased and Energy industries. The unique features of biological resources, like their carbon neutrality, their renewability, their potential for multiuse, not only reuse and the chances to offer new properties to products, like longer endurability, better stability, absence of toxicity or minimizing emissions make this new concept also with the help of plants and agrosience very attractive to give responses to the many so-called grand challenges. These challenges are ranging from increased demand for high quality food and sustainably food and feed production to overcoming the limited resources of raw materials and energy via a true resource efficiency (“more with less”). Last but not least the biobased economy might contribute to the transition from a fossil based chemical and energy industry into a more biobased oriented industry to successfully act towards climate and other global changes. The impact of such a paradigma change could however also affect many other industrial branches like building and construction, health care, fine chemicals, cosmetics, logistics and generally all so-called process industries. The European Commission has issued in 2012 its first European Strategy for Bioeconomy “Inno-vation for Growth”. More and more member states, like Germany, The Netherlands, Belgium, and here Flanders, Sweden, Finland, Denmark, Norway, Ireland, Austria, the Nordic Union, etc. have launched powerful national strategies to enter the new biobased world, and most recently France and Spain will soon come out with their own national ones. The two superpowers USA and the Russian Federation have also announced in April 2012 their own programs. Strong regional efforts are also underway in Finland, The Netherlands, Belgium, Germany, Scotland, Ireland, Italy and France to create Bioeconomy Regions as models. In Norway, a newly formed Bioeconomy Institute will take up its duties in July 2015. Ten years after its launch in Brussels the Biobased Economy, close to the Principle of sustainability and with so many affiliations to the potentials of the circular Economy, with its unique features of carbon neutrality, potentials for growth, renewability and resources efficiency, but also chances for new innovative products has turned out to become an important factor for our future.



NETWORK EPIDEMIOLOGY AND PLANT TRADE

M.J. Jeger¹, G. Stancanelli², M. Pautasso²

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Effective control of a plant disease by means of prohibition, eradication, containment, or ongoing disease management, requires information on all pathways that lead to the introduction, dissemination and subsequent impact of the causal pathogen. Globally, regionally and within-country, trade networks are major pathways leading to serious disease outbreaks and epidemics. Recent examples include *Phytophthora ramorum*, where the pathogen was first recorded in Europe in the ornamental nursery trade and has subsequently jumped to a previously unsuspected host, Japanese larch (*Larix kaempferi*), with devastating consequences in the United Kingdom. More recently, ash dieback has moved inexorably across Europe and threatens *Fraxinus excelsior* and its associated biodiversity, whereby the trade in ash saplings facilitated long-distance dispersal of *Hymenoscyphus fraxineus*. In many ways this aspect is common to both pathogens and insect pests such as *Dryocosmus kuriphilus* which has spread from NW Italy to widely-dispersed areas in Europe. Of particular concern in Italy and Europe is the current outbreak of a strain of *Xylella fastidiosa*, causing decline of olives in Puglia – the first occurrence of this quarantine organism in Europe. Epidemiology has long been the basis for disease management, but until recently techniques for analysis of disease spread on plants moving in trade networks (seeds, plants-for-planting and for direct retail/wholesale markets) had been poorly developed and little used. Methods for network epidemiology are now available and increasingly used in different contexts and spatio-temporal scales. These methods are based on graph-theoretic concepts applicable to (trade) networks, which essentially consist of a set of nodes with linkages between them representing an overall network structure. The nodes might be, for example, nursery sites with some imposed hierarchy, i.e. producers, wholesalers, and retailers; the linkages would represent the directed flow of plants from site to site. From mathematical analysis of the network structure it is possible to identify some key attributes determining the overall likelihood of disease spread on the network: these are the degree of connectedness across the network; the node/site at which the pathogen is first introduced; the correlation between in-out linkages; and the role played by “hubs”, or highly connected sites, within the network. From these attributes it is possible to determine a threshold for pathogen establishment and dissemination, whether in the long-term the pathogen is likely to persist, and as a consequence the disease control options that could be used in prevention or mitigation. Despite these recent developments in methods for epidemiological analysis of networks, there are some major challenges still to be resolved. The horticultural industry, particularly the nursery trade is fragmented in terms of size and scale of operations and connectedness, with an enormous range of traded commodities. Additionally, plant trade is dynamic which leads to network structures that are often changing because of commercial interests, but also sometimes as a consequence of a regulated disease affecting the trade.



FUNGICIDE DEVELOPMENT FOR THE 21ST CENTURY

G.M. Kemmitt

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Today as we move well into the second decade of the 21st Century we find ourselves at an interesting and challenging time with respect to the development of new fungicide solutions. Plant pathogens have the potential to cause significant crop losses in both pre- and post-harvest situations and fungicides remain a critical tool for protecting yield and quality. The UN estimates that by 2050 the world will need 70% more food to meet projected population growth and this increase will largely need to be covered by increased efficiency of production from the current finite area of agricultural land. Fungicides will play a key role in attempting to meet this challenging goal and it is critical that industry continues to bring new solutions to market whilst maintaining the registrations of important existing chemistries. The challenges facing R&D companies engaged in this endeavour are numerous. Ever more stringent regulatory safety margins make the identification of new fungicidally active molecules which have a high probability of being registered more difficult, whilst important existing chemistries such as the triazoles face uncertainty about their long term future in certain regions of the globe. One impact of increasing regulatory complexity is that the cost of development of new actives, approximately US \$256 million according to a 2010 European Crop Protection Association (ECPA) analysis, and maintenance of existing registrations continues to rise. Fungicide resistance continues to be a major challenge with a real need for new novel modes of action (MOAs). This is particularly acute in the context of the potential loss of some key chemistries in the future due to regulatory challenges thus further limiting the number of effective MOAs available to growers for integration into resistance management strategies. A further challenge is the uncertainty around how the drive towards sustainable crop protection will shape the future landscape for agrochemical development along with the potential impact of climate change on the prevalence and geographic spread of plant pathogens. Sources of new leads for fungicide development include natural products, compound collections from various institutions, combinatorial chemistry libraries and competitor chemistry. Traditional *in vivo* and *in vitro* screening of hits and their subsequent optimisation via classical Structural Activity Relationship (SAR) testing remain an important facet of new molecule discovery although the crop protection industry has also seen the introduction of structure based design. The characteristics which must be exhibited by a new fungicide are excellent efficacy and crop selectivity, a clean toxicological and environmental profile, freedom to operate and a synthetically accessible structure which can be manufactured at scale cost effectively. Additional desirable features include physiochemical properties allowing formulation flexibility and effective redistribution in or on the target crop as well as physiological effects on the crop such as greening and drought tolerance. Combining all the above characteristics into a single molecule remains an enormous technical challenge but one we must continue to achieve if we are to meet our needs for a sustainable increase in agricultural productivity as we move towards the middle of this Century.



ORAL PRESENTATIONS





EZIOLOGIA ED EPIDEMIOLOGIA

DETECTION, HOST PREFERENCE AND ROLE ON TREE STABILITY OF WOOD DECAY FUNGI IN URBAN ENVIRONMENT. L. Giordano, F. Sillo, P. Gonthier. *Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: paolo.gonthier@unito.it*

Wood decay fungi colonizing stem and root systems can predispose trees to the risk of windthrows or limb failures, thus resulting in significant damages especially in urban environment. Hence, an early detection and identification of hazardous wood decay agents may be pivotal during tree hazard assessment of urban trees. In this paper we report the results of a long lasting research performed in the city of Turin and in other urban contexts, based on the application of conventional diagnostic methods (visual inspection of trees) and molecular biology methods (e.g. multiplex PCRs) for the detection of the most harmful or widespread wood decay agents of both conifers and broadleaves. On average, visual inspection of trees underestimates >90% of infected trees compared with molecular methods. Lower rates of underestimation were observed for *Ganoderma* spp. and *Perenniporia fraxinea*; higher rates for *Armillaria* spp. and *Phaeolus schweinitzii*. Results of molecular biology methods show that the most frequent fungus in broadleaves was *Armillaria* spp., followed by *Ganoderma resinaceum* and *P. fraxinea* (14%, 5% and 4% of trees, respectively), while in conifers the frequency of *Armillaria* spp. was higher than that of *Fuscoporia torulosa* and *P. schweinitzii* (13%, 7% and 6% of trees, respectively). Furthermore, analyses show that the frequency of different fungal species greatly varied depending on the host species, suggesting relevant degrees of host preference. Finally, results suggest that wood decay fungi may play a more prominent role as factors of tree instability in the case of broadleaves compared to conifers.

PATHOGENICITY OF ETIOLOGICAL AGENTS OF CROWN ROT DISEASE ON ORGANIC BANANA IN DOMINICAN REPUBLIC. M.A.M. Kamel, P. Cortesi, M. Saracchi. *Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milano, Via Giovanni Celoria 2, I-20133 Milano, Italy. E-mail: mohamed.kamel@unimi.it*

Crown rot is a post-harvest disease affecting the fruits quality of banana. Different fungal pathogens are involved in the disease and may vary according to farming area. Fungi associated with crown tissues were isolated from five different organic farming areas in Dominican Republic over a period of two years (2013-2014) with the objective to assess the pathogenicity of the fungi associated to crown rot. We reproduce the conditions of natural infection by inoculating harvested green banana (*Musa* AAA, Cavendish) at the packinghouse. Experimental inoculations were carried out by spraying asymptomatic trimmed crowns with propagules suspensions of 24 strains of eight identified taxa. In addition, 5 mixed inoculations as well as untreated bananas as a control were included. Following inoculation, the bananas were packed and overseas shipped to Italy. Symptoms assessment was carried out 20 days after inoculation. Disease Incidence (DI) and Disease Severity Index (DSI from 0 to 7) were assessed and fungi were isolated from each treated crown. The results obtained showed that *Colletotrichum musae* was the most virulent species (100% DI and 7 DSI), followed by *Fusarium verticillioides* (100% DI and 6 DSI) and *Lasiodiplodia theobromae* (85% DI and 5 DSI). *Fusarium incarnatum*, which is considered the main pathogens in many Countries, in Dominican Republic reached a maximum of 50% DI and 4 DSI. Further strains

showed low level of pathogenicity but their role could be ancillary in the crown rot development.

RACE TYPING AND MOLECULAR CHARACTERIZATION OF *XANTHOMONAS CAMPESTRIS* pv. *CAMPESTRIS* STRAINS OCCURRING IN ITALY. P. Bella¹, C. Moretti², C.P. Strano¹, M. Zaccardelli³, F. Branca¹, R. Buonaurio², J.G. Vicente⁴, V. Catara¹. ¹Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 100, I-95123 Catania, Italy. ²Department of Agricultural, Food and Environmental Sciences (DSA3), University of Perugia, Borgo XX Giugno 74, I-06121 Perugia, Italy. ³Agricultural Research Council – Experimental Institute for Horticulture (CRA-ORT), Via Cavalleggeri 25, I-84098 Pontecagnano Faiano (SA), Italy. ⁴School of Life Sciences, University of Warwick, Wellesbourne Campus, CV35 9EF, United Kingdom. E-mail: patrizia.bella@unict.it

Xanthomonas campestris pv. *campestris* (*Xcc*) is the causal agent of black rot, a severe seed-borne systemic vascular disease of vegetable brassica crops. *Xcc* strains have been grouped into nine physiological races, with races 1 and 4 being the most widespread in *Brassica oleracea* crops. The characterization of *Xcc* races is important to identify resistance sources, establish breeding programs and to set up control strategies. To define *Xcc* race structure and distribution in Italy, a collection of 31 *Xcc* strains, isolated from six *B. oleracea* varieties, *B. napus* var. *napobrassica* and *Crambe maritima* in seven Regions, was established from a larger collection according to their geographic and host origin and PCR-based DNA fingerprints. These strains all managed to grow on the semi-selective medium FS, hydrolyzed starch, induced vascular symptoms on *B. oleracea* and were identified by *Xcc* specific primers based on the *brcC* gene. The race of each strain was determined by inoculating eight differential *Brassica* lines belonging to five species. *Xcc* strains from international collections and *Xcc* strain races 1, 4 and 6 were included as reference strains. On the basis of compatible interaction (susceptibility) or incompatible interaction (resistance), *Xcc* strains isolated in Italy were classified into races 1 (35.5%), 4 (54.8%), and 6 (9.7%). Multilocus sequence analysis (MLSA) based on four housekeeping genes (*dnaK*, *gyrB*, *fyuA* and *rpoD*) showed that *Xcc* strains in Italy are closely related to strains isolated worldwide. The results are discussed with relation to the strain source.

'CANDIDATUS PHYTOPLASMA PHOENICIUM' ASSOCIATED WITH ALMOND WITCHES'-BROOM DISEASE: INSIGHTS INTO THE STRAIN POPULATION STRUCTURE AND THE INTERACTIONS WITH HOSTS. F. Quaglino¹, M. Kube², M. Jawhari³, Y. Abou-Jawdah³, C. Siewert², E. Choueiri⁴, H. Sobh³, P. Casati¹, R. Tedeschi⁵, M. Molino Lova⁶, A. Alma⁵, P.A. Bianco¹. ¹Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DISAA), University of Milano, Via Giovanni Celoria 2, I-20133 Milano, Italy. ²Division Phytomedicine, Thaeer-Institute, Humboldt-Universität zu Berlin, Lentzeallee 55/57, Berlin, Germany. ³Faculty of Agricultural and Food Sciences, American University of Beirut, P.O. Box 11-0236, Riad El Solh, Beirut 1107 2020, Lebanon. ⁴Department of Plant Protection, Lebanese Agricultural Research Institute, Tal Amara, Lebanon. ⁵Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ⁶AVSI Foundation, Jounieh-Ghadir, Lebanon. E-mail: fabio.quaglino@unimi.it

Almond Witches'-Broom (AlmWB), a devastating disease of almond, peach and nectarine in Lebanon, is associated with 'Candidatus Phytoplasma phoenicium'. In the present study, we generated



a draft genome sequence of 'Ca. P. phoenicium' strain SA213, representative of phytoplasma strain populations from different host plants, and determined the genetic diversity among phytoplasma strain populations by phylogenetic analyses of 16S rRNA, *groEL*, *tufB* and *inmp* gene sequences. Sequence-based typing and phylogenetic analysis of the gene *inmp*, coding an integral membrane protein, distinguished AlmWB-associated phytoplasma strains originating from diverse host plants, whereas their 16S rRNA, *tufB* and *groEL* genes shared 100% sequence identity. Moreover, dN/dS analysis indicated positive selection acting on *inmp* gene. Draft genome analyses suggest a parasitism based on the import of glycerol-3-phosphate, a critical mobile inducer of plant systemic immunity. Additionally, integral membrane proteins, effector-like proteins and potential candidates for interaction with hosts were identified. One of the integral membrane proteins was predicted as BI-1, an inhibitor of apoptosis-promoting Bax factor. Bioinformatics analyses revealed the presence of putative BI-1 in draft and complete genomes of other 'Ca. Phytoplasma' species. The genetic diversity within 'Ca. P. phoenicium' strain populations in Lebanon suggested that AlmWB disease could be associated with phytoplasma strains derived from the adaptation of an original strain to diverse hosts. Moreover, the identification of BI-1 in 'Ca. P. phoenicium' draft genome and within genomes of other 'Ca. Phytoplasma' species suggested its potential role as a phytoplasma fitness-increasing factor by modification of the host-defense response.

THE NEW DEAL IN VIRUS DISCOVERY: A MAJOR CONCERN FOR "MINOR" CROPS. M. Morelli, M. Chiumenti, P. Saldarelli, A. Giampetruzzi, A. Minafra. *National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via G. Amendola 122/D, I-70126 Bari, Italy. E-mail: massimiliano.morelli@ipsp.cnr.it*

The use of NGS approach allowed the identification and the complete genome characterization of several plant virus species, previously unknown (*Persimmon cryptic virus*, PeCV; *Mulberry badnavirus-1*, MBV-1), or scarcely characterized and never associated to a specific symptomatology and/or host (*Persimmon rhabdovirus A*, PeVA; *Apple green crinkle associated virus*, AGCaV). Significantly, these findings arose from crops, respectively Japanese persimmon (*Diospyros kaki*) in the case of PeCV and PeVA, quince (*Cydonia oblonga*) for AGCaV and mulberry (*Morus alba*) for MBV-1, disregarded in traditional quest for viral agents and diseases. Routinely used bio- and molecular assays, however modern they are, always hide an *a priori* choice for target, influenced by evident symptomatology, diagnostic purposes prone to certification standard enquiries, availability of literature, etc. NGS analysis, being apart from such constraints, gives the opportunity to widen the target range, thus including also cultivated and wild crops, so far considered not agronomically relevant, or never characterized for their sanitary status. The new approach, accounting on a better feasibility and reliability of detection performance, is leading to noteworthy steps forward for plant virology studies addressed to characterize minor crop infections. A rising number of new species discovered, in once misinvestigated temperate and tropical fruit crops, should not be merely regarded in terms of basic research accomplishments. The meaningful contribution of NGS advent in this field relies in a potential of innovative knowledge to be expended, for instance, in upgrading the extant certification schemes, tracking unforeseen threats in propagative material exchanges and setting new attribution rules for a challenging taxonomical allocation of oncoming species or strains.

SYNTHETIC CLONES OF GRAPEVINE ALGERIAN LATENT VIRUS (GALV) DEVELOP A TYPICAL TOMBUSVIRUS

INFECTION IN NICOTIANA BENTHAMIANA INFECTED CELLS. A. Lovato¹, A. Polverari¹, D. Maffi², F. Faoro². ¹*Department of Biotechnology, University of Verona, Strada le Grazie 15, I-37134 Verona, Italy.* ²*Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DISAA), University of Milano, Via Giovanni Celoria 2, I-20133 Milano, Italy. E-mail: franco.faoro@unimi.it*

GALV is a 30 nm icosahedral virus (*Tombusvirus* genus), with a positive ssRNA genome of 4731 nucleotides including at least five Open Reading Frames (ORFs) encoding for replicase proteins (p33 and p92), the coat protein (p40), the movement protein (p24) and the multifunctional p19 protein, which also functions as a silencing suppressor. We have previously produced a synthetic GALV construct complying with the available genome sequence of a GALV isolate from nippelfruit (GALV-nf). This clone systemically infected both grapevine and *Nicotiana benthamiana* plants causing severe symptoms. In order to use GALV-nf as a VIGS (Virus Induced Gene Silencing) vector, we modified it by a single nucleotide substitution on the p19 ORF, to reduce the silencing suppressor activity of the virus, hence increasing the plant silencing efficiency against a target gene. The cytopathology of this mutant (GALV-nf-Δ) and the unmodified clone have been compared to verify if a less functional p19 allowed a correct, though reduced, virus synthesis. Ultrastructural analysis revealed the presence of isometric virus particles and multivesicular bodies (MVBs), typical of tombusviruses, in the apical leaves infected with either GALV clones but more consistent in GALV-nf infection. MVBs mainly originated from peroxisomes, though it cannot be excluded that also mitochondria could be involved in their formation. Immunogold labelling using an anti p33 serum showed that viral replicase is mainly located in the peripheral membrane of MVBs in both synthetic virus constructs, demonstrating that p19 modification does not affect GALV localization and replication but strongly interferes in terms of virus virulence.

OPIFICIO DELLE IDEE

DIPLODIA SAPINEA AND CLIMATE CHANGE: SPECIES DISTRIBUTION MODELS OF THE MOST IMPORTANT PINE SHOOT PATHOGEN IN ITALY. L. Bosso¹, H. Rebelo^{2,3}, N. Luchi⁴, G. Maresi⁵, D. Russo^{1,3}, G. Cristinzio¹. ¹*Department of Agricultural Sciences, University of Napoli "Federico II", Via Università 100, I-80055 Portici (NA), Italy.* ²*CIBIO-Centro de Investigacao em Biodiversidade e Recursos Genéticos da Universidade do Porto, Campus Agrário de Vairão, R. Padre Armando QuintasVairão, Portugal.* ³*School of Biological Sciences, University of Bristol 24, Tyndall Avenue, BS8 1TQ, Bristol, United Kingdom.* ⁴*National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via Madonna del Piano 10, I-50019 Sesto Fiorentino (FI), Italy.* ⁵*Technology Transfer Centre, Edmund Mach Foundation, Via E. Mach 1, I-38010 San Michele all'Adige (TN), Italy. E-mail: luciano.bosso@unina.it*

Species Distribution Models (SDMs) provide realistic scenarios to explain the influence of bioclimatic variables on plant pathogens distribution. In this study, we develop a maximum entropy model for *Diplodia sapinea* in Italy to reach the following goals: i) to carry out the first geographical distribution analysis in Italy and determine which Eco-Geographical Variables (EGVs) may affect its distribution; ii) to detect the effect of climate change on the species' geographic range by 2050 and 2070. To develop SDMs for *D. sapinea* we used Maxent vers. 3.3.3k, the most popular approach to model species distributions with scarce presence-only data. Future climate projections for *D. sapinea* were derived from six Global Climate Models (GCMs) (BCC-CSM1-1, CCSM4, GISS-E2-R, MIROC5,

HadGEM2-ES, MPI-ESM-LR) for two representative concentration pathways (RCP 4.5 and RCP 8.5) and two time projections: 2050 and 2070. The most important EGVs for the current distribution were found to be land cover, altitude and mean temperature of wettest quarter. The distribution of *D. pinea* essentially increased in Central and Southern Italy and shifted upwards by 90m. Moreover, this fungus expanded its range in response to an increase in mean temperature of wettest and driest quarter in all GCMs of 1.9 and 5°C, respectively. Validation statistics (AUC, AUC_{diff} TSS) showed that our models achieved high performances (>0.8). Our study shows that under different climate change scenarios *D. sapinea* damages will likely affect larger areas of pine forests in the country probably causing heavy effects on dynamics and evolution of these stands or perhaps playing as constrains factor to their survival.

RAPID SPREAD AND GENETIC DIVERSITY OF PEPINO MOSAIC VIRUS IN TOMATO CROP IN SICILY. S. Panno¹, S. Davino^{1,2,3}, G. Iacono⁴, M. Davino⁴. ¹Euro-Mediterranean Institute of Science and Technology (IEMEST), Via E. Amari 123, I-90139 Palermo, Italy. ²Department of Agricultural and Forest Sciences (SAF), University of Palermo, Viale delle Scienze Ed. 5, I-90123 Palermo, Italy. ³National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Strada delle Cacce 73, I-10135 Torino, Italy. ⁴Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 100, I-95123 Catania, Italy. E-mail: pannostefano@virgilio.it

Pepino mosaic virus (PepMV), belong to the genus *Potexvirus* of the family *Alphaflexiviridae* cause one of the most destructive diseases of tomato (*Solanum lycopersicum*) crops worldwide. In Sicily, the first outbreak of PepMV has been detected in a single greenhouse in the year 2005 and has been quickly eradicated. After this first report, PepMV has not been detected in Sicily until the end of 2008, when, in this case the disease was impossible to eradicate. The purpose of this study was to assessed the dispersion and the genetic diversity of PepMV in Sicily and to compare it to other PepMV isolates in order to know what factors are determinant for the evolution and epidemiology of this virus. A total of 1,800 samples from symptomatic and asymptomatic plants were randomly collected in Sicily during the period 2001-2013. The incidence of the virus increased rapidly from 13% in 2011 to 63% in 2013. Based on the molecular analysis and host range we can highlight two subgroups of PepMV isolates belonged to the clade CH2: one composed exclusively from sicilian isolates that was extremely virulent and cause symptoms on tomato fruits and another composed from foreign isolates that cause mild symptoms on tomato plants. From an epidemiological point of view more restrictive controls are required to avoid PepMV spreading to other Italian Regions.

COMPARATIVE GENOMICS BETWEEN THE INVASIVE FOREST PATHOGEN *HETEROBASIDIUM IRREGULARE* AND THE NATIVE SIBLING SPECIES *H. ANNOSUM* PROVIDE A GLIMPSE INTO THEIR DIVERGENT ADAPTIVE EVOLUTION. F. Sillo¹, M. Garbelotto², P. Gonthier¹. ¹Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Environmental Science, Policy and Management, Forest Pathology and Mycology Laboratory, University of California Berkeley, 54 Mulford Hall, 94720 Berkeley, California, USA. E-mail: paolo.gonthier@unito.it

The fungal plant pathogens *Heterobasidion irregulare* and *H. annosum* have been evolving allopatrically for 34-41 million of

years. *Heterobasidion irregulare* was recently introduced from North America to Italy, within the natural range of *H. annosum*, generating hybrid swarms. Divergent adaptive evolution affecting the genomes of these pathogens is still poorly studied. Here, a comparative genomic approach was used to determine which gene groups were affected by divergent positive selection during the allopatric phase. In particular, it was tested the hypothesis that genes involved in pathogenicity are not as divergent between the two species compared to genes involved in saprobic ability and sporulation, as previously demonstrated in phenotypic observations. Results based on the whole-genome sequencing of three genotypes per species confirmed their status as sister taxa, despite a large macrosynteny was observed. Genes involved in pathogenicity appeared to be more conserved between the two species compared to genes involved in saprobic growth and sporulation. This finding provided genomic evidence that differences in fitness are more likely to be determined by these two last functions, as previously documented by *in vitro* experiments. A large fraction of genes under positive selection was described as involved in transcriptional functions and mitochondrial factors. Genes in interspecific structural variations were also found to be related to these two categories and to transposable element activity. The study has shown at the genomic level that factors related to transmission rather than those related to pathogenicity might explain the invasiveness of exotic pathogens.

LASER MICRODISSECTION OF GRAPEVINE LEAVES HIGHLIGHTS SITE-SPECIFIC TRANSCRIPTIONAL CHANGES AT THE EARLY STAGES OF DOWNY MILDEW INFECTION. L. Lenzi^{1,2}, C. Caruso², P.L. Bianchedi³, I. Pertot¹, M. Perazzoli¹. ¹Research and Innovation Centre, Edmund Mach Foundation, Via E. Mach 1, I-38010 San Michele all'Adige (TN), Italy. ²Department of Agrobiologia and Agrochimica (DABAC), University of Tuscia, Via San Camillo de Lellis s.n.c., I-01100 Viterbo, Italy. ³Technology Transfer Centre, Edmund Mach Foundation, Via E. Mach 1, I-38010 San Michele all'Adige (TN), Trento, Italy. E-mail: luisa.lenzi@fmach.it

Grapevine (*Vitis vinifera*) is one of the world's major fruit crops, but most of the commercial cultivars are susceptible to downy mildew, caused by *Plasmopara viticola*. Transcript profiling has largely been used to investigate gene expression changes of the interaction between grapevine and *P. viticola*, but these studies have generally involved the use of RNA from whole grapevine leaves. *Plasmopara viticola* infects grapevine leaves and young berries by stomata and develops intercellular mycelium in the mesophyll. Only a small fraction of leaf cells is in contact with the pathogen at the early stages of infection and the large portion of not-infected cells could mask the transcriptional changes related to defence activation. Laser microdissection (LMD) technique allows the isolation of specific cell types from heterogeneous tissue. LMD was used to specifically collect cells at the site of *P. viticola* infection or at the adjacent layers from inoculated leaves of *in vitro*-grown grapevines. Protocols for sample fixation, laser microdissection and RNA isolation from group of cells were optimized and the expression of ten genes involved in the grapevine defence response was analysed by Real-time RT-PCR. The expression level of the selected genes was generally greater at the site of infection compared to the whole infected leaf, and expression profiles in infected and adjacent cells differed according to the tested genes. These results get new insights on the activation of specific processes at the sites of *P. viticola* infection, which were masked in the whole-leaf analysis, and the optimized protocols will be further used for site-specific transcriptomic studies.

GENOME PERTURBATION CONSEQUENT TO GENE DELETION BY HOMOLOGOUS RECOMBINATION. A. Grottoli¹, M. Beccaccioli¹, W. Sanseverino², C. Fanelli¹, M. Reverberi¹, V. Scala¹. ¹*Department of Environmental Biology, Sapienza University of Roma, Piazzale Aldo Moro 5, I-00185 Roma, Italy.* ²*Sequentia Biotech SL, Bellaterra, Cerdanyola del Vallès, 08193 Barcelona, Spain.* E-mail: grottoli.alessandro@gmail.com

Fusarium verticillioides causes ear rot disease in maize and produces fumonisins, mycotoxins toxic to humans and livestock. Gene deletion is a molecular approach effective in identifying genes or gene clusters function. In this study, the linoleate diol synthase-coding gene, *lds1*, was deleted into an Italian strain of *F. verticillioides*. Since significant difference in the genome emerged between our isolate and the reference strain (Fv7600), deposited in the BROAD Institute, we decide to re-sequence our wild type as well as the *lds1*-deleted mutants originated from our strain. Significant differences in genome sequences emerged between the wild type and the two *lds1*-mutants further than the trivial deletion of the *lds1* locus. Tests performed through a bioinformatic approach, highlighted significant differences in the three genotypes, such as Single Nucleotide Polymorphisms (SNPs), small Deletion/Insertion Polymorphisms (DIPs) and Structural Variations (SVs). These differences have been validated through a double approach: 1) by Amplification-Refractory Mutation System (ARMS) and RT-qPCR; 2) sequencing samples obtained from successive clonal generations of the wild type strain in order to evaluate intrinsic variability of *F. verticillioides*. The results led us to consider the possibility that the effect of a punctual transformation event might have caused an overall genomic instability, and that recombination practices may potentially be responsible of unexpected, stochastic and henceforth off-target rearrangements throughout the genome.

UNRAVELLING PLANT-MICROBE INTERACTIONS: SUCCESSFUL COLONIZATION OF LETTUCE BY TAGGED BIOCONTROL STREPTOMYCES. X. Chen¹, M. Bonaldi¹, C. Pizzatti¹, A. Kunova¹, M. Saracchi¹, A. Erlacher², G. Berg², P. Cortesi¹. ¹*Department of Food, Environmental and Nutritional Science (DeFENS), University of Milano, Via Giovanni Celoria 2, I-20133 Milano, Italy.* ²*Institute of Environmental Biotechnology, Graz University of Technology, Petersgasse 12, 8010 Graz, Austria.* E-mail: chen.xiaoyulong@unimi.it

The ability of the Biological Control Agents (BCAs) to colonize plant tissues is an important feature involved in microbe-assisted plant protection. Plant-microbe interaction research increased especially in the last decade thanks to technological revolution. Molecular methods and the development of advanced microscopic techniques allow researchers to explore gene expression and localization of beneficial microorganisms within plants. The Green Fluorescent Protein (GFP) and its modified version, Enhanced GFP (EGFP), more adapt for expression in mammalian cells and GC-rich actinomycetes like *Streptomyces*, have been widely used as markers to study gene expression, as well as plant-microbe interactions. We transformed five *Streptomyces* strains which showed strong inhibition activity against *Sclerotinia sclerotiorum*, with the EGFP construct to study their interactions with lettuce. The fitness of transformed strains was similar to wild-type; the transformants maintained similar sporulation, mycelium growth rate, and the ability to produce important secondary metabolites and lytic enzymes. Two strains, *Streptomyces cyaneus* ZEA17I and *Streptomyces* sp. SW06W, were used to study lettuce colonization dynamics by seed coating method. Streptomycete colonies were visualized in three-day-old seedlings. In addition, the colonization and spatio-temporal dynamics were studied in sterile and in non-sterile substrates. The strains were recovered from rhizosphere and inner root tissues

from up to six-week-old lettuce plants and showed the ability to compete with the natural microflora. The antagonistic activity, and rhizosphere and root competence showed by *S. cyaneus* ZEA17I and *Streptomyces* sp. SW06W conferred the potential to act as BCAs and help us to get insights into the mechanisms of microbiome-mediated plant protection.

HOPAI' A NEW PUTATIVE T3SS EFFECTOR OF PSEUDOMONAS SAVASTANOI pv. SAVASTANOI STRAIN DAPP-PG722, THE CASUAL AGENT OF OLIVE KNOT DISEASE. C. Cortese¹, M.P. Castañeda-Ojeda², C. Ramos², R. Buonaurio¹, C. Moretti¹. ¹*Department of Agricultural, Food and Environmental Sciences (DSA3), University of Perugia, Borgo XX Giugno 74, I-06121 Perugia, Italy.* ²*Área de Genética, Facultad de Ciencias, Universidad de Málaga, Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Málaga, Spain.* E-mail: chiaracortese88@libero.it

Recently, we reported the draft genome sequence of the Italian DAPP-PG722 strain of *Pseudomonas savastanoi* pv. *savastanoi* (*Psv*), which shows about 85% identity with the *Psv* genome of the French strain NCPPB3335. Since the Italian strain is less virulent than the French strain in both lignified and young micropropagated olive plants, bioinformatic analyses were performed to verify whether the difference in virulence is dependent on the effector repertoire of the two strains. Results showed that the two strains shared an identical T3SS repertoire, except for a new putative effector, named *hopA1'*, which is absent in the NCPPB3335 strain. In the DAPP-PG722 *Psv* genome, the *hopA1'* gene is present in an incomplete *hrc/hrp* gene cluster. Phylogenetic analysis between the amino acid sequence of HopA1' and those of the HopA1 and HopA2 present in several strains of the *P. syringae* complex, revealed a high identity of HopA1' with HopA2 of *P. syringae* pv. *aesculi* strain 2250. Dot blot analysis and southern hybridization were carried out to verify the presence of *hopA1'* among 35 *Psv* strains and 26 strains of the *P. syringae* complex isolated from herbaceous and woody plants. HopA1' is present in a single copy in the chromosomal DNA of the DAPP-PG722 strain as well as in others 12 *Psv* strains and 5 strains of the *P. syringae* complex attacking woody plants. To investigate the role of *hopA1'* in *Psv* virulence the mutant of this gene in the strain DAPP-PG722 and its ectopic expression in NCPPB3335 strain are in progress.

DOWNY MILDEW CONTROL AND VEGETATIVE GROWTH OF GRAPEVINES UNDER ALTERNATIVE TREATMENTS TO SYNTHETIC FUNGICIDES. V. Mancini, E. Feliziani, A. Servili, G. Romanazzi. *Department of Agricultural, Food and Environmental Sciences (D3A), University Politecnica delle Marche, Via Brecce Bianche 10, I-60131 Ancona, Italy.* E-mail: g.romanazzi@univpm.it

Grapevine Downy Mildew (GDM) is one of the most serious diseases of grapevines. With limitations in the use of copper-based products imposed for organic agriculture by the European Union, research for alternatives is encouraged. The aim of this research was to follow a two-year trial to evaluate the control of GDM using some alternative compounds, and to determine their effects on shoot growth, vigour of the vegetation and grape quality and quantity. Under low disease pressure, Bordeaux mixture, copper hydroxide, laminarin combined with low copper, and 0.5% and 0.8% chitosan showed the lowest GDM incidence. With high disease pressure, Bordeaux mixture, laminarin combined with *Saccharomyces* extracts, and 0.5% and 0.8% chitosan showed the lowest GDM incidence. Chitosan at 0.8% induced the lowest leaf area, and

dry weight, with no negative effects observed on the quantity of the grapes and the quality parameters of their juice. Among the alternatives to copper, chitosan ensured the best protection and reduced the vigour of the vegetation, without negative impacts on grape production. Laminarin used in combinations with *Saccharomyces* extracts or low copper also decreased GDM infection.

GENOMICA E INTERAZIONE PIANTA-PATOGENO

GENOME PLASTICITY MEDIATED BY TRANSPOSABLE ELEMENTS DRIVES THE EVOLUTION OF VIRULENCE IN THE VASCULAR WILT PATHOGEN *VERTICILLIUM DAHLIAE*. L. Faino, M.F. Seidl, B.P.H.J. Thomma. *Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands. E-mail: luigi.faino@wur.nl*

Verticillium dahliae is a soil-borne pathogen that aggressively colonizes hundreds of host plants, including high-value crops such as tomato and potato, leading to the formation of vascular wilt disease. Resistance factors in the host population exert selective pressure on the pathogen forcing the rapid evolution of adaptive traits to participate in the arms race with the host. By comparative genomics on a *V. dahliae* population, we recently revealed extensive genomic rearrangements that facilitate the gain and loss of genetic material and the establishment of highly dynamic Lineage-Specific (LS) regions. LS regions are enriched for Transposable Elements (TEs) and *in planta*-induced effector genes encoding secreted proteins that significantly contribute to aggressiveness towards the host, and thus have been hypothesized to contribute to the genome plasticity required for adaptive genome evolution. However, factors that drive genome plasticity in *V. dahliae* remain enigmatic. Using single molecule Real-time sequencing, we re-sequenced two *V. dahliae* strains and analyzed the previously identified genomic rearrangements in unprecedented detail, revealing multiple genomic breakpoints at the nucleotide level. We established that genomic breakpoints are flanked by multiple TEs, suggesting that these elements play essential roles in their formation. Moreover, we show that TEs are highly active at LS regions, which makes these regions highly unstable. Ultimately, we can show that LS regions were formed by genomic segmental duplications.

FUNGAL β -GLUCANS PROTECT THE FUNGAL CELL FROM PLANT THAUMATIN-LIKE PROTEINS AND CHITINASE. R. Marcato¹, L. Sella¹, S. Vincenzi², M. Sturlese³, S. Moro³, F. Favaron¹. ¹Department of Land, Environment, Agriculture and Forestry (TESAF), University of Padova, Viale dell'Università 16, I-35020 Legnaro (PD), Italy. ²Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), Via dell'Università 16, I-35020 Legnaro (PD), Italy. ³Department of Pharmaceutical and Pharmacological Sciences (DSF), University of Padova, Via Marzolo 5, I-35131 Padova, Italy. E-mail: francesco.favaron@unipd.it

Thaumatin-Like Proteins (TLP) and chitinases are plant antimicrobial proteins representing a general mechanism of plant defense against plant pathogens. On fungi these two proteins target the cell membrane and the cell wall, respectively. We observed that the necrotrophic fungal pathogens *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Sclerotinia minor* and *Sclerotium rolfsii* are barely or not at all affected by TLP and chitinase from grape. The fungal protease activity was investigated as a first mechanism of detoxification developed by these fungi. However, we observed that the fungal proteases are only weakly active against these proteins, with the grape chitinase being more susceptible to degradation than TLP. Instead,

we observed that the β -glucan components of the fungal cell wall absorb these proteins. In particular, the scleroglucan, that is the main extracellular component of *S. rolfsii*, displays a high affinity for TLP and chitinase. Docking analysis showed that scleroglucan has a structural affinity for both TLP and chitinase thus preventing these proteins from reaching their cellular targets. In particular competing with chitin for the binding site of chitinase, scleroglucan could prevent the enzymatic hydrolysis of the fungal cell wall. The observation that a fungal β -1,3 glucanase is unable to detach the grape proteins from scleroglucan shows that this complex carbohydrate polymer is particularly effective in protecting the fungal mycelium from the activity of some plant PR proteins.

ANALYSIS OF *PHYTOPHTHORA* DIVERSITY IN ORNAMENTAL NURSERIES USING METABARCODING APPROACHES BASED ON CLONING/SANGER SEQUENCING AND 454 PYROSEQUENCING. M.I. Prigigallo¹, A. Abdelfattah¹, A. Biasi¹, S. Mosca¹, M.G. Li Destri Nicosia¹, S.O. Cacciola², G. Magnano di San Lio¹, L. Schena¹. ¹Department of Agriculture, Mediterranean University of Reggio Calabria, Via Salita Melissari, Località Feo di Vito, I-89122 Reggio Calabria, Italy. ²Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 100, I-95123 Catania, Italy. ³The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, United Kingdom. E-mail: lschena@unirc.it

The genetic diversity of *Phytophthora* spp. was investigated in soil and root samples from ornamental nurseries using metabarcoding approaches based on Cloning/Sanger Sequencing (CSS) and on 454 Pyrosequencing (454P). According to CSS, 9 different species were detected: *P. nicotianae*, *P. citrophthora*, *P. meadii*, *P. taxon Pgcblamydo*, *P. cinnamomi*, *P. parvispora*, *P. cambivora*, *P. niederhauserii* and *P. lateralis*. Three phylotypes were associated to two or more taxa (*P. citricola* taxon E or III; *P. pseudosyringae*, *P. ilicis* or *P. nemorosa*; and *P. cryptogea*, *P. erythroseptica*, *P. himalayensis* or *P. sp. 'kelmania'*). Furthermore, three additional phylotypes were considered as representatives of novel *Phytophthora* taxa. The 454P provided higher resolution than CSS. In addition to above species, eleven phylotypes, including *P. cactorum*, *P. citricola s.str.*, *P. palmivora*, *P. palmivora*-like, *P. megasperma* or *P. gonapodyides*, *P. ramorum* and 5 putative new *Phytophthora* species were detected only with the 454P approach. Furthermore, the 454P made possible the identification of 18 new phylotype/nursery combinations as compared to the CSS approach. Although the CSS provides more reliable sequences, several aspects confirmed the trustworthiness of both methods: i) many identical Sequence Types (STs) were identified in different nurseries, ii) most STs identified with 454P were identical to those from the CSS approach and/or perfectly matched GenBank deposited sequences, and iii) the divergence noted between STs of putative new *Phytophthora* species and all other detected sequences was sufficient to rule out sequencing errors. Data revealed the existence of very complex populations and reinforced the primary role of plant nurseries in favoring the introduction, dissemination and evolution of *Phytophthora* species.

CA²⁺ TRANSPORT IN *PSEUDOMONAS SAVASTANOI* pv. *SAVASTANOI*-OLIVE PATHOSYSTEM. C. Moretti, L. Granieri, C. Cortese, M. Mazzoni, A.M. Del Pino, R. Buonauro, C.A. Palmerini. *Department of Agricultural, Food and Environmental Sciences (DSA3), University of Perugia, Borgo XX Giugno 74, I-06121 Perugia, Italy. E-mail: chiara.luce.moretti@unipg.it*

Pseudomonas savastanoi pv. *savastanoi* (Psv) penetrates olive tissues through wounds and once inside plant tissue colonizes

apoplast giving raise to knots formation. Little is known about the molecular signals early perceived in the apoplast by plant pathogenic bacteria, *Psv* included. Since Ca^{2+} is considered an important secondary messenger early involved in a number of bacterial processes, we determined *in vitro* changes in cytosolic Ca^{2+} levels in *Psv* (strain DAPP-PG722) cells, using the fluorescent probe FURA2AM. When bacterial cells were incubated in the absence of sugars, an increase in the cytosolic Ca^{2+} level coming from the extracellular medium was observed. A relationship between a possible energy deficiency state in the cells and Ca^{2+} signals was therefore hypothesized. Since the Ca^{2+} entry was inhibited by Li^+ ions, employed in place of Na^+ , and nifedipine, an inhibitor of the L-voltage channels in mammals, we supposed the Ca^{2+} entry is mediated by an L-voltage $\text{Na}^+/\text{Ca}^{2+}$ exchanger. To demonstrate this hypothesis, we analysed *in silico* the genome of *Psv* strain DAPP-PG722 and a gene coding for a $\text{Na}^+/\text{Ca}^{2+}$ exchanger was found. In the relative mutant obtained the Ca^{2+} entry did not occur. Studies are in progress to characterise phenotypically the *Psv* mutant and to verify if the mutation affects the virulence of the bacterium.

THE MAPK CASCADE IN PLANT INNATE IMMUNITY IS REGULATED BY PEROXYNITRITE-MEDIATED TYROSINE NITRATION. B. Sottocornola¹, D. Bellin¹, L. Tengfang¹, A. Amoresano², M. Delledonne¹, E. Vandelle¹. ¹Department of Biotechnology, University of Verona, Strada Le Grazie 15, I-37134 Verona, Italy. ²Department of Chemical Sciences, University of Napoli "Federico II", Cupa Nuova Cintia 21, I-80126 Napoli, Italy. E-mail: elodiegenevieve.vandelle@univr.it

The Hypersensitive Response (HR) triggered by an avirulent pathogen in resistant plants is characterized by the simultaneous production of Nitric Oxide (NO) and Reactive Oxygen Species (ROS), both involved in the onset of cell death. Among other things NO can react with O_2^- in a diffusion-limited reaction to produce peroxynitrite, the increase of which has been recently demonstrated in *Arabidopsis* plants challenged with an avirulent pathogen with a timing that correlates with an increase in tyrosine-nitrated proteins. However, although till now the physiological function of peroxynitrite in plants is poorly understood, it is emerging as a potential signaling molecule during the induction of defense responses against pathogens. We observed that peroxynitrite scavengers compromise MAPK activity dynamics induced by avirulent pathogens. Within the AtMKK4/AtMKK5-AtMPK3/6 cascade, known to be involved in plant defense mechanisms, we further identified AtMKK4 as specific target of nitration by peroxynitrite, leading to an inhibition of its activity *in vitro*. Surprisingly, *in planta*, the long-lasting exposure of AtMKK4 to the peroxynitrite, released by the donor SIN1 or induced by avirulent pathogens, leads to its degradation via nitration. We also found that this mechanism, which discriminates between AtMKK4 and AtMKK5, regulates the dynamics of MAPK activity in response to avirulent pathogens, controlling *PR1* defense gene expression. This demonstrates the signaling function of peroxynitrite in plants and the physiological role of tyrosine nitration in plant innate immunity.

NUCLEOLAR LOCALIZATION, PATHOGENIC EFFECTS AND SILENCING SUPPRESSOR ACTIVITY OF THE P15 PROTEIN FROM GRAPEVINE VIRUS B. S. Davino¹, S. Ruiz-Ruiz², P. Serra², R. Flores². ¹Department of Agricultural and Forest Sciences (SAF), University of Palermo, Viale delle Scienze Ed. 5, I-90123 Palermo, Italy. ²Instituto de Biología Molecular y Celular de

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RNA silencing is one of the most effective defenses of plants against viruses. To overcome this barrier viruses encode proteins that inactivate it. *Grapevine virus B* (GVB), a filamentous single-stranded positive RNA virus (genus *Vitivirus*), encodes p15, an RNA-binding protein with a putative zinc finger domain preceded by a motif rich in basic amino acids. To determine the subcellular localization of GVB-p15, the gene of the Green Fluorescent Protein (GFP) was fused to the 3'-terminus of gene *p15* and this construct, driven by the 35S promoter from *Cauliflower mosaic virus*, was agroinfiltrated in *Nicotiana benthamiana*. Confocal laser-scanning microscopy disclosed the preferential accumulation of p15-GFP in the nucleolus and perinucleolar bodies. Supporting this view, co-agroinfiltrations of the construct expressing p15-GFP with another expressing the nucleolus marker fibrillarin, fused to the red fluorescent protein, revealed a perfect image overlay. These results suggested that GVB-p15 has a Nucleolar Localization Signal (NoLS), and analysis of six substitution mutants showed that the basic amino acids and the Zn-finger contained in the N-terminal region of GVB-p15 form part of its NoLS. Moreover, GVB-p15 is a suppressor of sense-mediated RNA silencing as denoted by co-agroexpressing p15 with a plasmid expressing GFP in the transgenic line 16c of *N. benthamiana* (constitutively expressing GFP). Finally, when launched from a *Potato virus X* vector, p15 enhanced its pathogenicity in *N. benthamiana*. Examination of the effects of the p15 mutants should clarify whether RNA silencing suppression and pathogenicity are related to one another and to the nucleolar localization.

MALATTIE POST-RACCOLTA E MICOTOSINE

BIOLOGICAL CONTROL OF POST-HARVEST FUNGAL PATHOGENS BY AUREOBASIDIUM PULLULANS: COMPETITION ASPECTS. A. Di Francesco¹, L. Righetti², S. D'Aquino³, M. Mari¹. ¹Department of Agricultural Sciences (DipSA), University of Bologna, Via Gandolfi 19, I-40057 Cadriano (BO), Italy. ²Agricultural Research Council – Research Centre for Industrial Crops (CRA-CIN), Via di Corticella 133, I-40128 Bologna, Italy. ³National Research Council of Italy, Institute of Sciences of Food Production (ISPA), Traversa La Crucca 3, Regione Balduca, I-07040 Li Punti (SS), Italy. E-mail: alessand.difrancesc3@unibo.it

The yeast *Aureobasidium pullulans*, strains L1 and L8, were studied to evaluate their effectiveness against *Penicillium expansum* and *Monilinia laxa* in pome and stone fruits respectively and also to identify the possible mechanisms of action involved in the pathogen control. *Aureobasidium pullulans* showed a great attitude to compete for nutrients (glucose, fructose and sucrose) and space (as observed by SEM micrographs). Both strains showed a high antagonistic potential *in vivo*, competing especially for sucrose and glucose in wounded apples and in presence of *P. expansum*. Electron microscopy also showed that the yeast colonized the fruit surface obstructing the pathogen access to fruit. Moreover *A. pullulans* showed a great ability to keep a higher pH (pH 6) in the medium in presence of pathogens than when it is alone (pH 3). This ability could contrast the pathogen action in fruit colonization and induce the rot development. *Aureobasidium pullulans* seems to produce siderophores, competing for essential nutrients such as iron. According to our results, the competition for nutrients and space represent an essential mechanism of action that could play an essential role in the antagonistic activity of two *A. pullulans* strains against *P. expansum* and *M. laxa*.

FUSARIUM INCARNATUM-EQUISETI SPECIES COMPLEX FROM CEREALS: PHYLOGENY AND VARIABILITY OF TRICHOHECENE BIOSYNTHETIC GENE CLUSTER.

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Fusarium incarnatum-equiseti species complex (FIESC) includes mycotoxigenic species associated with several diseases mainly of cereals. Although these species are considered moderately aggressive, they are able to produce multiple mycotoxins, including beauvericin, zearalenone, equisetin, fusarochromanone, butenolide and a wide range of trichothecenes, potent inhibitors of proteic synthesis. Thus, members of FIESC are potential contributors to mycotoxin contamination of cereals. FIESC includes high levels of cryptic speciation as most species within the complex cannot be distinguished from one another by morphological traits. A previous DNA-based analysis of human isolates resolved FIESC into 28 phylogenetically distinct lineages, or multilocus sequence types (MLSTs). Here, we investigated the phylogenetic diversity of 69 FIESC isolates recovered from cereals grown in Europe and North America by comparison to the previously described MLSTs. In phylogenetic analyses of the four housekeeping genes *EF-1a*, *RPB2*, *CaM* and *TUB2*, 4 isolates were resolved within the *F. incarnatum* clade of FIESC, and all other isolates were resolved within *F. equiseti* clade. However, 8 isolates were resolved into a lineage that is distinct from all previously described MLSTs, suggesting that they constitute novel MLST within FIESC. Phylogenomic analysis of 12 isolates, representing one novel and 11 previously described MLSTs, inferred a phylogeny that was consistent with but more highly resolved than the phylogeny inferred from four genes. Comparative analysis of the genome sequences revealed variation in distribution of mycotoxin biosynthetic gene clusters. These data indicate that different FIESC MLSTs vary in their genetic potential to produce and contaminate cereal crops with different mycotoxins.

EFFICACY OF COLD PLASMA IN THE REDUCTION OF AFLATOXINS ON HAZELNUTS.

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Cold atmospheric pressure plasma has been widely used within the areas of food safety, medicine and environmental remediation. During the last years, plasma technology was used for degradation of fungal spores, but it could also be used for the degradation of mycotoxins. Aflatoxins, produced by *Aspergillus flavus* and *A. parasiticus*, can be present in food products, such as nuts and dried fruits, as a result of contamination that occurred before and after harvest. Aflatoxin B1 (AFB1) is the most common in food and it is the most genotoxic and carcinogenic. In this study, the operating parameters of cold atmospheric pressure plasma were optimized to reduce the presence of aflatoxins on hazelnuts. First, the effect of different gases was tested (N₂, air, 0.1% O₂ and 1% O₂), then power (400, 700, 1000, > 1000 W) and exposure time (1.12, 2.48, 4, 12 minutes) were optimized. In preliminary tests on aflatoxin standard solutions, this method allowed to obtain a complete detoxification using a high power for a few minutes. On hazelnuts,

in similar conditions (1000 W, 12 minutes), a reduction in the concentration of total aflatoxins and AFB1 of over 70% was obtained. Cold atmospheric pressure plasma could be a promising method for degradation of aflatoxins on food, though operating conditions must be optimized according to the matrix.

CHARACTERIZATION OF MONILINIA spp. POPULATIONS IN SOUTH ITALY AND DETERMINATION OF THEIR SEXUAL BEHAVIOR.

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Monilinia species are responsible of brown rot decay of stone and pome fruit in orchards as well as in post-harvest. *Monilinia laxa* and *M. fructigena* are common in Europe, while *M. fructicola* is a quarantine pathogen, included in the EPPO A2 List. In Italy, it was first reported in 2009 in Piemonte (North Italy). The results of a monitoring program on the occurrence of *M. fructicola*, *M. fructigena* and *M. laxa* in South Italy are reported along with a comparative characterization of the three fungal species. Out of the tested 513 isolates from 24 orchards located in South Italy, 388 were identified as *M. fructicola*, 98 were *M. laxa* and 10 *M. fructigena*. A direct PCR assay was set up to identify the *Monilinia* species on rotted fruits. *Monilinia fructicola* colonies grew faster and showed an optimum temperature higher than those of the other two species. Responses to different classes of fungicides in a colony growth assay revealed high sensitivity to fludioxonil, tebuconazole and SBI-III fungicides. The three species showed different responses to cyflufenamid and *M. fructicola* was the species more sensitive to the fungicide. A PCR-based protocol was set up to investigate on the sexual behavior of the three species. All the three species proved heterothallic, and both the *MAT1-1* and *MAT1-2* idiomorphs were detected in samples of their populations suggesting that sexual process is a potential source of genetic variability in *Monilinia* species.

DIFESA

COMPETITIVE MULTITROPHIC INTERACTIONS IN THE BIOCONTROL OF FUSARIUM HEAD BLIGHT.

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In view of using a multitrophic approach to improve its efficacy of control of Fusarium Head Blight (FHB), *Trichoderma gamsii* T6085 was exploited in combination with a selected *Fusarium oxysporum* strain (Fox7121) in order to evaluate the competitive ability of these two beneficial isolates in comparison with those of *Fusarium graminearum* and *F. culmorum*. The Biolog phenotype protocol has been extended as a high throughput system to calculate the Niche Overlapping Index. The ability to metabolize the 96 carbon sources included into the FF plates, expressed as growth rate, has been utilized to define whether fungi co-exist in the same niche or if one strain nutritionally dominates another one. Results showed that T6085 occupies separate niches than the pathogens and, partly, than Fox7121. These three last isolates are dominant, with Fox as a good competitor of pathogens. Then the competitive ability of the two potential biocontrol isolates has been studied on wheat debris vs. *F. graminearum*. Both T6085 and Fox significantly reduced, alone and co-inoculated, wheat straw colonization by the pathogen, without

affecting each other but with a stronger effect of T6085. Finally, the recently sequenced and annotated genome of T6085 has been compared with those of other *Trichoderma* species and of saprotrophic and plant pathogenic fungi, including *F. oxysporum* and *F. graminearum*. Data concerning proteins expansions, particularly of specific CaZs and peptidases, will help to support data herewith collected on the beneficial aptitude of T6085 and represent a potent tool to better investigate the ecological characters of this isolate.

CONTROL OF SOIL-BORNE PATHOGENS ON POTTED VEGETABLES WITH MICROORGANISMS ISOLATED FROM SUPPRESSIVE COMPOST. M. Pugliese^{1,2}, G. Castella¹, L. Battisti¹, M.L. Gullino^{1,2}, A. Garibaldi¹. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: massimo.pugliese@unito.it

Compost suppressiveness depends on chemical, physical and microbiological composition and antagonists can be isolated from high quality composts. The objective of the present work was to isolate microorganisms from a suppressive compost and to test them for their activity against soil-borne pathogens on vegetable crops. A compost from green wastes that showed a good suppressive activity in previous trials was used as source of microorganisms. Serial diluted suspensions of compost samples were plated on different media for isolation of fungi and bacteria. Colonies were isolated from plates and tested in greenhouse on potted plants against *Fusarium oxysporum* f. sp. *basilica*-basil, *Pythium ultimum*-cucumber and *Rhizoctonia solani*-bean. Antagonistic microorganisms were blended into a peat substrate at 10 g L⁻¹ fungal biomass or 10 mL L⁻¹ liquid culture 14 days before seeding. Pathogens were mixed into the substrate at 1 g of wheat kernels L⁻¹ 7 days before seeding. Seeds of basil, cucumber and bean were sown into 2 L pots in greenhouse. The number of alive plants and above ground biomass were measured 20-30 days after seeding. Four fungi and three bacteria were able to significantly control *P. ultimum* and *F. oxysporum* f. sp. *basilici*. None of them was effective against *R. solani*. Among all isolated microorganisms, bacteria significantly controlled the pathogens better than *Trichoderma* and other fungi.

INFLUENCE OF THINNING TREATMENT ON THE OCCURRENCE OF TREE DECLINE IN DECIDUOUS OAK FORESTS. N. Anselmi, A. Saraceni. Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF), University of Tuscia, Via San Camillo de Lellis s.n.c., I-01100 Viterbo, Italy. E-mail: anselmi@unitus.it

Decline of the deciduous oak forests, often encountered in Mediterranean regions, seems to be the result of prolonged and repeated severe droughts, often increased by competition for lack of thinning. A survey was undertaken in a declining *Quercus cerris* and *Q. pubescens* forest to evaluate the effects of thinning treatments on the occurrence of decline. Four plots of approximately half a hectare each were selected. Two of them were subjected to thinning in June 2007, leaving in each plot 26 trees. The other two plots, with similar health conditions, were left untreated. Through specific monitoring sheets, all trees of the four plots were subjected to phytosanitary assessment just before and after thinning, repeated in 2009, 2011 and 2015. Stem diameter, number of dead trees and incidence of decline symptoms, such as dead branches, bark necrosis and stromata on stem by *Biscognauxia mediterranea* were recorded. During the period 2007-2011, characterized by intense

summer drought, plant health conditions in the thinned plots were all significantly improved over time, with diameter increment and significant decrease of canopy damage from 33.46 to 16.73%, the control trees faced a substantial decline, of which six were dead, and the others exhibited increase canopy damage from 30.76 to 44.03%. On the contrary, in the period 2011-2015, characterized by rainy summers, the condition of the trees improve both in thinned and in non thinned plots. In any case, trees in thinned plots grew better and were healthier than those in non thinned ones. Thinning is therefore a valuable silviculture technique to counteract the oak decline phenomenon.

CONTROL OF GRAPEVINE DOWNY MILDEW BY PROTEIN HYDROLYSATES. N. Lachhab¹, S.M. Sanzani¹, M. Adrian², A. Chiltz³, S. Balacey², M. Boselli⁴, A. Ippolito¹, B. Poinssot². ¹Department of Soil, Plant and Food Sciences (DiSSPA), University of Bari "Aldo Moro", Via G. Amendola 165/A, I-70126 Bari, Italy. ²Université de Bourgogne, UMR 1347 Agroécologie, Pôle Interactions Plantes Micro-organismes, ERL CNRS 6300, Dijon, France. ³INRA, UMR 1347 Agroécologie, Pôle Interactions Plantes Micro-organismes, ERL CNRS 6300, Dijon, France. ⁴Department of Biotechnology, University of Verona, Via della Pieve, I-37020 S. Floriano di S. Pietro in Cariano (VR), Italy. E-mail: simonamarianna.sanzani@uniba.it

Downy mildew, caused by *Plasmopara viticola*, is one of the most important grape pathogen in Europe and North America. Although the control is traditionally performed with fungicides, the appearance of resistant pathogen populations and the possible adverse effects on human and environment health are spurring the search for alternative means. In the present investigation, two protein hydrolysates of soybean (*soy*) and casein (*cas*) origin were successfully tested against *P. viticola*. On *Vitis vinifera* cv. Marselan plants, the application of *soy* and *cas* reduced the infected leaf surface by 76 and 63%, as compared to the untreated control, respectively. Since both hydrolysates seemed to trigger the plant immunity, we investigated their effect on selected grapevine defence responses. On treated grapevine cell suspensions, a different free cytosolic calcium signature was recorded for each hydrolysate, whereas a similar transient phosphorylation of two MAP kinases of 45 and 49 kDa was observed. These signalling events were followed by transcriptome reprogramming, including the up-regulation of genes encoding Pathogenesis-Related (PR) proteins and the enzyme stilbene synthase responsible for the biosynthesis of resveratrol, the main grapevine phytoalexin. Liquid chromatography analyses confirmed the production of resveratrol and its dimer metabolites, δ - and ϵ -viniferins. Overall, *soy* effect was more pronounced than *cas* one. Both hydrolysates proved to be able to enhance grapevine immunity against pathogen attack.

NEW FUNGAL HYBRIDS THAT COMBINE IN A SINGLE STRAIN THE PRODUCTION OF DIFFERENT BIOACTIVE SECONDARY METABOLITES AND ENHANCED BENEFICIAL EFFECTS ON PLANTS. G. Manganiello¹, S. Lanzuise^{1,2}, M. Ruocco^{1,2}, F. Vinale^{1,2}, R. Marra^{1,2}, N. Lombardi^{1,2}, A. Pascale¹, F. Lacatena¹, A. Djella², L. De Vito¹, S.L. Woo^{1,2}, M. Lorito^{1,2}. ¹Department of Agricultural Sciences, University of Napoli "Federico II", Via Università 100, I-80055 Portici (NA), Italy. ²National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via Università 133, I-80055 Portici (NA), Italy. E-mail: lorito@unina.it

A few strains of *Trichoderma*, made many years ago by protoplast fusion, have been considered hybrids and registered for agricultural

use worldwide. However, they were obtained by heavily mutagenizing the parental strains in order reach a state of auxotrophy that facilitate the selection of the resulting fusants. We have developed a method that allow to avoid the mutagenesis, thus producing true hybrids from natural, wild type strains. Our result opens a variety of new possibilities for designing new fungal hybrids for application in agriculture, bio-remediation and other industrial uses.

ROLE OF SINGLE SITE-SPECIFIC ALLELE REPLACEMENT INTO *SVHK1* LOCUS IN THE STUDY OF *STEMPHYLIUM VESICARIUM* DICARBOXIMIDE AND PHENYLPYRROLE FUNGICIDES RESISTANCE. K. Gazzetti, A. Ciriani, A. Brunelli, M. Collina. *Department of Agricultural Sciences (DipSA), University of Bologna, Viale G. Fanin 46, I-40127 Bologna, Italy. E-mail: marina.collina@unibo.it*

Stemphylium vesicarium is the fungal agent of pear brown spot and its resistance to dicarboximide fungicides has been a known concerning phenomenon since the 1990s. Henceforward, pear orchards have been monitored and field strains have been tested by

mycelial growth inhibition assays to understand the sensitivity to dicarboximide and phenylpyrrole fungicides. Four phenotype classes were recognized according to *in vitro* responses to procymidone and iprodione: S (sensitive), S+ (low resistance), R1 (moderate resistance), R2 (high resistance). Cross-resistance to fludioxonil was only detected in R2 phenotype. Previous molecular studies correlated dicarboximide resistance class with single amino acid substitutions observed in a two-component histidine kinase (HK1), corresponding to single nucleotide polymorphism (SNPs) in the nucleotidic sequence of *SvHK1* gene. The goal of this ongoing study is to define the role of known SNPs in *SvHK1* sequence on dicarboximide resistance by the replacement of the S allele with S+, R1 or R2 alleles. A reference sensitive strain was selected through biological and molecular assays and DNA was properly extracted. Fusion PCR technique was used to build the linear disruption vector (KOSvHK1). Fungal protoplast were obtained by enzymatic lysis of cell wall and transformed. KOSvHK1 replacement of *SvHK1* gene produced null mutants which were able to grow up on Hygromycin B. Transformants will be screened for unique and site-specific insertion of KOSvHK1 using PCR and Southern Blotting assays. Interesting mutants will be transformed with linear complementation vectors and complemented strains will be tested for the expected acquired resistance level.



POSTERS
(in alphabetical order)



Eziologia ed epidemiologia

1. DETECTION AND QUANTIFICATION OF COLLETOTRICHUM GODETIAE AND C. ACUTATUM S.STR. IN THE OLIVE CANOPY BY QPCR. A. Abdelfattah¹, S. Mosca¹, S. Pangallo¹, G.E. Agosteo¹, I. Antelmi¹, S.O. Cacciola², L. Schena¹.

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Many *Colletotrichum* species have been associated to olive anthracnose but *C. godetiae* and *C. acutatum* s.str. may be regarded as the most important. A duplex quantitative PCR (qPCR) method based on TaqMan[®] MGB chemistry was developed and used to simultaneously detect and quantify both pathogens in different olive organs collected in Gioia Tauro plain, Southern Italy, an olive growing area where epidemic anthracnose outbreaks occur yearly. Species-specific primers were designed on β -tubulin and histone 3 genes and labelled with FAM and VIC reporter dyes. The analysis of 70 different olive samples (green and senescent leaves, floral residues, symptomatic and asymptomatic fruits, and fertilized fruits) collected in four phases (May, June, October and December) revealed the presence of both pathogens in all organs and assessment times, although populations sharply increased in October and December. *Colletotrichum godetiae* was more frequently detected with a percentage of positive samples ranging from 25 (May green leaves) and 100% (December senescent leaves and symptomatic and asymptomatic fruits). Samples infected by *C. acutatum* s.str. ranged from 8.3 (green leaves collected in May and June) and 100% (October symptomatic fruits). However, in positive samples, a higher population of *C. acutatum* s.str. was revealed as compared to *C. godetiae* in terms of total quantity of target DNA and corresponding estimated number of cells. Indeed, between 33 (May green leaves) and 3777400 (December symptomatic fruits) and between 8 (June green leaves) and 671566 (December symptomatic fruits) cells mg⁻¹ of olive tissues were detected for *C. acutatum* s.str. and *C. godetiae*, respectively.

2. A NEW DISEASE OF LAVANDULA X ALLARDII CAUSED BY FUSARIUM OXYSPORUM. D. Bertetti¹, G. Ortu¹, P. Pensa¹, M.L. Gullino^{1,2}, A. Garibaldi¹.

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Several potted plants of *Lavandula* × *allardii* (hybrid of *Lavandula dentata* and *L. latifolia*), *Lamiaceae* family, were recently affected by a new vascular wilt. The disease was detected in a nursery located near Albenga (Savona province, Northern Italy). Chlorosis and yellowing appeared on leaves that wilted with branches. Brown discoloration was observed in the vascular tissues of stems from which *Fusarium oxysporum* was consistently isolated on Potato Dextrose Agar (PDA). The microorganism was identified by the morphological features of microconidia, macroconidia and chlamidospores observed *in vitro*. The Translation Elongation Factor (TEF) 1 α analysis and the Intergenic Spacer Region (IGS) analysis, carried out on a single-spore culture, confirmed the identification. In the pathogenicity test, a single isolate was artificially inoculated on healthy plants of *L. × allardii* by dipping roots in a conidial suspension prepared in Potato Dextrose Broth (PDB). Control plants were dipped in sterilized water. Then, all plants were transplanted in 2L

pots, containing a steamed mixed soil and maintained in a growth chamber, at 30°C. First symptoms of *Fusarium* wilt appeared on inoculated plants, 21 days after the artificial inoculation. *Fusarium oxysporum* was constantly re-isolated from affected tissues. Controls remained symptomless. *Fusarium oxysporum* is reported on *L. × allardii* for the first time in Italy, as well as worldwide. Preliminary molecular studies suggest the presence of a new *forma specialis*: phylogenetic analysis based on polygalacturonase genes are necessary to confirm this hypothesis, as well as the susceptibility trials on several *Lavandula* species and cultivars.

3. FUSARIUM WILTS RECENTLY OBSERVED ON SUCCULENT PLANTS GROWN IN ITALY. D. Bertetti¹, P. Pensa¹, G. Ortu¹, M.L. Gullino^{1,2}, A. Garibaldi¹.

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New wilts were recently detected in farms located in Liguria Region (Northern Italy) on succulent plants belonging to two families: *Astrophytum myriostigma*, *Cereus florida*, *C. marginatus* var. *crispata* and *Mammillaria zeilmanniana* (*Cactaceae* family); *Euphorbia mammillaris* (*Euphorbiaceae* family). In the case of *C. florida*, the disease progressed very slowly on affected plants that stunted and withered, however they didn't die. In the other cases, main symptoms consisted in collar and/or stem rot of affected plants that eventually died. The fungal causal agents of all the diseases were isolated from affected tissues and identified as *Fusarium oxysporum* by morphological characteristics of the colonies grown *in vitro*. The ITS (Internal Transcribed Spacer) analysis or the Translation Elongation Factor (TEF) 1 α analysis conducted on the isolates confirmed the identifications. In pathogenicity tests, little fragments of mycelium taken from pure culture of the pathogens grown on Potato Dextrose Agar (PDA) were inoculated through small wounds produced on stems of healthy plants. Controls were wounded without inoculum. In all cases, inoculated plants were affected, whereas controls remained symptomless. *Fusarium oxysporum* was consistently re-isolated from inoculated plants, fulfilling the Koch's postulates. Since *F. oxysporum* has been reported on these hosts for the first time in Italy, as well as in the world, the presence of new *formae speciales* of this pathogen should be investigated with molecular analysis on the single-spore isolates, together with pathogenicity assays.

4. VIRAL INFECTIONS IN ONE COLLECTION FIELD OF POMEGRANATE (PUNICA GRANATUM) IN ITALY. R. Bichieri¹, A. D'Anniballe¹, C. Lanzoni¹, A.R. Babini², A. Mirotti², C. Ratti¹, C. Poggi Pollini¹.

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Pomegranate (*Punica granatum*) has gained considerable popularity in many countries worldwide due to its health benefits. The competitive nature of fruit marketing forces improving the quality attributes, while keeping yield at an optimum level. To pursue this objective, in 2012 in the Ravenna province of the Emilia-Romagna Region (Italy) was established one collection field, wherein 57 cultivars from different countries of the world are maintained. As pomegranate is propagated by hardwood cuttings, healthy propagation material is required to avoid virus epidemics. We therefore focused our research on this collection field, which may play a pivotal role to spread new pomegranate propagation

material. Moreover, we recently (2014) reported *Cherry leaf roll virus* (CLRV) in the collection field and in a consistent amount of ornamental pomegranate plants in public gardens in Bologna (almost 10% of the analyzed samples, two expressing leaf deformation symptoms). Field inspections during spring 2015 showed several cultivars exhibiting virus-like symptoms on the leaves, such as calico mosaic, chlorotic rings and malformations suggesting infection by some viral agent. Serological (ELISA) and molecular analysis (RT-PCR) are ongoing on leaf extracts from all 57 cv., most symptomless, in order to detect the presence of several viruses such as: *Alfa alfa mosaic virus* (AMV), *Arabid mosaic virus* (ArMV), CLRV, *Cucumber mosaic virus* (CMV), *Strawberry latent ringspot virus* (SLRV), *Tobacco ring spot virus* (TRSV) and *Tomato ring spot virus* (ToRSV).

5. OUTBREAK OF *BOTRYOSPHAERIA DOTHIDEA* AND ITS ANAMORPH *DIPLODIA AFRICANA* ON *PINUS PINEA* IN THE VESUVIUS NATIONAL PARK (CAMPANIA REGION, SOUTHERN ITALY). L. Bosso, G. Cristinzio. *Department of Agricultural Sciences, University of Napoli "Federico II", Via Università 100, I-80055 Portici (NA), Italy. E-mail: luciano.bosso@unina.it*

In some municipalities, located within the boundaries of the Vesuvius National Park (Campania Region, Southern Italy), several areas with forest cover of *Pinus pinea* showed severe withering of the crowns and damage to pine cones. In the present study, we have isolated in the period May 2013-May 2014 from Ercolano, San Sebastiano, Terzigno, Torre del Greco and Trecase an anamorphic form of *Botryosphaeriaceae*. Morphological and cultural characteristics as well as DNA sequence data (5.8S rDNA, ITS-1 and ITS-4) were made on 30 isolates obtained from 5 municipalities. All strains belonged to the species: *Botryosphaeria dothidea* and its anamorph, *Diplodia africana*. These fungi were present on all pine cones collected and analyzed. Finally, we carried out growth assays at different temperatures: 8°C, 18°C, 28°C and 32°C. All fungi found the optimum of growth at 28°C while at 8°C and 32°C we noted the lowest growth. This seems to be the first report of *D. africana* on *Pinus* species in Campania Region.

6. NICHE-BASED MODEL AND *XYLELLA FASTIDIOSA*: DRAWING THE POTENTIAL SPREAD OF A NEW EMERGING PLANT PATHOGEN IN ITALY. L. Bosso¹, D. Russo^{1,2}, M. Di Febbraro³, G. Cristinzio¹, A. Zoina¹. ¹Department of Agricultural Sciences, University of Napoli "Federico II", Via Università 100, I-80055 Portici (NA), Italy. ²School of Biological Sciences, University of Bristol, 24 Tyndall Avenue, BS8 1TQ, Bristol, United Kingdom. ³EnvixLab, Department of Biosciences and Territory, University of Molise, Contrada Fonte Lappone, I-86090 Pesche (IS), Italy. E-mail: luciano.bosso@unina.it

Spatial Distribution Models (SDMs) may provide realistic scenarios to explain the influence of bioclimatic variables in the context of emerging plant pathogens. *Xylella fastidiosa* is a xylem-limited Gram-negative bacterium causing a high number of severe diseases to many plants. We developed a maximum entropy model (Maxent vers. 3.3.3k) for *X. fastidiosa* in Italy in order to carry out a preliminary analysis of its potential geographical distribution and to determine which Eco-Geographical Variables (EGVs) may affect its spreading. The analysis of single variable contribution showed that precipitation of driest month (40.3%) and precipitation of wettest month (30.4%) were the main factors influencing model performance. Altitude, precipitation of warmest quarter, mean temperature of coldest quarter, and land cover provided a

total contribution of 19.5%. Based on the model predictions, *X. fastidiosa* has a high settling probability (>0.8) in areas characterized by: i) relatively low altitude (0-150 m a.s.l.); ii) precipitations in the driest month (<10 mm), wettest month (80-110 mm) and warmest quarter (<60 mm); iii) mean temperature of coldest quarter higher than 8°C; iv) agricultural areas comprising intensive agriculture, complex cultivation patterns, olive groves, annual crops associated with permanent crops, orchards and vineyards; forest (essentially oak woodland); and Mediterranean shrubland. SDMs showed high probability of presence in Apulia, Calabria, Basilicata, Sicily, Sardinia, Campania, Lazio and south Tuscany. Maxent models achieved excellent levels of predictive performance as can be seen from AUC, TSS and AUC_{diff} values. Our study highlights that *X. fastidiosa* will likely overcome the current boundaries and affect larger areas in Italy outside Apulia.

7. SURVEY OF JACKFRUIT (*ARTOCARPUS HETEROPHYLLUS*) PLANTATIONS IN THE MEKONG RIVER DELTA FOR DECLINE INCITED BY *PHYTOPHTHORA PALMIVORA*. S.O. Cacciola¹, A. Pane¹, M. Evoli¹, R. Faedda¹, L. Schena², M.G. Li Destri Nicosia², L.M. Van Tri³, N. Van Hoa³, N. Minh Chau³, C. Olsson⁴, S. Wright⁵, M. Ramstedt⁶. ¹Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 100, I-95123 Catania, Italy. ²Department of Agriculture, Mediterranean University of Reggio Calabria, Via Salita Melissari, Località Feo di Vito, I-89122 Reggio Calabria, Italy. ³Southern Horticultural Research Institute, Box 203, My Tho, Tien Giang, Vietnam. ⁴Department of Biological and Environmental Sciences, Gothenburg University, 40530 Gothenburg, Sweden. ⁵Department of Electronics, Mathematics and Natural Sciences, University of Gävle, 80176 Gävle, Sweden. ⁶Department of Forest Mycology and Plant Pathology, Swedish Agricultural University (SLU), 75007 Uppsala, Sweden. E-mail: olgacacciola@unict.it

A new disease of jackfruit (*Artocarpus heterophyllus*) caused by *Phytophthora palmivora* was recently reported in Southern Vietnam. Symptoms included root rot, bleeding cankers at the base of the trunk, leaf chlorosis, defoliation, wilt, leaf blight and occasionally fruit brown rot. In the 2012 and 2013, a survey of jackfruit plantations was performed in four provinces (Ba Ria-Vung Tau, Binh Duong, Binh Phuoc and Dong Nai) of the Mekong River Delta Region (Southern Vietnam) to determine the incidence of this disease and study the variability of the causal agent. The disease was found in 10% of surveyed farms with an incidence varying from 2 up to near 60% of the trees in each farm. The total numbers of surveyed trees and proportions of symptomatic trees per province were as follows: 2126 trees in Ba Ria-Vung Tau province with 19% of symptomatic trees, 1540 in the Binh Duong province with 14.5% of symptomatic trees, 1892 in the Binh Phuoc province with 20.5% of symptomatic trees and 1962 in the Dong Nai province with 34% of symptomatic trees. The analysis of the ITS1-5.8S-ITS2 sequences of all isolates from jackfruit revealed 100% of homology with reference isolates of *P. palmivora* and *P. arecae*, which is a synonymous of *P. palmivora*. All isolates were A1 mating type and did not group separately from the isolates of *P. palmivora* sourced in the same geographic area from trees of durian (*Durio zibethinus*), one of the most valuable fruit crops in Southern Vietnam.

8. FIRST REPORT OF *PESTALOTIOPSIS* IN HAZELNUT IN ITALY. R. Cappelli¹, A. Prodi¹, F. Cavina¹, M. Vibio², P. Nipoti¹, A. Pisi¹. ¹Department of Agricultural Sciences (DipSA), University of Bologna, Viale G. Fanin 44, I-40127 Bologna, Italy. ²Centro Attività

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Italy is the first European producer of hazelnuts (*Corylus avellana*) and second in the world after Turkey. The orchards are mainly located in Piedmont, Lazio, Campania and Sicily. In 2013 a sample of hazelnut leaves from Piedmont, showing necrotic spots, has been analyzed. The aims of this work have been to identify the causal agent and verify Koch's postulates, since specific references in literature are not present. Colonies with a white aerial, dense mycelium with black acervula close to center, similar to ink drops, grew really fast from hazelnut leaf samples plated on Potato Dextrose Agar (PDA). At light microscope the conidia appeared ellipsoid, 4-septate, with three brown central cells, the basal and apical cells hyaline and with appendages. Morphologically the fungus was identified as *Pestalotiopsis* spp. Molecular identification has still not been successfully applied for species-level differentiation. Furthermore pathogenicity tests on leaves, both *in vitro* and *in vivo*, were set up at different temperatures. Positive response and a better infection resulted at 20°C. *Pestalotiopsis* was re-isolated from the leaf tissues artificially infected. *Pestalotiopsis* spp. on hazelnut has been reported in different countries, in particular *P. macrospora* was found to be pathogenic on leaves and twigs in Iran and *P. guepinii* in Turkey. Furthermore in 2015 *Pestalotiopsis* spp. was reported to cause disease on hazelnut fruit clusters in Turkey, causing yield reduction. This work is the first report of *Pestalotiopsis* in hazelnut in Italy. Due to the economic importance of this crop in Italy, the incidence of this fungus should be monitored.

9. MILD CLIMATIC CONDITIONS INDUCE THE REPLACEMENT OF GREMMENIELLA ABIETINA BY DIPLODIA SAPINEA, IN PINE PLANTATIONS. P. Capretti¹, A.L. Pepori², D. Migliorini^{1,2}, L. Botella³, N. Luchi². ¹Department of Agrifood Production and Environmental Sciences (DISPAA), University of Firenze, Piazzale delle Cascine 28, I-50144 Firenze, Italy. ²National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via Madonna del Piano 10, I-50019, Sesto Fiorentino (FI), Italy. ³Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 1, 61300 Brno, Czech Republic. E-mail: paolo.capretti@unifi.it

The ascomycetous fungus *Gremmeniella abietina* responsible of Scleroderris canker causes several damages on pine plantations in the northern hemisphere. The disease described in the northern part of the Italian peninsula has been studied since the 1970s. Outbreaks were always related to climatic unfavourable conditions for the hosts. The fungus described in Italy was characterized by two forms that can be distinguish according conidial morphology and molecular methods: Alpine and continental. They have different host preference and grow under different environmental conditions. In the Alpine region this pathogen mainly affect *Picea abies*, *Pinus cembra*, *P. sylvestris* and *P. nigra*, while in the Mediterranean region it occurs on *P. pinea*, *P. pinaster*, *P. halepensis* and *P. nigra*. The damage consist in shoot death and affect plants in reforestation, but also necrosis of terminal shoots on adult trees. During the last years the occurrence of damages in affected areas, and particularly in Mediterranean region, was less severe. Changing of cultural conditions in plantations and climate change reduced the opportunity for the pathogen to survive in previously affected area. The mild temperatures and less severe winter seasons, allowed the plants to recover. In many cases the pathogen was replaced by *Diplodia sapinea* and *Sirococcus conigenum*, more thermophilic fungal pathogens. These finding suggest that the effect of climate change play a relevant role on the incidence of Gremmeniella canker, creating a new scenario to other pathogens mainly related to environmental stresses.

10. MOLECULAR DETECTION OF FUSARIUM FUJIKUROI ON RICE SEEDS BASED ON A TAQMAN QPCR. G.A. Carneiro^{1,2}, D. Spadaro^{1,2}, M.L. Gullino^{1,2}, A. Garibaldi². ¹Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: greice.amaralcarneiro@unito.it

The Italian rice production covers 50% of the European production. In the last decades the Bakanae disease spread throughout the country, growing in its importance year after year. Seeds treatment controlled *Fusarium fujikuroi*, the causative agent of Bakanae disease, for a long time. The reduction of availability of agrochemicals for seed treatments caused increasing losses in crops. Infected plants can be identified at an early stage of growth, showing an abnormal elongation of the stem and its progressive thinning and yellowing. The possibility to detect the presence of the pathogen directly from seed is of great interest. In this work, we developed a quantitative PCR using a specific *F. fujikuroi* TaqMan probe designed on the polymorphic regions of the TEF gene (Translation Elongation Factor 1 α). The deletion of six nucleotides was identified only in the gene sequence of *F. fujikuroi* and not in other species of the *Gibberella fujikuroi* species complex (GFSC). Probe specificity was confirmed by analysing the DNA of different species of *Fusarium* spp. The specific probe was subsequently used to detect *F. fujikuroi* on rice seeds, providing a rapid tool for the seeds companies to verify presence of pathogen on rice seeds.

11. TWO PECTOBACTERIUM CAROTOVORUM SUBSPECIES INVOLVED IN STEM ROT AND PITH NECROSIS OF GRAFTED TOMATO PLANTS. A. Caruso, P. Bella, R. La Rosa, V. Catara. Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 100, I-95123 Catania, Italy. E-mail: vittoria.catara@unict.it

During winter 2014-2015 grafted tomato plants grown on coconut fiber in heated greenhouse in east Sicily were affected by a stem rot disease. Symptoms occurred in December during ripening of the first fruits and consisted of dark brown longitudinal discoloration of the basal part of the stem and petioles. Longitudinal sections through the stem revealed brown water-soaked to soft-rotted pith tissues, both of the scion and rootstock, as well as xylem discoloration. From symptomatic tissues numerous bacterial colonies were obtained on nutrient dextrose agar. Based on two different colony morphologies, eight bacterial strains were purified. They were Gram-negative, oxidase-negative, facultative anaerobic and pectolytic, and did not fluoresce on King's B agar. Strains were PCR positive with *Pectobacterium* spp. primers and negative with *P. atrosepticum* primers. *Pectobacterium* species and *P. carotovorum* subspecies reference strains were used as a comparison. BLAST analysis of four strain 16S rRNA gene sequences showed two strains with the highest identity with *P. carotovorum* subsp. *carotovorum* and the remaining two with *P. carotovorum* subsp. *brasiliensis*. Pathogenicity assays were conducted using tomato seedlings by stem prick inoculation of a 24-hr old bacterial colony or by injecting a bacterial suspension (10⁶ cfu mL⁻¹) into the stem at the axil of first true leaf. Two virulence groups were identified. The first group induced the rotting and collapse of the stems within 3 days when prick inoculated, with browning, soft rotting and hollowing of the whole stem pith in 15 days. When the strains of the second group are inoculated using the two methods of inoculation, they show firm, brown, hollowed 1-4 cm lesions at the inoculation sites.

12. SURVEY ON SANITARY STATUS OF NATIVE *VITIS VINIFERA* VARIETIES IN GEORGIA. P. Casati¹, D. Maghradze², F. Quaglino¹, A. Ravasio¹, O. Failla¹, P.A. Bianco¹. ¹Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DISAA), University of Milano, Via Giovanni Celoria 2, I-20133 Milano, Italy. ²Institute of Horticulture, Viticulture and Oenology, Agricultural University of Georgia, David Aghmashenebeli Alley 13-th Km, 0159 Tbilisi, Georgia. E-mail: piero.bianco@unimi.it

In September 2013, a survey was carried out in three collections representing the native Georgian *Vitis vinifera* germplasm, located in Saguramo (Shida Kartli Region), Shumi and Kindzmaraulis (Khaketi Region). Leaf samples were collected from 37 plants of 12 white and 13 red berry varieties showing symptoms of viral diseases, such as leaf rolling and chromatic alterations, with different symptom severity. Total RNAs were extracted from leaves and PCR-based reactions were performed with specific primer pairs to identify *Grapevine rupestris stem pitting-associated virus* (GRSPaV), *Grapevine leaf roll-associated virus 1, 2, and 3* (GLRaV-1, GLRaV-2 and GLRaV-3), *Grapevine virus A* (GVA), and *Grapevine pinot gris virus* (GPGV). Obtained results revealed the prevalence of GRSPaV (mainly strains of groups 2a and 2b, associated with vein necrosis of 110R, identified in 29 out of 37 plants) and GLRaV-3 (identified in 26 out of 37 plants). Surprisingly, GLRaV-1 and GVA were detected in few plants (three out of 37), always in mixed infection with GLRaV-3. As expected, GLRaV-2 was identified only in two plants. Moreover, GPGV, a *Trichovirus* recently reported in Europe and Korea, was detected in 7 plants of six varieties (Goruli mtsvane, Khikvi, Mtsvane kviteli, Saperavi pachkha, Tavkveri, Korkaula), always in mixed infection with GRSPaV, GLRaV-3 and GVA. Since the response of the Georgian grapevine varieties to the pathogens has not been accurately described, further investigation will be needed to determine the typical symptoms associated with grapevine viruses and to evaluate their potential effects on yield and wine quality.

13. DETECTION AND MOLECULAR CHARACTERIZATION OF A *BADNAVIRUS* IN AN AUTOCHTHONOUS GRAPE FROM APULIA (ITALY). M. Chiumenti¹, M. Morelli¹, A. Giampetruzzi¹, F. Palmisano², V.N. Savino^{2,3}, P. La Notte^{1,2}, G.P. Martelli³, P. Saldarelli¹. ¹National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via G. Amendola 122/D, I-70126 Bari, Italy. ²Centre for Research, Experimentation and Education in Agriculture (CRSFA) "Basile Caramia", Via Cisternino 281, I-70010 Locorotondo (BA), Italy. ³Department of Soil, Plant and Food Sciences (DiSSPA), University of Bari "Aldo Moro", Via G. Amendola 165/A, I-70126 Bari, Italy. E-mail: michela.chiumenti@ipsp.cnr.it

Vegetative propagation of grapevines favours the perpetuation and dissemination in nature of intracellular pathogens (over than 70 considering viruses, viroids and phytoplasmas). Once in the field, their presence may affect in various ways the plant's yield and its quality. Preventing infection spreading through a prompt identification, characterization and exclusion approach, is still the most effective action of containment. To this aim, in 2014, registered native grapevine cultivars from Apulia were analyzed to determine their "absolute" sanitary status (virome), i.e. the totality of the infectious agents present in any single accession using a High Throughput Sequencing (HTS) approach. Small RNA libraries were therefore synthesized and analyzed for the presence virus/viroid sequences. Among these, a symptomless accession D205 of cv. "Bombino nero" growing in a foundation block showed the presence of 21 contigs (ranging from 329 to 56 nt in length) identified as *Badnavirus*-like. Using the closest *Badnavirus* found by BLASTX search as a reference sequence, i.e. *Fig Badnavirus 1* (FBV-1, accession No. NC017830), a set of specific primers was designed. Amplification

with these primers produced the expected amplicon, which has 91% nucleotide identity with a *Badnavirus* recently discovered in Greece and denoted *Grapevine roditis leaf discoloration-associated virus* (GRLDaV). For a preliminary assessment of the incidence of this virus in the field, a preliminary survey in the same foundation block was conducted. A total of 11 samples from different autochthonous cultivars were checked by PCR but none of them tested positive.

14. CHARACTERIZATION AND PATHOGENICITY OF FUNGAL SPECIES ASSOCIATED WITH EUCALYPT DIEBACK IN SARDINIA (ITALY). A. Deidda, B.T. Linaldeddu, B. Scanu, A. Franceschini. Department of Agriculture, University of Sassari, Viale Italia 39, I-07100 Sassari, Italy. E-mail: adeidda@uniss.it

Due to their rapid growth and adaptability to different environmental conditions, *Eucalyptus* species have been widely introduced in both hemispheres for pulpwood production. In Sardinia (Italy), eucalypt plantations were established in the 20th Century primarily in areas reclaimed from marshland and subsequently all over the island where they are currently cultivated as ornamental plants, windbreaks and for honey production. In recent years, a severe and unusual disease of unknown aetiology has been observed in several artificially established plantations of *Eucalyptus camaldulensis* throughout the island. The affected plants showed leaf chlorosis, crown thinning, shoot and branch dieback, sunken cankers, epicormic shoots and exudations of Kino. Since there is no information about this unusual disease and given the high ecological and economic relevance of these ecosystems, in 2013 a survey was carried out to establish the causal agents involved in the aetiology of the disease. Isolations from 510 symptomatic woody samples yielded a total of 489 fungal isolates belonging to three distinct families, namely *Botryosphaeriaceae*, *Diaporthaceae* and *Valsaceae*. On the basis of morphological features and DNA sequence data (ITS), seven distinct species: *Diaporthe foeniculina*, *Neofusicoccum australe*, *N. luteum*, *N. mediterraneum*, *N. parvum*, *N. vitifusiforme* and *Valsa fabianae* were identified. In addition, two putative new species of *Cytospora* were obtained. *Neofusicoccum australe* was the only species recovered in all surveyed sites and its isolation frequencies ranged from 51 to 95%. Pathogenicity trials on *E. camaldulensis* trees showed that only the *Neofusicoccum* species, with the exception of *N. vitifusiforme*, are pathogenic on this host.

15. *XANTHOMONAS EUVESICATORIA* IN PEPPER SEEDS: IMPLEMENTATION OF ITS DETECTION AND PRELIMINARY STUDY ON ITS GENETIC FINGERPRINTS. M. Ferrari¹, B. Xhemali^{1,2}, D. Giovanardi¹, F. Valentini², M. Ignjatov³, R. Jevtić³, E. Stefani¹. ¹Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2, I-42122 Reggio Emilia, Italy. ²Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM-IAMB), Via Ceglie 9, I-70010 Valenzano (BA), Italy. ³Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia. E-mail: michele.ferrari@unimore.it

The bacterial spot of pepper is a destructive disease and its causal agent was formerly known as *Xanthomonas campestris* pv. *vesicatoria*, later reclassified in four different species: *X. vesicatoria*, *X. euvesicatoria*, *X. perforans* and *X. gardneri*. All four species are seed-borne and regulated. *Xanthomonas euvesicatoria* is particularly aggressive on pepper and its detection and identification, particularly in seed lots, is the key for a safe pepper production. We compared a conventional serological detection method (ELISA) with the direct isolation and identification of the pathogen, and with a specific molecular detection (simplex-PCR), but following two

different DNA extraction and purification procedures in parallel: 1) treatment of the seed concentrate by a heat shock or 2) use of the DNeasy Plant Mini Kit columns (Qiagen). Thirteen ELISA positive samples were found: from those seed samples, 5000 seeds for each extraction method were taken and extracted according to the two procedures mentioned above. Results highlighted that ELISA performed quite well, but the most sensitive and reliable pathogen detection was done by seed extraction with the DNeasy Plant Mini Kit, followed by simplex-PCR. Direct isolation and simplex-PCR following heat shock gave several false negative results. Genotyping of a *X. euvesicatoria* isolate collection was attempted through rep-PCR, using the BOX, REP and ERIC primers to specifically amplify repetitive elements dispersed throughout the bacterial genome. Results highlighted that *X. euvesicatoria* is quite a uniform population, taxonomically not so distant from *X. perforans*, but clearly distinguished from the other two xanthomonads. Analysis of BOX-PCR profiles supports the fact that a possible *X. euvesicatoria* subgroup is present in the main population, which might be related to a different geographical origin of seeds.

16. CHARACTERIZATION OF SELECTED CITRUS-ASSOCIATED *ALTERNARIA* ISOLATES. F. Garganese¹, S.M. Sanzani¹, L. Schena², A. Ippolito¹. ¹Department of Soil, Plant and Food Sciences (DiSSPA), University of Bari "Aldo Moro", Via G. Amendola 165/A, I-70126 Bari, Italy. ²Department of Agriculture, Mediterranean University of Reggio Calabria, Via Salita Melissari, Località Feo di Vito, I-89124 Reggio Calabria, Italy. E-mail: simonamarianna.sanzani@uniba.it

Alternaria brown spot is one of the most important diseases of tangerines and their hybrids worldwide. Recently, a disease outbreak in Southern Italy refocused the attention on the disease. Twenty representative cultures of *Alternaria* were selected from a collection of more than 100 isolates from leaves and fruits of cvs. Fortune, Nova, Valencia, and Tangerine. Then, they were characterized along with specimen strains of *A. tenuissima*, *A. alternata*, *A. arborescens*, *A. citri*, *A. toxicogenica*, and *A. limoniasperae* ("small-spored" *Alternaria* species) to determine the etiology of the disease and evaluate the virulence of different isolates/species. Morphological characteristics and sporulation patterns separated most *Alternaria* isolates into three main groups corresponding to *A. alternata*, *A. arborescens*, and *A. tenuissima*, of which the first was the most abundant one. Phylogenetic analyses based on endopolygalacturonase (endoPG) and β -tubulin genes, two anonymous genomics regions (OPA 1-3 and OPA 2-1), and the Internal Transcribed Spacer (ITS) region produced a clustering of isolates largely confirming morphological results. The OPA 1-3 region was more suitable than other tested regions for separating closely related "small-spored" *Alternaria* species and revealed the existence of intra-species molecular variability. Investigated isolates showed different levels of virulence on leaves and fruits but it was not possible to identify a direct correlation between virulence and genetic/morphological groupings of isolates.

17. ADOPTION OF HIGH THROUGHPUT SEQUENCING FOR ASSESSING THE OUTRIGHT SANITARY STATUS OF CERTIFIED GRAPEVINE CLONES AND ROOTSTOCKS. A. Giampetruzzi¹, M. Morelli¹, M. Chiumenti¹, V.N. Savino², G.P. Martelli², P. La Notte^{1,3}, F. Palmisano³, P. Saldarelli¹. ¹National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via G. Amendola 122/D, I-70126 Bari, Italy. ²Department of Soil, Plant and Food Sciences (DiSSPA), University of Bari "Aldo Moro", Via G. Amendola 165/A, I-70126 Bari, Italy. ³Centre for Research, Experimentation and Education in Agriculture (CRSFA)

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For a thorough characterization of their virome, 20 selected clones of grapevine cultivars and rootstocks, maintained in the premultiplication plots of the CRSFA (Centre for Research, Experimentation and Education in Agriculture "Basile Caramia", Locorotondo, Italy), were subjected to High Throughput Sequencing (HTS). Prior to the present study, which was developed in the frame of the Apulian Regional project Re.Ge.Vi.P. ("Recovery of Apulian grape germplasm"), certified grapevine accessions were known to be free from viruses and diseases regulated in the Italian certification system, as assessed by time-consuming and narrow-range specific assays, i.e. Wood Indexing (WI) and serological or RT-PCR molecular tests. The HTS of small RNA (sRNA) libraries, performed on an Illumina HiScan SQ™ apparatus, and data processing through a custom-developed bioinformatic pipeline, confirmed the absence of the following regulated viruses: *Grapevine virus A* (GVA), *Grapevine virus B* (GVB), *Grapevine leafroll-associated virus 1, 2 and 3* (GLRaV-1, -2, -3), *Grapevine fanleaf virus* (GFLV), *Grapevine fleck virus* (GFkV) and *Arabidopsis mosaic virus* (ArMV), allowing an in-depth analysis also of viral and viroidal infections. This unbiased approach disclosed the presence of agents that are not targeted in routinely performed assays, like *Grapevine rupestris stem pitting-associated virus* (GRSPaV), *Grapevine rupestris vein feathering virus* (GRVfV), *Hot stunt viroid* (HSVd) and *Grapevine yellow speckle viroid 1* (GYSVd-1), in addition to hitherto unknown viruses, such as a new badnavirus-like species, showing similarities to *Grapevine roditis leaf discoloration-associated virus* (GRLDaV). Our findings, although requiring further standardization efforts, highlight the potential benefits of using the HTS approach in grapevine certification schemes, in substitution for, or in synergy with WI and conventional laboratory tests.

18. NEW *COLLETOTRICHUM* spp. ON ORNAMENTAL PLANTS IN NORTHERN ITALY. G. Gilardi^{1,2}, G. Ortu¹, S. Franco Ortega¹, M.L. Gullino^{1,2}, A. Garibaldi¹. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: giovanna.gilardi@unito.it

Anthraco-nose caused by *Colletotrichum* spp. is one of the most common disease on many ornamentals. During the summer-fall 2014, previously unreported leaf spots were observed on plants of *Salvia leucantha* and *S. greggii* grown in a garden located near Biella (Northern Italy) at an altitude of 800 m. All plants were affected, though at different level. The first symptoms consisted in small necrotic spots interesting a large percentage of the leaf, which eventually wilted. Generally, the disease started from basal leaves on plants grown in shadow and at higher RH. On the basis of several isolations carried out on Potato Dextrose Agar (PDA) amended with 25 mg L⁻¹ of streptomycin sulphate from infected tissues, *Colletotrichum* sp. was consistently recovered from infected tissues of *S. leucantha*, and *S. greggii*. The pathogenicity of the different strains of *Colletotrichum* were confirmed under controlled conditions and re-isolation of fungal isolates showing the same morphological characteristics as *Colletotrichum*, fulfilling Koch's postulate. Internal Transcribed Spacer (ITS) analysis permitted to confirm such identification. This is to our knowledge the first report of anthracnose caused by different strains of *Colletotrichum* on *S. leucantha* and *S. greggii* in Italy, as well as in the world.

19. NEW FOLIAR AND SOIL-BORNE PATHOGENS OF LEAFY VEGETABLES FOR FRESH CUT MARKET. G. Gilardi^{1,2}, G. Ortu¹, M.L. Gullino^{1,2}, A. Garibaldi¹. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGRO-INNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: giovanna.gilardi@unito.it

New diseases caused by soil-borne and foliar pathogens were recently observed for the first time in Italy on fresh-cut vegetable crops, as a consequence of its dynamism, specialization and use of intensive cultivation techniques. Among soil-borne pathogens, *Pythium aphanidermatum* on leaf beet (*Beta vulgaris* subsp. *vulgaris*) and spinach (*Spinacea oleracea*), and *Pythium irregulare* on lamb's lettuce (*Valerianella olitoria*), were observed. Wild rocket (*Diploaxis tenuifolia*) showed symptoms of new leaf spots caused by *Alternaria japonica*, *Plectosphaerella cucumerina* and *Fusarium equiseti*. *Fusarium equiseti* was also consistently isolated from affected tissues of lettuce grown under greenhouse at 20-30°C. Seed transmission of *A. japonica* and *P. cucumerina* has been confirmed in wild rocket. Identifying the primary sources of inoculum is of critical importance to prevent the spread of new pathogens in field as well as to adopt effective disease management. Quick and sensitive diagnostic tools are needed.

20. EVALUATION IN PHYTOTRONS OF THE EFFECT OF INCREASED CO₂ AND TEMPERATURE ON BASIL DOWNY MILDEW. G. Gilardi^{1,2}, M. Pugliese^{1,2}, M.L. Gullino^{1,2}, A. Garibaldi¹. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: giovanna.gilardi@unito.it

Research conducted over the past decades has shown how global climate change is associated with higher concentrations of atmospheric CO₂, rise in temperatures, extreme weather events. Since both CO₂ and temperature are key variables that can affect plants and their diseases, climate changes are influencing plant growth, plant diseases and, consequently, the global food supply. In the last decade, phytotrons have been used to study the effects of CO₂ enrichment and temperature increases on infection rates for several pathosystems. The pathosystem basil (*Ocimum basilicum*)-downy mildew (*Peronospora belbahrii*) was chosen in this study to evaluate six different combinations of temperature and CO₂: 1) 400-450 ppm CO₂, 18-22°C; 2) 800-850 ppm CO₂, 18-22°C; 3) 400-450 ppm CO₂, 22-26°C; 4) 800-850 ppm CO₂, 22-26°C; 5) 400-450 ppm CO₂, 26-30°C; 6) 800-850 ppm CO₂, 26-30°C. In the presence of the standard conditions (18-22°C and 400-450 ppm of CO₂), the mean disease incidence was 43.8%, while, affected leaf area was 22.2%. A doubled level of CO₂ at the same temperature ranges of 18-22°C, caused a significant and notable increase on disease incidence and severity with 70.9% of infected leaves and 41.6% of affected leaf area, respectively. At the highest temperatures tested of 26-30°C, the increase in CO₂ caused a significant increase only in downy mildew severity. In conclusion, climate change simulated under phytotrons conditions had a significant influence on the incidence and severity of basil downy mildew.

21. SPREAD OF BEGOMOVIRUS SPECIES IN TOMATO CROPS IN PANAMA. J.A. Herrera Vásquez¹, S. Panno^{2,3}, C. Carpino⁴, A.B. Romero¹, G. Iacono³, M. Davino^{3,4,5}.

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Begomoviruses are DNA viruses with monopartite or bipartite genomic components. To date, in Panama, was reported only one *Begomovirus* species with bipartite genome, *Potato yellow mosaic Panama virus* (PYMPV), to affect tomato crops in the main Panamanian provinces. In the years 2011 and 2012, in the central provinces of the country, 319 samples were collected from symptomatic plants, with various combinations symptoms. DNA was extracted from each sample for PCR analysis, using two sets of degenerate primers able to detect members of the genus *Begomovirus*. 135 samples showed a positive signal to PCR reactions and were directly sequenced in both direction in order to identify the affecting *Begomovirus* species. Three *Begomovirus* species were detected: PYMPV, *Tomato leaf curl Sinaloa virus* (ToLCSinV), and *Tomato yellow mottle virus* (TYMoV). Our results show that the *Begomovirus* species studied are more geographically widespread than previously in the Country. To our knowledge this is the first report of ToLCSinV and TYMoV in tomato cultivated crops in Panama. These findings should help to establish a basis for the control of begomoviral epidemics in tomato crops and other many species present in the Panamanian areas.

22. SEVERE DECLINE OF LOCAL GARLIC (*ALLIUM SATIVUM*) LANDRACES IN LATIUM, ITALY. A. Infantino¹, A. Taglienti¹, S. Vitale¹, R. Rea², P. Taviani², S. Paoletti², L. Tomassoli¹. ¹Agricultural Research Council – Plant Pathology Research Centre (CRA-PAV), Via C.G. Bertero 22, I-00156 Roma, Italy. ²Regional Agency for Development and Innovation of Agriculture in Lazio (ARSIAL), Via Rodolfo Lanciani 38, I-00162 Roma, Italy. E-mail: alessandro.infantino@entecra.it

Garlic (*Allium sativum*) is a niche production (67 ha) in Latium (Italy), mostly grown in two provinces (Frosinone and Viterbo). Two red-clove landraces (“Aglione rosso di Proceno” and “Aglione rosso di Castelliri”) are particularly appreciated due to their organoleptic properties and they are protected by the Regional Law n. 15 (1st March 2000 “Protection of autochthonous genetic resources of agricultural interest”). During 2015, surveys were performed in 10 and 13 garlic-growing farms in Proceno and Castelliri, respectively, where growers tell on a progressive decline of the crop. Plants showing stunting, crown and root rots, yellowing and twisted leaves were observed and collected. Samples were examined for both soil fungal and viral pathogens. Isolations of the most important fungi were attempted by surface disinfecting (NaOCl 2% of active Cl₂) symptomatic tissues and placing them on Potato Dextrose Agar (PDA) plates amended with streptomycin sulphate and ampicillin (100 ppm). *Fusarium* spp. were present on 70% and 38% of the sampled farms in Proceno and Castelliri, respectively. *Fusarium proliferatum* seems to be the prevalent species observed. For virus analyses, total RNA was extracted from leaf tissues and used for RT-PCR tests performed to detect the most common garlic viruses: *Leek yellow stripe virus* (LYSV), *Garlic common latent virus* (GCLV), *Onion yellow dwarf virus* (OYDV) and the group of allelixiviruses. In all farms, except one in Castelliri, plants infected by one or more

viruses were found. GCLV was predominant and “Proceno” landrace was more severely infected. The accumulation of viruses in cloves and the high incidence and severity of several phytopathogenic fungi observed in this study are of serious concern for the maintenance of the quality of the two garlic landraces.

23. IDENTIFICATION AND PATHOGENICITY OF BOTRYOSPHAERIACEAE SPECIES ASSOCIATED WITH LENTISK DIEBACK IN ITALY. B.T. Linaldeddu, A. Deidda, B. Scanu, L. Maddau, A. Franceschini. *Department of Agriculture, University of Sassari, Viale Italia 39, I-07100 Sassari, Italy. E-mail: ben@uniss.it*

Lentisk (*Pistacia lentiscus*) is an evergreen shrub that is widespread over the Mediterranean region. Since spring 2012, a severe and unusual disease of unknown aetiology has been observed on lentisk in six islands (Budelli, Caprera, Mortorio, Santa Maria, Santo Stefano and Spargi) belonging to the La Maddalena archipelago (Italy). The affected plants showed leaf chlorosis, crown thinning, branch dieback and sunken cankers. When branch with sunken cankers were cross-sectioned, internal wood symptoms included characteristic V-shaped necrotic sectors. Frequently, the necrotic lesions girdled the branches, causing rapid death of the upper crowns. Since there is no information about the aetiology of this disease and given the high ecological importance of these natural ecosystems, from spring 2012 to summer 2014, 36 samples of twigs and branches of lentisk showing sunken cankers were collected and processed. Based on morphology, colony appearance and DNA sequence data, three *Botryosphaeriaceae* species were isolated and identified. These included *Diplodia olivarum*, *Neofusicoccum cryptoaustrale* and *N. luteum*. In addition, another *Diplodia* species morphologically distinct from all known species was isolated. Phylogenetic analyses based on nucleotide sequences of ITS and *tef1- α* regions placed this new species within the *Diplodia* Clade 3. Phylogenetically this putative new *Diplodia* species is most closely related to *D. pseudoseriata* and *D. alatafructa*. Pathogenicity trials carried out in field conditions on asymptomatic branches of lentisk showed that all four species are aggressive pathogens on this host and therefore directly involved in the severe dieback that is currently threatening this typical shrub of the Mediterranean maquis.

24. TESTING THE SPATIAL DISTRIBUTION OF PLANT DISEASES THROUGH PERMUTATION AND RANDOMIZATION METHODS. G. Lione, L. Giordano, P. Gonthier. *Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: paolo.gonthier@unito.it*

The analysis of the spatial distribution of plant diseases is a pivotal issue in plant pathology. A series of geostatistical tests based on permutation and randomization is proposed to assess the spatial distribution of plant diseases when the variable of phytopathological relevance is categorical. Monte Carlo simulations run to provide estimates of power and type I error showed the good performances and the reliability of the tests (power > 0.80; type I error < 0.05). The tests were successfully validated by verifying the consistency between their output and previously published results on the spatial distribution of spores of two fungal pathogens causing root rot on conifers, i.e. *Heterobasidion annosum* and *H. irregulare*. The tests were also carried out to analyze the influence of plantation density on the distribution of sweet chestnuts infected by *Gnomoniopsis castanea*, an emerging fungal pathogen causing nut rot. Trees carrying nuts infected by *G. castanea* were randomly distributed in patches with different plantation densities, suggesting that the distribution

of the pathogen was unrelated to the plantation density. These geostatistical tests could be applied in the analysis of the spatial distribution of plant diseases both in agriculture and in forestry. A user-friendly software embedding the algorithms that perform the tests is also available.

25. CANKER DISEASE ON PINUS RADIATA CAUSED BY CALICIOPSIS PINEA IN ITALY. N. Luchi¹, A.L. Pepori¹, C. Aglietti², D. Migliorini^{1,2}, A. Santini¹, P. Capretti². ¹National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via Madonna del Piano 10, I-50019 Sesto Fiorentino (FI), Italy. ²Department of Agrifood Production and Environmental Sciences (DISPAA), University of Firenze, Piazzale delle Cascine 28, I-50144 Firenze, Italy. E-mail: nicola.luchi@ipsp.cnr.it

Caliciopsis pinea is an ascomycetous fungus that causes bleeding cankers in conifers in North America and Europe in several different native pine species. It has been reported since 1960's in France and later in Italy in Tuscany (*Pinus pinea*, *P. pinaster*, *P. nigra* and *P. halepensis*). More recently, the fungus has been collected in Spain on *P. radiata* affected by *Gibberella circinata*, the causal agent of pitch canker disease. A survey to detect its occurrence was recently organized in *P. radiata* and *P. pinaster* plantations in central Italy. To assess the impact of *Caliciopsis* on native and exotic pine hosts two different experiments were carried out: 1) fungal growth using Pine Needle Agarized (PNA) media; 2) pathogenicity test on young pine seedlings. Additionally the evaluation of climatic parameters suitable for disease occurrence was studied. Results showed that there are differences in fungal growth according the substrate origin. Significant differences in lesion lengths were also found on pine species tested. As regard on climatic parameters *C. pinea* growth on Mediterranean conditions but its needs some periods humidity during the growing seasons. Our findings confirm that *C. pinea* could be considered as a dangerous pathogen of *P. radiata* stands in central Italy, where it causes serious failure in plantations. Despite the pitch canker was not found in Tuscany, the presence of *C. pinea* could be considered as potential bioindicator of the most suitable site for *G. circinata*.

26. SIMULTANEOUS DETECTION OF 'CANDIDATUS PHYTOPLASMA PRUNORUM' AND PLUM POX VIRUS IN PRUNUS spp. BY CRUDE RNA-DNA EXTRACTION AND RT-qPCR. S. Minguzzi, F. Terlizzi, C. Lanzoni, C. Poggi Pollini, C. Ratti. *Department of Agricultural Sciences (DipSA), University of Bologna, Viale G. Fanin 44, I-40127 Bologna, Italy. E-mail: claudio.ratti@unibo.it*

Many efforts have been made to develop a rapid and sensitive method for phytoplasma and virus detection. Taking our cue from previous works, different rapid sample preparation methods have been tested and applied to 'Candidatus Phytoplasma prunorum' ('Ca. P. prunorum') detection by RT-qPCR. A duplex RT-qPCR has been optimized using the crude sap as a template to simultaneously amplify a fragment of 16S rRNA of the pathogen and 18S rRNA of the host plant. The specific plant 18S rRNA internal control allows comparison and relative quantification of samples. A comparison between DNA and RNA contribution to qPCR detection is provided, showing higher sensitivity for the latter. The method presented here has been validated on more than a hundred samples of apricot, plum and peach trees in field and nursery since 2010. Finally, a triplex RT-qPCR assay has been optimized to simultaneously detect 'Ca. P. prunorum' and Plum pox virus (PPV) in *Prunus*, as these pathogens may occur in the same plant species, are both detrimental and localized to the phloem. Therefore sampling can

be done from the same tissue and RT-qPCR can be performed on the same crude sap, without the need to extract nucleic acids. The described triplex test could decrease costs, time and labour of stone fruit trees screening in areas susceptible to both pathogens.

27. RECENT EMERGENCE OF *SPHACELOMA CORYLI* AS THE CAUSE OF A PREMATURE HAZELNUT FRUIT DROPPING IN SOUTHERN ITALY. M. Minutolo, B. Nanni, F. Scala, D. Alioto. *Department of Agricultural Sciences, University of Napoli "Federico II", Via Università 100, I-80055 Portici (NA), Italy. E-mail: minutolm@unina.it*

Since 2013, a premature hazelnut fruit drop off associated to necrotic spots on fruit bracts and shells, necrosis or abort of nuts and necrotic spots and vein necrosis of leaves caused severe crop losses in several growing areas of Campania Region (Southern Italy). To identify the pathogen responsible of the disease, symptomatic samples were collected from different areas of Campania. A fungus has been consistently isolated from symptomatic tissues. It forms irregular, strongly folded, convoluted, compact and elastic colonies with branched, septate and hyaline hyphae. Along the mycelium, swollen, rounded, polygonal, thick-walled cells have been observed. Hyaline, cylindrical and unicellular conidiophores carried hyaline and unicellular conidia of about $5.5\text{-}6.5 \times 2.8\text{-}3.6 \mu\text{m}$. These morphological characteristics are similar to those shown by *Sphaceloma coryli*, a fungus which has been already described on hazelnut in Campania more than 30 years ago, but never caused any significant damage. The teleomorph of *S. coryli* has been recorded for the first time on overwintering tissues of hazelnut trees. It has been also induced *in vitro*. Asci are spherical, bitunicate and harbor eight hyaline, oblong bicellular ascospores with a central septum. Internal Transcribed Spacer (ITS) sequences of isolates from diseased hazelnuts (KT001427-KT001450) showed the closest similarity to *Elsinoe* spp. Ascospore morphology together with sequence analysis indicated that the teleomorph is an undescribed species within the genus *Elsinoe* for which the name of *E. coryli* is here proposed. Koch's postulates have been fulfilled by pathogenicity tests carried out on *Corylus avellana* plants.

28. *RANUNCULUS ASIATICUS* A NEW NATURAL HOST FOR BROAD BEAN WILT VIRUS-2. M. Minutolo¹, R. Sorrentino¹, V. Masenga², D. Alioto¹. ¹*Department of Agricultural Sciences, University of Napoli "Federico II", Via Università 100, I-80055 Portici (NA), Italy.* ²*National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Strada delle Cacce 73, I-10135 Torino, Italy. E-mail: minutolm@unina.it*

Plants of *Ranunculus asiaticus* with leaf deformation and mosaic symptoms were observed in a commercial greenhouse in Campania (Southern Italy). Electron microscope observations of sap extracts from symptomatic leaves showed the presence of isometric particles about 25 nm. Occasionally, filamentous particles of about 800 nm were also observed. Symptomatic leaves were tested by DAS-ELISA with antisera to *Broad bean wilt virus-1* (BBWV-1), *Broad bean wilt virus-2* (BBWV-2), *Cucumber mosaic virus* (CMV), *Impatiens necrotic spot virus* (INSV), *Potato virus Y* (PVY), *Turnip mosaic virus* (TuMV) and *Tomato spotted wilt virus* (TSWV) and by fast lateral flow against *Ranunculus leaf distortion virus* (RanLDV-RN122), *Ranunculus mild mosaic virus* (RanMMV-RN129), and *Ranunculus mosaic virus* (RanMV-RN136). All symptomatic buttercup plants resulted positive to BBWV-2, some resulted also positive to RanMMV-RN129. These results were confirmed by RT-PCR and

sequencing using primer pairs Fab5R1F/Fab5R1R and Sprimer/M4 against *Fabavirus* and *Potyvirus*. Blast analysis of BBWV-2 (KJ715961) showed 97% nucleotide identity with a Korean isolates from pepper (JX183225) and with an isolate from tomato in Xinjiang (FN985164). RanMMV (KJ715960) showed 97% and 98% nucleotide identity with isolates from Italy and Israel (DQ152191 and EF445546). Five *R. asiaticus* plants mechanically inoculated with BBWV-2 reacted positively, showing symptoms identical to those observed on the natural infected buttercup plants. The role of RanMMV in symptom expression remain to be clarified since the virus has not been isolated on indicators. To our knowledge, this is the first report of *R. asiaticus* as a natural host of BBWV-2 and the first report of RanMMV infecting this species in Campania, Southern Italy.

29. GRAPEVINE PINOT GRIS DISEASE: AN ULTRASTRUCTURAL STUDY. R. Musetti¹, G.L. Bianchi², A. Loschi¹, P. Ermacora¹, N. Loi¹. ¹*Department of Agricultural and Environmental Sciences (DISA), University of Udine, Via delle Scienze 206, I-33100 Udine, Italy.* ²*Regional Agency for Rural Development (ERSA), Plant Health and Agricultural Chemistry Service, Research, Experimentation and Technical Assistance, Via Sabbatini 5, I-33050 Pozzuolo del Friuli (UD), Italy. E-mail: rita.musetti@uniud.it*

The presence of *Grapevine pinot gris virus* (GPGV), a new member of *Trichoviridae* family, has been reported in different grapevine cultivars both showing symptoms (such as stunting, chlorotic mottling, and leaf deformation) and symptomless, for which the aetiology of the disease is still a matter of debate. To give insight about this disease, ultrastructural investigations have been carried out to study the localization of the virus particles and the cytopathologic effects on the grapevine leaf tissues. Leaves from grapevines (cultivar Pinot gris grown in Farra d'Isonzo, Friuli Venezia Giulia, Italy), showing different symptom severity (severe, moderate, or mild symptoms) and symptomless were sampled and prepared for Transmission Electron Microscopy (TEM) observations. Selected samples were previously tested by Real-time PCR to confirm the presence of GPGV and by ELISA to exclude material affected by viruses included in the Italian certification programme (ArMV, GLRAV-1, GLRAV-2, GLRAV-3, GVA, GVB and GFKV). Filamentous flexuous virus particles have been detected in all types of samples, only in the Phloem Parenchyma Cells (PPC). In the PPC, the main cytopathic features consisted of tonoplast-associated vesicles and vesiculating mitochondria, in mesophyll cells, besides tonoplast-associated vesicles, irregular/distorted cell walls and starch accumulation in the chloroplasts have been detected. No necrosis or other cytological modifications due to other pathogens have been observed. Cytopathological alterations were particularly evident in tissues from plants showing severe symptoms but they also were detected in the other samples, including symptomless PCR-positive plants, even if to a lesser extent. Our observations are in accordance to what previously reported about the most widespread trichoviruses affecting grapevine.

30. PHYLOGEOGRAPHY AND GENETIC DIVERSITY OF *CITRUS TRISTEZA VIRUS* REVEAL THE HISTORY OF THE DISEASE IN SICILY. S. Panno¹, G. Iacono², G. Scuderi^{1,2,3}, M. Davino², S. Davino^{1,4,5}. ¹*Euro-Mediterranean Institute of Science and Technology (IEMEST), Via E. Amari 123, I-90139 Palermo, Italy.* ²*Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 100, I-95123 Catania, Italy.* ³*Agrobiotech, ZI Blocco Palma I, Via V. Lancia 57, I-95121 Catania, Italy.* ⁴*Department of Agricultural and Forest Sciences (SAF), University of*

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Citrus tristeza virus (CTV), genus *Closterovirus* and family *Closteroviridae*, is the causal agent of some of the most economically important diseases in citrus worldwide. The first outbreak in Sicily was detected in 2002 and after that many foci were detected in the island during the last decade. During the period 2002-2014 a survey was conducted in the nine provinces of Sicily and about 80,000 samples were randomly collected from symptomatic and asymptomatic plants. Samples were tested by DAS-ELISA using monoclonal antibody 3DF1+3CA5. About 200 samples, resulted positive from DAS-ELISA test, were utilized for subsequently molecular analysis. Bayesian phylogenetic analysis showed five CTV lineages composed by mild and virulent isolates, suggesting that CTV was introduced in Sicily by at least five independent events. Phylogenetic structure, statistical tests of neutrality and comparison of synonymous and nonsynonymous substitution rates suggest weak negative selection and genetic drift, as a consequence of a rapid spread due to illegal exchange of scions and plants. As a consequence, the genetic variation of CTV was not structured according to geographical location or sampling time, likely due to the multiple introduction events and a complex migration pattern with intense co- and recirculation of different lineages in the same area.

31. RECENT FINDINGS OF TOMATO SPOTTED WILT VIRUS INFECTING ORNAMENTAL PLANTS IN LIGURIA.

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Tomato spotted wilt virus (TSWV) is the most economically important viral pathogen for both open field and greenhouse ornamental productions in Liguria Region. Investigations of the presence and distribution of this *Tospovirus* in "Piana di Albenga" (Savona, Italy) were carried out during 2013 to 2015 including survey and collection of samples belonging to several ornamental species showing symptoms resembling those of TSWV infection. Mechanical inoculations on test plants and serological analysis (PAS-ELISA, LFT) proved TSWV presence in: *Alstroemeria* spp. (white rings and yellow spots on malformed leaves; asymptomatic flowers), *Cyclamen* spp. (necrotic concentric rings; malformed flowers), *Iberis semperflorens* (chloro-necrotic ringspots), *Senecio cruentus* (necrotic spots), *Zinnia elegans* (mottling and brown oak-leaf patterns on leaves; colour-breaking on malformed flowers; wilt). Tospoviruses presence was confirmed by RT-PCR using the BR60/BR65 universal primers, which amplify part of the nucleocapsid protein gene of several tospoviruses. Target amplicons of 454 bp were produced for all samples tested. PCR products were cloned and sequenced on both strands. The resulting sequences showed high percentage of identity, ranging from 96.0 to 99.5%, with several isolates of TSWV. *Iberis semperflorens* was confirmed as new natural host of TSWV, while the virus was for the first time detected in *Z. elegans* in Italy. Since TSWV is limiting factor of ornamental plant production and plays important role in the epidemiology of other crops (including aromatic and vegetable species), these results appear serious enough to require better control measures involving both thrips and propagation material.

32. CHARACTERIZATION OF PSEUDOMONAS POPULATIONS ISOLATED FROM HAZELNUT (*CORYLUS AVELLANA*) TREES IN SARDINIA (ITALY). C. Pinna¹, F. Fancellu¹, S. Oggiano¹, G. Marchi², M. Fiori¹. ¹Department of Agriculture, University of Sassari, Viale Italia 39, I-07100 Sassari, Italy. ²Department of Agricultural Biotechnology, University of Firenze, Piazzale delle Cascine 28, I-50144 Firenze, Italy. E-mail: fiorim@uniss.it

Studies on hazelnut in Sardinia showed the presence of different bacterial populations (*Xanthomonas arboricola* pv. *corylina*, *Pseudomonas syringae* pv. *syringae* and *P. syringae* pv. *coryli*) causal agents of dieback. In field surveys carried out in autumn and spring 2014-2015 in the central part of Sardinia (Barbagia di Belvi), on hazelnut cultivations, were observed plants showing atypical symptoms characterized by the presence of necrosis along leaf veins and young shoots and atypical canker lesions, with or without hypertrophies, on one/two years old branches. Twenty-five isolates obtained from tissues with symptoms were characterized, four, on sucrose nutrient agar, were levan positive, instead the others twenty-one were negative. All isolates induced a hypersensitivity reaction on tobacco leaves, were pathogens, with different degrees, on hazelnut plantlets and showed a different pathogenic reaction on tomato and bean seedlings. The isolates were assessed for serological slide agglutination test using an antiserum from *Pseudomonas syringae* van Hall: 20 of them showed a positive reaction. Metabolic profile performed by Biolog GEN III microplates showed that all the twenty-five isolates belong to *Pseudomonas* genus. In particular: three of them were *P. caricapapayae*, two *P. fluorescens*, five *P. syringae*, five *P. syringae* pv. *tabaci*, ten *P. syringae* pv. *anthirrhini*. These latter were characterized to grow on lincomycin, vancomycin, rifamycin sv, quinic acid, 1% sodium lactate, tetrazolium violet, tetrazolium blue, niaproof 4, potassium tellurite and pH 6. Molecular characterization of these bacteria as well as further tests to determine their pathogenic role are currently underway.

33. COMPLEXITY OF ALMOND WITCHES'-BROOM DISEASE CYCLE IN LEBANON. F. Quagliano¹, Y. Abou-Jawdah², R. Tedeschi³, M. Jawhari², L. Picciau³, E. Choueiri⁴, H. Sobh², P. Casati¹, M. Kube⁵, C. Siewert⁵, A. Cominetti¹, M. Molino Lova⁶, M. Beyrouthy⁷, A. Alma³, P.A. Bianco¹. ¹Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DISAA), University of Milano, Via Giovanni Celoria 2, I-20133 Milano, Italy. ²Faculty of Agricultural and Food Sciences, American University of Beirut, P.O. Box 11-0236, Riad El Solb, Beirut 1107 2020, Lebanon. ³Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ⁴Department of Plant Protection, Lebanese Agricultural Research Institute, Tal Amara, Lebanon. ⁵Division Phytomedicine, Thaeer-Institute, Humboldt-Universität zu Berlin, Lentzeallee 55/57, Berlin, Germany. ⁶AVSI Foundation, Jounieb-Ghadir, Lebanon. ⁷Faculty of Agricultural and Food Sciences, Holy Spirit University of Kaslik (USEK), Mount Lebanon, Lebanon. E-mail: piero.bianco@unimi.it

'*Candidatus* Phytoplasma phoenicium' ('*Ca. P. phoenicium*') is the etiological agent of almond witches'-broom (AlmWB) in Lebanon and in Iran. The aim of this study was to investigate its epidemiological cycle. During field surveys, conducted in the 4-year period 2010-2013 in AlmWB-infested almond and peach orchards and surroundings, leafhoppers (*Cicadellidae*) and cixiids (*Cixiidae*) were collected by means of yellow sticky traps, Malaise traps and mechanical aspirator. Moreover, leaf samples were collected from crops and wild plants where insects had been captured. *Asymmetrasca decedens* was the prevalent leafhopper, identified mainly on almond and nectarine plants; *Cixius* sp., *Tachycixius* spp., *Hyaletthes* spp., and *Eumecurus* spp. were the prevalent cixiids, identified

mainly on the weeds *Smilax aspera* and *Anthemis* sp. 'Ca. P. phoenicium' was identified in the insects *A. decedens*, *Cixius* sp., *Tachycixius* spp., and *Eumecurus* spp., and in crops and wild plants. Transmission trials demonstrated that *A. decedens* and *Tachycixius* spp. are able to transmit 'Ca. P. phoenicium' to plants. Such results revealed a complexity of AlmWB epidemiological cycle in Lebanon, involving: i) the leafhopper *A. decedens*, possibly responsible for the transmission of 'Ca. P. phoenicium' from almond (peach, nectarine) to almond (peach, nectarine), and ii) cixiids of the genus *Tachycixius*, possibly responsible for the transmission of 'Ca. P. phoenicium' from weeds to almond. For these insects, almond could be considered only end-host for the phytoplasma. Molecular characterization of 'Ca. P. phoenicium' strains identified in the hosts will help to corroborate and confirm this picture, and to understand how 'Ca. P. phoenicium' moves to peach.

34. PHENOTYPE AND GENOTYPE CHARACTERIZATION OF AN UNUSUAL CITRUS TRISTEZA VIRUS FROM SICILY. M. Russo^{1,2}, G. Scuderi^{1,2}, R. Ferraro¹, D. Raspagliesi¹, M. Bar-Joseph³, A. Catara¹, G. Licciardello^{1,2}. ¹Science and Technology Park of Sicily, ZI Blocco Palma I, Via V. Lancia 57, I-95121 Catania, Italy. ²Agrobiotech, ZI Blocco Palma I, Via V. Lancia 57, I-95121 Catania, Italy. ³Gimlaotec, 8 Hazanchanim St., Rehovot 76211 Israel. E-mail: glicciardello@agrobiotech.it

Deep sequencing of small RNAs of the most prevalent isolates of *Citrus tristeza virus* (CTV), SG29 and Bau282, from Sicily, revealed their clustering within the VT and the T30 lineages of the virus. Since the phenomena of super infection exclusion, which in practical terms means the ability to cross protect against severe CTV isolates, was found to operate only between CTV variants belonging to the same strain, these sequencing results, were suggesting that the majority of the naturally spreading mild isolates from Sicily could not be expected to provide cross protection against the locally spreading severe VT isolate. The present study describes the genomic characterization of a new potentially cross-protecting CTV isolate obtained from a local alemow (*Citrus macrophylla*) rootstock plot, which was found to be symptomless, when inoculated on sour orange, and to show only mild symptoms of vein clearing and stem pitting of infected alemow seedlings. Sequencing the small RNAs of the new isolate and phylogenetic analyses showed that it is sharing 99% and 98% sequence identities with the local SG29 and the Spanish T318A isolates, respectively, thus clearly clustering within the western VT subgroup of CTV. Furthermore when aligned against SG29, the new mild isolate was found to differ only by 13 nucleotides (7 of which were non-silent mutations). These results open now some interesting opportunities for the characterization of the molecular basis of the quick decline and seedling yellows reactions that are differentiating between the severe and the mild VT isolates.

35. CHARACTERIZATION AND EVOLUTIONARY SIGNIFICANCE OF A NEW PHYTOPHTHORA SPECIES PRODUCING CONIDIA. B. Scanu¹, S.O. Cacciola², B.T. Linaldeddu¹, A. Pane², A. Franceschini¹, G. Magnano di San Lio³. ¹Department of Agriculture, University of Sassari, Viale Italia 39, I-07100 Sassari, Italy. ²Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 100, I-95123 Catania, Italy. ³Department of Agriculture, Mediterranean University of Reggio Calabria, Via Salita Melissari, Località Feo di Vito, I-89122 Reggio Calabria, Italy. E-mail: bscanu@uniss.it

Since 1999, an unusual *Phytophthora* species has been found associated with root and collar rot, and occasionally stem lesions on young olive trees in Southern Italy. In all cases, this species was

obtained from new commercial plantations as well as seedlings in nursery. Morphologically these isolates were characterized by abundant production of subglobose, caducous conidia (sporocysts) and caducous, papillate sporangia with a short pedicel, in which they resemble *P. palmivora*. Based on the morphological characteristics of sporangia they were originally assigned to *P. palmivora*. In this study, the morphology and growth characteristics of fourteen of these isolates from olive sourced in Sicily and Sardinia were investigated, together with their breeding system and a combined mitochondrial and nuclear multilocus phylogeny. The proportion between sporocysts and true sporangia varied amongst isolates. Oogonia with amphigynous antheridia and plerotic oospores were produced in dual cultures with an A2 mating type strain of *P. palmivora* indicating all isolates were A1 mating type. Phylogenetically, these isolates grouped in a distinct well-supported clade sister to *P. palmivora* indicating they are a separate species. ITS and β -tubulin sequences of the putative new species differed from *P. palmivora* in three and eight nucleotides, respectively. By contrast, *cox1* sequences of both species were identical, suggesting that they share a common ancestor. The detection of this new *Phytophthora* species only from new plantings and nurseries suggests its recent introduction in Italy and emphasizes the need to study the host range, ecology, epidemiology and biogeography of this new pathogen of olive.

36. INFERRING THE INFECTION BIOLOGY OF THE WOOD DECAY FUNGUS PERENNIPORIA FRAXINEA THROUGH AN ANALYSIS OF GENOTYPIC DIVERSITY: A CASE STUDY IN NORTHERN ITALY. F. Sillo¹, L. Giordano¹, D. Astegiano², C. Girometta², E. Savino², A.M. Picco², P. Gonthier¹. ¹Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Earth and Environmental Sciences (DSTA), University of Pavia, Via S. Epifanio 14, I-27100 Pavia, Italy. E-mail: paolo.gonthier@unito.it

Perenniporia fraxinea is a fungal pathogen causing wood decay in roots and bole of a wide variety of broadleaf tree species. Despite the crucial role played by *P. fraxinea* in wood decay processes, little is known on how the fungus spreads from tree to tree. In order to clarify its ways of spread, genetic variation among *P. fraxinea* isolates collected from closely located trees was investigated coupling molecular analysis with vegetative incompatibility assays. Twenty samples were isolated from *P. fraxinea* fruiting bodies collected from different standing trees in the Parco della Vernavola (Pavia, Italy) and in several surrounding areas. All the isolates were genotyped by using Random Amplified Microsatellites (RAMS) and somatic incompatibility tests. Analysis through RAMS allowed to distinguish 19 different haplotypes. Somatic incompatibility tests allowed to detect 16 compatibility groups, thus failing to distinguish all haplotypes identified through molecular analysis. These results of genotyping suggest the presence of high intrapopulation diversity, even when isolates collected from closely located trees were compared. These findings may suggest that the spread through root contacts is unlikely for *P. fraxinea*, which rather may spread through basidiospores. In addition, a significant correlation between spatial distribution and genetic variation was observed for the isolates collected in the Parco della Vernavola. This is one of the first genetic population studies on *P. fraxinea* aimed at uncovering its spreading mechanisms.

37. DEVELOPMENT OF SSR MARKERS AND ASSESSMENT OF POLYMORPHISM BY HIGH RESOLUTION MELTING ANALYSIS IN POPULATIONS OF CHESTNUT NUT ROT AGENT GNOMONIOPSIS CASTANEA. F. Sillo, G. Lione,

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In the complex phytosanitary situation of European chestnut, a relevant role is played by the recently described chestnut nut rot agent *Gnomoniopsis castanea*. In order to elucidate the epidemiology of this pathogen through a population genetic study, five SSR loci were isolated starting from four genomic libraries enriched in SSR sequences prepared and screened through the Microsatellite Amplified Library (MAL) method. To assess their polymorphism, a recently developed analysis called High Resolution Melting Analysis (HRMA) was used on 132 *G. castanea* isolates, collected in Italy, France and Switzerland. This approach allowed to distinguish different alleles based not only on repeat number but also on melting temperature differences among amplicons. Based on HRMA results, isolates were grouped in different clusters, each representing an allelic variant. Clusterization was confirmed by sequencing and alignment of the representative alleles. These results indicate that HRMA is an efficient, rapid and sensitive alternative to traditional electrophoresis-based method for SSR markers. It also allows to detect polymorphisms present in the SSR flanking regions, thus allowing to discriminate a larger number of haplotypes in fungal genetic population studies.

38. CURRENT GEOGRAPHICAL DISTRIBUTION OF *CITRUS TRISTEZA VIRUS* (CTV) IN SICILY AND *CITRUS* REPLANTING CONSEQUENCES. **G. Sorrentino¹, M. Guardo¹, M.C. Strano¹, S. Bella¹, G. Massimino Cocuzza², F. Ferlito¹, S. Di Silvestro¹.** *¹Agricultural Research Council – Research Center for the Citrus Crops and the Mediterranean (CRA-ACM), Corso Savoia 190, I-95024 Acireale (CT), Italy. ²Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 100, I-95123 Catania, Italy. E-mail: guido.sorrentino@entecra.it*

Citrus tristeza virus (CTV) is the current key pathogen of citrus in the Mediterranean regions. CTV is specific of many commercial varieties of *Citrus* on sour orange (*C. aurantium*) rootstocks. CTV is transmitted in a semi-persistent manner by a few aphid species (*Aphis gossypii*, *A. spirecola*), becoming endemic in a *Citrus*-growing area. In Sicily, CTV distribution was located mainly in Catania. A recent monitoring (2013-2014) has highlighted the diffusion of the disease in other Sicilian zones. The analysis were carried out with serological tests and molecular methods like test ELISA and Real-time PCR. The zones in which, the virus was found are Campobello di Mazzara and Ribera with an average of infection of 3%. Samplings were effectuated also in Caltanissetta that resulted free from infection, Ragusa, and Messina provinces. In Ragusa province infect samples were found in Comiso, Acate, Chiamonte Gulfi and Vittoria with infection between 4% and 6%. In Messina province, infections were among 5% and 13% in Barcellona P.G., San Piero Patti, Capo D'Orlando, Terme Vigliatore and Taormina. In Catania outbreak of infection was found between Mascali and Fiumefreddo with an average of 3.5%. An isolated case showed a value of 44%. Different strains of the virus were found, CTV-DS2 severe strain, Seedling Yellows (SY) kind, CTV-DS6 mild strain up to now find only in Tuscany and CTV-DS1 another mild strain. In citrus culture grafted on Citrange after reconversion were found, viroids (CEVD, HpSVd and III group) with infection among 3% and 66%, and *Fusarium* spp.

39. IRESINE VIROID 1 DIAGNOSED ON ORNAMENTAL PLANTS FOR THE FIRST TIME IN ITALY. **R. Sorrentino¹,**

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In 2014, during a survey to verify the presence of pospiviroids in ornamental nurseries in the frame of the project URCOFI (Campania Region, Southern Italy), 60 symptomless ornamental species were tested by tissue printing hybridization with a POSPIprobe designed to detect all pospiviroids. Most samples tested negative, except nine plants of the species *Portulaca* spp., *Celosia cristata* and *C. plumosa*. These findings were further confirmed by Northern-blot hybridization using the POSPIprobe that showed the presence of a circular RNA of about 370 nt and its corresponding linear form. The same RNA preparations were also examined by RT-PCR using primers designed to detect a broad range of pospiviroids. A cDNA of 220 bp was amplified, which after sequencing was shown to be a partial cDNA of *Iresine viroid 1* (IrVd-1, NC003613). The presence of IrVd-1 was confirmed in *Portulaca* spp., *C. plumosa* and *C. cristata* by RT-PCR using separately two IrVd-1-specific primer pairs (Ir1F: 5'-GGAGCTCGTCTCCTTCCTTTC-3' and Ir2R: 5'-TCCTGTTTCTTCCGCCGCG-3'; Ir5R: 5'-CCAG-GTTTTCCCGGGGATC-3' and Ir6F: 5'-GGAGCGAACTCG-GCAAGGAGG-3'). Sequences of 22 full-length cDNA clones showed high sequence identities (97-100%) to each other and to IrVd-1 reference variant (96-97%). Five IrVd-1 new variants were deposited in GenBank (KR020037-KR020041). No significant clustering according to the geographic origin or the host species was observed when a phylogenetic analysis was performed using IrVd-1 sequence variants reported in this and previous studies. To our knowledge, this is the first report of IrVd-1 in Italy and of *C. cristata* as a natural host of this viroid.

40. FIRST OBSERVATION ON A NEW FUNGAL INFECTIOUS DISEASE OF FIG TREES (*FICUS CARICA*) IN THE EAST COAST OF SICILY. **M.C. Strano, S. Di Silvestro, G. Sorrentino, C. Rocuzzo, A. Leonardi, M. Guardo, F. Ferlito.** *Agricultural Research Council – Research Center for the Citrus Crops and the Mediterranean (CRA-ACM), Corso Savoia 190, I-95024 Acireale (CT), Italy. E-mail: mariaconcetta.strano@entecra.it*

The fig (*Ficus carica*) is one of the oldest minor fruit crop well adapted to different environmental areas and soils. Fig trees grow in most of the Mediterranean basin countries, for both fresh and dried fruit consumption. In the last few years a new decline syndrome in fig plants has been observed in the East coast of Sicily (Italy). In the adult trees symptoms consist on a rapid leaf necrosis and twig dieback that affecting the neck area, the trunk and all of the plant organs. The infection leads to a fast death of the tree within few years. No previous reports on these symptoms or about a specific pathogen have been made in Italy. This study was carried out to identify the exact distribution of the disease in East Sicily and the pathogen of the described disease. Samples of leaves and branches, from different affected areas, showing the symptoms, were evaluated and the presence of a reddish coloration along the dissected vascular tissues was observed. Microorganisms isolation from samples was undertaken. Samples were surface sterilized with 2% NaOCl, washed twice with sterile water, cut into small pieces (2 to 3 mm) and placed onto Potato Dextrose Agar (PDA) medium. The isolated samples formed a white-pink mycelium and a dark grey mycelium at 25°C, after 7-10 days. A preliminary identification, based on the macroscopic and microscopic morphological characteristics of the mycelium and fruiting bodies was obtained. *Fusarium* and *Alternaria* species were consistently observed.

GENOMICA E INTERAZIONE PIANTA-PATOGENO

41. GRAPEVINE DOWNY MILDEW RESISTANCE FROM A WILD RELATIVE: STABLE TRANSFORMATION OF *VITIS VINIFERA* WITH AN ATL GENE FROM *VITIS RIPARIA*.

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Grapevine is one of the most important fruit crop in the world, with a high economic impact mainly due to the wine production. The European *Vitis vinifera* produces high quality grapes but is prone to several pathogens, which cause significant losses to viticulture worldwide. Among these, *Plasmopara viticola* (downy mildew) is commonly considered one of the most threatening fungal disease and treatments for its control are responsible for relevant economic costs and environmental concerns. Therefore alternative ways to enhance the resistance of *V. vinifera* towards this pathogen is still a major objective of grapevine research. Our research group found a set of genes supposedly involved in the resistance to downy mildew in *V. riparia*, a naturally resistant wild relative. Interestingly, a subset of uncharacterised genes, sharing a common RING-H2 finger domain, were found to be specifically induced by *P. viticola* in the resistant grape. Further studies revealed that they are members of the ATL gene family, a group of E3-ubiquitin ligases induced in response to common elicitors. One of these genes, the ortholog of *Arabidopsis thaliana* ATL2, was selected and used to stably transform *V. vinifera* cv. Shiraz by *Agrobacterium*-mediated transformation. Transgenic plants were regenerated from transformed grapevine embryogenic calli and molecularly characterised. Greenhouse acclimatised plants were then phenotyped for their resistance against *P. viticola*. Results showed that the resistant phenotype is positively correlated with the level of transgene expression and some transgenic lines revealed a significant resistant phenotype with respect to wild type Shiraz.

42. THIRD-GENERATION SEQUENCING TECHNOLOGY ALLOWED TO OBTAIN THE COMPLETE GENOME OF THE FUNGAL TOMATO PATHOGEN *PYRENOCHAETA LYCOPERSICI*.

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Pyrenochaeta lycopersici is a soil-borne fungus that causes Corky Root Rot (CRR) disease in tomato and other Solanaceous crops, reducing yield by up to 75%. We recently sequenced the genome of one isolate of this species by short reads-based Illumina technology and were able to assemble a 54.9 Mb draft sequence. Here we present the genome sequence of a *P. lycopersici* isolate belonging to a biotype different from that first sequenced obtained using the PacBio SMRT sequencing, the so-called third generation technology. This technology allowed a high-quality genome assembly with contiguous coverage, which is particularly important for large genomes, as in the case of fungi, which contain long repeats. In our case, we were able to assemble a 62 Mb genome sequence, with no gap and an N50 of 1 Mb, compared to the N50 of 73.4 kb of the first isolate. Moreover, the repeat sequence fraction has been identified, corresponding to 35% of the genome, in agreement with other fungal genomes, while for the first *P. lycopersici* isolate we were not able to assemble this part because of the short read length generated by Illumina. The sequenced isolates are representatives of the two biotypes identified in *P. lycopersici*, they differ for growth rate

and morphology in culture. Nevertheless, the genome sequencing showed high level of similarity at this level: the number of total genes is similar, though the genome size is different and the two isolates differ for about 10%. Work is in progress for identifying other differences between the two biotypes.

43. TRANSCRIPTIONAL STUDY OF CERATO-PLATANIN ENCODING GENES IN HOMOKARYOTIC AND HETEROKARYOTIC ISOLATES OF THE FOREST PATHOGEN *HETEROBASIDIUM IRREGULARE*.

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The forest pathogen *Heterobasidium irregulare* (Basidiomycota) was recently sequenced and three cerato-platanin encoding genes were found in its genome (*HiCPs*). Cerato-Platanin Proteins (CPPs) are a family of proteins well known for their resistance-inducing ability when applied on plants. What is still poorly understood is their role in fungal life. These proteins seem to play both a role in the fungal cell wall and a role in the fungus-plant interaction, but most data available to date on CPPs derive from studies performed on Ascomycetes. In the present study, we investigated the expression of *HiCPs* in three homokaryotic isolates and two heterokaryotic isolates of the plant pathogen *H. irregulare*. Homokaryotic and heterokaryotic mycelia not only play different roles in the infectious process but also differ in their biology: clamp connections are formed in the heterokaryotic mycelia. Transcription of *HiCPs* was analysed both at the edge and at the centre of the fungal colony and compared between homokaryon and heterokaryon. Results showed *HiCP1* to be the gene with the highest transcript abundance among *HiCPs*. *HiCP1* did not show any preferential expression in different sections of the fungal colony, while *HiCP2* was significantly more expressed at the colony centre, thus suggesting a link with the production of conidia. The level of expression of *HiCPs* in heterokaryons was generally comparable to that of one or both the parental homokaryons, irrespective of the colony section, thus demonstrating that *HiCPs* are not transcriptionally influenced by the heterokaryotic stage.

44. PHYTOALEXINS DYNAMIC IN GLSD AFFECTED GRAPEVINES (ESCA COMPLEX).

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Grapevine Leaf Stripe Disease (GLSD), a disease of grapevine within the esca complex, is the cause of reduction in the production and longevity of vineyards. The relationship between the development of the leaves symptoms – a typical interveinal necrosis with a yellow or red margin – and the production and accumulation of phytoalexins like stilbenic substances was investigated. We extracted and identified in the leaves of grapevines affected by GLSD: resveratrol, pterostilbenes, ϵ , δ , and ω -viniferin. The leaf content of such detected substances was evaluated during different phenological stages. In most of the cases all phytoalexins showed a higher

level in symptomatic leaves compared to the asymptomatic leaves of infected or apparently healthy vines. Resveratrol showed to be the stilbene with a higher concentration, reaching the highest values in pre-bunch closure, often with high values also of pterostilbenes and/or ϵ -viniferin. In all types of leaves a decrease in the level of the different substances at colour change was recorded, together with a general increase at harvest time or during the maturation. In the whole, the formation of phytoalexins showed to be strictly related with the phenological phases, but also to be different in the symptomatic and the asymptomatic leaves. The development of the leaf symptoms during the growth season in relation with the phytoalexins concentration and the different phenological stages is discussed.

45. DEEP SEQUENCING ANALYSIS OF THE MYCOVIROME IN *BOTRYTIS CINEREA*. R.M. De Miccolis Angelini¹, D. Sardaro¹, A. Spadoni¹, C. Rotolo¹, S. Pollastro¹, A. Minafra², F. Faretra¹. ¹Department of Soil, Plant and Food Sciences (DiSSPA), University of Bari "Aldo Moro", Via Amendola 165/A, I-70126 Bari, Italy. ²National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via Amendola 122/D, I-70126 Bari, Italy. E-mail: francesco.faretra@uniba.it

Deep sequencing on an Illumina HiScan SQ system was used to obtain a comprehensive characterization of the virome and identify novel viruses in *Botrytis cinerea* (*Botryotinia fuckeliana*), the fungal pathogen causing grey mould on several crops. Double stranded RNAs (dsRNAs) was extracted and purified from 12 pooled mycelia from a total of 60 isolates, collected from different host plants and geographical areas, and sequenced to generate about 64 million of high-quality short sequence single reads (50 bp). Contigs obtained from *de novo* assembly of the short reads were analysed by sequence similarity searching into nucleotide collection of viruses to identify putative mycoviral sequences. A great abundance and diversity of viruses infecting *B. cinerea* were found. At least 23 different viruses were detected and identified in one or more the analysed pools of dsRNA samples. They included mycoviruses already identified in *B. cinerea*, such as *Botrytis virus F*, *Botrytis cinerea mitovirus 1*, *Botryotinia fuckeliana partitivirus 1* and *Totivirus 1*, together with viruses reported in other fungal species, such as *Sclerotinia sclerotiorum* (*Mitovirus 3*, *Mitovirus 4*, *Hypovirus 2*, *dsRNA mycovirus-L*) and *Fusarium graminearum* (*Mycovirus 4*), or associated to host plants, such as the grapevine-associated *Narnavirus 1*, *Totivirus 1* and *Partitivirus 2*. The remaining 14 sequences were novel viruses homologous to the coding sequences of partitiviruses, mitoviruses, hypoviruses, totiviruses, endornaviruses, umbraviruses or negative-stranded RNA viruses. Sequence analysis of the identified viruses evidenced a remarkable variability in viral genomic sequences. For selected viruses, the results were validated by PCR and Sanger sequencing.

46. FULL GENOME SEQUENCING OF CITRUS YELLOW VEIN CLEARING VIRUS ISOLATE HU FROM CHINA. R. Ferraro¹, G. Scuderi^{1,2}, M. Russo^{1,2}, Z.N. Deng³, A. Catara¹, G. Licciardello^{1,2}. ¹Science and Technology Park of Sicily, ZI Blocco Palma I, Via V. Lancia 57, I-95121 Catania, Italy. ²Agrobiotech, ZI Blocco Palma I, Via V. Lancia 57, I-95121 Catania, Italy. ³National Center for Citrus Improvement (Changsha), Hunan Agricultural University, Hunan 410128, P.R. China. E-mail: licciardello@agrobiotech.it

A new isolate of *Citrus yellow vein clearing virus* (CYVCV) has been isolated and identified in a *Citrus* sweet orange grafted on trifoliolate orange in Hunan Province (China). Propagation of the plant on sour orange revealed leaf crinkling, associated to yellow

flecks and vein clearing, clearly distinctive of the disease first reported in Pakistan. Alemow, Marsh seedless grapefruit, Troyer citrange, Volkamer lemon, Mexican lime and Etrog citron were symptomless, but back inoculation on sour orange and lemon was successful. Deep sequencing analysis of the virus-induced small RNA fractions allowed the CYVCV isolate HU genome reconstruction. For the *de novo* genome assembly a reference-guided approach has been used. Reads were first aligned against the CYVCV isolate CQ genome, coming from China, by Bowtie2 and 171.075 reads were selected as belonging to CYVCV. The reads were assembled using Velvet in 16 contigs which were ordered by alignment with SAMtool software. The full nucleotide sequence of CYVCV-HU is 7530 nt in length. BLASTn analysis showed 99% of sequence identity with CQ and YN isolates from China, 98% with PK isolate from Pakistan, and 97% with Y1 isolate from Turkey. It showed a 75% sequence identity with *Indian citrus ringspot virus* isolate K1 (ICRSV). Previous phylogenetic analysis have shown that CYVCV and ICRSV belong to the same genus, *Mandarivirus*. Analyses of putative coding regions show that CYVCV-HU genome encodes for six Open Reading Frames (ORFs) and is structurally identical to known CYVCV isolates.

47. IN SILICO AND IN VIVO ANALYSIS OF LYSM GENES OF A NEW ISOLATE OF *TRICHODERMA HARZIANUM*. L. Fiorini¹, R. Baroncelli², M. van Damme³, D.E. Cook³, E.R. Padilla³, S. Sarrocco¹, G. Vannacci¹, B.P.H.J. Thomma³. ¹Department of Agriculture, Food and Environment (DISAAA-a), University of Pisa, Via del Borghetto 80, I-56124 Pisa, Italy. ²Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, Université de Bretagne Occidentale, Brest, France. ³Laboratory of Phytopathology, Wageningen University, 6708 PB Wageningen, The Netherlands. E-mail: lisa.fiorini@wur.nl

The fungus *Trichoderma harzianum* strain T6776 is a recently discovered beneficial isolate that modulates plant hormonal homeostasis and photosynthesis and increases growth and pathogen resistance of tomato plants. As with many endophytic *T. harzianum* isolates, T6776 can colonize the roots of tomato plants, acting as a non-pathogenic plant symbiont. Pathogenic and non-pathogenic fungi use effectors to establish interactions with their hosts, and as such, effectors help establish diverse symbioses with plants. In plant pathogenic fungi, a class of secreted LysM proteins has been shown to function as effector by dampening plant immune responses. The LysM domain is a carbohydrate-binding module that can bind to several types of carbohydrates, including peptidoglycan and chitin. The T6776 genome is predicted to encode eleven LysM domain-containing proteins, of which nine have a secretion signal. Sequence analysis of the predicted proteins shows that three candidates belong to Subgroup C of chitinases, where the LysM domains are associated with a chitinase domain (GH18), while six candidates have only a variable number of LysM domains. These latter six are candidate LysM effectors. Transcripts for four of the nine secreted candidates increase during the early stage of the interaction between T6776 and tomato plants. These results for the first time show the possible involvement of LysM effectors in the interaction between a *Trichoderma* spp. and host plants. We are currently analyzing the importance of these four LysM effector candidates in the T6776-tomato interaction by generating targeted deletion strains.

48. COMPARATIVE STUDIES ON ANTAGONISTIC EFFECTS BETWEEN INVASIVE AND NATIVE FUNGAL PATHOGENS AND ECTOMYCORRHIZAL FUNGI. L. Giordano, G. Leone, E. Zampieri, P. Gonthier. Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino,

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The North American pine-associated root rot agent *Heterobasidium irregulare* was introduced in central Italy during World War II and is currently distributed in forest stands along 103 km of coastline west of Rome. Although many papers dealing with the ecological impacts associated with the invasion have been published, little is known on the consequences that this non-native pathogen may have on host plant symbionts such as ectomycorrhizal (ECM) fungi. Here we tested, through dual culture technique, whether *H. irregulare* and the native Eurasian species *H. annosum* differ in their antagonistic effects against the ECM fungus *Suillus luteus*. Morphological observations and measurements were performed during the experiments and, for each genotype in dual culture, the Inhibition Growth Rate (IGR in %) of average mycelium surface relative to the control was calculated. Results showed that *S. luteus* was considerably and significantly inhibited by native and non-native species of *Heterobasidium* spp. (*S. luteus* IGR>70%; P<0.05) indicating that the pathogens can modulate the growth of the symbiont. Nevertheless, it was not possible to distinguish the effects of the non-native pathogen from that of the native one on the ECM fungus, suggesting that the IGRs observed depend on the genotypes rather than on the species of *Heterobasidium*.

49. INFLUENCE OF DIFFERENT AGRICULTURAL PRACTICES ON STRAWBERRY RHIZOSPHERE BACTERIAL MICROBIOME. D. Lamorte, A.M. Ciarfaglia, P. Lo Cantore, N.S. Iacobellis. School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), University of Basilicata, Viale dell'Ateneo Lucano 10, I-85100 Potenza, Italy. E-mail: nicola.iacobellis@unibas.it

In the context of a project, which aim is to isolate rhizobacteria useful for strawberry crops growth induction and protection, the possible effect of the rhizosphere microbial variability was also considered. In this regard, the influence of different strawberry cultural practices was evaluated on the rhizobacteria communities. Bacteria were isolated from plants in conventional, organic and biodynamic commercial farms located in Basilicata (Southern Italy). Bacterial communities were analyzed using Biolog 96-well Eco-Microplates (AES Laboratoire, France). Data were used to determine metabolic diversity indices, including Average Well Color Development (AWCD), Shannon's substrate diversity index (H'), substrate evenness (E) and substrate richness (S). The carbon substrate of the Biolog plates were clustered into eight main groups of compounds and radial diagrams of nutritional profiles were obtained. All data were subjected to analysis of variance. Significant differences (P<0.05) were observed at the pre-blooming period, but not at the ripening stage. It appeared that bacterial microbiome of strawberry rhizosphere is mostly influenced by the plant roots exudates rather than by cultural practices suggesting that the former may select microbial species conditioning the structure and diversity of soil bacterial communities. However, further studies are needed to confirm the latter hypothesis.

50. CROSS TALK BETWEEN PLANTS AND BIOCONTROL AGENTS CAN REGULATE THE RESPONSE TO BIOTIC AND ABIOTIC STRESSES. N. Lombardi^{1,2}, S.L. Woo^{1,2}, M. Ruocco^{1,2}, D. Turrà³, F. Vinale^{1,2}, R. Marra^{1,2}, A. Pascale¹, S. Lanzuise^{1,2}, G. Manganiello¹, F. Lacatena¹, L. De Vitto¹, L. Boso¹, A. Djella¹, A. Di Pietro³, M. Lorito^{1,2}. ¹Department of Agricultural Sciences, University of Napoli "Federico II", Via Università 100, I-80055 Portici (NA), Italy. ²National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via Università 133,

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Plants live in close association with microbes that inhabit the soil and the presence of this complex plant-associated microbial community (plant microbiome) is crucial for plant health and growth. The latest discoveries clearly indicate that the presence of the microbiome is important for providing an equilibrium in the soil for many biological and nutrient processes, particularly in the root zone (rhizosphere). The root microbiome composition may be modified by specific stresses in the environment, both biotic including pathogen attack, and abiotic such as a lack of water or nutrients, and this makeup may assist the plant to respond and possibly overcome the constraint. In this work tomato plants were grown in a split root system, with one part in water and the other part subjected to stress (a pathogenic *Fusarium* strain or salt). Root exudates were collected from each side, then used to test their attractiveness to a *Trichoderma* biocontrol agent. A microscope assay was used to observe the directionality of the germinating spores of *Trichoderma*. It was observed that the spores germinated more towards the root exudates released by the stressed tomato plants than towards the root exudates released by the healthy tomato plants. This evidence indicates that specific compounds released by the root system of stressed plants are able to attract and stimulate beneficial root-colonizing microbes, such as *Trichoderma*. Future work will focus on the characterization of these signaling molecules that allow the plant to recruit or activate the "helper" microbes.

51. WHOLE-TRANSCRIPTOME RNA-SEQ ANALYSIS OF GRAPEVINE BOTRYTIS CINEREA INTERACTION DURING LATENT INFECTION OF BERRIES ("NOBLE ROT"). A. Lovato, T. Colombo, G.B. Tornielli, A. Polverari. Department of Biotechnology, University of Verona, Strada le Grazie 15, I-37134 Verona, Italy. E-mail: arianna.lovato@univr.it

High throughput sequencing technologies provide a unique opportunity to deeply investigate the molecular mechanisms involved in plant-pathogen interaction. *Botrytis cinerea*, is the agent of grapevine grey mould, but in yet uncharacterized environmental conditions, a latent infection can occur determining favourable metabolic and physico-chemical berry modifications which possibly contribute to the typical aromas of "passito" wines ("noble rot"). The present project aims at the identification of the genes deployed by *B. cinerea* during grape berries colonization in the latent form, in comparison to the saprophytic growth *in vitro*. A total of 100 healthy berries have been artificially inoculated one by one with *B. cinerea* by injecting conidia under berry skin, in controlled conditions, reproducing the *pourri plein* stage of noble rot. Control samples have been inoculated with water. The saprophytic growth was obtained in liquid nutrient medium in laboratory flasks, and the mycelium collected by filtration. The RNA-sequencing experiments on healthy or infected samples in biological triplicate resulted in 27 data sets to be analyzed (Illumina HiSeq1000 paired-end sequencing; 533,779,730 total reads, 150 Gb of data). Results of the statistical and bioinformatic analyses will be presented, providing new insights into both plant and pathogen genes modulated by their interaction in the specific conditions under study.

52. GENE EXPRESSION, PHYTOHORMONE AND PHYTOALEXIN RESPONSES OF TWO RICE GENOTYPES TO FUSARIUM FUJIKUROI. S. Matić^{1,2}, I. Siciliano², G.A. Carneiro^{1,2}, P. Bagnaresi³, C. Biselli³, L. Orrù³, G. Valé⁴, M.L. Gulino^{1,2}, A. Garibaldi², D. Spadaro^{1,2}. ¹Department of Agricultural,

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In the last decades Bakanae disease spread throughout Northern of Italy, becoming a greater problem every year. The causative agent is the fungus *Fusarium fujikuroi*, the most significant seed-borne disease of rice. Twelve rice genotypes were screened against Bakanae disease leading to the identification of Selenio and Dorella as the most resistant and susceptible cultivars, respectively. In order to understand the basis of rice resistance, a RNA-seq comparative transcriptome profiling was conducted on infected seedlings of selected rice genotypes at one and three weeks post germination (wpg). In parallel, a quantitative method to detect simultaneously phytohormones and phytoalexins was developed by using HPLC-MS/MS. More abundant transcriptional changes occurred at 3 wpg in both cultivars, suggesting that this infection stage is more important for studying the rice Bakanae resistance mechanisms. Results showed that genes related to defence responses to pathogens (PR1 genes, germin-like protein genes, glycoside hydrolases, MAP kinases, and WRKY transcriptional factors) were up-regulated in the resistant genotype, on the contrary down-regulation was observed in susceptible genotype. In the resistant genotype Selenio the presence of pathogen induced high production of phytoalexins, mainly sakuranetin, and symptoms of Bakanae were not observed. In the susceptible genotype Dorella, the pathogen induced the production of gibberellin and abscisic acid, inhibited jasmonic acid production, phytoalexins were very low and Bakanae symptoms were observed. In conclusion, results elucidate the rice molecular and cellular processes occurring during *F. fujikuroi* infection and could be useful to develop Bakanae resistant rice germplasm.

53. GENOME SEQUENCE OF *BACILLUS AMYLOLIQUEFACIENS* S499 HIGHLIGHTED PRESENCE OF GENES INVOLVED IN RHIZOSPHERE INTERACTIONS. G. Molinatto¹, G. Puopolo¹, P. Sonogo², M. Moretto², K. Engelen², M. Ongena³, I. Pertot¹. ¹Department of Sustainable Agro-Ecosystems and Biore-sources, Research and Innovation Centre, Edmund Mach Foundation, Via E. Mach 1, I-38010 San Michele all'Adige (TN), Italy. ²Department of Computational Biology, Research and Innovation Centre, Edmund Mach Foundation, Via E. Mach 1, I-38010 San Michele all'Adige (TN), Italy. ³Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés 2, 5030 Gembloux, Belgium. E-mail: giulia.molinatto@fmach.it

Several strains of *Bacillus amyloliquefaciens* (Gram-positive aerobic spore-forming bacteria) can colonize plant roots and stimulate plant growth. Among them, the strain S499 is known to have good biocontrol properties because it produces antibiotics, such as cyclic lipopeptides, and can trigger induced systemic resistance against several plant pathogens. Completion of its genome sequence, made up of a 3927922 bp chromosome and an 8008 bp plasmid, allowed to explore the genetic arsenal of S499. Annotated coding sequences are 3974, which encompasses eight ribosomal RNA operons and 81 tRNA genes. Ten regions of hypothetical horizontal origin, including four large prophagic islands, carry genes for antibiotic resistance, enzymes involved in stress responses, detoxification systems and various transcriptional regulators. We assessed the presence of genes known to be involved in motility and biofilm formation, which are processes that favour root colonization. Moreover, S499

owns genes for the synthesis of auxin, phytase and volatile compounds (e.g. acetoin and 2,3-butanediol) that contribute to plant growth promotion. Regarding biocontrol activity, we identified eight gene clusters for polyketides and non-ribosomally synthesized peptides, including cyclic lipopeptides of surfactin, iturin and fengycin families. The genome mining for secondary metabolites also retrieved genes related to the secretion of amylocyclicin (bacteriocin) and amylolysin (lantibiotic). These two molecules were not detected previously by chemical analyses, underlining the importance of sequencing the genomes to fully characterize the potential metabolic profile of bacterial strains.

54. GENE EXPRESSION OF NECROSIS-INDUCING PHYTOPHTHORA PROTEINS NPPS IN *PHYTOPHTHORA CAPSICI* DURING INFECTION PROCESS ON DIFFERENT HOST PLANT SPECIES. G. Ortu¹, U. Gisi¹, G. Gilardi¹, M.L. Gullino^{1,2}, A. Garibaldi¹. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: giuseppe.ortu@unito.it

Phytophthora capsici is a plant pathogen able to causes root, crown, foliar and fruit rot on a high number of economically important vegetables crops. Plant infection can be initiated by an hypha generated from a germinated oospore or by zoospores. *Phytophthora capsici* present two distinct metabolisms during the colonization of plant tissues, in the first steps his biotrophic approach don't kill the plant cells and the tissues it seems healthy but when infection proceeds *P. capsici* shift, her metabolism from biotrophic to necrotrophic producing necrosis and the cells die. Necrosis-inducing Phytophthora Proteins (NPPs) are a group of secreted toxins found particularly in oomycetes. *Phytophthora capsici* present 18 different NPPs proteins that are responsible to produce necrosis in infected tissues. Through gene expression analysis *in vivo*, we analyzed the modulation of 10 different NPPs during the infection process on three different *P. capsici* isolates on pepper, zucchini and tomato plants. Results obtained showed the activation of different genes encoding for these proteins in function on the host plant. Moreover, different isolates of *P. capsici* are able to activate different genes to infect the same host plant.

55. LONG TERM EFFECT OF *TRICHODERMA* AND SILICON ON METHYL JASMONATE PRODUCTION IN LEAF OF POTTED GAPEVINES INOCULATED WITH *PLASMOPARA VITICOLA*. F. Osti¹, C. Ratti², S. Di Marco¹. ¹National Research Council of Italy, Institute of Biometeorology (IBIMET), Via Gobetti 101, I-40129 Bologna, Italy. ²Department of Agricultural Sciences (DipSA), University of Bologna, Viale G. Fanin 40, I-40127 Bologna, Italy. E-mail: f.osti@bimnet.cnr.it

Downy mildew is a serious disease in organic viticulture as only copper is allowed for its control. Despite this already great difficulty the European Community, and is probably soon going to decrease the allowed quantity to 3 kg ha⁻¹ year⁻¹. Thus, an improvement in copper efficiency, for example enhancing plant response to the disease, is urgently needed. Two days after infection by *Plasmopara viticola*, resistant produce a peak of methyl-jasmonate higher than the sensitive grapevines. Jasmonate production could be triggered in plants applying *Trichoderma* or silicon. In this frame *Trichoderma*, silicon or their combination were tested on potted vines to evaluate the ability of treatments to induce, far from the time of application, plant response such as production of methyl-jasmonate and

reduction of the leaf symptoms. The study was carried out analyzing the expression of 13-lipoxygenases LOXA and LOXO, the 9-LOX and the coronatine insensitive protein 1 (COI1) as genes involved in the synthesis of the jasmonic acid and in the plant response to this hormone. The LOXO activation precede or is contemporary to methyl-jasmonate peak and is probably involved in its synthesis, while the activation of LOXA follows the peak, as a responsive gene. *Trichoderma* activate 9-LOX response while silicon activates the COI1. Since the two compounds activate different pathways, they may integrate each to other. The higher effect observed for the combination against artificial infection seems to confirm this hypothesis.

56. EXPRESSION OF A WHEAT XYLANASE INHIBITOR AND OF A *FUSARIUM GRAMINEARUM* XYLANASE IN PLANTS INCREASE RESISTANCE TO PATHOGENS.

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During host plant infection, pathogens produce many Cell Wall Degrading Enzymes (CWDE) in order to colonize the host tissue and also to obtain nutrients. Xylanases are hydrolytic enzymes with a dual role: they catalyze the hydrolysis of xylan, the largest structural polysaccharide of plant cell wall, and some of them can cause necrosis in the host tissue. Since a wheat xylanase inhibitor (TAXI-I) has been shown to inhibit a *Botrytis cinerea* xylanase, a well known virulence factor of the fungus, we transiently expressed TAXI-I and TAXI-III inhibitors, which has similar inhibitory capability, in tobacco leaves by agroinfiltration. Total leaves protein extracts expressing TAXIs inhibited fungal xylanase activity and TAXIs agroinfiltrated tobacco plants were less susceptible towards *B. cinerea* by about 20-25%. Recently, we have identified a *Fusarium graminearum* xylanase (FGSG_03624) shown to cause H₂O₂ accumulation in wheat tissues and induction of defense genes in *Arabidopsis thaliana*; we therefore tested its ability to increase resistance against bacterial and fungal pathogens. Exogenous treatment with the xylanase showed a slight reduction of symptoms caused by *Pseudomonas syringae* pv. *maculicola*, while the treatment was ineffective against *B. cinerea*. To further verify this result we also transiently expressed the xylanase in tobacco plants through agroinfiltration; preliminary infection experiments seem to confirm previous results. Finally, we also produced by floral dip transformation *Arabidopsis* transgenic plants constitutively expressing TAXI-I, TAXI-III and the xylanase FGSG_03624. Infection experiments of these plants with *B. cinerea* and *P. syringae* pv. *maculicola* are in progress.

57. SEO1 AND SEO2 GENES ARE INVOLVED IN THE RESPONSE OF *ARABIDOPSIS* TO PHYTOPLASMA INFECTION. **L. Pagliari¹, S.V. Buxa², M. Martini¹, R. Musetti¹.** ¹Department of Agricultural and Environmental Sciences (DISA), University of Udine, Via delle Scienze 206, I-33100 Udine, Italy. ²Department of Phytopathology and Applied Zoology, Justus Liebig University, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany. E-mail: laura.pagliari@uniud.it

Phytoplasmas are plant-pathogenic prokaryotes restricted to the sieve elements and transmitted from plant to plant by phloem-feeding insects. Curative strategies are not available so far, therefore plant natural defence mechanisms, mainly those occurring in the sieve elements, should be studied more in depth. One of the first

responses of the sieve elements undergoing pathogen attack (or abiotic stimuli) is the aggregation of the Phloem protein (P-protein) filaments. P-proteins are able to plug affected sieve elements likely to avoid pathogen diffusion, moreover they seem to have a role also in the systemic defence signalling. Nevertheless, the role of the P-proteins during plant-pathogen interactions is still matter of debate. Among the genes encoding for P-proteins, AtSEOR1 and AtSEOR2 are reported to be essential for filament formation in *Arabidopsis thaliana*. To evaluate the effective role of P-proteins in the limitation of phytoplasma spread through the sieve elements, wild type and mutant (knockout for AtSEOR1 or AtSEOR2 or both) *A. thaliana* plants have been used and infected with '*Candidatus* Phytoplasma asteris'. Lacking of AtSEOR1 and AtSEOR2 in healthy plants caused the absence of P-protein filaments: this did not affect plant phenotype, tissue morphology nor sieve-element ultrastructure. Both wild type and knockout infected plants developed disease symptoms, but in knockout plants they were more severe. Surprisingly, infected knockout plants evidenced filaments in the sieve elements, morphologically identical to the ones observed in wild type plants. On the other hand, filaments should not be as effective as the ones in wild type plants. Experiments carried out using CFDA and confocal microscope demonstrated that sieve-element mass flow was significantly reduced only in infected wild type plants.

58. A MICROBIAL CONSORTIUM IN THE RHIZOSPHERE OF TOMATO PLANTS SUPPRESSES DISEASE INCIDENCE CAUSED BY *FUSARIUM OXYSPORUM* f. sp. *LYCOPERSICI*.

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Antagonist microorganisms that suppress plant diseases have co-evolved with plants and are primary factors in determining plant health. Biological control of *Fusarium* wilt using soil-borne microbial antagonists has been reported, but the relatively narrow spectrum of single biocontrol agents activity against plant pathogens is a major constraint for their commercial exploitation in field conditions. The deployment of strain mixtures containing microbial antagonists and plant growth promoting rhizobacteria increases the efficacy of biocontrol, resulting in synergistic effects. The goal of this study was to select and optimize a microbial consortium against *F. oxysporum* f. sp. *lycopersici*. Bacterial strain selected from the chickpea rhizosphere and organic amendments were tested for bioactivity against this pathogen. The best results were obtained with a mixture of isolates of *Serratia marcescens*, *Pseudomonas fluorescens*, *Rahnella aquatilis* and *Bacillus amyloliquefaciens*. In biocontrol assays carried out on tomato plants the selected microbial consortium efficiently controlled *Fusarium* wilt, displaying a synergistic effect with respect to the activity of each bacterial strain applied individually. The possible mechanisms of action involved in the biocontrol activity of our bacterial consortium are currently being investigated.

59. IDENTIFICATION OF *LYCHNIS RINGSPOT VIRUS* IN *MENTHA PIPERITA* IN ITALY BY NEXT GENERATION SEQUENCING (NGS) TECHNOLOGY.

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A sap-transmissible virus was detected since early 2013 in peppermint (*Mentha piperita*) plants growing in commercial nursery of Northwest Italy (Liguria Region). Susceptible host species were found in the family *Chenopodiaceae* (*Chenopodium amaranticolor* and *C. quinoa*), *Fabaceae* (*Pisum sativum* and *Vigna unguiculata*), *Lamiaceae* (*Ocimum basilicum* and *Salvia splendens*) and *Solanaceae* (*Nicotiana benthamiana*, *N. clelandii*, *N. tabacum*, *Petunia hybrida*, *Solanum lycopersicum* and *Solanum melongena*), whereas species belonging to *Asteraceae* (*Zinnia elegans*), *Brassicaceae* (*Brassica rapa*) and also to *Fabaceae* (*Phaseolus vulgaris* and *Vicia faba*) did not show any symptoms. Electron microscope observations of crude-saps from peppermint leaves with bright yellow mosaic symptoms, revealed the presence of rod shaped virus particles of different lengths. In order to identify the virus, dsRNAs were extracted from inoculated *N. tabacum* and submitted to deep sequencing. Five scaffolds were reconstructed: two mapped in the RNA beta of *Lycobis ringspot virus* (LRSV, Acc. Z46351), with percentages of identity of 95.4 and 96.4% (of 410 and 1036 nts, respectively), two in the RNA alpha of LRSV (Acc. Z46630), with percentages of identity of 96.9 and 92.4% (of 357 and 392 nts, respectively), while last scaffold mapped in the RNA alpha of *Barley stripe mosaic virus* (67% identity with BSMV, Acc. KJ433977), because the corresponding sequence is not available for LRSV. LRSV was previously described in 1972 in *M. longiflora* in Hungary. By NGS technology we were able to identify the same *Hordeivirus* specie in *M. piperita*; however some differences in host symptomatology and some genomic sequence variations suggest that it should represent a new variant of LRSV.

60. A CHIMERIC CONSTRUCT OF PVY INDICATES THE HC-PRO PROTEIN OF PVY^{NTN} ISOLATE MK AS RESPONSIBLE FOR VEIN CLEARING IN TOBACCO. G. Parrella¹, C. Vovlas², B. Moury³. ¹National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via Università 133, I-80055 Portici (NA), Italy. ²Department of Plant Protection and Applied Microbiology, University of Bari "Aldo Moro", Via Amendola 165/A, I-70126 Bari, Italy. ³INRA, UR407 Pathologie Végétale, Domaine Saint Maurice, CS60094, F-84143, Montfavet Cedex, France. E-mail: giuseppe.parrella@ipspp.cnr.it

Based on symptoms induced in tobacco, the PVY isolates can be classified into two major strain groups: those inducing vein necrosis (VN), i.e. PVY^N, PVY^{NTN}, PVY^{N:O} and PVY^{N-Wi}, and those inducing vein clearing and mottling, i.e. PVY^O and PVY^C. The isolate MK is a PVY isolate found in 2003 in Apulia (Southern Italy), naturally infecting *Datura metel*. Near Whole Genome Sequencing (WGS), revealed that MK belongs to the NTN strain group. However, in contrast to almost all PVY^{NTN} isolates, MK induces vein clearing and mild mottling in tobacco. Previously, two amino acids of the PVY^N HC-Pro protein were shown to be major determinants for VN induction using reverse genetics on the infectious N605 genetic system: a lysine at position 400 and a glutamic acid at position 419. Surprisingly, despite the MK isolate has the same amino acids, it does not induce VN. In order to demonstrate that the MK HC-Pro is involved in the atypical symptomatology observed, we have created and biologically tested genetically modified PVY genomes corresponding to *in vitro* HC-Pro recombinants between the necrotic PVY isolate N605 and the MK isolate. Results confirmed the involvement of the HC-Pro in the symptomatology observed in tobacco and suggested that additional amino acid substitutions, found only in the MK HC-Pro protein, could be responsible for the

unexpected symptomatology. Taken together, these data confirm that the VN genetic determinant of PVY in tobacco, as already demonstrated for the L26 isolate, is complex and includes other element(s), in addition to the K-400 and E-419 residues of HC-Pro.

61. COMPARATIVE IMPACT ON ECOPHYSIOLOGICAL PROPERTIES, VOLATILE TERPENOIDS AND TRANSCRIPTS OF ALIEN AND NATIVE FUNGAL TREE PATHOGENS ON PINUS PINEA. A.L. Pepori¹, M. Michelozzi², L. Calamai³, G. Cencetti², A. Santini¹, P. Bonello⁴, N. Luchi¹. ¹National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via Madonna del Piano 10, I-50019 Sesto Fiorentino (FI), Italy. ²National Research Council of Italy, Institute of Biosciences and Bioresources (IBBR), Via Madonna del Piano 10, I-50019 Sesto Fiorentino (FI), Italy. ³Department of Agrifood Production and Environmental Sciences (DISPAA), University of Firenze, Piazzale delle Cascine 28, I-50144 Firenze, Italy. ⁴Department of Plant Pathology, The Ohio State University, 201 Kottman Hall, 2021 Coffey Rd, Columbus, OH 43210, USA. E-mail: alessia.pepori@ipspp.cnr.it

Heterobasidion irregulare was introduced accidentally into Italy and now is slowly spreading in several *Pinus pinea* (Italian stone pine) stands along the Tyrrhenian coast, sharing the same ecosystem with the native pathogen *H. annosum*. Aim of this study was to investigate the impact of *H. irregulare* in comparison with the native pathogen on ecophysiology, volatile terpenoids and transcripts on Italian stone pine. Seedlings of *P. pinea* were inoculated with both fungal pathogens through stem wounds. Differences in host susceptibility were assayed by measuring the lesions length at different sampling time from two locations. Transcripts analysis on bark samples was carried out by using six different genes involved in pathogenesis by using RT-qPCR. The attack by the native and alien *Heterobasidion* species strongly affected photosynthesis and stomatal conductance. Biochemical host responses were studied by the analyses of constitutive and induced terpenoids following the invasion of both *Heterobasidion* species. In both site bark collected, the expression of all six gene tested did not reveal significant differences between two pathogens. In the distal portion, we found that almost all genes were more up-regulated in comparison with the transcripts levels in inoculation site. Fungal infections strongly affected the production of volatile terpenoids: differences in the relative proportions of constitutive and induced terpenes with *Heterobasidion* spp. were observed. In general, no significant differences were observed in the response of pine trees to the infection of the invasive *H. irregulare* and the native *H. annosum*.

62. CERATO PLATANINS (CP) OF FUSARIUM GRAMINEARUM INDUCE DEFENSE RESPONSES IN PLANT AND ARE NOT ESSENTIAL FOR FUNGAL VIRULENCE. A. Quarantin, F. Favaron, L. Sella. Department of Land, Environment, Agriculture and Forestry (TESAF), University of Padova, Viale dell'Università 16, I-35020 Legnaro (PD). E-mail: alessandra.quarantin@gmail.com

Cerato Platanins (CP) belong to a family of small secreted fungal proteins with phytotoxic activity which seem to induce defense responses and necrosis in plants and contribute to *Botrytis cinerea* and *Magnaporthe grisea* virulence. In the genome of *Fusarium graminearum*, a necrotrophic fungal pathogen which causes Fusarium Head Blight (FHB) disease of wheat, barley and other cereal grains, there are two genes (*fsgs_10212* and *fsgs_11205*) putatively encoding for CP-like proteins that we cloned and heterologously expressed in *Pichia pastoris*. The recombinant proteins, native and boiled, resulted able to reduce the viscosity of carboxymethyl

cellulose, in particular the FGSG_11205. Treatments of *Arabidopsis thaliana* leaves with the two *F. graminearum* CPs induced necrotic symptoms, accumulation of H₂O₂ and expression of defense genes, specifically PR1, marker of salicylic acid signaling, and ERF1b, a transcriptional regulator of some ethylene-responsive genes. Being these CPs able to induce defense responses, we tested their effectiveness in increasing the resistance of *A. thaliana* to the fungal pathogen *B. cinerea*; treatments with both CPs determined a reduction of lesion size of about 30-40%. The expression of the two *cp* genes was analyzed by qPCR in the early stages of wheat spike infection and during *in vitro* growth and only the *fgsg_10212* gene resulted strongly transcribed. To verify the contribution of *F. graminearum* CPs to fungal virulence, single and double gene knock-out mutants were produced and used to infect host plants such as wheat and soybean but their virulence resulted comparable to that of the wild-type strain.

63. TRANSCRIPTOMIC AND BIOCHEMICAL APPROACHES TO UNRAVEL THE INTERACTION BETWEEN *ACTINIDIA* AND *PSEUDOMONAS SYRINGAE* pv. *ACTINIDIAE*. A. Regaiolo, A. Lovato, T. Colombo, A. Allegrini, E. Vandelle, A. Polverari. Department of Biotechnology, University of Verona, Strada le Grazie 15, I-37134 Verona, Italy. E-mail: alice.regaiolo@univr.it

Pseudomonas syringae pv. *actinidiae* (Psa) is a Gram-negative plant pathogenic bacterium that affects the foliage and the trunk of *Actinidia* plants. Currently, this pathogen has a worldwide distribution and it is the major cause of economic losses in kiwifruit production for different countries such as New Zealand, Korea, Japan and Italy, where kiwi is one of the most cultivated fruit crops. In the frame of a recent Regional project (Regione Veneto), we started researches aimed at the evaluation of the transcriptomic profiles of *Actinidia* and *Psa* during the plant infection. *In vitro* plants of *Actinidia chinensis* infected by *Pseudomonas syringae* pv. *actinidiae* and collected after 4, 24 and 48 hours will be analyzed by RNA-sequencing (Illumina HiSeq1000). Moreover, growth of *Pseudomonas syringae* pv. *actinidiae* in different media (KB, M9 minimal medium and hrp-inducing medium) supplemented with plant extracts were measured, in order to set the conditions for the analysis of differentially expressed bacterial genes in different media and growing conditions, by a microarray experiment. Finally, investigations are in progress to identify plant molecules that may be recognized by bacterial LuxR solos and trigger pathogen virulence. This study can help to understand the mechanisms involved in the interaction of *Pseudomonas syringae* pv. *actinidiae* with a susceptible host plant, and to develop new control strategies against the pathogen.

64. POLYKETIDE SYNTHASES IN *DIAPORTHE HELIANTHI*. M. Ruocco¹, R. Baroncelli², M. Vergara², G. Vannacci², F. Scala^{1,3}. ¹National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via Università 133, I-80055 Portici (NA), Italy. ²Department of Agriculture, Food and Environment (DISAAA-a), University of Pisa, Via del Borghetto 80, I-56124 Pisa, Italy. ³Department of Agricultural Sciences, University of Napoli "Federico II", Via Università 100, I-80055 Portici (NA), Italy. E-mail: michelina.ruocco@ipsp.cnr.it

Diaporthe helianthi (anamorph *Phomopsis helianthi*) is a phytopathogenic fungus which causes stem canker and leaf shedding in sunflower (*Helianthus annuus*). The mechanisms of pathogenicity and symptom induction by this fungus are poorly understood. The early phases of *D. helianthi* pathogenesis on sunflower are characterized by the production of polyketidic phytotoxins that probably

open the way to host colonisation. In order to investigate about the role of polyketide synthases in the genetic supply of *D. helianthi*, a draft genome of the highly virulent isolate 7/96 was obtained. A remarkable number (at least 40) of genes putatively coding for PKSs were found. The phylogenetic analysis revealed that most PKS genes are highly reducing PKSs, whereas only eight PKSs lack reducing domains and clustered with non-reducing PKSs. The newly genome sequenced and the data provided represent new resources useful for further research into the genetics of this pathogen.

65. KNOCKOUT OF A *TRICHODERMA VIRENS* POLY-GALACTURONASE GENE AFFECTS THE INTERACTION WITH TOMATO. S. Sarrocco¹, F. Matarese¹, R. Baroncelli¹, V. Seidl-Seiboth², G. Vannacci¹, M. Vergara^{1,3}. ¹Department of Agriculture, Food and Environment (DISAAA-a), University of Pisa, Via del Borghetto 80, I-56124 Pisa, Italy. ²Molecular Biotechnology, Institute of Chemical Engineering TU, Getreidemarkt 9/166, A-1060 Wien, Austria. ³Scuola Normale Superiore of Pisa, Piazza dei Cavalieri 7, I-56126 Pisa, Italy. E-mail: mariarosaria.vergara@sns.it

The antagonistic attitude of *Trichoderma* spp. against plant pathogens and their ability to promote plant growth and to induce plant resistance has been extensively shown. The direct interaction *Trichoderma*-plant is as a precondition for reciprocal advantages. In this beneficial cross-talking a relevant role is taken by Polygalacturonases (PGs) produced by *Trichoderma* spp. which assist root penetration and play a pre-eliciting role in Induced Systemic Resistance (ISR). Two PG genes were previously identified and characterised in an antagonistic *T. virens* isolate (I10): *Tvpg1* was inducible and *Tvpg2* was constitutively expressed in early stages of fungal colonisation of tomato roots. A tomato PGIP (*Lepgip1*) was also induced at the same time as *Tvpg1* expression suggesting a functional correlation between the two gene products. The expression analysis was then performed using the same system with a *Tvpg2* deleted mutant ($\Delta pg2$) and no inducible expression of *Tvpg1* or *Lepgip1* was detected. This implies a *Tvpg2* role in triggering the induction of *Tvpg1* in *T. virens* and indirectly of *Lepgip1* in plant. The ISR in tomato plants pre-treated by *T. virens* wt or $\Delta pg2$ and then infected by *Botrytis cinerea* was then *in vivo* checked. A radically reduced resistance was shown by the deleted mutant by comparison with the wt, confirming results from the expression analysis. Metabolic patterns shown by the wt compared to the $\Delta pg2$ mutant, both grown on 95 Carbon sources (Biolog Phenotype MicroArray System), are under investigation.

66. COMPLETE NUCLEOTIDE SEQUENCE OF A T36-LIKE *CITRUS TRISTEZA VIRUS* DETECTED IN SICILY. G. Scuderi^{1,2}, R. Ferraro¹, M. Russo^{1,2}, D. Raspagliesi¹, A. Lombardo¹, A. Catara¹, G. Licciardello^{1,2}. ¹Science and Technology Park of Sicily, ZI Blocco Palma I, Via V. Lancia 57, I-95121 Catania, Italy. ²Agrobiotech, ZI Blocco Palma I, Via V. Lancia 57, I-95121 Catania, Italy. E-mail: glicciardello@agrobiotech.it

The sequence of the entire genome of Mac25 *Citrus tristeza virus* (CTV) isolate, a Sicilian T36-like isolate, was completed. The virus was isolated from a symptomatic alemow seedling found in a highly infected area in Catania province. Biological analysis highlighted that six months alemow seedlings showed vein clearing and a degree of stem pitting, whereas sour orange displayed a variable shortening of internodes and zinc deficiency leaf discoloration. Real-time PCR revealed that Mac25 reacts positively with CPi-T36 and T36-like probes, specifically designed to discriminate T36 genotype. Moreover, CE-SSCP profile analysis on a variable region targeting Open Reading Frames (ORFs) 1b and 2, evidenced

a pattern different from those already described in Sicily. The full genome of Mac25 (KR263170) was obtained by sequencing small RNAs using Illumina GAIIX platforms and assembly of overlapping sequences with reference genomes. The complete Mac25 genome is 19,293 bp in length and encodes 12 ORFs. The isolate is structurally identical to known CTV isolates, including 107 nt in the 5'-UTR and 272 nt in the 3'-UTR. Phylogenetic analysis based on 44 full CTV genomes showed that Mac25 clustered with the T36-lineage in which FS701-T36, FS703-T36, FS674, FS577, T361C, T36QD, T36-FS2-2, (Florida), Severe (Mexico), and Qaha (Egypt) isolates segregate and has the highest homology identity with T36-FS2-2 (99%). To the best of our knowledge, this is the first report of a T36 CTV infection in Italy and the second from the Mediterranean area (after Qaha isolate in Egypt).

67. THE ACTIVITY OF THE *BOTRYTIS CINEREA* ENDO-POLYGALACTURONASE PG1 IS DETECTED IN BERRY SKINS AND IS REQUIRED FOR FULL VIRULENCE DURING GRAPE INFECTION. L. Sella¹, S. Odorizzi¹, S. Lengyel¹, A. Quarantin¹, C. Castiglioni¹, J.A.L. van Kan², F. Favaron¹. ¹Department of Land, Environment, Agriculture and Forestry (TESAF), University of Padova, Viale dell'Università 16, I-35020 Legnaro (PD). ²Laboratory of Phytopathology, Wageningen University, PO Box 8025, 6700EE Wageningen, The Netherlands. E-mail: luca.sella@unipd.it

The necrotrophic fungal pathogen *Botrytis cinerea* is the causal agent of grey mould or Botrytis bunch rot in grapes. During the infection process, this fungus secretes several cell-wall degrading enzymes, in particular endo-polygalacturonases (PGs) which are involved in the depolymerization of pectin, the main constituent of primary cell wall and middle lamella. The genome of *B. cinerea* contains six endo-PG encoding genes. Aim of the present work was to characterize the role of these enzymes during infection of grape berries. First, we studied the expression of the corresponding genes on berries of cv. Pinot blanc and Pinot noir. Among the genes analyzed by qPCR, only *Bcpg2* was not expressed, while *Bcpg1*, encoding a basic isoform, showed the highest transcript levels at all time points analyzed and in both cvs. We also analyzed the PG activity produced by *B. cinerea* during grape berries infection by loading on a gel activity assay grinded berry skins: the BcPG1 isoform was detected but only as a weak band compared to other PGs produced by the fungus. However, since the BcPG1 has been previously demonstrated to be an important virulence factor in several host tissues although its role has never been investigated on grape berries, we performed infection experiments with a Δ BcPG1 knock-out mutant. A 20% reduction of symptoms caused by the Δ BcPG1 mutant was observed on both Pinot cv. and also on cv. Italia table grape, thus indicating that BcPG1 is required for full virulence on grape berries.

68. IN VITRO EFFECTS OF INVASIVE AND NATIVE FUNGAL PATHOGENS ON GENE EXPRESSION OF AN ECTOMYCORRHIZAL FUNGUS. E. Zampieri¹, F. Sillo¹, L. Giordano¹, J.V. Colpaert², R. Balestrini³, P. Gonthier¹. ¹Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Hasselt University, Centre for Environmental Sciences (CMK), Agoralaan gebouw D, 3590 Diepenbeek, Belgium. ³National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Viale P.A. Mattioli 25, I-10125 Torino, Italy. E-mail: paolo.gonthier@unito.it

Non-native invasive organisms stand among the main elements of global change and are playing a role in the biodiversity loss, ecosystem degradation, and impairment of ecosystem services. The

effects of biological invasions have been extensively investigated in terms of environmental, economic, and human health impacts. However, little is known on the consequences that non-native plant pathogens may cause on host plant symbionts, such as ectomycorrhizal (ECM) fungi. In this work, we tested the hypothesis that non-native invasive fungal pathogen may have greater effects on ectomycorrhizal fungi than native fungal pathogens using *Heterobasidion irregulare*/*H. annosum* and the ECM fungus *Suillus luteus* as a model system. Secondly, the effects of the symbiont on the pathogens were also investigated in dual culture by expression analyses on putative cell wall related genes. The up- and down-regulated genes both in the symbiont and in the pathogens confirmed the rewiring of the transcriptional machinery related to cell wall hydrolytic enzymes and hydrophobins, putatively involved in the fungus-fungus interaction. Despite it was not possible to distinguish the effects of the invasive pathogen from that of the native one on the ECM fungus from a wide gene expression perspective, a single *S. luteus* gene encoding a putative chitinase was found to differentially perceive the two pathogens, thus showing a diverse expression trend.

MALATTIE POST-RACCOLTA E MICOTOSINE

69. CONTROL OF POST-HARVEST PATHOGENS USING PLANT ESSENTIAL OILS AND THEIR POTENTIAL IN INDUCING RESISTANCE IN APPLE FRUIT. H. Banani^{1,2}, D. Spadaro^{1,2}, M.L. Gullino^{1,2}, A. Garibaldi¹. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGRO-INNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it

Considerable losses are caused by post-harvest diseases during fruit transportation and storage. To control post-harvest diseases of fruits, few synthetic fungicides are admitted. Moreover, the public demands to reduce pesticide use on food and to use safer and eco-friendly treatments, have generated interest in the development of alternative non-chemical methods to reduce post-harvest losses. In recent years, there has been increasing interest in the use of natural compounds including essential oils treatments to control post-harvest pathogens and different mechanisms of action have been proposed, based on the interaction between oil components, target organism, and host fruit. In the present work we evaluated the efficacy of essential oils application at different concentrations to control post-harvest pathogens on artificially inoculated fruits, and we investigated at molecular level the effects of the post-harvest treatments on fruit, by characterizing the expression of pathogenesis-related genes on fruit peel tissue. The results obtained showed that thyme (*Thymus vulgaris*) and savory (*Satureja montana*) essential oils were effective on controlling the pathogens in shelf-life conditions and at a low concentration. Moreover, thyme oil applied at a concentration of 1% on apples cv. Golden Delicious led to an increase in the transcription level of pathogenesis-related protein 8 (*Pr8*) known to play a significant role in the defense mechanism of the fruits against the pathogens. These findings raise the potential of these natural products for the control of post-harvest rots, not only during storage but also in shelf life conditions.

70. POST-HARVEST APPLICATION OF A NOVEL CHITINASE *MFCHI* CLONED FROM *METSCHNIKOWIA FRUCTICOLA* AP47 AGAINST BROWN ROT OF PEACHES. H. Banani, D. Spadaro, M.L. Gullino, A. Garibaldi. Centre of

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Brown rot caused mainly by *Monilinia laxa* and *M. fructicola* is considered the main post-harvest disease of stone fruits, and in the European Union, no chemical fungicides are allowed for post-harvest treatment of stone fruits. *Metschnikowia fructicola* strain AP47 was isolated from the carposphere of apple fruits and showed a high efficacy in controlling *Monilinia* spp., however its mechanism against post-harvest pathogens is still unclear. AP47 was able to produce chitinase enzymes in the presence of *Monilinia* spp. cell wall. A novel chitinase gene *MfChi* (GenBank accession number HQ113461) was amplified from the genomic DNA of AP47. *Mfchi* sequence analysis shows lack of introns and an open reading frame of 1,098 bp encoding a 365 amino acid protein with a calculated molecular weight of 40.9 kDa and a predicted pI of 5.27 was identified. The chitinase gene *MfChi* was highly induced in the yeast AP47 in response to *Monilinia* spp. cell wall, suggesting its possible involvement in the antagonistic activity of the yeast. *MfChi* gene was overexpressed in the heterologous expression system of *Pichia pastoris* KM71, then the antifungal activity of the recombinant chitinase was investigated against *M. fructicola* and *M. laxa* *in vitro* and on peaches. *MfChi* significantly controlled *Monilinia* spp. spore germination and germ tube length *in vitro* and successfully reduced brown rot severity on peaches especially during the first days of the treatment. This work shows that the chitinase *MfChi* could be developed as a post-harvest treatment with antimicrobial activity for fruit undergoing a short shelf life.

71. REGULATION OF FATTY ACIDS METABOLISM IN THE FUNGAL MAIZE PATHOGEN *FUSARIUM VERTICILLIOIDES*. M. Beccaccioli, A. Grotoli, D. Magali, C. Fanelli, M. Reverberi, M. Ludovici, V. Scala. *Department of Environmental Biology, Sapienza University of Roma, Piazzale Aldo Moro 5, I-00185 Roma, Italy. E-mail: marzia.beccaccioli@uniroma1.it*

Fusarium verticillioides is a fungal maize pathogen producer of fumonisins, harmful to human health. As shown in previous works, mycotoxin production is related to fatty acids processing and lipid mediator synthesis, such as oxylipins. Fungal lipids play a crucial role in determining the outcome of the interaction between the pathogen and its host. The aim of this work is to study the expression of some genes encoding for enzymes involved in the production of these lipid compounds. We focused our attention on the expression of Linoleate Diol Synthase 1 (*Lds1*) and the transcription factor *Crz1*. *Crz1* consensus motif is present in the promoter of several oxylipin-related genes. *Crz1* is a calcineurin-responsive zinc-finger transcription factor. We also analysed the expression of genes involved in fatty acid desaturation, i.e. those involved in unsaturated fatty acids production. Alternatively, the fatty acids desaturase *Ole1* and the acyltransferase *Sct1* are involved in fatty acids desaturation. Gene expression was analysed in *F. verticillioides* (wild type) and in the $\Delta Fvlds1$ strains (deleted for *lds1*) grown in different conditions. Gene expression profile was compared with the lipidomic one. It emerges a close link between fatty acids desaturation and oxylipin synthesis.

72. PRE-HARVEST ALTERNATIVE TREATMENTS TO CONTROL POST-HARVEST ROTS ON Highbush BLUEBERRY cv. DUKE. M.P. Bustos-López¹, D. Spadaro^{1,2}, A. Garibaldi¹, M.L. Gullino^{1,2}. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University

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Worldwide blueberry production increased over the last years becoming the second most important soft fruit species, after strawberry. Among the commercial cultivars, “Duke” is the most common and widely cultivated in Italy because of its commercial qualities. Post-harvest rots caused by different fungal pathogens can compromise quality and economic value of blueberries. Alternative disease control methods, such as natural compounds and biocontrol agents are attracting increasing interest. Different pre-harvest treatments, including two potassium silicates and two antagonistic microorganisms, were evaluated for their efficacy in controlling post-harvest rots on blueberry in 2012 and 2013. Disease severity, firmness, soluble solids content, titratable acidity and ripening index were evaluated at harvest and the end of the storage. Post-harvest rots on blueberries were caused mainly by *Botrytis cinerea*, *Penicillium expansum* and *Alternaria* spp. Results of experimentation showed a positive effect of treatments with potassium silicates against post-harvest diseases on blueberries respect to the other treatments. The decrease of rot incidence due to potassium silicates could be related to the beneficial effects of silicon on plant growth, development and disease resistance. Among the antagonistic microorganisms evaluated, *Aureobasidium pullulans* showed a significant control of the rot incidence on the berries compared to the untreated control. Both potassium silicates and formulations of *A. pullulans* could be considered as effective tools to control post-harvest rots on blueberries.

73. DEVELOPMENT OF A PROTOTYPE PREDICTIVE MODEL FOR OCHRATOXIN A CONTAMINATION IN GRAPE. M. Camardo Leggieri, P. Battilani. *Department of Sustainable Crop Productions (DIPROVES), Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, I-29122 Piacenza, Italy. E-mail: marco.camardoleggieri@unicatt.it*

Aspergillus carbonarius is considered the main responsible for ochratoxin A (OTA) contamination in grapes, even if *A. niger* and *A. tubingensis* are confirmed as dominant on berries. OTA is a possible carcinogen fungal metabolite and the European Commission set 2 $\mu\text{g Kg}^{-1}$ as maximum OTA content allowed in wine, must and grape juice being wine an important potential source of OTA in the human diet. Therefore, it is crucial for the grape-wine chain stakeholders to predict the risk of OTA contamination in berries at harvest. The aim of this work was to develop a mechanistic predictive model (OTA-grapes) based on the infection cycle of *A. carbonarius* on grapes. A relational diagram was drawn; data available in literature were used to develop mathematical equations for each step of the infection cycle, in relation to grape growth stages. For sporulation and infection, few quantitative data were available and only limiting conditions for their occurrence were established, while fungal germination, growth and OTA production were modelled based on temperature and water activity regimes. The model uses weather data as input; the *A. carbonarius* sub-model was run from setting to ripening. Crop growth stages were predicted by the phenological sub-model, that uses meteorological data as input as well. The prototype model OTA-grapes is now available, the next mandatory step is the collection of a suitable set of vineyard data, geo-referenced OTA contamination and related meteorological data, to manage the validation, crucial step before OTA-grapes model delivery to support stakeholders in the grape-wine chain.

74. FUSARIUM HEAD BLIGHT ON QUENCH BARLEY MALT CULTIVAR: PRODUCT QUALITY. F. Cavina¹,

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Fusarium Head Blight (FHB) is a cereal disease widespread throughout the world and mainly studied in wheat. In barley (*Hordeum vulgare*) its fungal agents can affect yield crops and reduce the quality of grains for beer manufacture due to the production of several mycotoxins. Furthermore, during the malting process, some *Fusarium* species can produce special proteins called “hydrophobins” responsible of “beer gushing” that influences beer quality. Artificial inoculations on cv. Quench with *Fusarium graminearum*, *F. culmorum* and *F. poae* have been performed in experimental field trials in the years 2013-2014, to evaluate the effects of FHB on barley malt. Disease incidence and severity were assessed. Fusarium Damaged Kernels (FDK), the presence of Deoxynivalenol (DON), T-2 and HT-2 mycotoxins were evaluated. In addition, the species of *Fusarium* isolated were morphologically and molecularly identified. The results showed that the average incidence of mixed inoculum (*F. graminearum* and *F. culmorum*) was 15% while *F. poae* alone 28%. These low values were confirmed by FDK. *Fusarium* spp. was re-isolated on 2% of the tested kernels, and *F. culmorum* was the prevalent species. The levels of DON, T-2 and HT-2 mycotoxins were lower than the limits set by the European regulations and recommendations. We can conclude that the trends of the temperature and precipitation, in the investigated area and at the time of the study, were not favorable for the establishment of FHB disease. Then the grains of barley malt maintained their quality avoiding the gushing risk.

75. SAMPLING SEEDS IN STORAGE FACILITIES: HUNTING FOR *FUSARIUM* INFECTION. M. Dal Prà¹, I. Alberti², S. Tonti³, M. Montanari³, E. Stefani¹. ¹*Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2, I-42122 Reggio Emilia, Italy.* ²*Agricultural Research Council – Center for Experimentation and Certification of Seed (CRA-SCS), Via Ca' Nova Zampieri 37, I-37057 San Giovanni Lupatoto (VR), Italy.* ³*Agricultural Research Council – Center for Experimentation and Certification of Seed (CRA-SCS), Via di Corticella 133, I-40128 Bologna, Italy. E-mail: mauro.dalpra@unimore.it*

Fusarium graminearum is one of the most studied fungal pathogens in the world. A great number of scientific publications are available that describe morphology, physiology, toxicology and genome of this species. On the other hand, *F. poae* is less widely studied but its importance as toxigenic fungus has been recently recognized. In Italy, *F. poae* is rapidly becoming one of the most widespread *Fusarium* species on small seeds cereals heads. Soft wheat is often colonized by these species. So far, Fusarium Head Blight (FHB) symptoms have been always investigated on soft wheat heads or on seeds collected in the field during harvesting. This poses a problem, as the seed industry and the national seed certification services would be interested to know the percentage of infection of seeds stored into facilities after processing and how infection is distributed inside seed lots. In order to study these aspects, we performed a mycological screening on soft wheat seed samples collected inside a facility, sampling directly from bags intended for seed trading. Sampling was performed during the years 2013 and 2014. The screening, carried out on Potato Dextrose Agar (PDA), was limited to organic soft wheat coming from Northern and Central Italy. Putative *F. graminearum* and *F. poae* colonies were selected and single spores purified and confirmed through microscope observations and through a molecular identification.

Seed samples resulted to be infected at a very low level. Though some lack of homogeneity was observed among the lots, this was sufficiently small to allow statistical studies.

76. BIOMOLECULAR CHARACTERIZATION OF *FUSARIUM LANGSETHIAE* STRAINS ISOLATED ON OAT SEEDS IN ITALY. M. Dal Prà¹, S. Tonti², M. Montanari², E. Stefani¹, I. Alberti³. ¹*Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2, I-42122 Reggio Emilia, Italy.* ²*Agricultural Research Council – Center for Experimentation and Certification of Seed (CRA-SCS), Via di Corticella 133, I-40128 Bologna, Italy.* ³*Agricultural Research Council – Center for Experimentation and Certification of Seed (CRA-SCS), Via Ca' Nova Zampieri 37, I-37057 San Giovanni Lupatoto (VR), Italy. E-mail: mauro.dalpra@unimore.it*

Fusarium langsethiae, a recently described fungus, is a high-level producer of T-2 and HT-2 toxins. These trichotecenes are related to alimentary toxic aleukia in humans and haematotoxicity and immunotoxicity in animals. *Fusarium langsethiae* has been reported on different cereal crops in Central and Northern Europe, but the most problematic is oat. Little is known about the occurrence of this fungus in Italy, where the demand for oat products is rising. During a 5-years survey, 64 organic seed samples were tested for the presence of *F. langsethiae*. The screening was carried out on Potato Dextrose Agar (PDA). Putative *Fusarium* colonies were selected and single spores purified and identified by microscope observation and by amplification and sequencing of the Translation Elongation Factor (TEF) 1- α . The most represented *Fusarium* species detected on oat was *F. poae*. *Fusarium langsethiae* was recovered from 13 samples and was usually associated with low levels of infected seeds. Interestingly, the main causal agents of Fusarium Head Blight (FHB), *F. graminearum* and *F. culmorum*, were almost absent. In order to detect the presence of T-2 and HT-2 toxins on samples, oat seeds were grinded to a fine powder in a blender and toxins were extracted with 70% methanol. The extracts were analysed by Rapid One Step Assay (ROSA) T2-HT2 Quantitative Test. Seed samples resulted to be contaminated by T-2 and HT-2 toxins but, so far, it was not possible to find a statistical correlation between *F. langsethiae* occurrence, infection levels and toxin contamination.

77. TRANSCRIPTOMIC APPROACH TO ELUCIDATE THE MOLECULAR MECHANISMS ACTIVATED BY *SPOROBOLOMYCES* SP. IN RESPONSE TO THE MYCOTOXIN PATULIN. G. Ianiri¹, A. Idnurm², R. Castoria¹. ¹*Department of Agricultural, Environmental and Food Sciences, University of Molise, Via Francesco De Sanctis s.n.c., I-86100 Campobasso, Italy.* ²*School of BioSciences, University of Melbourne, VIC 3010, Australia. E-mail: giuseppe.ianiri@unimol.it*

Patulin (PAT) is produced by *Penicillium expansum*, the causal agent of blue mold of stored pome fruits. This mycotoxin has genotoxic, teratogenic and immunotoxic effects, and its presence in pome fruits and derived products represents a serious health hazard. Biocontrol agents (BCAs) such as *Rhodosporidium kratochvilovae* and *Sporobolomyces* sp., are able to degrade PAT into the less toxic compounds desoxyapatulinic acid and ascladiols. In this work, we investigated the changes of gene expression in *Sporobolomyces* sp. exposed to PAT through RNAseq. PAT treatment causes cellular redox homeostasis alteration that results in oxidative stress, which leads to the activation of stress response mechanisms controlled by transcription factors. Up-regulated *Sporobolomyces* genes were those involved in oxidation-reduction process, transport, and glutathione and thioredoxin systems, indicating the activation of

defense mechanisms to resist PAT toxicity, restore the cellular redox homeostasis and expel the mycotoxin out of the cells. Conversely, PAT treatment decreased the expression of genes involved in the processes of protein synthesis and modification, ions transport, cell division and cell cycle. This indicates a reduction of metabolic activity probably due to the high energy requirement of the yeast cells, which need to recover from insult caused by PAT toxicity. Although PAT degradation needs to be further investigated through gene-protein discovery, this study outlines the complex mechanisms activated by a BCA in response to the mycotoxin and set the basis for i) the biodegradation of PAT in fruit juices, and ii) the development of a user-friendly biosensor for its rapid and cost-effective detection.

78. FUSARIUM GRAMINEARUM AND FUSARIUM CULMORUM IN WHEAT: SPME-GC-MS OF VOLATILE COMPOUNDS FOR IDENTIFICATION OF INFECTION INDICATORS. F. Lupi¹, C. Palermo^{1,2}, M. Quinto^{1,2}, D. Nardiello^{1,2}, A. Mentana¹, A. Moretti³, S. Frisullo^{1,2}, D. Centonze^{1,2}. ¹Department of Agriculture, Food and Environmental Sciences, University of Foggia, Via Napoli 25, I-71100 Foggia, Italy. ²Centro Servizi di Ricerca Applicata, University of Foggia, Via A. Gramsci 89/91, I-71100 Foggia, Italy. ³National Research Council of Italy, Institute of Sciences of Food Production (ISPA), Via Amendola 122/O, I-70126 Bari, Italy. E-mail: francesca.lupi@unifg.it

Fusarium spp. are the most dangerous mycotoxigenic fungi that could contaminate wheat. In particular, *Fusarium graminearum* and *F. culmorum* are among the main agents of Fusarium Head Blight (FHB), a worldwide disease causing accumulation of trichothecenes, especially deoxynivalenol and its acetylated form, in wheat grains at the harvest. Several analytical methods have been developed to determine the presence of mycotoxins in wheat grains and by-products. If the mycotoxins content is higher than the legal limit, the products must be destroyed causing huge economical damages. The aim of this study was to characterize the metabolites produced during the interaction between *F. graminearum* and *F. culmorum* and wheat plants, in order to identify possible metabolites eventually produced during the early stages of infection. Considering that many fungi and plants could produce Volatile Organic Compounds (VOCs), the headspace solid-phase microextraction coupled to gas chromatography with MS detector (HS-SPME-GC-MS) has been used. Healthy and artificially infected plants by *F. graminearum* and *F. culmorum* have been monitored until the grain ripening. In addition to VOCs analysis, the degree of the fungal contamination has been evaluated by fungal re-isolation on plate. The results have been treated by proper chemiometric approaches, such as the principal components analysis, in order to identify substances produced in the fungi-plant interaction. The approach developed in this study has provided promising results for the control of both *Fusarium* species and the reduction of the mycotoxins contamination. Further studies are in progress aimed at the identification of non-volatile metabolites eventually useful for an early diagnosis of fungal infections.

79. FUNGITOXIC ACTIVITY OF EUGENOL AGAINST BOTRYTIS CINEREA MAY BE MEDIATED BY FUNGAL PEROXIDASE. R. Marcato¹, S. Lengyel¹, M. Lucchetta², L. Sella¹, F. Favaron¹. ¹Department of Land, Environment, Agriculture and Forestry (TESAF), University of Padova, Viale dell'Università 16, I-35020 Legnaro (PD), Italy. ²Cocitech s.r.l., Via Treviso 38, I-31020 San Vendemiano (TV), Italy. E-mail: riccardo.marcato@unipd.it

Plant essential oils have shown antifungal activity against several plant pathogenic fungi in pre- and post-harvest. Their activity is due to monoterpene constituents that probably target cell membrane

and wall components. We studied the effect of the monoterpene eugenol on *Botrytis cinerea* (strain B05.10). The eugenol concentration that inhibits mycelium growth and conidia germination was about 0.3-3 mM and the minimum effective dose of eugenol to protect grape leaves from *B. cinerea* infection was about 10 mM. Experimental data showed that in *B. cinerea* culture filtrate, eugenol causes a release of potassium ions and of intracellular material indicating a damage to cell membrane possibly due to an oxidative stress. However, in comparison to untreated control, no accumulation of reactive oxygen species (measured by xylenol orange assay) was observed in *B. cinerea* mycelia treated with eugenol, while the concentration of antioxidant molecules such as glutathione and cysteine decreased. *Botrytis cinerea* produces high level of peroxidase activity and we observed that eugenol competes for the catalytic site of peroxidase. However, differently from other peroxidase cellular substrates, eugenol releases hydroxyl radicals as products of the reaction with hydrogen peroxide. Besides, oxidized eugenol showed the same fungitoxic activity of the not oxidized eugenol. The possible effect of complexes of eugenol with antioxidant molecules is under investigation.

80. ISOLATION AND PHYLOGENETIC ANALYSIS OF ALTERNARIA SPECIES ISOLATED FROM ALTERNARIOL CONTAMINATED DURUM WHEAT SAMPLES OF DIFFERENT GENOTYPES GROWN IN BOLOGNA AREA, SHOWING BLACK POINT SYMPTOMS. M. Masiello¹, S. Somma¹, A. Susca¹, V. Ghionna¹, A.F. Logrieco¹, M. Franzoni², S. Ravaglia², G. Meca³, A. Moretti¹. ¹National Research Council of Italy, Institute of Sciences of Food Production (ISPA), Via G. Amendola 122/O, I-70126 Bari, Italy. ²S.I.S. Società Italiana Sementi S.a.A, Via Mirandola 5, I-40068 San Lazzaro di Savena (BO), Italy. ³Department of Preventive Medicine, Nutrition and Food Science Area, University of Valencia (Spain), Avenida Vicent Andres Estelles s/n, 46100 Burjassot, Valencia, Spain. E-mail: antonio.moretti@ispa.cnr.it

Black point is a complex fungal disease of wheat mainly associated to *Alternaria* species, ubiquitous mycotoxigenic fungi causing different diseases on a high number of economically important crops. Affected wheat kernels are characterized by dark brown discolouration of the embryo region. The potential risk for wheat production is the reduction of kernel quality due to decreased nutritional value and the possible accumulation of *Alternaria* mycotoxins. One-hundred-twenty durum wheat samples belonging to 30 different genotypes grown in Bologna and Modena areas and affected by black point disease symptoms were analyzed for fungal contamination and *Alternaria* mycotoxin occurrence. *Alternaria* was the main genus identified on all genotypes with a contamination range of 5.5-14.3%. Among 382 strains isolated, 84 were identified as *A. alternata*, 64 as *A. arborescens*, 40 as *A. infectoria*, 150 as *A. tenuissima* and 44 isolates were not assigned to any of these species. Strain identification was also confirmed by sequencing the allergen alt1a (*alt1a*), glyceraldehyde-3-phosphate dehydrogenase (*gpd*) and translation elongation factor 1 α (*tef*) genes, according with a multi-locus gene sequence approach. One-hundred representative strains were sequenced and the data showed a high level of genetic variability among and within species, a variable genetic relationship among species based on the gene target, and the lack of correlation between morphological and molecular identification for some cases. Finally, the contamination of *Alternaria* mycotoxins was evaluated, showing a low level of toxigenic risk since only alternariol was detected at low level (range 25-265 ppb) in 69 out of 120 samples.

81. ROLE OF A SECONDARY METABOLISM GENE CLUSTER IN THE PATHOGENIC INTERACTION BETWEEN

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Aspergillus flavus is an opportunistic and saprophytic crops pathogen mostly known as an effective mycotoxins producer. Starting from previous studies aimed to identify gene clusters encoding for secondary metabolites, involved in pathogenicity of *A. flavus*, we focused on Cluster32 and specifically on a Zn₂Cys₆ transcription factors, present inside the cluster. Our purpose is to understand its role in the regulation of Cluster32 expression and to clarify finally its significance within the process of pathogenesis. To achieve this, we designed a knockout mutant for Zn₂Cys₆ via the TOPO cloning method: we have assembled a construct containing the *argD* gene, coding for the enzyme acetyl ornithine aminotransferase, flanked by 3'UTR and 5'UTR, regions homologous to Zn₂Cys₆. Once obtained, we used the deletion construct to transform AFC-1, a double auxotroph mutant incapable of producing Arginine and Uracil. Simultaneously, to characterize better the metabolic profile related to the cluster 32, we produced overexpression mutants of Zn₂Cys₆ fused to GFP. Thus, mutants were screened by fluorescence emission. Such mutant, have been tested to assay pathogenicity and fitness in different environmental conditions, compared to the wild type.

82. MECHANISMS OF FOOD ISOLATED BIOCONTROL YEASTS TO CONTROL POST-HARVEST PHYTOPATHOGENIC MOULDS. L. Parafati¹, C. Restuccia¹, A. Vitale¹, G. Polizzi¹, M. Wisniewski², G. Cirvilleri¹. ¹Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 100, I-95123 Catania, Italy. ²United States Department of Agriculture, Agricultural Research Service (USDA-ARS), Kearneysville, WV 25430 USA. E-mail: gcirvil@unict.it

Biological control by antagonistic yeasts is a promising strategy for reducing the common use of fungicides to control post-harvest phytopathogenic moulds. The knowledge of their mode of action is an important step to improve their performance, to develop appropriate formulations, and to establish selection strategies of new biocontrol agents. In this study, food isolated yeast strains demonstrated antifungal activity against *Penicillium digitatum*, *P. italicum* and *Botrytis cinerea* at different level depending on species and commodity. Iron competition, ability to form biofilm and to colonize fruit wounds were the main mechanisms of action for *Metschnikowia pulcherrima*. The production of glucanase, chitinase and protease, and the ability to colonize the wounds were the most important mechanisms of action in *Aureobasidium pullulans* and *Wickerhamomyces anomalus*. Moreover, the biocontrol abilities of *Saccharomyces cerevisiae* and *W. anomalus* was proved to be correlated with killer phenotype, and the expression of *WaEXG1* and *WaEXG2* genes coding the killer toxins exoglucanases was studied in *W. anomalus* strain by RT-qPCR. The production of VOCs with *in vitro* and *in vivo* inhibitory effects was observed for all the tested species. Peroxidase and superoxide dismutase activity assays were conducted to evaluate the ability to induce host systemic resistance. In *in vivo* experiments, strains demonstrated a significant reduction of post-harvest green, blue and grey moulds of citrus, table grape and strawberries. It is concluded that the understanding of the multiple and different modes of action of the tested yeast species represents a key step to explain the excellent control of post-harvest *Penicillium* spp. and *Botrytis* spp. moulds of oranges, grapes and strawberries fruits.

83. BIOACTIVE METABOLITES FROM BASIDIOMYCETE TRAMETES VERSICOLOR IN THE CONTROL OF FUNGAL PATHOGENS AND MYCOTOXINS. A. Parroni, M. Scarpri, C. Pietricola, M. Reverberi, C. Fanelli. Department of Environmental Biology, Sapienza University of Roma, Piazzale Aldo Moro 5, I-00185 Roma, Italy. E-mail: alessia.parroni@uniroma1.it

Among pathogens that attack plants, fungi play a pivotal role. Pathogenic fungi (e.g. *Aspergillus*, *Penicillium* and *Fusarium*) may synthesize mycotoxins, dangerous metabolites with toxic effects on animals and humans. For this, Europe strictly regulated their presence in feed and food (e.g. 1881/2006). Mycotoxin contamination in foods and feeds is limited mainly by chemical control. Nevertheless, the use of chemicals led to a severe environmental pollution, to the emergence of resistant pathogen populations and presence of chemical residues in food products. The European Community has banned about 50% of chemicals commonly used in crop production (EC/129/2009). For this, new “green” approaches for controlling fungal contamination and preventing or detoxifying mycotoxins, are under study. The biological control, with GRAS (Generally Recognised As Safe) organisms and/or their products (namely, bio-control agents), is increasing. A promising approach is the use of bioactive compounds from *Trametes versicolor*, a “medicinal mushroom”, with healing properties toward some human diseases. These metabolites, non-toxic for humans and animals, are under study for enhancing plant defences and/or for inhibiting pathogen growth and/or toxin synthesis. Here we present some compounds, a purified polysaccharide (Trametano) and its oligosaccharides, that can inhibit mycotoxins synthesis such as aflatoxins, ocratoxin A, patulin and fumonisin B1. They can be considered a new eco-compatible tool for mycotoxins control, in line with EU directives.

84. EFFECT OF PSEUDOMONAS SYRINGAE pv. ACTINIDIAE ON POST-HARVEST STORAGE AND HEALTH OF KIWI FRUIT “HAYWARD”. S. Prencipe^{1,2}, M.L. Gullino^{1,2}, A. Garibaldi², D. Spadaro^{1,2}. ¹Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: simona.prencipe@unito.it

Pseudomonas syringae pv. *actinidiae* (*Psa*) is the causal agent of bacterial canker of kiwifruit causing large economic crop losses to the production of European and non-European *Actinidia deliciosa* and *A. chinensis* since 2008. The quality and the health of the fruits can be influenced by the management practices, storage techniques and climatic conditions. The objective of this work was to assess the influence of *Psa* on post-harvest quality of fruits harvested from diseased and healthy orchards affected by *Psa*, as a result of two storage methods, in Normal Atmosphere (NA) and in Controlled Atmosphere (CA) over a period of 120 days. The study has been developed, for two years, by monitoring physicochemical parameters: firmness, Total Soluble Solid (TSS), and Titratable Acidity (TA). Further analysis of Dry Matter (DM) and major nutrients, calcium and nitrogen, was performed to observed the changing responses of fruit in relationship with different disorders. The incidence of *Botrytis* rot after storage was also measured. A further trial in CA with 1-methylcyclopropene (1-MCP) was performed. Fruits harvested from orchards affected by *Psa* showed reduction in storage time with lower firmness and TA and higher TSS and susceptibility to *Botrytis* rot. The CA storage helps to make the fruits firmer, more if preceded by 1-MCP treatment, and reduces the values of TSS for both categories of fruits analyzed obtained by plants diseased and healthy. In conclusion, *Psa* infection in field influenced post-harvest rots and kiwifruits quality.

85. CALLOSE DEPOSITION IN APPLE (*MALUS DOMESTICA* cv. GOLDEN DELICIOUS) PEEL TREATED WITH RESISTANCE INDUCERS. M. Quaglia, T. Gatto, F. Baglivo. *Department of Agricultural, Food and Environmental Sciences (DSA3), University of Perugia, Borgo XX Giugno 74, I-06121 Perugia, Italy. E-mail: mara.quaglia@unipg.it*

Callose, a polysaccharide in the form of β -1,3-glucan with β -1,6-branches, is deposited between the plasma membrane and the wall as response of plant cell to biotic or abiotic stresses. Callose deposition in the form of papillae at sites where pathogenic fungi attempt penetration, is an early host response designed to hinder the development of the infectious process. In several plant species, callose deposition has been observed after treatments with the chemical resistance inducers dL- β -aminobutyric acid (BABA), acibenzolar-S-methyl (ASM) or methyl-jasmonate (MeJA), activators of the BABA Induced Resistance (BABA-IR), Systemic Acquired Resistance and Induced Systemic Resistance, respectively. Also chitosan, a polymer derived from the chitin forming the outer shell of crustaceans and capable to activate defense responses common to the different resistance pathways, has shown to induce callose deposition. Here, by fluorescence microscopy, we developed a protocol for an optimal viewing of callose in aniline blue stained apple peel and we demonstrated the ability of the treatments with BABA 20 mM, ASM 1 mM, MeJA 1 mM and chitosan 1% to induce callose deposition in apple peel. Therefore, callose deposition is a response common to the different resistance pathways in apple peel. Callose is associated with the reduction of incidence of lesions caused by *Penicillium expansum* in the fruits treated with the above reported resistance inducers. Also pathogen inoculation determined callose deposition. The deposition was significantly higher in BABA-treated apple inoculated with *P. expansum* than in only BABA-treated apples, confirming the validity of priming phenomenon also in BABA-IR.

86. AFLATOXIN DEGRADATION BY LACCASE OF *TRAMETES VERSICOLOR*. M. Scarpari, M. Reverberi, L. Bignozzi, A. Parroni, C. Fanelli. *Department of Environmental Biology, Sapienza University of Roma, Piazzale Aldo Moro 5, I-00185 Roma, Italy. E-mail: marzia.scarparsi@uniroma1.it*

Aspergillus flavus is a well-known cosmopolitan fungus able to contaminate both in pre- and post-harvest period different plant matrices. The requirement of products, able to limit aflatoxin showing a low impact on the environment and on human health, has increased. In this work, the effect of laccase, lignin-degrading enzymes produced by the basidiomycete *Trametes versicolor*, on the aflatoxin degradation was investigated. The goal was to propose an environmental-loyal tool for degrading aflatoxins, in order to obtain feedstuffs and feed with a high standard of quality and safety. The cultural filtrate of *T. versicolor* contains generally a main laccase and a number of isoforms some of which closely related, others differing for structural and catalytic properties. The production of different isozymes is due to the occurrence of multiple laccase genes. Laccase are very versatile enzymes, being able to oxidize an extensive list of aromatic compounds containing hydroxyl- or amino-groups, including pesticides, polycyclic aromatic hydrocarbons and dyes. The great interest that laccases aroused for biotechnological uses has promoted intense investigations for clarifying their oxidative mechanism. In this study, we characterized these oxidative enzymes through purification with FPLC and trying to express them in a heterologous system. The shuttle vectors pIB2 and pIB4 have been cloned and inserted into the yeast *Pichia pastoris* by electroporation. Treatments of contaminated maize with culture filtrates of *T. versicolor* containing ligninolytic enzymes or purified laccase showed a significant reduction of the content of aflatoxin B1.

87. MYCOTOXIN PRODUCTION IN LIQUID CULTURE AND ON PLANTS INFECTED WITH *ALTERNARIA* spp. ISOLATED FROM ROCKET AND CABBAGE. I. Siciliano¹, G. Ortu¹, G. Gilardi¹, M.L. Gullino^{1,2}, A. Garibaldi¹. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: giuseppe.ortu@unito.it

The genus *Alternaria* includes plant pathogenic species that may affect a wide range of crops, including cereals, vegetables, fruits, ornamentals and oil-seed crops. Some species are able to produce secondary metabolites with mutagenic and teratogenic potential, responsible for certain types of cancer. *Alternaria* spp. are among most common and destructive pathogens of cabbage and cauliflower and were recently detected on plants and seeds of wild and cultivated rocket. The toxigenic potential of twenty-eight *Alternaria* isolates from rocket and cabbage, belonging to different species (*A. alternata*, *A. arborescens*, *A. brassicicola*, *A. japonica*), was investigated by using HPLC-MS/MS. The isolates were first cultured in modified Czapek-Dox medium in order to investigate the production of tenuazonic acid, alternariol, alternariol monomethyl ether, altenuene and tentoxin *in vitro*. Under these conditions, more than 80% of the isolates showed the ability to produce at least one mycotoxin, generally with higher levels for tenuazonic acid. The isolates were also tested *in vivo*, on cabbage, cultivated rocket and cauliflower. Results show a good correlation between *in vitro* and *in vivo* production for alternariol, alternariol monomethyl ether, altenuene and tentoxin while tenuazonic acid was not produced by any strain *in vivo*. *In vitro* assay represents a potential tool to predict the possible mycotoxin contamination, except for tenuazonic acid, and it could be useful to prevent serious risk for human health.

88. *FUSARIUM* MYCOTOXINS AND RELATED SPECIES OCCURRENCE IN ITALIAN DURUM WHEAT IN DIFFERENT GEOGRAPHICAL AREAS. S. Somma, M. Haidukowski, V. Ghionna, A. Pastorella, M.T. Cimmarusti, A. Susca, A.F. Logrieco, A. Moretti. ¹National Research Council of Italy, Institute of Sciences of Food Production (ISPA), Via G. Amendola 122/O, I-70126 Bari, Italy. E-mail: stefania.somma@ispa.cnr.it

Fusarium Head Blight (FHB) is a worldwide economically devastating disease of durum wheat, caused by a complex of species belonging mostly to *Fusarium* genus. Many of these species can produce a wide range of mycotoxins that can be accumulated in wheat kernels at maturity, among which trichothecenes, potent inhibitors of proteic synthesis, and zearalenone (ZEA), an oestrogenic compound, are the most common. One-hundred-four samples of durum wheat, collected in Italy in 2013 and 2014, were analysed for the occurrence of *Fusarium* species and trichothecenes and zearalenone. *Fusarium* strains isolated from wheat kernels were identified. Mycotoxin analyses, performed by HPLC and confirmed by mass spectrometry (LC-MS/MS), revealed: higher level of contamination in 2014 compared to 2013; deoxynivalenol (DON) detected at relevant levels only in the samples collected in Central and Northern Italy; T-2 and HT-2 toxins and ZEA at higher levels in samples collected in Southern Italy. In particular, 36 out of 44 wheat samples from Southern Italy in 2013 and 2014 (range, 100-335 and 155-486 ppb, respectively) were over the recommended limits suggested by European Union for the sum of T-2 and HT-2 occurrence in wheat kernels. The most occurring species were *Fusarium graminearum sensu stricto* in samples in which DON occurred at high levels, and *F. langsethiae* when T-2 and HT-2 toxins were detected. Mycotoxin contamination occurring in the kernels was reflected in the

spectrum of *Fusarium* species isolated. This study shows that a real mycotoxin risk related to *Fusarium* does exist along the whole Italy.

89. FILAMENTOUS FUNGI DURING ENSILING AND FEED-OUT OF CORN SILAGE ARE AFFECTED BY POLYETHYLENE OR BIODEGRADABLE FILMS. D. Spadaro^{1,2}, M.P. Bustos-Lopez¹, M.L. Gullino^{1,2}, S. Piano², E. Tabacco², G. Borreani². ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it

Whole corn silage is widespread in North America and Europe as a fiber and energy source for dairy and beef cattle. Corn silage is particularly susceptible to aerobic deterioration caused by yeasts and molds. Molecular techniques are able to identify the predominant fungal species and their evolution in corn silage and during the feed-out phase. The aim of this study was to identify cultivable filamentous fungi before ensiling, after silage conservation, in different zones of a silo covered with two different plastic films (standard polyethylene *vs.* biodegradable), as well as after 7 and 14 days air exposure of whole-crop corn ensiled in farm-scale silos. The cultivable fungal population of the forage changed remarkably from harvesting to silo opening. Anaerobiosis, along with pH decrease, greatly reduced mold count and the presence of mycotoxigenic *Fusarium* species, particularly in the deeper part of the silos. In the peripheral areas of the silo, where air penetration could not be completely prevented, the fungal population did not decrease. When silages were exposed to air for 7 days, as usually occurs during feed-out, the mold count reached 6.5 log cfu g⁻¹, and *Aspergillus fumigatus*, a well-known human and animal pathogen, became the dominant species.

90. PENICILLIUM GRISEOFULVUM, AGENT OF BLUE MOULD ROT, COULD BE A SIGNIFICANT PATULIN PRODUCER ON POME AND STONE FRUIT. D. Spadaro^{1,2}, A. Loré¹, A. Garibaldi¹, M.L. Gullino^{1,2}. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it

Blue mould is the most common post-harvest disease of cold stored apples and pears. *Penicillium griseofulvum* has been reported as agent of blue mould, together with *P. expansum*, on apple in the United States, Brazil, and Italy. Both *P. expansum* and *P. griseofulvum* belong to the same section *Penicillium* of the genus *Penicillium* and they are morphologically and phylogenetically close. *Penicillium expansum* and *P. griseofulvum* can produce patulin, a secondary fungal metabolite toxic to humans and animals. The virulence and patulin production of eight isolates of *P. expansum* and two isolates of *P. griseofulvum* – all isolated from Italian apples – were evaluated on 17 cultivars of pome and stone fruit. The isolates of *P. griseofulvum* showed a higher virulence on apples, while their virulence was significantly lower on pear, peach, plum, and apricot. “Golden Delicious” and “Royal Gala” apples were the most susceptible to *P. expansum* and *P. griseofulvum*. Though not as strongly pathogenic as *P. expansum*, the isolates of *P. griseofulvum* were high patulin producers, particularly on apples and on apricots “Aurora”. “Golden Delicious” and “Royal Gala” apples were more conducive to patulin contamination. Also stone fruit, and particularly apricots

and peaches, were susceptible to patulin contamination. Our study found that *P. griseofulvum*, besides *P. expansum*, could be a significant patulin producer on pome and stone fruit, and particularly on apple.

91. HOW MENADIONE TRIGGERS AFLATOXIN SYNTHESIS IN A. FLAVUS. M. Zaccaria¹, M. Ludovici¹, M. Scarpari¹, S.M. Sanzani³, V. Scala¹, A.A. Fabbri¹, C. Fanelli¹, W. Sansaverino², M. Reverberi¹. ¹Department of Environmental Biology, Sapienza University of Roma, Piazzale Aldo Moro 5, I-00185 Roma, Italy. ²Sequentia Biotech, Carrer Comte d’Urgell 240, 08036 Barcelona, Spain. ³Department of Soil, Plant and Food Sciences (DiSSPA), University of Bari “Aldo Moro”, Via G. Amendola 165/A, I-70126 Bari, Italy. E-mail: massimo.reverberi@uniroma1.it

Aspergillus flavus is a saprophytic fungus responsible for worldwide spread harvest and post-harvest infections on cultivations. It is a producer of the most carcinogenic metabolite in nature: aflatoxin B1. Ongoing climate changes favor plant susceptibility to fungal attack with a consequent increase of aflatoxins into previously unexploited foodstuff. In order to address the economic and sanitary consequences of *A. flavus* contamination, an extensive knowledge of the pathogen metabolism and of the environmental conditions triggering the different biological processes, is of paramount importance. In this study, we focus on the effects of oxidative stress in the intracellular compartment, obtained by adding menadione 0.1 mM to the culture medium. Menadione is a chinone, its cytotoxicity has been investigated in several human and murine cellular lines as an intracellular ROS inductor. Chinones are very common in nature. As substrates for flavoenzymes they may incur in one electron reduction to semichinone which, conversely, reduce O₂ to superoxide anion in the intracellular environment, therefore providing a stressing condition. We evaluate *A. flavus* response via several analytical approaches: mycelial growth, conidia quantification, aflatoxin B1 synthesis, antioxidant enzymes activity, intracellular ROS quantification. To evaluate gene expression, we exploit RNAseq technology for transcriptome analysis, plus RT-PCR of markers for cellular respiration, pentose phosphate pathway, oxidative stress response. Lastly, we have extracted and evaluated oxylipins by an MRM based LC-MS/MS method, in order to ascertain if they may represent a stable reactive signal able to trigger aflatoxin synthesis and conidiogenesis through a remodulation of *A. flavus* metabolism in oxidative stress conditions.

DIFESA

92. COPPER SENSITIVITY OF ITALIAN PSEUDOMONAS SYRINGAE pv. ACTINIDIAE STRAINS. G. Battistini, M. Collina, A. Brunelli. Department of Agricultural Sciences (DipSA), University of Bologna, Viale G. Fanin 44, I-40127 Bologna, Italy. E-mail: marina.collina@unibo.it

Pseudomonas syringae pv. *actinidiae* (*Psa*) is the agent of the bacterial canker of green and yellow-fleshed kiwifruit. This pathogen was reported on *Actinidia deliciosa* in Italy for the first time in 1994 without yield losses up to 2008 when the disease infected most part of orchards located in Latium Region. *Psa* is currently a worldwide pandemic disease, threatening the kiwifruit producing countries, particularly Italy, France, New Zealand and Chile. The current chemical control of *Psa* in field is reliant on spraying of copper-based compounds. Unfortunately, copper may lead many bacteria to develop different strategies to overcome its toxicity. This study was undertaken to establish the copper sensitivity of many strains of *Psa*

coming from North of Italy isolated during 2009-2013 years. Fifty-three strains were evaluated for copper sensitivity by their ability to grow on a specific media supplemented with copper sulphate. The assessment was made as Minimal Inhibitory Concentration (MIC). The 54% of strains isolated from 2009 to 2011 (namely before the widespread and massive use of copper in field) showed $1.2 < \text{MIC}$ values $< 2.4 \text{ mM}$ of Cu^{++} while the remainder had a $\text{MIC} < 1.2 \text{ mM}$. The percentage of strains with $1.2 < \text{MIC}$ values $< 2.4 \text{ mM}$ of Cu^{++} increased up to 82.7% on isolates collected during 2012-2013. Nevertheless, this results lead to conclude that the *Psa* isolates sampled in northern Italy during 2009-2013 years show a normal sensitivity to copper ion also according to other studies.

93. USE OF MICROBIAL CONSORTIA FOR AN ECO-SUSTAINABLE MANAGEMENT OF SOME TOMATO DISEASES. V. Catalano¹, E. Verzelloni¹, D. Giovanardi¹, M. Ferrari¹, E. Casagrande Biasuz¹, A. Prodi², P. Nipoti², E. Stefani¹. ¹Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2, I-42122 Reggio Emilia, Italy. ²Department of Agricultural Sciences (DipSA), University of Bologna, Viale G. Fanin 44, I-40127 Bologna, Italy. E-mail: valecatalano@gmail.com

The use of beneficial microbial population in agriculture has proven to be important for organic productions and enhances biodiversity in agro-ecosystems. This study was aimed to evaluate the beneficial effects of commercial microbial consortia (Micosat F[®]) on the growth and productivity of tomato plants and, in particular, the ability of such consortia to elicit an endogenous protection against the bacterial spot, (*Xanthomonas vesicatoria*), and the root and crown rot (*Rhizoctonia solani*). Experiments were conducted under field conditions on tomato for industrial processing during two seasons, following a randomised plot design and in duplicate for a statistical evaluation of data. Micosat F[®] was applied 5 times fortnightly, from first fruit set to three weeks before harvest. Phytopathometrical assessments were done on plants during the whole season and the tomato production was also assessed. Results highlighted that Micosat F[®] consistently enhanced plant size by an average increase of 13.04%. In case on plots inoculated with *R. solani*, the use of Micosat F[®] led to an increase of tomato production by 18.3%, when compared with the untreated controls. In case of plots inoculated with *X. vesicatoria*, no significant difference was seen between treated and untreated plants, as regards the harvested fruits. The induction of a protective status in tomato plants, elicited by the use of Micosat F[®], was evaluated by measuring the increased activity of antioxidant enzymes (CAT, POD, SOD). Preliminary data are very promising and are indicating that microbial consortia, such as those contained in Micosat F[®], might have a significant role in both the biological and integrated tomato production.

94. EFFICACY OF DIMETHYL DISULFIDE (DMDS) FOR THE CONTROL OF VERTICILLIUM DAHLIAE ON CHRYSANTHEMUM IN ITALY. A. Franceschini¹, A. Santori², A. Myrta³, G. Puntoni⁴, S. Pecchia⁴. ¹Flora Toscana Soc. Agr. Coop., Via di Montecarlo 81, I-51017 Pescia (PT), Italy. ²Certis Europe B.V., Via Josèmaria Escriuà de Balaguer 6, I-21047 Saronno (VA), Italy. ³Certis Europe B.V., Boulevard de la Woluwe 60, 1200 Brussels, Belgium. ⁴Department of Agriculture, Food and Environment (DISAAA), University of Pisa, Via del Borghetto 80, I-56124 Pisa, Italy. E-mail: susanna.pecchia@unipi.it

Dimethyl disulfide (DMDS) was applied to control the vascular wilt fungus *Verticillium dahliae* in greenhouse experiments conducted in the two successive seasons of 2013 and 2014. The trials were carried out in Tuscany (Italy) on *Chrysanthemum* cv.

“Veneri”, according to a randomized block design with 3 replicates per treatment. The soil of the sites was loamy and naturally infected by *V. dahliae*. DMDS was applied at the rates of 600 kg ha⁻¹ via drip-irrigation (EC) or in shank application (AL). The treated plots were covered for 2 weeks with a very impermeable plastic film (VIF). After one week of soil aeration *Chrysanthemum* seedlings were transplanted in each plot. DMDS treatments were compared with metam-sodium (1400 kg ha⁻¹) or chloropicrin (500 kg ha⁻¹) and untreated controls and, at the end of the crop cycle, the effects of the treatments on disease incidence and crop performance were evaluated. The results showed a very good efficacy of DMDS against *Verticillium* wilt of *Chrysanthemum*. In both trials, vascular wilt symptoms showed significant differences among untreated and treated plots (<1% compared with 51% untreated in 2013 and 0-6% vs. 27% untreated, in 2014) with no difference among the treated plots. Treatments registered greater production in term of stem numbers per ground area and no difference occurred among them. Therefore, DMDS could be a new effective solution to control *Verticillium* wilt of *Chrysanthemum* and because of its favorable ecotoxicological profile, it could also be considered in sustainable floriculture programs.

95. ITALIAN BIOCONTROL AGENTS (BCAs) FROM PHYLLOSPHERE OF ACTINIDIA AGAINST PSEUDOMONAS SYRINGAE pv. ACTINIDIAE (PSA) ISOLATED IN DIFFERENT GEOGRAPHIC WORLD AREAS. L. Gallipoli¹, F. Mastrogiovanni¹, M.E. Virili¹, A. Tiezzi², G.M. Balestra¹. ¹Department of Agriculture, Forests, Nature and Energy (DAFNE), University of Tuscia, Via San Camillo de Lellis s.n.c., I-01100 Viterbo, Italy. ²Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF), University of Tuscia, Via San Camillo de Lellis s.n.c., I-01100 Viterbo, Italy. E-mail: balestra@unitus.it

Bacterial canker of *Actinidia*, currently, is the most problematic disease of this plant and is caused by *Pseudomonas syringae* pv. *actinidiae* (*Psa*). Since 2008 its presence was detected in all world's areas, where culture of *Actinidia* has economic relevance. In effect to date, the bacterial canker of *Actinidia* has become so widespread to be described pandemic, with incalculable damage to all main producer countries of kiwifruit in the world. Several systems, allowed in organic farming, such as copper compounds and natural substances have been tested, but biocontrol agents (BCAs) seem to be among the most effective against *Psa*. In order to search a system to contrast this pathogen, in this study, six BCAs were isolated from phyllosphere of *Actinidia* affected by bacterial canker and characterized with traditional and molecular methods. To evaluate their effectiveness, the BCAs were tested against twenty-eight strains of *Psa* isolated in different geographic world areas. The results indicated that these natural antagonists were capable of inhibiting the growth of the various *Psa*, both *in vitro* and *in planta* and showed also a good epiphytic survival on the host.

96. PRE-PLANTING TREATMENTS WITH RESISTANT INDUCERS AND BIOCONTROL AGENTS, AGAINST FUSARIUM WILTS OF LEAFY VEGETABLES. G. Gilardi^{1,2}, M.L. Gullino^{1,2}, A. Garibaldi¹. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: giovanna.gilardi@unito.it

Over recent years, lettuce and cultivated rocket have increased their economic relevance since many farms produce fresh cut and

ready-to-eat salads. *Fusarium* wilts on lettuce and cultivated rocket, incited by *Fusarium oxysporum* f. sp. *lactucae* on lettuce and by *Fusarium oxysporum* f. sp. *raphani* and *conglutinans* on rocket, cause serious losses. Several approaches to soil-borne disease management are intensively investigated to give an answer to the many practical problems associated with the loss or limitation of use of effective fumigants. Trials were carried out to evaluate the efficacy of preventive treatments based on plant defense activator products, biocontrol agents, microbial complex with arbuscular mycorrhizal fungi, and *Brassica carinata* pellets against *Fusarium* wilts of lettuce and cultivated rocket under greenhouse conditions. Such products were compared with fungicides known for their ability to induce host resistance (phosethyl-Al and acibenzolar-S-methyl), and with azoxystrobin. Four applications with the phosphites-based products provided a disease reduction on lettuce from 33 to 83% and of 44.8 to 67.7% on cultivated rocket; the biocontrol agents tested were partially effective, with a disease reduction from 21 to 45%. *Brassica carinata* pellets provided not always consistent results. Acibenzolar-S-methyl induced an increase control of *F. oxysporum* f. sp. *lactucae* from 50 to 68% and of *F. oxysporum* f. sp. *raphani* from 58 to 75%, showing results statistically similar to azoxystrobin (disease reduction from 50 to 75%). Disease resistance inducers based on phosphites and biocontrol agents can be considered interesting components of integrated disease management strategies.

97. SOIL DISINFESTATION WITH DIMETHYL DISULFIDE TO CONTROL FUSARIUM WILT ON LETTUCE. G. Gilardi¹, G. Martano^{1,4}, A. Santori², A. Myrta³, A. Garibaldi¹, M.L. Gullino^{1,4}. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Certis Europe B.V., Via J.M.E. de Balaguer 6, I-21047 Saronno (VA), Italy. ³Certis Europe B.V., Bld. Woluwedal 60, 1200 Brussels, Belgium. ⁴Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: giovanna.gilardi@unito.it

Fusarium wilt of lettuce has been detected for the first time in Europe in Northern Italy in 2002. Lettuce varieties which are resistant or at least tolerant to *Fusarium* wilt are available, but their use is complicated by the presence at least of 3 races of the pathogen. At present, the management of soil-borne pathogens is complicated by the limited number of registered chemicals and by the restrictions in the use of pre-plant fumigants, including metham sodium and dazomet. Field trials were conducted in order to evaluate the efficacy of soil disinfestation treatments based on dimethyl disulfide (DMDS) applied by shank injection under virtually impermeable transparent films for 14 days. DMDS at 30, 40, 60 g m⁻² alone or combined with metham sodium (DMDS 40 g m⁻² + Metham 14.1 mL m⁻²) was compared with dazomet against *Fusarium oxysporum* f. sp. *lactucae* race 1 on lettuce. At the end of each crop cycle, the severity of *Fusarium* wilt of lettuce was evaluated according to rating scales of 0-100. In the presence of a disease incidence ranging from 23.4 to 78%, DMDS at 40 and 60 g m⁻² showed the best effectiveness in *Fusarium* wilt control. DMDS, at the dosage of 30 g m⁻², did not always ensure satisfactory reduction of *Fusarium* wilt on lettuce compared to dazomet. DMDS at 40 g m⁻², applied in combination of lettuce plants showing a low susceptibility to the pathogen, is also effective.

98. CHEMICAL AND PHYSICAL SEED TREATMENTS FOR BASIL DOWNY MILDEW MANAGEMENT. G. Gilardi^{1,2}, I. Pintore¹, S. Demarchi¹, M.L. Gullino^{1,2}, A. Garibaldi¹. ¹Centre of Competence for the Innovation in the Agro-Environmental

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Downy mildew of basil caused by *Peronospora belbahrii* has been observed in several countries wherever basil is grown as herb crop as well as for pesto sauce production. Such broad diffusion has been probably favored by the fact that the pathogen is seed transmitted. Different approaches of disease management need to be evaluated by considering the epidemiology of the seed transmitted *P. belbahrii* and its repercussions on the control measures. Trials were carried out under controlled conditions in greenhouse in order to evaluate the effect of different products (chemicals, resistance inducers and natural products) as well as heat air treatment applied to basil seeds naturally infested by *P. belbahrii*. Seed quality, as vigour index and disease incidence (number of infected plants and percentage of affected plants) were evaluated. Results showed as seed quality was not affected by chemical treatments, with fungicides at the lower dosage, thyme oil and dry heat air treatment. Although many of the fungicides, resistance inducers, thyme oil treatments and heat air (65°C for 10 min) tested showed a significant disease reduction compared with the untreated control, the protection offered was only partial. Moreover, the effectiveness of the tested seed treatments varied considerably among trials. Further evaluation need to be carried out in a full integrated approach for downy mildew management.

99. EMPHASIS, A HORIZON 2020 PROJECT FOR EFFECTIVE MANAGEMENT OF PESTS AND HARMFUL ALIEN SPECIES. M.L. Gullino^{1,2} on behalf of EMPHASIS consortium. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: marialodovica.gullino@unito.it

EMPHASIS (Effective Management of Pests and Harmful Alien Species – Integrated Solutions) is an international project funded by the European Commission under Horizon 2020 Research and Innovation Program addressing native and alien pests threats (insect pests, pathogens, weeds) for a range of both natural ecosystems and farming systems. The project consortium gathers 22 Partners from 10 Countries and it includes research institutes, enterprises, SMEs and international organizations bringing cross-sectorial and complementary expertise. The project aims to ensure a European food security system and the protection of biodiversity and of ecosystems services while developing integrated mechanisms of response measures (practical solutions) to predict, to prevent and to protect agriculture and forestry systems from native and alien pests threats. A cross-cutting approach to participatory research and technology transfer is adopted, in order to strengthen the connectivity between agricultural research and other system actors. Thus, on-farm testing and participatory learning activities are being developed since the beginning of the project, in order to facilitate co-design, co-development and co-implementation. The project is not focused on a single management system but the plant-pest ecosystems dealt with are treated with a multi-method approach to design true IPM methodology that will be developed for key systems with portability to other similar systems, thereby having a large impact.

100. EUCLID, AN EU-CHINA LEVER FOR IPM DEMONSTRATION. M.L. Gullino^{1,2}, N. Desneux³ on behalf of EUCLID

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More sustainable pest management methods are needed to reduce the negative effects of pesticides on human health and the environment. EUCLID (EU-China Lever for IPM Demonstration) is a project funded by the European Commission under Horizon 2020 Research and Innovation Program aiming to contribute to secure the production of food for the increasing worldwide population while developing sustainable production approaches to be used in the European and Chinese agriculture. The choice of the crops of interest in EUCLID, i.e. fresh tomatoes, grapes and leafy vegetables, is based on their economic importance for both European and Chinese fruit and vegetable production, but also for their exemplarity in representing different production systems. This means that the solutions of the project could be used as models for developing similar actions for other crops. The project will exploit the thorough knowledge developed in the last years on IPM to adapt and optimize those tools and approaches which did not reach the field/market (yet). In addition, the consortium will work on further development of high potential innovation pest management solutions. The consortium is coordinated by INRA and it integrates in the research process, from the very beginning, the main end-users of the project's results: farmers associations, SMEs, economists, experts in policy. The consortium also has a good coverage of both European and Chinese experts, in order to take advantage of the experience of each region and to more efficiently adapt the pest management solutions to the specific problems of European and Chinese farmers.

101. CROSS-RESISTANCE STUDIES OF METRAFENONE-RESISTANT ISOLATES OF *ERYSIPHE NECATOR*. A. Kunova, C. Pizzatti, M. Bonaldi, P. Cortesi. Department of Food, Environmental and Nutritional Science (DeFENS), University of Milano, Via Giovanni Celoria 2, I-20133 Milano, Italy. E-mail: andrea.kunova@unimi.it

Erysiphe necator is one of the major fungal diseases of cultivated grapevine worldwide and it is managed almost exclusively by fungicides. It has already developed resistance to several fungicide groups including Quinone outside Inhibitors (QoI) and Sterol Biosynthesis Inhibitors (SBI). Metrafenone is a fungicide belonging to benzophenones (FRAC group U8), and it is used specifically to control powdery mildews. Although its exact mode of action is not known, it is different from other fungicides used in powdery mildew management and is therefore a valuable choice in fungicide rotation programs. Recently, we have described metrafenone resistance in a population of *E. necator* in northern Italy. Thirteen strains were obtained from Franciacorta area and analyzed for their sensitivity to metrafenone in terms of mycelium growth and sporulation. All isolates grew abundantly on control plants; after 14 days of growth their average colony area was 89.4 mm² (Standard Deviation, SD 39.9) and they produced on average 2212.1 spores cm⁻² (SD 1668.8). Two strains were sensitive, and they did not grow at the metrafenone concentration used in field (125 mg L⁻¹ a.i.). The resistant strains grew and sporulated similarly to control at metrafenone field concentration, and even at metrafenone concentration of 1250 mg L⁻¹. Moreover, we studied the cross-resistance of *E. necator* metrafenone-resistant strains to pyriofenone, which belongs to the same FRAC group and two fungicides representative of QoI and SBI groups, azoxystrobin and myclobutanil. The leaf surface

of pyriofenone-treated plants was 100% colonized by metrafenone-resistant strains, confirming cross-resistance between metrafenone and pyriofenone, whereas they were fully inhibited by azoxystrobin and myclobutanil, indicating absence of cross-resistance with QoI and SBI.

102. ENTOMOPATHOGENIC FUNGI: A SOURCE OF SECONDARY METABOLITES USEFUL TO CONTROL INSECT PESTS. F. Lacatena¹, S. Woo^{1,2}, F. Vinale², R. Marra², N. Lombardi², S. Lanzuise^{1,2}, L. Bosso¹, M. Pascale^{1,2}, A. Djella^{1,2}, G. Manganiello¹, A. Pascale¹, L. De Vitto¹, S. Caira³, M. Ruocco², M. Giorgini², M.C. Digilio¹, M. Lorito^{1,2}. ¹Department of Agricultural Sciences, University of Napoli "Federico II", Via Università 100, I-80055 Portici (NA), Italy. ²National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via Università 133, I-80055 Portici (NA), Italy. ³National Research Council of Italy, Institute of Food Sciences, Via Roma 64, I-83110 Avellino, Italy. E-mail: matteo.lorito@unina.it

The traditional methods of pest management with the employment of chemical pesticides are caused a big accumulation of pollution in environmental matrixes. In order to reduce this risk, there have been an increasing for utilization of biocontrol agents against the pest insects in the last ten years. The aim of this research was to find and identify new fungal metabolites that may be used as formulation for bio-insecticides. We started from a collection of entomopathogenic fungi belonging to the genera *Paecilomyces*, *Beauveria*, *Pochonia*, and *Metarhizium* that we cultured in Potato Dextrose Broth (PDB) in static conditions for 1 month. We tested the culture broth in combination with hydrophobin produced by the known biocontrol agent *Trichoderma* sp. After the extraction, we proceeded to fractioning by using extensive chromatographic techniques (CC, TLC, HPLC). The metabolites were tested against aphids (*Acyrtosiphon pisum*) on bean and whitefly (*Trialeurodes vaporariorum*). The better result on aphids was obtained with the genera *Pochonia* and *Beauveria* in combination with hydrophobin, in which the mortality was respectively 53 and 45%, and with a purified fraction from *Metarhizium anisopliae*. This active compound, a cyclodepsipeptides characterized by using LC/MS-qTOF analysis, caused the 55% of mortality in 72 hours. We continue our research with the utilization of other fungal species in different growth conditions. Expected results from this study may open new possibilities of using secondary metabolites from entomopathogenic fungi as bio-pesticides for the insect pest management.

103. RHIZOBACTERIA AND ENDOPHYTES FROM STRAWBERRY FOR PLANT GROWTH INDUCTION AND PROTECTION. D. Lamorte, A.M. Ciarfaglia, P. Lo Cantore, N.S. Iacobellis. School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), University of Basilicata, Viale dell'Ateneo Lucano 10, I-85100 Potenza, Italy. E-mail: iacobellis@unibas.it

Strawberry is attacked by a large number of pathogens that can cause qualitative and quantitative losses. Rhizosphere and endophyte microorganisms are increasingly being used for plant protection as biocontrol agents. It is known that some of the above bacteria are able to protect plants through the induction of resistance (ISR), the competition for niches and substrates, and the production of hydrolytic enzymes and antimicrobial compounds. 445 bacteria were isolated from the rhizosphere soil and from the outside and the inside of the roots of strawberry plants (cvs. "San Andreas" and "Candonga"). Bacterial isolates were evaluated for colonies morphology, the Gram character, the production of fluorescent pigments, indoles and siderophores, as well as nitrogen fixation

and phosphate solubilization. Furthermore, bacterial isolates were also evaluated for their ability to inhibit *in vitro* the growth of *Xanthomonas fragariae* and of fungi belonging to the genus *Pythium* and *Fusarium* spp. 29 bacterial isolates were selected according to the above results for further studies in order to test their ability to promote plant growth and to protect strawberry plants artificially infected with the bacterial pathogen *X. fragariae* under field and greenhouse condition, respectively. The results appear of interest for eight of them whose molecular identification is in progress.

104. STRAWBERRY GROWTH STIMULATION BY BACTERIA ISOLATED FROM THE RHIZOSPHERE. D. Lamorte, A.M. Ciarfaglia, P. Lo Cantore, N.S. Iacobellis. *School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), University of Basilicata, Viale dell'Ateneo Lucano 10, I-85100 Potenza, Italy. E-mail: nicola.iacobellis@unibas.it*

A project with the aim to isolate rhizobacteria useful for the strawberry growth induction and protection was recently started. Among 445 bacteria isolated from the rhizosphere, 29 appeared, on the basis of metabolic features as determined in this project, potential Plant Growth Promoting Bacteria (PGPB) and for that they were evaluated for their ability to promote strawberry plant growth under field conditions. Aliquots (100 mL) of bacterial suspension (10^8 cfu mL⁻¹) were applied twice (15 and 30 days after planting) in the rhizosphere soil of strawberry plants. 20 plants per treatment were used. No bacterized plants were used as control. Growth-promoting effects were evaluated 15, 30, 45 and 130 days after the second bacterial treatment by determining the number of buds, leaves, flowers and fruits for each plant. Data were subjected to ANOVA and the above characters in the case of plants treated with 8 out of 29 tested isolates resulted highly significant ($P < 0.05$) when compared to the control. For these 8 bacterial-treated plants the number, the fresh and dry weight of leaves, stems and roots as well as the volume of the latter organs were further determined at the end of crop production. ANOVA of the above data showed once again the positive highly significant ($P < 0.05$) effect of bacterial inoculations on the above plant growth parameters. The molecular identification of the 8 above PGPB is in progress.

105. RHIZOBACTERIA FROM STRAWBERRY FOR THIS CROP PROTECTION. D. Lamorte, A.M. Ciarfaglia, P. Lo Cantore, N.S. Iacobellis. *School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), University of Basilicata, Viale dell'Ateneo Lucano 10, I-85100 Potenza, Italy. E-mail: nicola.iacobellis@unibas.it*

Strawberry is challenged by a large number of pathogens that can cause qualitative and quantitative crop losses. The use of rhizosphere and endophyte microorganisms for plant protection is increasing and it is known that some of the above bacteria are able to protect plants through the induction of resistance (ISR), the competition for niches and substrates, and the production of hydrolytic enzymes and antimicrobial compounds. 445 bacteria were isolated from the rhizosphere soil, from the outside and the inside (endophytes) of the roots of strawberry plants (cvs. "San Andreas" and "Candonga") grown in conventional, organic and biodynamic commercial farms. Bacterial isolates were evaluated for colonies morphology, the Gram character, the production of fluorescent pigments, indoles and siderophores, as well as nitrogen fixation and phosphate solubilization. Furthermore, bacterial isolates were also evaluated for their ability to inhibit *in vitro* the growth of *Xanthomonas fragariae*, *Pythium* and *Fusarium* spp. pathogens of strawberry. The interactions and ability of 29 selected bacterial isolates to

protect strawberry plants artificially infected with a virulent strain of *X. fragariae* under greenhouse conditions will be presented.

106. EVALUATION OF BACTERIAL AND FUNGAL BIOCONTROL AGENTS AS TREATMENTS AGAINST *FUSARIUM OXYSPORUM* f. sp. *LACTUCAE* ON LETTUCE SEEDS. J.G. Lopez-Reyes¹, G. Gilardi¹, A. Garibaldi¹, M.L. Gullino^{1,2}. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: giovanny.lopez@unito.it

Five antagonistic strains of *Pseudomonas* spp., and two strains of *Fusarium oxysporum* were evaluated on lettuce cv. Crispilla Blanca as seed treatment against *F. oxysporum* f. sp. *lactucae*. The effectiveness of the treatments with biocontrol agents was compared to seed treatment with two different fungicides (prochloraz and thiram), a resistance inducer (acibenzolar-S-methyl), and biological products available in commerce (including *Bacillus subtilis*, *Glomus* spp., *Streptomyces* spp., *Trichoderma* spp. applied alone or in a commercial mixture). The efficacy of the seed treatments was assessed *in vivo* under controlled conditions in glasshouse. Seed treatments with *Pseudomonas* strains showed in most cases an efficacy limited, but their effectiveness was statistically comparable with that obtained by treatments with prochloraz and thiram, in terms of the number of infected plants and disease index. If compared with the inoculated and untreated control, the treatment with the *Pseudomonas* strain FC9B reduced the plant infection about 76.2% and the disease index about 79.7%. Seed treatments with the strain MSA35 of *F. oxysporum* were slightly phytotoxic as they reduced slightly the germination rate of treated seeds. The efficacy of the tested seed treatments with biocontrol agents for controlling the pathogen was not totally satisfactory, however it is amenable to improvements and combination with other strategies of seed sanitization.

107. EFFICACY OF BIOCONTROL AGENTS AND ESSENTIAL OILS APPLIED AFTER HOT WATER TREATMENTS AGAINST *ALTERNARIA RADICINA* ON CARROT SEEDS. J.G. Lopez-Reyes¹, G. Gilardi¹, A. Garibaldi¹, M.L. Gullino^{1,2}. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: giovanny.lopez@unito.it

Seed-dressing with essential oils from savory (*Satureja montana*) and thyme (*Thymus vulgaris*) and with antagonistic strains of *Pseudomonas* sp. were tested after a hot water treatment (seed immersion in pre-conditioned water at 55°C for 10 min) against *Alternaria radicina* inoculated on carrot seeds. The seed treatment were evaluated *in vivo* under controlled conditions in glasshouse, in comparison to treatments with a resistance inductor (acibenzolar-S-methyl), two different fungicides (iprodione and thiram), and a biological product commercially available (containing an antagonist strain of *Bacillus subtilis*). Seed treatments by immersion in hot water improved the efficacy on pathogen control of the treatments with biocontrol agent strains more than the efficacy of those with essential oils, reducing the plant infection from 52% to 8% for the treatments with the strain FC7B, from 43% to 12% for the treatments with the strain FC8B, and from 37% to 8% for the treatments with the strain FC9B. Pretreatment with hot water generally increased the germination rate of the tested carrot seeds. A mild phytotoxic effect was observed on the germination rate and the

fresh biomass obtained from seeds treated with both essential oils. Treatments with essential oils and antagonistic bacteria presented positive results when combined with hot water dipping of seeds on control of *A. radicina*, but the effect was not completely additive.

108. NOVEL STRATEGIES FOR AGRONOMIC BIOFORTIFICATION OF FOOD CROPS BY USING *TRICHODERMA* BIOCONTROL AGENTS AND THEIR SECONDARY METABOLITES. R. Marra^{1,2}, R. Varlese², A. De Martino², F. Scognamiglio², N. Lombardi^{1,2}, F. Vinale^{1,2}, M. Ruocco^{1,2}, S. Lanzi^{1,2}, G. Manganiello², A. Pascale², F. Lacatena², A. Djella², A. Piccolo², M. Lorito^{1,2}, S.L. Woo^{1,2}. ¹National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via Università 133, I-80055 Portici (NA), Italy. ²Department of Agricultural Sciences, University of Napoli "Federico II", Via Università 100, I-80055 Portici (NA), Italy. E-mail: woo@unina.it

Lentil (*Lens culinaris*) is a crop of great agronomic and commercial interest widely used for human consumption worldwide. From a nutritional standpoint, lentils are an excellent source of micronutrients, particularly iron and zinc. Nutritional deficiencies of these elements are among the leading causes of MicroNutrient Malnutrition or "hidden hunger". The aim of this work was to use biocontrol fungi of the genus *Trichoderma* and their Secondary Metabolites (SMs) to augment the nutritional value, specifically increase iron and/or zinc content, in lentil plants. Up to 11 different strains and 3 SMs were tested. The bioformulations (strains and/or SMs) were applied under different conditions and in diverse combinations to lentil seeds and plants. The mineral content of some micronutrients (Fe, Zn, Ca, Cu and Mg) in lentil plants was determined by atomic absorption spectrometry in flame (FAAS). The combinations of *Trichoderma* seed treatments and watering to the soil of diverse strains or SMs had varying effects on lentil. Particularly regarding Fe content, we found that the best treatments were those consisting of SMs applied to the seeds and *Trichoderma* strains watered to the soil, singly or in combinations, particularly when Fe and Zn solutions were supplemented to the soil. The results obtained indicated that the application of selected *Trichoderma* strains or SMs can increase the nutritional value of foods by improving the absorption or assimilation of important minerals micronutrients, such as iron and zinc. This microbial biotechnology could be an effective strategy to overcome micronutrient deficiencies and improve human nutrition.

109. LEAF EXTRACT OF *MYRTUS COMMUNIS* AGAINST *PSEUDOMONAS SYRINGAE* pv. *ACTINIDIAE* (PSA). F. Mastrogiovanni¹, L. Gallipoli¹, M.E. Virili¹, G.M. Balestra¹, A. Tiezzi². ¹Department of Agriculture, Forests, Nature and Energy (DAFNE), University of Tuscia, Via San Camillo de Lellis s.n.c., I-01100 Viterbo, Italy. ²Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF), University of Tuscia, Via San Camillo de Lellis s.n.c., I-01100 Viterbo, Italy. E-mail: antoniot@unitus.it

Myrtus communis is a common plant used in traditional medicine for its antimicrobial and antibacterial properties. *Pseudomonas syringae* pv. *actinidiae* (Psa) is the pathogen that causes bacterial canker of *Actinidia*. Currently, Psa is the leading cause of economic losses and severe damages to kiwifruit crops in the world. One way to counteract bacterial diseases, as bacterial canker of *Actinidia*, is based on the use of natural substances that are also usable in organic farming. For this reason, the object of research was to test dried extract and molecular fractions of *M. communis*, against Psa. *Myrtus communis* leaves were collected from plants and air dried; dried leaves were reduced as dust and ethanol added for extraction for 50 min in the dark. The ethanol extract was then centrifuged,

the pellet discarded and the supernatant dried by a nitrogen flow. The residue was dissolved in a solution of DMSO 10% in distilled water to a concentration of 20 mg mL⁻¹ and filtered through a 0.22 µm cellulose syringe filter. The tested extract, *in toto*, showed activity able to contrast the Psa growth. Subsequently, the extract was processed by Thin Layer Chromatography (TLC) in order to detect which fraction was active *vs.* Psa. Each fraction singularly obtained didn't show any antibacterial activity. Thus, was showed that effectiveness towards Psa is probably attributable to a synergistic effect of the identified fractions.

110. VIRUS SURVEY AND SANITATION FOR THE RECOVERY OF ANCIENT AUTOCHTHONOUS APULIAN GRAPES. M. Morelli¹, S. Zicca², G. Bottalico², A. Campanale¹, M. Calderaro³, G. Donatelli², P. Saldarelli¹, V.N. Savino^{1,3}, P. La Notte^{1,3}. ¹National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via G. Amendola 122/D, I-70126 Bari, Italy. ²Department of Soil, Plant and Food Sciences (DiSSPA), University of Bari "Aldo Moro", Via G. Amendola 165/A, I-70126 Bari, Italy. ³Centre for Research, Experimentation and Education in Agriculture (CRSFA) "Basile Caramia", Via Cisternino 281, I-70010 Locorotondo (BA), Italy. E-mail: pierfederico.lanotte@ipsp.cnr.it

In the frame of the Apulian Regional project Re.Ge.Vi.P. ("Recovery of Apulian grape germplasm"), in which 152 ancient autochthonous grape biotypes and cultivars were identified, a survey was conducted to assess the presence and incidence of eight viruses regulated in the Italian certification system: *Grapevine virus A* (GVA), *Grapevine virus B* (GVB), *Grapevine leafroll-associated virus-1, -2, -3* (GLRaV-1, -2, -3), *Grapevine fanleaf virus* (GFLV), *Grapevine fleck virus* (GFkV) and *Arabis mosaic virus* (ArMV). Dormant canes from 80 table and wine grape accessions were ELISA-tested prior to their introduction in a repository of Apulian native germplasm. A great deal (85%) of the tested mother plants resulted positive for single or multiple infections. GLRaV-3 was the most represented virus (71.3%), followed by GFkV (45%) and GVA (42.5%). The presence of infectious degeneration agents was limited to GFLV (28.8%). GLRaV-1 was detected in 22.5% of the vines, whereas other viruses had either a low incidence (GLRaV-2) or were absent (GVB, ArMV). Infected accessions were submitted to sanitation, through *in vitro* meristem tip culture, in combination with heat therapy. To test the efficacy of virus elimination, a pool of 119 plantlets, deriving from micropropagation of treated explants was analysed by RT-PCR. Successful virus elimination occurred in 79% of the sanitized plants. GFLV and GFkV, which were both still present in eight treated explants, and GVA, were the viruses most recalcitrant to eradication. Results of this study allowed the addition of a number of minor autochthonous grape cultivars to the Apulian grapevine germplasm collection, thus contributing to the conservation of the hitherto neglected agro-biodiversity of Apulia.

111. SCREENING *CAPSICUM* spp. FOR TOLERANCE TO A RESISTANCE-BREAKING STRAIN OF TOMATO SPOTTED WILT VIRUS BY ARTIFICIAL INOCULATION. M. Parisi¹, F. Di Dato¹, M. Minutolo², G. Festa¹, D. Alioto². ¹Agricultural Research Council – Experimental Institute for Horticulture (CRA-ORT), Via Cavallleggeri 25, I-84098 Pontecagnano Faiano (SA), Italy. ²Department of Agricultural Sciences, University of Napoli "Federico II", Via Università 100, I-80055 Portici (NA), Italy. E-mail: mario.parsi@entecra.it

Pepper (*Capsicum annuum*) and other *Capsicum* species are severely affected by *Tomato spotted wilt virus* (TSWV). The highly polyphagous nature of TSWV and the efficiency of transmission

by thrips (*Frankliniella occidentalis*) lead to a severe spread of the virus. The most effective strategy for controlling the virus damages is the use of durable resistance genes. However, the frequent appearance of Resistance-Breaking (RB) strains that overcome the resistance conferred by *Tsw* gene, makes search of new viral resistance/tolerance sources necessary. In this work, an isolate of TSWV isolated from a commercial resistant pepper hybrid in Sele Valley, was biologically characterized and identified as able to break the genetic resistance. This RB-TSWV was mechanically inoculated in greenhouse conditions onto 26 accessions of *Capsicum* spp. belonging to *C. baccatum* var. *pendulum* (20 accessions), *C. annuum* (3), *C. chinense* (2) and *C. frutescens* (1). Percent of plants showing systemic infection was very high already 15 days post inoculation for all the tested accessions except for CAPBACP04 that showed a low incidence of infected plants (20%) up to 30 days after inoculation. The ELISA test confirmed this trend. Healthy plants of CAPBACP04 have been self pollinated in greenhouse and the resulting progenies mechanically inoculated with the RB-TSWV isolate. Five out of six progenies pointed out a high incidence of infected plants while the CAPBACP04-R2 progenies showed only 22% of infected plants. In conclusion, CAPBACP04 and CAPBACP04-R2 accessions could be source of novel resistance genes. Different screenings on these genotypes are currently in progress.

112. USE OF TRAMETANO® FOR ELICITING DEFENCE REACTIONS IN WHEAT AND MAIZE PLANTS AGAINST FUNGAL PATHOGENS. C. Pietricola¹, A. Iori², V. Farina¹, V. Scala¹, M. Scarpari¹, M. Scrosati³, F. Quaranta², M. Fornara², M. Reverberi¹, C. Fanelli¹. ¹Department of Environmental Biology, Sapienza University of Roma, Piazzale Aldo Moro 5, I-00185 Roma, Italy. ²Agricultural Research Council – Research Unit for Cereal Quality (CRA-QCE), Via Cassia 132, I-00100 Roma, Italy. ³Agricola 2000 S.c.p.A., Via Trieste 9, I-20067 Tribiano (MI), Italy. E-mail: chiara.pietricola@uniroma1.it

Durum and common wheat and maize are amongst the most cultivated cereals worldwide. They are the base of human and animal food and used in livestock industry. Up to 20-30% of worldwide cereals production is wasted because of foliar diseases due to fungi such as *Zymoseptoria tritici* and *Parastagonospora nodorum*. Another important aspect to consider is the contamination of cereals with mycotoxins. These substances are secondary metabolites produced by fungi that can have several toxic effects in both humans and animals. *Aspergillus flavus* and *Fusarium verticillioides*, mycotoxins producers, are the main cause of maize diseases. Phytochemicals treatment in field can partially control these diseases but generating pollution and health hazards. Moreover, starting from 2014 EC has banned several pesticides (EC/129/2009) posing severe constraint to cereal farmers for using such products. If confirmed, the application of this directory will worsen the current situation concerning the wheat production leading to concrete and severe losses. The aim of our study is to exploit the eliciting aspect of Trametano®, an exo-polysaccharide produced by the edible mushroom *Trametes versicolor*, for priming the defences of wheat and maize against pathogens of these cereals.

113. FIELD EFFICACY OF SOME PRODUCTS AGAINST THE BACTERIAL CANKER OF KIWIFRUIT. M. Preti¹, M. Scannavini¹, F. Franceschelli¹, F. Cavazza¹, L. Antoniacci², R. Rossi², R. Bugiani², M.G. Tommasini³, M. Collina⁴. ¹ASTRA Innovazione e Sviluppo, Via Tebano 45, I-48018 Faenza (RA), Italy. ²Emilia-Romagna Regional Plant Protection Service, Via di Saliceto 81, I-40128 Bologna, Italy. ³Centro Ricerche Produzioni Vegetali (CRPV), Via dell'Arrigoni 120, I-47522 Cesena (FC), Italy. ⁴Department of

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Pseudomonas syringae pv. *actinidiae* (Psa) is the causal agent of the bacterial canker on *Actinidia* spp. This pathogen was first recorded in Japan and in China. It was first found in Italy in 1992, but only since 2008, Psa has spread with devastating virulence in all kiwifruit producing countries such as Italy, Chile and New Zealand. In this work are presented the results of 4 trials conducted in open field conditions in Emilia-Romagna Region to verify the efficacy of different active ingredients. The field trials confirmed, as already obtained in previous studies, the efficacy of copper-based products and of acibenzolar-S-methyl (Bion® 50 WG). Interesting results have also been achieved by a formulation of polyglucosamine (Hendophyt®ps) and a formulation which combines the action of polyglucosamine with copper and boron (Kodens Cu®). The inorganic compound LMA – based on aluminium potassium sulphate dodecahydrate – confirmed in field the good results showed *in vitro* and in greenhouse assays. To date one field trial is in progress to evaluate the effectiveness of different control strategies based on copper compounds and acibenzolar-S-methyl, verifying the importance of autumn-winter applications in containing the outbreaks of Psa infections.

114. COMPOST APPLIED TO SUBSTRATES INDUCE RESISTANCE IN LAMB'S LETTUCE AGAINST PHOMA VALERIANELLAE IN SOILLESS CONDITIONS. M. Pugliese^{1,2}, G. Gilardi¹, M.L. Gullino^{1,2}, A. Garibaldi¹. ¹Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: massimo.pugliese@unito.it

Phoma valerianellae is causing increasing crop losses on lamb's lettuce (*Valerianella olitoria*). The efficacy of compost to control the pathogen has been evaluated under soilless conditions. The following combinations have been tested: 20% compost mixed with a growing media (50% peat + 50% perlite); 20% compost mixed with a growing media in combination with 2 or 3 applications of a bacterial mix (*Pseudomonas* sp. strains 7B, 8B and 9B) in the nutrient solution. All the treatments had a significant effect on the number of leaf spots caused by *P. valerianellae* in the six trials, and the percentage of infected leaves was reduced from 31 up to 94% compared to the control. The best control was obtained using three applications of the bacterial mix, around the base of lamb's lettuce, growing in a substrate containing 20% compost. The *Pseudomonas* sp. strains and the compost together constitute a promising strategy to control leaf spots caused by *P. valerianellae* on *V. olitoria* as it induces resistance to the pathogen.

115. THE GROWING MEDIA “HORTOFAN” FOR SUPPRESSING SOIL-BORNE PATHOGENS ON POTTED PLANTS. M. Pugliese^{1,2,3}, M. Marengo¹, M.L. Gullino^{1,2,3}, A. Garibaldi^{1,3}. ¹AgriNewTech s.r.l., Via G. Quarello 15/A, I-10135 Torino, Italy. ²Centre of Competence for the Innovation in the Agro-Environmental Field – AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. ³Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. E-mail: massimo.pugliese@unito.it

Compost-based growing media are expected to suppress plant diseases, according to the type of wastes, the composting process, the

chemical and microbiological composition. The aim of this research was to evaluate the suppressiveness of the growing media "Hortofan", which is made of 20% v/v suppressive compost "Ant's Compost", peat and pumice. Suppressiveness was tested in greenhouse on potted plants against *Pythium ultimum* on cucumber, *Phytophthora nicotianae* and *Fusarium oxysporum* f. sp. *lycopersici* on tomato. Pathogens were mixed into the substrate at 1 g of biomass on wheat kernels L⁻¹ 7 days before seeding or with chlamydo-spores talc at 1×10⁴ cfu g⁻¹ of substrate. Seeds of cucumber and tomato were sown into 2 L pots in greenhouse and five pots were used for each treatment. A peat based commercial substrate was used as control. The number of alive plants and above ground biomass were measured 20-30 days after seeding. Cucumber and tomato biomass significantly increased up to 40-50% in "Hortofan" compared to control. The number of diseased tomato plants in substrates inoculated with *P. nicotianae* was significantly reduced by 40% and the number of cucumber plants by 30% compared to the peat substrate. Fusarium wilt of tomato was reduced by 60% in plants grown on with "Hortofan".

116. CERCOSPORA BETICOLA SENSITIVITY TO FUNGICIDES IN ITALY. C. Turan, G. Battistini, A. Brunelli, M. Collina. Department of Agricultural Sciences (DipSA), University of Bologna, Viale G. Fanin 46, I-40127 Bologna, Italy. E-mail: marina.collina@unibo.it

Commercial mixtures of difenoconazole, fenpropidin, tetraconazole, trifloxystrobin and azoxystrobin are the most frequent fungicides used in Italy to control Cercospora Leaf Spot (CLS), caused by the fungus *Cercospora beticola*. Recently, chlorothalonil and thiophanate-methyl have been introduced to the market for CLS control. This study was aimed to evaluate the sensitivity of *C. beticola* isolates collected from Northern Italy to difenoconazole, tetraconazole, prochloraz, trifloxystrobin and thiophanate-methyl. About 200 isolates were obtained from experimental plots, commercial fields and a garden in September 2012 and 2013. Conidial germination tests were carried out using trifloxystrobin as technical grade and commercial formulation. Technical grade of all other fungicides were tested for mycelial growth inhibition. All the isolates were found highly sensitive to thiophanate-methyl, showing mean EC₅₀ from 0.03 to 0.37 mg L⁻¹. Sensitivity tests indicated that QoI resistance developed in all isolates with EC₅₀ > 100 mg L⁻¹ except in that coming from the garden (EC₅₀ 0.17 mg L⁻¹). As for DMI, some cases of decreased sensitivity were observed. However, this decreased sensitivity had lower intensity with respect to strobilurins. For samples coming from experimental and commercial field, difenoconazole, prochloraz and tetraconazole showed EC₅₀ values ranging from 0.13 to 5.65 mg L⁻¹, from 0.8 to 4.69 mg L⁻¹ and from 0.78 to 6.23 mg L⁻¹, respectively. All isolates sampled from the garden presented high sensitivity to DMI (mean EC₅₀ from 0.13 to 0.7 mg L⁻¹).

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