ORAL PRESENTATIONS

BENEFICIAL MICROBE–PATHOGEN-PLANT INTERACTIONS: THE RECOGNIZED ROLE OF SECONDARY METABOLITES. F. Vinale[1*], M. Ruocco[1], R. Marra[1,2], N. Lombardi[1,2], A. Pascale[1,2], S. Lanzuise[1,2], R. Varlese[2], G. Manganiello[2], D. Stellitano[1], F. Zecca[3], S.L. Woo[1,2], M. Lorito[1,2]. *[1]CNR – Istituto per la Protezione Sostenibile delle Piante, Via Università 133, 80055 Portici, Italy. [2]Dipartimento di Agraria, Università degli Studi di Napoli 'Federico II', Via Università 100, 80055, Portici, Italy.[3]Dipartimento di Management, Università di Roma "La Sapienza", Via del Castro Laurenziano 9 - 00161 Roma. E-mail: f.vinale@ipp.cnr.it*

Microbial secondary metabolites are chemically different natural compounds of low molecular weight produced during processes of competition with other micro- and macro-organisms, symbiosis, parasitism or pathogenesis. Beneficial microbes typically produce bioactive molecules involved in the interactions with plant and pathogens. Some secondary metabolites with antibiotic activity have been associated to the biological control of plant pathogens and pests. The production of secondary metabolites depends on: i) the compound considered; ii) the species and the strain; iii) the occurrence of other microorganisms; iv) the equilibrium between elicited biosynthesis and biotransformation rate; and v) the growth conditions. In addition to direct toxic activity against phytopathogens, biocontrol-related metabolites may also increase disease resistance, and/or enhance root, stem, shoot and leaf vegetative growth.

Metabolomic analysis of the interactions between plants, fungal phytopathogens and beneficial microbes has aided in the identification of new bioactive metabolites that positively affect plant metabolism. Recently, we have found a new *Trichoderma* polyketide, named cremenolide, with strong antifungal activity. This compound alone or in combination with other known metabolites (i.e. 6-penthyl-apyrone or harzianic acid) improve growth and/or health of different plant species. We have effectively tested several *Trichoderma* metabolites from *in vitro* conditions all the way to the field, and developed new active ingredients for use as biostimulators and disease inhibitors, both alone and in combination with the living beneficial microbes. Agrobiochemicals of this new class should be available on the market in few years and become very useful IPM tools.

FIMICOLOUS FUNGI: AN UNDEREXPLORED RESERVOIR OF NEW COMPOUNDS TO BE EMPLOYED FOR THE CONTROL OF PLANT PATHOGENS. S. Sarrocco[1], S. Diquattro[1], F. Avolio[2], A. Cimmino[2], A. Andolfi[2], G. Puntoni[1], F. Doveri[1], A. Evidente[2], G. Vannacci[1]. *[1] Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, Università di Pisa, Via del Borghetto 80 - 56124 Pisa, Italy. [2] Dipartimento di Scienze Chimiche, Università degli Studi di Napoli Federico II, Via Cintia 4 - 80126 Napoli, Italy. E-mail: sabrina.sarrocco@unipi.it*

Herbivorous mammals dung support a large variety of fimicolous fungi and the number of new genera and species is constantly increasing. Dung is a complex ecosystem and to win the struggle for life, these fungi produce a plethora of bioactive secondary metabolites to compete with other organisms. Fimicolous fungi and their bioactive metabolites are mostly evaluated for a possible use in medicine. Very few information are available about their possible exploitation in agriculture against plant pathogens.

Five coprophilous (*Auxarthron umbrinum*, *A. concentricum*, *Cleistothelebolus nipigonensis*, *Neogymnomyces virgineus* and *Rodentomyces reticulatus*) Italian isolates collected from dung of different herbivores, were investigated. "*In vitro*" antibiosis tests showed positive results against several fungal plant pathogens on different growth media. A solid culture on rye flour was extracted by methanol-water (5% sodium chloride) and this latter, successively, with n-hexane and dichloromethane. Extracts and the corresponding aqueous fractions were tested on PDA against eight plant pathogenic fungi. A very good inhibitory activity of some *C. nipigonensis* and *N. virgineus* extracts was recorded and further fractions obtained by column chromatography were successfully tested against *Alternaria* sp., *Botrytis cinerea*, *Fusarium graminearum* and *Sclerotinia sclerotiorum*. A further chromatography of the selected bioactive fractions will allow to detect the compounds responsible of this activity and to determine their structures.

Data here obtained suggest the possibility to discover novel metabolites to be used in the management of plant disease and highlight how fimiculous fungi could represent a marvellous unexplored reservoir of new bioactive compounds.

MINING THE GENOME OF *LYSOBACTER CAPSICI* AZ78 TO IDENTIFY THE MECHANISMS INVOLVED IN THE BIOLOGICAL CONTROL OF *PHYTOPHTHORA INFESTANS* AND *PLASMOPARA VITICOLA*. G. Puopolo[1], P. Sonego[2], K. Engelen[2], I. Pertot[1]. *[1]Department of Sustainable Agro-Ecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, 38010 S. Michele all'Adige (TN), Italy. [2]Department of Computational Biology, Research and Innovation Centre, Fondazione Edmund Mach, 38010 S. Michele all'Adige (TN), Italy. E-mail: gerardo.puopolo@fmach.it*

The use of biological control microorganisms to protect plants against pathogenic oomycetes may contribute to make crop production more sustainable. Recently, we showed that *Lysobacter capsici* AZ78 effectively controls grapevine downy mildew and tomato late blight incited by *Plasmopara viticola* and *Phytophthora infestans*, respectively. Furthermore, we proved that this bacterial strain can be combined with copper based fungicides for controlling both the plant pathogenic oomycetes. In order to investigate the mechanisms that can be involved in the antagonism of *L. capsici* AZ78, the genome of this bacterium was sequenced through Illumina GAIIX system.

The *L. capsici* AZ78 draft genome is constituted of 6.3 Mb with a 66.43% GC content. Mining the annotated *L. capsici* AZ78 genome showed the presence of genes coding for chitinases, glucanases, lipases, xilanases and a high amount of proteases, lytic enzymes that can be potentially involved in the control of plant pathogenic microorganisms. Furthermore, the analysis revealed also that *L. capsici* AZ78 genome contains genes responsible for the production of non-ribosomally synthesized peptides with antibiotic activity. Interestingly, *L. capsici* AZ78 genome encompasses genes involved in resistance to drugs and heavy metals as cadmium, cobalt, copper and zinc.

The availability of the whole genome of *L. capsici* AZ78 represents a first step in unravelling the biology of the biological control bacteria belonging to the genus *Lysobacter* and, moreover, will provide the basis for analysing the interaction between this biocontrol bacterium and plant pathogenic oomycetes.

BIOCONTROL ACTIVITY OF A NOVEL ENDOCHITINASE FROM *METSCHNIKOWIA FRUCTICOLA* EXPRESSED IN *PICHIA PASTORIS* AGAINST POSTHARVEST PATHOGENS OF FRUITS. H. Banani, D. Spadaro, M.L. Gullino, A. Garibaldi. *AGROINNOVA – Centro di Competenza per l'Innovazione in Campo Agroambientale, Università di Torino, Largo Paolo Braccini 2* (ex- *Via L. da Vinci 44), Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it*

Metschnikowia fructicola strain AP47 was isolated from the carposphere of temperate fruits and showed a high efficacy in controlling *Monilinia* spp. on stone fruits, however its mechanism against postharvest pathogens is still unclear.

M. fructicola AP47 was able to produce high chitinase activity in the presence of *Monilinia* spp. cell wall. A novel endochitinase gene *MfChi* (GenBank n° HQ113461) was amplified from the DNA and cDNA of *M. fructicola* AP47. 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 characterized.

Mfchi gene expression was significantly elevated in response to *Monilinia* spp. cell wall, suggesting a MfChi chitinase role in the antagonistic activity of the yeast.

Therefore, *MfChi* gene was transferred into *Pichia pastoris* KM71 for high heterologous expression. The antifungal activity of the recombinant chitinase was investigated against *Monilinia fructicola* and *Monilinia laxa in vitro* and on peaches cv. Redhaven. The recombinant chitinase MfChi reduced significantly spore germination and germ tube length of the tested pathogens in PDB medium and successfully reduced rot diameter on peaches. However, the efficacy was dependent on the enzyme concentration and the length of fruit storage.This study provided the direct evidence that extracellular chitinase Mfchi secreted by *Metschnikowia fructicola* AP47 plays an important role in the biocontrol activity against some postharvest pathogens of peaches, therefore MfChi could be developed as an efficient postharvest treatment with antimicrobial activity for fruits undergoing a short shelf life.

PRODUCTION OF VOLATILE ORGANIC COMPOUNDS FROM *AUREOBASIDIUM PULLULANS* STRAINS AS A MECHANISM OF ACTION AGAINST POSTHARVEST PATHOGENS. A. Di Francesco[1], L. Ugolini[2], C. Martini[1], M. Mari[1]. *[1]CRIOF, University of Bologna, Via Gandolfi, 19 40057, Cadriano, Bologna (Italy). [2]CRA-CIN, Consiglio per la Ricerca e la Sperimentazione in Agricoltura-Centro di ricerca per le Colture Industriali, Via di Corticella 133, 40128, Bologna (Italy). E-mail: marta.mari@unibo.it*

Aureobasidium pullulans L1 and L8 strains were previously tested for their antagonistic activity against some postharvest pathogens. In order to study the mechanisms of action of L1 and L8 strains, the production of volatile organic compounds (VOCs) was evaluated in the growth suppression of *Penicillium expansum*, *Botrytis cinerea* and *Colletotrichum acutatum* on apple fruit, *Penicillium italicum* and *Penicillium digitatum* on orange fruit. VOCs production was verified by *in vitro* and *in vivo* trials against the pathogens listed above and their characterization was made by SPME GC-MS. Dual cultures results showed that VOCs emitted by *A. pullulans* strains inhibited completely the conidia germination of *Penicillium* spp. while conidia germination of *B. cinerea* and *C. acutatum* was controlled at 60% and 50% for L1 and L8 respectively. *In vivo* tests were performed on apples inoculated with *P. expansum*, *B. cinerea* and *C. acutatum* and oranges with *P. italicum* and *P. digitatum.* Fruit artificially inoculated were placed in glass boxes saturated 48 h before, with L1 and L8 VOCs and stored for a week. The results showed a high reduction (> 60%) of decays caused by all three *Pencillium* spp. While a lower efficacy of VOCs was observed against *B. cinerea* and *C. acutatum* (<50%). A total of 11 yeast VOCs was identified by SPME GC-MS. The most representative molecules were 2-phenylethanol, 3-methyl-1-butanol, 2-methyl-1-butanol and 2-methyl-1-propanol. Furthermore they tested to find relatives ED_{50} values. This study suggests that VOCs emission by L1 and L8 could be considered a mode of action of these antagonists; however, more investigations are required to better understand the real involvement of the compounds in the inhibition of pathogens.

POSTHARVEST TREATMENTS WITH RESISTANCE INDUCERS STIMULATE THE EXPRESSION OF DEFENCE GENES IN STRAWBERRY FRUITS. L. Landi, E. Feliziani, G. Romanazzi. *Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche, 60131 Ancona. E-mail g.romanazzi@univpm.it*

The application of resistance inducers controls postharvest decay of strawberry and promotes physiological changes in host tissues. The RT-qPCR was used to analyze the expression of 18 plant defense genes in strawberry fruit treated with elicitors: chitosan, benzothiadiazole (BTH), and calcium plus organic acids (COA), at 0.5, 6, 24 and 48 h post treatment. These genes were differentially up-regulated, according to elicitor. Expression of a K^+ channel gene increased in response to all tested elicitors, while expression of a calcium-dependent protein kinase gene increased only after BTH treatment. Chitosan and COA treatments promoted expression of key phenylpropanoid pathway genes, for synthesis of lignin and flavonoids; only those associated with flavonoid metabolism were up-regulated by BTH. For antioxidative systems, the ascorbate peroxidase and glutathione-S-transferase genes were up-regulated by BTH. Several genes related to cell-wall degradation, including those for polygalacturonase, polygalacturonase-inhibiting protein, and b-1,3-glucanase, were similarly enhanced after all resistance inducer treatments, while endo-b 1,4-glucanse gene expression increased only with BTH treatment. Class III chitinase and pathogen-related protein-1 genes were up-regulated using chitosan and COA. The enzyme activities of phenylalanine ammonia lyase, b-1,3-glucanase, and guaiacol peroxidase correlated with gene expression, while that of chitinase increased with all tested elicitors. Similarity of gene expression was >72% between chitosan and COA treatments, starting from 6 h post treatments, while BTH showed lower similarity (38%) with the other elicitors. These resistance inducer-strawberry fruit interactions led to up-regulation of different gene expression pathways. This study provides a useful resource to identify specific elicitor-responsive genes.

***COLLETOTRICHUM ACUTATUM* SPECIES COMPLEX: LINKING BIOLOGY, EVOLUTION AND GENOMICS. R. Baroncelli[1,2], S. Sreenivasaprasad[3], S. Sukno[1], E. Holub[2], M. Thon[1].** *[1]Centro Hispano-Luso de Investigaciones Agrarias (CIALE), Departamento de Microbiología y Genética, Universidad de Salamanca, C/ Río Duero 12, Villamayor, 37185 Salamanca, Spain. [2]School of Life Sciences, University of Warwick, Coventry, CV4 7AL. United Kingdom. [3]University of Bedfordshire, Park Square, Luton, Bedfordshire LU1 3JU, United Kingdom. E-mail: riccardobaroncelli@gmail.com*

Colletotrichum acutatum sensu lato includes a number of important pathogens that cause economically significant losses of various crops. *C. acutatum* has a wide host range in both domesticated and wild plant species, and its capability to infect different types of hosts such as insects has also been described. Members belonging to this complex are able to develop three different types of interaction with plant hosts including biotrophic, necrotrophic and hemibiotrophic infections and are also capable of surviving on weeds and non-hosts without causing visible symptoms. They are mainly asexual, but some have a teleomorphic state called *Glomerella* and can be either homothallic or heterothallic. The wide host range and the different lifestyles (necrotrophic, biotrophic, hemibiotrophic and quiescent) suggest *C. acutatum* is a suitable system for studying evolution, speciation process and host association through whole genome comparisons. Four representative isolates has been sequenced, assembled and annotated. The isolates were chosen based on its wide host range and the phylogeographic position. Resulting data have been used for a wider *Colletotrichum* comparative genomics with the aim to investigate gene family expansions in non-specificity host. The data suggest an interesting expansion of

several gene families, such as those encoding carbohydrate-active enzymes, peptidases, secondary metabolites pathways and genus-specific effectors that could be associated with the host range. The new knowledge and resources developed with the genome analyses along with the results of the population level diversity studies provide a platform for functional genomics investigations to advance this research.

RELATIONS BETWEEN THE CLIMATE AND THE INCIDENCE OF THE NUT ROT OF CHESTNUTS CAUSED BY *GNOMONIOPSIS CASTANEA*. L. Giordano, G. Lione, F. Sillo, P. Gonthier. *Department of Agricultural, Forest and Food Sciences, University of Torino, Grugliasco, Italy. E-mail: paolo.gonthier@unito.it*

Gnomoniopsis castanea is the causal agent of an emerging nut rot on chestnut trees. A Partial Least Squares Regression (PLSR) analysis was performed to model the incidence of this fungal pathogen based on climate. The analysis involved four steps: I) assessment of the disease incidence, II) pre-selection of predictors, III) models fitting, IV) external validation.

I) In 2011 the incidence of *G. castanea* was assessed as percentage of infected nuts in 12 sites located in the north-west of Italy. Both isolations and molecular analyses revealed that disease incidence ranged from 20% to 93%.
II) Geostatistical analyses showed that, despite the geographical clustering of sites ($P < 0.05$), the incidence of *G. castanea* was not spatially autocorrelated ($P > 0.05$). This finding suggests that site-dependent factors may influence the disease. A Principal Coordinates Analysis (PCoA) and a Hierarchical Cluster Analysis (HCA) on maximum, mean and minimum temperatures and on rainfalls showed that warmer temperatures were associated to a significant increase of disease incidence (+10.4%; $P < 0.05$).
III) The temperatures of the months before nut harvesting were selected as predictors in the PLSR models. Bootstrap analyses and cross-validation were performed for models selection.
IV) External validation carried out on data collected from additional sites showed the good predictive abilities of the models ($\rho > 0.70$; $P < 0.05$).

All the above findings indicate that the climate and the incidence of *G. castanea* are related, and that PLSR equation provide a useful statistical tool to forecast the incidence of the disease at site level.

INCUBATION TIME, TEMPERATURE AND WATER ACTIVITY EFFECTS ON *ASPERGILLUS CARBONARIUS* GERMINATION ON GRAPE SKIN AND FLESH. M. Camardo Leggieri[1], P. Battilani[1], D. Mitchell[2], R. Withe[3], D. Aldred[2], N. Magan[2]. *[1]Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84 – 291224- Piacenza, Italy. [2]Applied Mycology Group, Cranfield Soil and Agrifood Institute, Cranfield University, Bedford MK43 0AL, United Kingdom and [3]Rothamsted Research, Harpenden, Herts, AL5 2JQ, United Kingdom. E-mail: paola.battilani@unicatt.it*

Aspergillus carbonarius is confirmed as the main species responsible for ochratoxin A (OTA) in grapes and products based on vine fruits. Several studies have provided information on the ecology of *A. carbonarius*, in particular growth and OTA production. To date, no detailed studies have been addressed to quantify the germination rate on grape skin and flesh. The objective of this study was to compare the ability of *A. carbonarius* spores to germinate on grape skin and flesh in relation to temperature (15-40°C) and relative humidity (85-100% RH), in time course studies (4-36 hours). A spore suspension of *A. carbonarius* (10^6 spores ml^{-1}) was inoculated onto the skin and on the flesh of cut berries of white organic grapes. For comparison, spores were spread plated onto an artificial grape juice medium. Time to 5% germination was significantly shorter on grape flesh than *in vitro* and on grape skin (6 *vs* 9 *vs* 24 hours in optimal conditions). Fifty percent germination was reached on grape skin at 25-30°C and RH ≥ 90% and on grape flesh in almost all the tested conditions. This suggests that damaged skin may be the main pathway which is conducive to *A. carbonarius* spore germination providing an easier and rapid infection route. Data on the combined effect of temperature and a_w on spore germination were fitted with Bete and polynomial equations providing a good fit of the biological processes ($R^2 \geq 0.98$). These functions can contribute in a predictive model to estimate the risk of OTA occurrence in grapes.

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AFLATOXIN CONTROL IN FEED BY *TRAMETES VERSICOLOR*. M. Scarpari[2], M. Reverberi[2], C. Fanelli[2], A. Fabbri[2], C. Bello[1], C. Dall'Asta[3], F. Righi[3], A. Angelucci[1], L. Bertocchi[1]. *[1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Ubertini", via Bianchi 9, 25124 Brescia. [2]Dipartimento di Biologia Ambientale, Sapienza Università di Roma, Largo Cristina di Svezia 24, 00165 Roma. [3]Dipartimento di Scienze degli Alimenti, Università di Parma, Parco Area delle Scienze, 59/A.*

Aspergillus flavus are well known widely diffused fungi able to contaminate, already in the field, food commodities like seeds. Once the crop is contaminated, these fungi can develop and produce aflatoxins, secondary metabolites which are carcinogenic, teratogenic and mutagenic for animals and humans. These mycotoxins can enter the human food chain by the direct ingestion of contaminated seeds or processed food and by the consumption of animal products coming from livestock fed with contaminated silages. The requirement of products with low impact on the environment and on human health, able to control aflatoxin production, has increased. Several papers report the use of extracts from fungi to inhibit fungal development and mycotoxin production. In this work the effect of bioactive compounds produced by the basidiomycete fungus *T. versicolor* on the aflatoxin production by *A. flavus* both in vitro and in maize, was investigated. The goal was to propose an eco-friendly tool for a significant control of aflatoxin production, in order to obtain feedstuffs and feeds with a high standard of quality and safety to enhance the wellbeing of dairy cows. The presence of *T. versicolor*, grown on sugar beet pulp, was able to inhibit the production of aflatoxin B1 in maize by *A. flavus*. Furthermore, treatment with culture filtrates of *T. versicolor* containing ligninolytic enzymes, showed a significant reduction of the content of aflatoxin B1 in contaminated maize. Moreover, treated and control maize samples were also compared under in vitro ruminal digestive condition to simulate the possible releasing of aflatoxins upon cow's digestion. Finally, the effect of the bioactive compounds has been verified in vivo. Feed, contaminated with aflatoxin B1 and treated with *T. versicolor* has been administered to dairy cows and the treatment effect assessed by examining carry-over of aflatoxin B1 to Aflatoxin M1 in milk.

TWO *FUSARIUM CULMORUM* GENES INVOLVED IN FOOT AND ROOT ROT AND HEAD BLIGHT PATHOGENICITY ON DURUM WHEAT. F. Spanu[1], I. Camboni[1], B. Scherm[1], V. Balmas[1], M. Pasquali[2], Q. Migheli[1]. *[1]Dipartimento di Agraria and Unità di ricerca Istituto Nazionale di Biostrutture*

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Fusarium culmorum is a cereal pathogen with widespread distribution, able to produce type B trichothecene mycotoxins. Knowledge on pathogenicity factors of this fungus is very limited. A transposon tagging approach based on the *mimp1/impala* double component system has allowed us to select mutants altered in their metabolic or morphological processes and/or impaired in their aggressiveness. *In vitro* bioassays were carried out to identify altered phenotypic characters in putative mutants growing on potato dextrose agar amended with 2 M sorbitol, 1 M NaCl (osmotic stress), 30 mM potassium persulphate (oxidative stress), 0.02% sodium dodecylsulphate and 0.5 ppm tebuconazole. An *in vitro* pathogenicity assay was performed by placing one durum wheat seed onto each one of ten mycelium plugs in a Petri dish and incubating 3-6 days in the dark at 25°C. To confirm the result obtained *in vitro*, *in planta* assays were performed in greenhouse and field conditions. Two *F. culmorum* mutants (coded R38 and R386) were selected for altered phenotypic characters, including metabolic or morphological processes and complete loss of pathogenicity in both foot and root rot (FRR) and head blight (FHB). The flanking regions of *mimp1* were used to seek homologies in the *F. graminearum* database. In R38, the *mimp1* reinsertion was inside an hypothetical gene with orthologs only in the fungal domain, while in the R386 a conserved hypothetical protein localized in the cytoplasm and endoplasmic reticulum was identified. These are the first two *mimp1*-tagged genes involved in both FRR and FHB pathogenicity.

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TRICHODERMA HARZIANUM 6776 ON TOMATO: A SINGLE BENEFICIAL ISOLATE FOR TWO CULTIVATION SYSTEMS.

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Trichoderma harzianum strain T6776 is a potential beneficial isolate to be employed as inoculant of tomato plants since it can promote plant growth and can colonize roots as endophyte. In addition, its ability to protect plants against soil-borne and air-borne pathogens, such as *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *radicis-lycopersici* and *Alternaria solani* has been documented.

In order to study the interaction between this isolate and tomato plants (cv MicroTom) we evaluated the beneficial effects of T6776 in two cultivation systems: a peat based tomato growth substrate inoculated by a pre-fermented fungal biomass and in soilless condition, inoculated by a spore suspension. Different physiological and biochemical parameters have been measured such as growth rate, carbohydrate source-sink portioning, photochemical efficiency of photosystem II, pigment composition, photochemical and non-photochemical quenching of the chlorophyll fluorescence and hormonal status of different treated plants in both cultural conditions. Moreover, we also evaluated the effects of T6776 on PSII under the abiotic stress conditions of salinity and anoxia.

This study shows that in both culture conditions T6776 is able to positively affect some of the selected physiological and biochemical parameters in treated plants, in comparison with the control. Some of these parameters, like hormonal status, are potentially involved in the induction of systemic resistance. Under abiotic stress conditions, *T. harzianum* T6776 positively influenced plants response, even if more studies are needed to better characterize the associated mechanisms.

Moreover, to better understand the genetic potential of *T. harzianum* 6776, its genome has been recently sequenced and it's now under annotation.

NETWORKING BY STRESS SIGNALLING PATHWAYS: IDENTIFICATION OF NOVEL REGULATORS OF COMBINATORIAL STRESS TOLERANCE.

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Plant responses to multiple biotic/abiotic stresses employ finely tuned regulatory mechanisms, largely orchestrated by phytohormones. In particular, salicylic acid (SA) plays a central role in defense responses against biotrophic and hemi-biotrophic pathogens, whereas jasmonic acid (JA) and ethylene (ET) are usually associated with defense against necrotrophic pathogens and herbivorous insects. In addition, abscisic acid (ABA) is the main signalling molecule of abiotic stress responses. To coordinate the complex interactions occurring during multiple stresses, an intense cross-talk among the regulatory networks is necessary.

To obtain new insights into the selective capacity of plants to adapt to combinatorial stresses, their response to multiple phytohormones was explored. In particular, the effect of ABA, SA, ET on JA responses was analyzed using a collection of 360 *Arabidopsis thaliana* accessions. This collection represents a population of globally collected plants that have been genotyped for 250000 SNPs, allowing for genome-wide association studies. The obtained data, reflecting a great natural variation in response to the mimicked combinatorial stresses were used to identify novel genes playing roles in the adaptation of plants to different stress conditions.

ADVANTAGES AND LIMITATIONS OF USING NGS IN PLANT VIRUS DIAGNOSIS: A CASE STUDY OF FOUR CV PRIMITIVO CLONES.

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The pace of advancement of Next Generation Sequencing (NGS) applied to plant virus diagnosis and detection raises several issues on the acceptance of the technology in crop productions. Advantages of its adoption are undisputable since it relies on an unbiased approach which theoretically allows the detection of all viruses and viroids infecting a plant sample, and the discovery of variants of known or unknown pathogens. The approach, which overcomes the drawbacks of molecular and serological diagnosis, besides being attractive in certification programs, discloses several issues, regarding the adoption of standardized protocols and, particularly for woody perennial plants frequently showing multiple infections, the attitude to assume with "background" viruses not clearly associated to a disease.

We tested a NGS diagnostic approach on four virus-infected cv Primitivo clones and the corresponding heathy offspring obtained after sanitation (3 clones under certification and 1 clone already certified). Small RNAs were purified and eight different libraries o were constructed according to the Illumina small RNA protocol and deep-sequenced on HiScanSQ machine in two separate runs of 50bp. Analysis of data, using an optimized bioinformatic pipeline, confirmed the degraded sanitary status of 3 out of 4 clones, since sequences homologous to several viruses and viroids were found, few of them listed in the Italian protocol for the production of certified plant propagation material. After sanitation, three out of four clones were still infected by two viroids and one by *Grapevine rupestris stem pitting-associated virus.*

Comparative results with RT-PCR and implications with current diagnostic techniques will be discussed.

SNF1 PROTEIN KINASE IS IMPORTANT FOR GROWTH AND FULL VIRULENCE OF *BOTRYTIS CINEREA*. Sz. Lengyel[1,2], C. Rascle[2], L. Sella[1], F. Favaron[1], M. Choquer[2]. *[1]Department TeSAF, University of Padova, viale dell'Università 16, 35020 Legnaro (PD), Italy. [2]Laboratoire mixte de génomique fonctionnelle des champignons phytopathogènes, Université Lyon 1/Cnrs/Bayer CropScience, Lyon, France. E-mail: szabina.lengyel@studenti.unipd.it*

At early stages of infection, *Botrytis cinerea* secretes a wide spectrum of plant cell wall degrading enzymes (CWDEs) to facilitate penetration into host. So far, only the polygalacturonases PG1 and PG2 and the xylanase XYN11A were proved by reverse genetics to be required for virulence. Verification of the function of single members of the different CWDE classes is indeed difficult, due to gene redundancy in multigenic families.

In various plant pathogenic fungi, production of these enzymes is under catabolic repression and positively regulated by the snf1 (sucrose non-fermenting 1) gene which is expressed when glucose is depleted.

To examine the function of the *snf1* gene in *B. cinerea*, knockout mutants were obtained by targeted mutagenesis. The growth of the Δsnf1 mutants was compared with the wild type strain on minimal medium enriched with different simple and complex carbon sources (fructose, sucrose, glucose, xylan, xylose, pectin, polygalacturonic acid, cellulose). A significant reduction of growth was observed on some carbon sources except with glucose and xylan. Microscopic studies verified, that the sporulation of the mutants was almost abolished and unusually shaped mycelia were found. Pathogenicity tests performed on apple fruits displayed a strong defect in colonization by the Δsnf1 mutants with a 60% decrease in virulence.

Pathogenicity tests on plant systems are in progress together with the characterization of possible effects on secretion and expression of CWDEs.

A NEW MEMBER OF THE FAMILY *CLOSTEROVIRIDAE* IDENTIFIED FROM SYMPTOMATIC KIWIFRUIT (ACTINIDIA spp) PLANTS SHOWING MULTIPLE VIRAL INFECTION. R. Biccheri[1], A. G. Blouin[2], C. Poggi Pollini[1], D. Cohen[2], M.N. Pearson[3], C. Ratti[1]. *[1]DipSA, Università di Bologna, Viale G. Fanin, 40 - 40127 Bologna, Italy. [2]Plant & Food Research Limited, Private Bag 92169, Auckland, New Zealand. [3]The University of Auckland, Private Bag 92019, Auckland, New Zealand. E-mail: roberta.biccheri2@unibo.it*

Kiwifruit (*Actinidia spp*) is an important crop in China, Italy, New Zealand and Chile that collectively contribute for 80% of the world's production. Recent studies demonstrated that *Actinidia spp.* can be infected by a wide range of pathogens and currently twelve different viral species have been identified on kiwifruit plants. Our studies recently focused on plants of *A. chinensis* cv. Hort 16a showing chlorotic and necrotic rings on leaves followed by a general decline and death of the scion but not of the rootstock (*A. deliciosa* cv Hayward). *Pelargonium zonate spot virus*, *Actinidia virus A* and *Actinidia virus B* viruses have been detected in samples analyzed and etiology dissection of this complex disorder has been investigated by NextGeneration Sequencing approach.

De-novo assembly of raw data from 454, Illumina and Ion Torrent platforms generated a sequence of about 17 kb containing one large ORF carrying two papain-like leader proteases, the methyltransferase, the helicase and the RNA-dependent RNA polymerase. Additional ORFs contain homologues to a heat shock protein 70, a heat shock protein 90, and a silencing suppressor protein and a minor coat protein. However no homologue of a major coat protein has been detected. The genomic organization and the phylogenetic analysis showed that this sequence is related to the *Olive leaf yellow associated virus*, a tentative member of the *Closterovirus* genus. The sequence has been confirmed by cloning of overlapping fragments sequenced by the classical Sanger sequencing.

The new viral species identified contributes to the understanding of a disorder observed that has already been associated with significant consequences for yield on kiwifruit plants.

THE ENDOPHYTES *PANTOEA AGGLOMERANS* AND *ERWINIA OLEAE* COLOCALIZE WITH *PSEUDOMONAS SAVASTANOI* PV. *SAVASTANOI* IN THE OLIVE KNOTS. C. Cortese[1], I. Pérez-Martìnez[2], C. Ramos[2], R. Buonaurio[1], C. Moretti[1]. *[1]Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università degli Studi di Perugia, 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: chiaraluce.moretti@unipg.it*

Many endophytic bacterial species such as *Pantoea agglomerans*, *Erwinia toletana* and *Erwinia oleae*, have been reported to be associated within the olive knots caused by *Pseudomonas savastanoi* pv. *savastanoi*. We have investigated the co-localization of *P. agglomerans* and *E. oleae* with *P. savastanoi* pv. *savastanoi*, to preliminarily verify whether they form a bacterial consortium in the development of olive knots.

Plasmids containing GFP and DsRedExpress were transferred to *P. savastanoi* pv. *savastanoi* and *P. agglomerans* or *E. oleae*, respectively. Fluorescently labeled bacterial strains were inoculated on *in vitro* micropropagated olive plants (derived from a cv. Arbequina seed), either independently or in combination with the other species. Bacterial localization was evaluated 7, 14, 21 and 28 days post-inoculation (dpi) by stereoscopic epifluorescence microscopy. To further study the localization and distribution of bacterial cells, transections of knots were analyzed by confocal laser scanning microscopy, 28 dpi.

Acquired images showed that when *P. agglomerans* or *E. oleae* were co-inoculated with *P. savastanoi* pv. *savastanoi*, the cells of the two endophytes were more abundant and visible at the inoculation site than when inoculated alone. *P. agglomerans*- or *E. oleae*-DsRedExpress bacterial cells were localized close to *P. savastanoi* pv. *savastanoi*-GFP cells, suggesting that the two endophytes require a close interaction with *P. savastanoi* pv. *savastanoi* for growth and persistence in olive knot.

Further analyses are in progress to determine the role of quoeum sensing regulation in the interaction established between *P. savastanoi* pv. *savastanoi* and *P. agglomerans* or *E. oleae* during infection of olive plants.

EARLY DETECTION OF *PSEUDOMONAS SYRINGAE* pv. *ACTINIDIAE* FROM 'BLEEDING SAP' IN CHILE. S. Ardizzi[1], E. Biondi[1], S. Perez[1], Ca. Lucchese[1], P. Minardi[1], J. Carrasco Figueroa[3], C. Ureta Olivares[3], C. Soto Pereira[3], C. Cerpa[3], J.A. Molina[3], E. Vega Berroeta[3], A. Zamorano[2], X. Gonzalez[2], N. Fiore[2], A.Bertaccini[1]. *[1]DipSA, Patologia vegetale, Università di Bologna, v.le Fanin, 44, Italia. [2]Departamento de Sanidad Vegetal, Faculdad de Ciencias Agronomicas, Universidad de Chile, Chile. [3]Servicio Agricola y Ganadero de Chile, Chile. E-mail: stefano.ardizzi2@unibo.it*

In Italy, during the boreal spring of 2012, a useful method was evaluated to detect the causal agent of kiwifruit bacterial canker (*Pseudomonas syringae* pv. *actinidiae*, Psa) from 'bleeding sap'. In Chile, during the austral spring of 2013, such method was applied to confirm its efficiency in Psa early detection and to evaluate the best sampling period in the austral hemisphere. Almost 30 bleeding sap samples were collected from asymptomatic plants located in two regions, in three different Psa-free fields.

Microbiological and molecular analyses (nested PCR) were performed on collected bleeding sap samples to assay the presence of the pathogen in any field. Traditional sampling and analysis were also carried out on plant material collected from the same plants used for bleeding saps collection. The isolation of Psa was possible from approx. 20% total bleeding sap samples, while the detection of the pathogen using nested PCR directly on processed bleeding saps was achievable from more than 70%. When analysis on traditional samples was applied, *ca.* 30% total samples were found positive for the presence of Psa. During the next vegetative season only 15% part of the analyzed plants became symptomatic. Bleeding sap analysis revealed the presence of the pathogen in any of the three fields visited; the analysis on traditional samples confirmed those results. The analysis achieved in Chile on bleeding saps confirmed its efficacy in the early detection of Psa. The rapidity of the method may become useful in detecting latent infections.

OXYLIPIN INVOLVEMENT IN THE CROSS-TALK *FUSARIUM VERTICILLIOIDES* AND MAIZE. V. Scala[1], C. Dall'Asta[2], P. Giorni[3], R. Gregori[3], M. Cirlini[2], M. Reverberi[1], M. Ludovici[4], E. Camera[4], C. Fanelli[1], P. Battilani[3]. *[1]Dipartimento di Biologia Ambientale, Università Sapienza, Largo Cristina di Svezia, 24, 00165 Roma. [2]Dipartimento di Scienze degli Alimenti, Università degli Studi di Parma, Parco Area delle Scienze 17/A, 43124 Parma, Italy. [3]Istituto di Entomologia e Patologia vegetale, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Piacenza 29100, Italy. [4]Laboratorio di Fisiopatologia Cutanea e Centro Integrato di Metabolomica, Istituto Dermatologico San Gallicano IRCCS, Roma, Italy. E-mail: paola.battilani@unicatt.it*

Fusarium verticillioides became an interesting fungus for maize crop in 1989 when fumonisins were discovered. The detection of hidden fumonisins (2009) and their occurrence in raw maize (2010) increased health concerns. The involvement of fatty acids, both in hybrid susceptibility to contamination and in fumonisin esterification were recently also described. Therefore, oxylipins and their role in host plant-fungus cross-talk were considered in this project. A multifaceted approach was followed to describe the cross-talk that included: 1) artificial inoculation to study in detail the role of biological and ecological parameters; 2) field sampling to clarify the role of maize hybrids and the growth stage where their behavior can be highlighted. All samples were managed with biological (fungal isolation, identification, mutant generation), chemical (fumonisin determination, lipidomic analysis) and molecular (fungal and plant gene expression, focus on LOX and LDS) approaches.

The expected involvement of lipid composition of maize kernels in fungal infection and toxin accumulation was confirmed. Four lipid entities differentiated high-contaminated from low-contaminated samples (cut-off of 2000 μg Kg^{-1} of fumonisins), confirming that sphingolipid and oxylipin metabolism in maize kernels interfere with *F. verticillioides* growth and fumonisin production. *FvLDS1 and ZmLOX12* in FB_1 seem the genes most involved.

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THE WHEAT XYLANASE INHIBITOR TAXI-III INTERACTS WITH A XYLANASE SECRETED BY *FUSARIUM GRAMINEARUM* AND LIMITS WHEAT CELL DEATH. I. Moscetti[1], L. Sella[2], F. Faoro[3], S. Moro[4], F. Favaron[2], R. D'Ovidio[1]. *[1]Dipartimento di Scienze e tecnologie per l'Agricoltura, le Foreste, la Natura e l'Energia (DAFNE), Università della Tuscia, Via S. Camillo de Lellis snc, 01100 Viterbo (Italy). [2]Dipartimento TeSAF, Università degli studi di Padova, viale dell'Università 16, 35020 Legnaro (PD). [3]Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy. [4]Dipartimento di Scienze del Farmaco, Università degli studi di Padova, via Marzolo 5, 35131 Padova. E-mail: ilariamoscetti@gmail.com*

Cereals contain xylanase inhibitor proteins (XIs) which inhibit microbial xylanases from glycoside hydrolase families 10 and 11. In wheat, three types of XIs have been identified: *Triticum aestivum* XI (TAXI), xylanase inhibitor protein (XIP) and thaumatin-like XI (TLXI). These inhibitors are considered part of the defence mechanisms that plants use to counteract microbial pathogens: recently, we provided *in planta* evidences for the protective role of TAXI-III, a member of the TAXI type XIs. To elucidate the molecular mechanism underlying the capacity of the transgenic wheat plants expressing TAXI-III to limit Fusarium Head Blight (FHB) disease symptoms caused by *Fusarium graminearum*, we performed infiltration experiments on wild-type and transgenic wheat tissues with a xylanase strongly expressed by *F. graminearum* during wheat spike infection, which we have previously demonstrated to induce cell death and hydrogen peroxide accumulation. Experiments performed on glumes of flowering wild-type wheat spikes showed that the co-infiltration with TAXI-III significantly decreased cell death and hydrogen peroxide accumulation. Most interestingly, similar results were also obtained by infiltrating the same xylanase on glumes of transgenic wheat plants expressing TAXI-III. Molecular modelling studies predict an interaction between the TAXI-III and the active site of the xylanase, thus the formation of this complex might prevent the recognition of the xylanase by a plant receptor possibly involved in cell death elicitation. Therefore these results suggest that the reduced FHB symptoms on transgenic TAXI-III plants can be due to the direct inhibition of xylanase activity secreted by the pathogen but also to the capacity of TAXI-III to block the xylanase necrotizing activity.

TRANSCRIPTOME ANALYSIS OF TWO RICE GENOTYPES IN RESPONSE TO *FUSARIUM FUJIKUROI*. S. Matic[1,2], P. Bagnaresi[3], C. Biselli[3], G. Valé[4], A. Garibaldi[1], M.L. Gullino[1,2], D. Spadaro[1,2]. *[1]AGROINNOVA – Centre of Competence, Università di Torino, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy. [2]DISAFA - Dip. Science Agrarie, Forestali ed Alimentari, Università di Torino, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy. [3]CRA – GPG Centro di ricerca per la genomica vegetale,Via S. Protaso 302, 29017 Fiorenzuola d'Arda (PC), Italy. [4]CRA – RIS Unità di ricerca per la risicoltura, Strada Statale 11 km 2,5, 13100 Vercelli, Italy. E-mail: davide.spadaro@unito.it*

Rice (*Oryza sativa* L.) is one of the most important crop worldwide. *Fusarium fujikuroi* is the causal agent of bakanae disease on rice. Due to the reduction in pesticide availability, in recent years the disease incidence has increased also in Italy, becoming a serious problem. Commercial rice cultivars show different levels of resistance to *F. fujikuroi*. Out of 12 genotypes screened against bakanae disease, genotype Selenio resulted as the most resistant, while genotype Dorella was the most susceptible. RNA-seq was used to dissect the early molecular processes deployed during the resistance response of 'Selenio' at 1 and 3 weeks post germination (wpg). The number of differentially expressed genes (DEGs) during 1 wpg was 80 in Selenio and 1285 in Dorella. The number of DEGs was higher during 3 wpg in both cultivars: 3119 in Selenio and 5095 in Dorella. The defense response for both genotypes was more active in 3 wpg then in 1 wpg when a much higher number of DEGs was identified. Gene ontology (GO) enrichment analyses revealed a set of GO terms enriched in both cultivars but, despite this commonality, the gene sets contributing to common GO enriched terms were dissimilar.

MULTI-TOXIN AND RELATED FUNGAL CONTAMINATION OF CEREAL KERNELS IN ITALY. A.F. Logrieco[1], M. Sulyok[2], R. Krska[2], G. Mulè[1], A. Susca[1], L. Mugnai[3], A. Moretti[1]. *[1]Institute of Sciences of Food Production, National Research Council, CNR-ISPA, Via Amendola 122/O, 70126 Bari, Italy. [2]Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Applied Life Sciences, Vienna, Konrad Lorenzstr. 20, A-3430 Tulln, Austria. [3]Università degli Studi di Firenze, Dipartimento di Scienze Produzioni agroalimentari e dell'Ambiente, Piazzale delle Cascine 28, 50144 Firenze. E-mail: antonio.logrieco@ispa.cnr.it*

Cereals are commonly colonized by a mixture of spoilage fungi in pre- and post-harvest conditions, where the dominance of those species depends on several abiotic and biotic factors. Grains can be colonized competitively by species of *Aspergillus*, including those in section *Flavi*, *Nigri* and *Circumdati*, and by several species of *Alternaria*, *Fusarium* and *Penicillium*. Considering this wide range of moulds, a large variety of mycotoxins can be found under specific conditions in cereal products. The most common mycotoxins that contaminate cereals in the field in Italy are the *Fusarium* toxins, especially trichothecenes, zearalenone and fumonisins, while little is known about other *Fusarium* mycotoxins. Moreover, other harmful mycotoxins produced by species of *Alternaria*, *Aspergillus* and *Penicillium*, can also contaminate grains in the field. Although the European Commission has set up the maximum levels of most important mycotoxins, there are no rules or recommendations for the co-occurrence of more than one toxin. Therefore, the risk of multi-toxin contamination of food commodities is still poorly recognized even if it is well known that each of these toxic fungal metabolites has a specific target and the complex of mycotoxins could have a wide range of effects on human and animal health. We report here the results of a large screening carried out in Italy on the multi-toxin contamination of grains and related producing species. Data showed that the risk that several mycotoxins from diverse fungi occur together on cereal kernels in Italy is realistic and need further investigations.

INVESTIGATION ON THE RESISTANCE-INDUCING ABILITY OF CERATO-PLATANIN ON CROPS. I. Baccelli[1], S. Luti[2], L. Lombardi[3], C. Comparini[1], R. Bernardi[4], P. Picciarelli[4], L. Pazzagli[2], A. Scala[1]. *[1]Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Firenze Italy. [2]Dipartimento di Scienze Biomediche, Sperimentali e Cliniche, Università degli Studi di Firenze, Viale Morgagni 50, 50134 Firenze, Italy. [3]Dipartimento di Biologia, Università di Pisa, Via Luca Ghini 13, 56124 Pisa, Italy. [4]Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, Università di Pisa, Via Matteotti 1/B, 56124 Pisa, Italy. E-mail: ivan.baccelli@unifi.it*

Microbe- or pathogen-associated molecular patterns (MAMPs/PAMPs) lead to the activation of the first line of plant defence. Few fungal molecules are universally qualified as MAMPs, and the protein cerato-platanin (CP) has shown experimental evidence of this quality.

CP is produced by *Ceratocystis platani*, the causal agent of the canker stain disease of plane trees (*Platanus* spp.), and when applied on the leaf surface it acts as an elicitor of defence responses. In the model plant *Arabidopsis thaliana*, we have recently elucidated the key steps of the signalling process triggered by CP and we have shown the central role played by the stomata in this process. In summary, guard cells were the first on the epidermis to respond to CP with the production of H_2O_2, and CP rapidly induced stomatal closure, MAPK phosphorylation, overexpression of salicylic acid (SA)- and ethylene (ET)-signalling genes, and camalexin biosynthesis. After 24 h of treatment, the leaves also increased the resistance to the infection with *Botrytis cinerea* and *Pseudomonas syringae* pv. *tomato* DC3000 similarly to what occurred by using chitosan.

In order to evaluate the commercial potential of this protein as resistance inducer in plant protection, we are currently investigating the ability of CP and 4 different CP-derived synthetic peptides to protect crops against pathogens. Laboratory trials are in progress on tomato, bean, and grape plants against *B. cinerea*.

***PHYTOPHTHORA* SPECIES EMERGE AS A SERIOUS THREATH TO MEDITERRANEAN ECOSYSTEMS. B. Scanu, B. T. Linaldeddu, A. Deidda, L. Maddau, A. Franceschini.** *Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia (SPaVE), Università degli Studi di Sassari, Viale Italia 39 – 07100 - Sassari, Italy. E-mail: bscanu@uniss.it*

The Mediterranean basin is recognized as a global biodiversity hotspot accounting for more than 25,000 plant species that represent almost 10% of the world's vascular flora. In particular, the maquis vegetation on Mediterranean islands and islets constitutes an important resource of the Mediterranean plant diversity due to its high rate of endemism accounting for 4.3% of all plant species worldwide. Since 2009, a severe and widespread dieback and mortality has been observed in natural forests located in the National Park of La Maddalena archipelago (northeast Sardinia, Italy). Main plant species affected included *Arbutus unedo*, *Asparagus albus*, *Juniperus phoenicea*, *J. oxycedrus*, *Pistacia lentiscus*, *Quercus ilex* and *Q. suber*. In order to clarify the symptomatology and aetiology of these epidemic events, field surveys and isolations from symptomatic trees/shrubs were carried out between 2010 and 2013. A total of 13 *Phytophthora* species were isolated from fine roots and rhizosphere soil samples, including *P. alticola*, *P. asparagi*, *P. bilorbang*, *P. cinnamomi*, *P. cryptogea*, *P. gonapodyides*, *P. melonis*, *P. nicotianae*, *P. parvispora*, *P. psychrophila*, *P. quercina*, *P. syringae* and two informally designated taxa, *P. aplerotica* prov. nom. and *P. ornamentata* prov. nom. Isolates were identified using morphological analysis, growth–temperature relations and DNA-based techniques. Pathogenicity tests for all *Phytophthora* species using a soil infestation method are currently underway. The possible implications of such a high number of *Phytophthora* species, some of them unknown to science, detected in a typical Mediterranean ecosystem are discussed.

ANALYSIS OF THE POPULATION STRUCTURE OF *PHYTOPHTHORA NICOTIANAE* USING MICROSATELLITE MARKERS. **A. Biasi[1], F.N. Martin[2], S.O. Cacciola[3], N.J. Grünwald[4], M. Evoli[3], L. Schena[1].** *[1]Dipartimento di Agraria, Università degli Studi Mediterranea, Località Feo di Vito, 89124 Reggio Calabria, Italy. [2]United States Department of Agriculture- Agricultural Research Service, 1636 East Alisal Street, Salinas, CA 93905. [3]Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali, Universita degli Studi, Via Santa Sofia, 100, 95123 Catania, Italy. [4]Horticultural Crops Research Laboratory, US Department of Agriculture Agricultural Research Service, Corvallis, OR 97330. E-mail: lschena@unirc.it*

A panel of single sequence repeats (SSRs) was screened taking advantage of the recently sequenced genomes of *Phytophthora nicotianae* from 6 different isolates and selected markers were accurately evaluated using *in silico* and experimental approaches. The best 9 performing SSRs were used to characterize a wide population of *P. nicotianae* (268 isolates) from a broad range of hosts and geographic localities. Primers were optimized to be used in a duplex approach with two differently labeled fluorescent dyes, in order to reduce costs and time of the analyses. A total of 129 different multilocus genotypes (MLG) were identified, with a different rate of polymorphism within the markers used. Analyses revealed a prevalence of clonality in productive orchards, while sexual recombination seemed to be more widespread in nurseries. A strong association between genetic groups and host was revealed for most *Citrus* isolates, while a significant geographical structuring was recovered for isolates from tobacco (sourced in Australia and United States) and from pommelo (*Citrus maxima*) sourced in Vietnam. For other *Citrus* species a typical panmictic distribution was revealed. Differences in the population structure were likely to be influenced by propagation and cultivation systems. Isolates from potted ornamental and citrus (excepted pommelo) are probably diffused worldwide with infected plant materials. In contrast tobacco is propagated by seeds which do not contribute to the spread of the pathogen. As regards to *C. maxima*, this species is a native plant of Vietnam and plant materials were not introduced from other countries suggesting a specific co-evolution with *P. nicotianae*.

VIRUS AND VIROID COMMUNITY DETECTION BY METAGENOMIC ANALYSIS. **G. Licciardello[1], R. Ferraro[1], M. Russo[1], S. M. Dai[2], Z.N. Deng [2], A. Catara[1].** *[1]Parco Scientifico e Tecnologico della Sicilia, z.i. Stradale Lancia 57, 95121 Catania, Italy. [2]National Center for Citrus Improvement (Changsha), Hunan Agricultural University, Hunan 410128, P.R. China. E-mail: glicciardello@pstsicilia.it*

A source plant inducing symptoms of tristeza seedling yellows in sour orange, yellow vein clearing in lemon and mottled and tattered leaves in Carrizo citrange was analysed by deep sequencing of sRNA (18-26 nt) using Illumina technology. The abundance of virus- and viroid-derived siRNAs was estimated by aligning the filtered reads on the genomes of 17 *Citrus tristeza virus* (CTV) isolates, 24 viroids, one *Citrus yellow vein clearing virus* (CYVCV) genome and one *Citrus tatter leaf virus* (CTLV) genome. The analysis showed a prevalent reads abundance of 17,604,200 with CTV isolates belonging to the VT genotype. A typical structural organization with 12 open reading frames (ORFs) and two untranslated regions (UTRs) was identified in the resulting consensus sequence, approximately 19.3 kb in length. When the sRNA library was aligned against the CYVCV isolate Y1, a total of 159,585 reads were able to reconstruct the full genome with an overall nucleotide identity of 98%. Computational analysis of the RNA genome sequence, 7529 nt in length, predicted six ORFs and two UTRs. The complete genome sequence of CTLV, constituted by two ORFs and a poly (A) tail at the 3' end, was also reconstructed. The alignment with viroid genomes showed the presence of CDVd, HSVd, CVdII, CVdIII and CVdIV with an abundance of about 30,000-40,000 reads. The study demonstrated that deep sequencing and bioinformatic analyses can identify a viral community in a host in agreement with indexing results, although in some cases bioindexing and PCR detection are necessary to confirm the results.

***BEET NECROTIC YELLOW VEIN VIRUS* AND *BEET SOIL-BORNE MOSAIC VIRUS*: A PRELIMINARY STUDY OF RNAs REASSORTMENT.** **M. Dall'Ara[1], A. Delbianco[1], S. Bouzoubaa[2], E. Klein[2], D. Gilmer[2], C. Ratti[1].** *[1]DipSA - Plant Pathology, University of Bologna, Viale G. Fanin, 40 - 40127 Bologna, Italy. [2]Institut de Biologie Moléculaire des Plantes du CNRS, Université de Strasbourg, 12 rue du Général Zimmer, 67084 Strasbourg Cedex, France. E-mail: david.gilmer@ibmp-cnrs.unistra.fr*

Beet necrotic yellow vein virus (BNYVV) and *Beet soil-borne mosaic virus* (BSBMV) (*Benyviridae* family) are distinct but closely related species possessing the same host range, vector and multipartite genome of four to five ssRNAs (+) that represent an interesting model to study plant-virus interactions.

RNA-1 and -2 combination (helper strain) is essential for the infection and the replication of both viruses, while RNA-3 and -4 are accessory RNAs playing important roles in plant-virus and virus-vector interactions, respectively.

Previous work demonstrated on the one hand the ability of BNYVV/BSBMV helper strains to handle the replication and packaging of cognate small RNA segments, on the other hand, the availability of a functional exchange of genomic RNA-1 between both viral species. Interestingly, one of the two chimeric combinations induced a strong necrosis of the infected *Chenopodium quinoa* leading to a dead end for the recombinant virus. Such dramatic cell death or incompatible reaction could explain why no natural chimeras have ever been found in plant naturally infected with both viruses.

As both viruses could co-exist in the same plant, a mixture of both genomic RNAs in the same cell should be avoided, particularly when the virus moves at a long distance.

The unavailability of benyviridaes genomic RNA reassortment, in particular for RNA-1, has been tested in *C. quinoa* and *Nicotiana benthamiana* local and systemic host, respectively, using BNYVV/BSBMV full-length infectious cDNA clones and agroclones in a genetic reverse approach.

SEQUENCING AN UNCONVENTIONAL VIRUS GENOME: THE MULBERRY BADNAVIRUS-1 CASE. **M. Chiumenti[1], M. Morelli[1], T. Elbeaino[2], L. Stavolone[3], A. De Stradis[3], A. Minafra[3], G.P. Martelli[1].** *[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, 70126 Bari, Italy. [2]Istituto Agronomico Mediterraneo di Bari (IAMB),70010 Valenzano (Bari), Italy. [3]Istituto di Protezione Sostenibile delle Piante (CNR-IPSP), UOS Bari, 70126 Bari, Italy. E-mail: michela.chiumenti@gmail.com*

Mulberry is a deciduous tree belonging to the *Moraceae* family. Although its economical importance and spreading all over the world, due mainly to the domesticated silkworm (*Bombyx mori* L.) breeding, to date, only few information are available about viral and virus-like diseases affecting this plant.

In 2012 a small fragment of a *Badnavirus* DNA genome was sequenced after a DOP-PCR assay conducted on a mulberry plant,

originated from Lebanon, showing symptoms of leaf mottling and vein yellowing. The virus, whose particles were observed with electron microscopy from a partially purified preparation and in tissue thin sections, was provisionally named Mulberry badnavirus-1 (MBV-1). Since different attempts of completing the full-length genome sequence through a conventional approach failed, a small RNA library was constructed for deep sequencing and run according to Illumina protocol.

sRNAs analysis allowed the design of a set of primers used in PCR for the achievement of the full length sequence. The complete genome contains all the sequence features and the characteristic functional domains of the genus *Badnavirus* (highest nucleotide similarity shared with FBV-1 at 54%). By contrast to the badnaviruses, with genomes encoding for 3-4 ORFs, MBV-1 resembles genome organization of *Petunia vein clearing virus* (PVCV), which bears a single ORF.

The study of the distribution of sRNA on the MBV-1 complete genome showed a tidy prevalence of 21- and 22-nt reads, differently from other viruses in the *Caulimoviridae* family, featuring a nuclear replication and typically supporting an accumulation of the 24-nt sRNAs involved in methylation processes.

EVIDENCES OF THE EXISTENCE OF GRAPEVINE PINOT GRIS VIRUS ISOLATES SHOWING DIVERSE PATHOGENICITY. **M. Morelli[1], A. Giampetruzzi[1], P. Bianchedi[2], P. Saldarelli[1], V. Gualandri[2].** *[1]Istituto per la Protezione Sostenibile delle Piante del C.N.R. UOS Bari, Via Amendola, 165/A, 70126 Bari, Italy. [2]FEM-IASMA, Centre for Technology Transfer, via E. Mach, 1 38010, San Michele all'Adige (TN) Italy E-mail: p.saldarelli@ba.ivv.cnr.it*

Grapevine Pinot gris virus (GPGV) is a recently described trichovirus, discovered by next generation sequencing (NGS) in the cv Pinot gris, seemingly associated to a new grapevine disease. To define the aetiology of the disease, the virome of two additional Pinot gris accessions, showing or not symptoms, were analysed by NGS. Virus content consistently reproduced the already known virome with GPGV, *Grapevine rupestris stem pitting virus* (GRSPaV), *Grapevine Rupestris vein feathering virus* (GRVFV) and the two viroids *Hop stunt viroid* (HSVd) and *Grapevine yellow speckle viroid 1* (GYSVd-1), infecting either the symptomatic or the symptomless plants. Whole genome phylogenetic analysis on seven GPGV isolates, including three isolates originating from Czech Republic and Slovak Republic and consensus sequences assembled by NGS, clearly clustered those infecting symptomatic vines. We therefore extended the analysis to two genomic regions, comprising the RNA polymerase RNA dependent domain of the replicase gene and part of the movement protein, on a group of accessions from Trentino, free from relevant viruses involved in leafroll, infectious degenerations and rugose-wood associated grapevine diseases. Maximum likelihood and Bayesian phylogenetic analyses of nucleotide sequences, proved that isolates from vines with symptoms form a clade distinct from those of symptomless vines, thus confirming studies on the whole genome. These results showed that GPGV virus populations from Trentino underwent, in the infected vines, a different evolutionary dynamic, not related with the vine variety, which selected for isolates with different pathogenicity.

VALIDATION OF A RAPID AND SENSITIVE DIAGNOSTIC PROTOCOL FOR THE IDENTIFICATION OF *PLUM POX VIRUS*: LAMP RT-PCR. **G. Pasquini [1], L. Ferretti[1], S. Lopriore[2], G. Airoldi[2], M. Barba[1].** *[1]Consiglio per la Ricerca e la sperimentazione in Agricoltura – Centro di Ricerca per la Patologia Vegetale CRA-PAV – Roma. [2]Teltec Srl - Vimercate (MB). E-mail: graziella.pasquini@entecra.it*

The development of innovative rapid and reliable detection methods 'ready to use' directly in open field or at the borders, is an important aspect for the phytosanitary control of quarantine pathogens as *Plum pox virus* (PPV), a virus negatively impacting on stone fruits cultivation.

The use of the rapid and sensitive loop-mediated isothermal amplification (LAMP) technology with a portable device allows to perform the analysis directly in the field, combining the sensitivity of the technique with a practical use for a rapid interception of pathogens.

A commercial kit for the diagnosis of PPV based on LAMP technology (Bioteltec) has been validated by the calculation of the performance criteria (ISO 17025) compared with those obtained for a real time RT-PCR in a national Italian ringtest. All experiments were performed with a Bioteltec commercial kit and the Genie® II Instrument (Optigene Ltd.), starting from total RNAs (TRNA) extracted from target and no-target reference samples by a simple and rapid extraction kit (Bioteltec) and by RNeasy Plant minikit (Qiagen).

LAMP RT-PCR resulted to be a very efficient and rapid technology for PPV detection, showing a higher analytical sensitivity than RT-PCR, both with sensitive or rapid TRNA extraction methods, with a comparable diagnostic accuracy and repeatability, resulting very useful for the diagnosis of the virus, also directly in the field.

DRAFT GENOMES OF AN ITALIAN STRAIN OF *PSEUDOMONAS SAVASTANOI* PV. *SAVASTANOI* AND OF TWO ENDOPHYTES LIVING IN THE OLIVE KNOTS. **C. Moretti[1], C. Cortese[1], D. Passos da Silva[2], G. DeVescovi[2], E. Torelli[3], C. Ramos[4], V. Venturi[2], G. Firrao[3], R. Buonaurio[1].** *[1]Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università di Perugia, Via Borgo XX Giugno 74 – 06121- Perugia, Italy. [2]Centre for Genetic Engineering and Biotechnology, Trieste, Italy. [3]Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Udine, Udine, Italy. [4]Área de Genética, Facultad de Ciencias, Universidad de Málaga, Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Málaga, Spain. E-mail: chiaraluce.moretti@unipg.it*

Knots caused by *Pseudomonas savastanoi* pv. *savastanoi* in olive trees provide an interesting niche for studying bacterial multispecies interactions. Among the high number of bacterial endophytes detected inside the olive knots by metagenomics, those belonging to the genera *Pantoea*, *Pectobacterium*, *Erwinia* and *Curtobacterium* are the most represented. Our attention is focused on two endophytic bacterial species, *Pantoea agglomerans* and *Erwinia oleae*, which cause an increase in disease severity when co-inoculated with *P. savastanoi* pv. *savastano*i in olive plants. To better understand the molecular basis of the interaction between the pathogen and the endophytes, we sequenced their genomes. The comparison of the *P. savastanoi* pv. *savastanoi* genome with that of the previously sequenced strain NCPPB 3335 provides useful information to explain the different extend of virulence the two strains show. In the *E. oleae* genome, the quorum sensing system is present and knockout mutants of *luxI/R* genes homologs were obtained. We are verifying whether *E. oleae* undergoes inter-species communication with *P. savastanoi* pv. *savastanoi* through this system. *In silico* analysis of *P. agglomerans* genome reveals the presence of a complete *hrp/hrc* gene cluster, which explains the capability of this strain to induce hypersensitive reaction in tobacco plants. This cluster shows remarkable synteny and high sequence similarity with *Erwinia amylovora* and *Erwinia pyrifoliae* homologs. Mutants of *hrpN*, *hrpY* and *hrpJ* genes were obtained and their phenotypes will be described.

A *XYLELLA FASTIDIOSA* STRAIN WITH UNIQUE BIOLOGY AND PHYLOGENY IS ASSOCIATED WITH A SEVERE DISEASE OF OLIVE IN SOUTHERN APULIA. G. Loconsole[1], D. Boscia[1], F. Palmisano[2], V. Savino[3], O. Potere[3], G.P. Martelli[3], M. Saponari[1]. *[1]Istituto per la Protezione Sostenibile delle Piante UOS Bari, CNR, via Amendola 165/A, 70126 – Bari, Italy. [2]Centro di Ricerca, Formazione e Sperimentazione in Agricoltura, Via Cisternino 281, 70100 - Locorotondo (BA), Italy. [3]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 - Bari, Italy. E-mail: g.loconsole@ba.ivv.cnr.it*

In October 2013, an outbreak of *Xylella fastidiosa* was detected in the Salento peninsula (Apulia, south-eastern Italy) in olive groves that were severely affected by a disease denoted "olive quick decline syndrome". Surveys carried out throughout the region revealed the presence of new outbreaks, all restricted to the province of Lecce, the site of the first *X. fastidiosa* discovery. Investigations carried out showed that olive is the prevailing susceptible hosts, but infections were also found in oleander, some stone fruits and ornamentals. By converse, grapevines and citrus trees growing in the heavily contaminated area, were always negative in laboratory assays and free from symptoms of Pierce's disease and citrus variegated chlorosis. Preliminary genetic analysis based on 13 housekeeping genes, including those used for multi locus sequence typing (MLST), demonstrated that the olive isolate of *X. fastidiosa* is genetically related with the subspecies *pauca*, but clearly distinct from the other strains comprised this cluster. A novel, hitherto undescribed "Sequence Type" profile (http://pubmlst.org/xfastidiosa/), was therefore assigned to the olive strain based on MLST analysis. Multiple alignments of DNA sequences of bacterial isolates recovered from all susceptible hosts so far known, showed high nucleotide identity, suggesting that the infections are caused by the same strain. In conclusion, the data so far collected about the natural host range and the genetics of the bacterial isolates under study, strongly support the notion that the olive isolate of *X. fastidiosa* represents a novel strain, for which the name "strain CoDiRO" has been proposed.

BACTERIAL COMMUNITIES OF CITRUS PLANTS AFFECTED BY HUANGLONGBING AND *CANDIDATUS* LIBERIBACTER ASIATICUS CONCENTRATION. A. Campisano[1], C.P. Strano[2], P. Bella[2], G. Licciardello[3], Z. Deng[4], X. Deng[5], R. La Rosa[2], V. Catara[2]. *[1]Research and Innovation Centre, Fondazione Edmund Mach (FEM), S. Michele all'Adige (TN), Italy.[2]Department of Agricultural and Food Science, University of Catania, Italy.[3]Science and Technology Park of Sicily, Catania, Italy. [4]National Center of Citrus Improvement, Hunan University of Agriculture, Changsha, P.R. China. [5]Laboratory of Citrus Huanglongbing Research, Department of Plant Pathology, South China Agricultural University, Guangzhou, P.R. China. E-mail: vcatara@unict.it*

Huanglongbing (HLB) is the most devastating disease of citrus worldwide. This vector-transmitted disease is associated with phloem-limited fastidious alphaproteobacteria of the genus "*Candidatus* Liberibacter" that are of quarantine concern for the European and Mediterranean region. Investigation in the countries where the disease is present is a way to improve our knowledge in view of a potential invasion. In China HLB is associated to "*Ca.* L. asiaticus" (Las). During field surveys in Guandong province, China, we collected young shoots of Buddha's hand (*Citrus medica* var. sarcodactylis) and 'Shatangju mandarin' (*Citrus reticulata*) in orchards with a HLB infection history. Bulk samples of leaf midribs and bark from single plants were separately processed. Total genomic DNA, including microbial DNA, was extracted using the DNeasy plant mini kit, Qiagen. Las presence was investigated by real-time PCR and bacterial titer estimated. Las mean concentration was higher in DNA extracted from bark samples rather than those obtained from midribs. The same DNAs were used for analysis of bacterial community composition by 454 pyrosequencing of the 16S rDNA gene amplicons. We determined the composition of bacterial communities in the two plant species and in the different tissues. Proteobacteria dominated the bacterial communities in all but one sample (where Bacilli were dominant). Among these, *Alphaproteobacteria* were the most abundant. *Actinobacteria* and *Acidobacteria* were also abundant in all samples. Special attention was devoted to the analysis of the *Rhizobiales* order to which Las belong to investigate the presence of taxonomically related bacteria that could influence Las detection.

HOST-PATHOGEN INTERACTION IN *ACTINIDIA*-BACTERIAL CANKER PATHOSYSTEM: INVESTIGATION ON THE LATENCY PERIOD IN ASYMPTOMATIC PLANTS. P. Minardi[1], S. Ardizzi[2], A. Bertaccini[2]. *[1]Dipartimento di Scienze Mediche Veterinarie, (DIMEVET), Alma Mater Studiorum, Università di Bologna, Via Tolara di Sopra, 50, 40064 Ozzano Emilia (BO), Italy. [2]Dipartimento di Scienze Agrarie (DipSA) Alma Mater Studiorum, Università di Bologna,Viale Fanin 42, 40127 Bologna, Italy. E-mail: paola.minardi@unibo.it*

Bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) still results in serious yield losses in Italy and, despite the studies conducted hitherto to deal with this devastating disease, some epidemiological aspects are still to be clarified. In particular, the available information on life-cycle of Psa is still incomplete on the latency period of Psa within the susceptible host plant. To elucidate this dangerous endophytic latent phase, the survival of a virulent Psa *gfp*-expressing/Rif-resistant strain (Psa::*gfp*) within asymptomatic *Actinidia* spp. plants was studied. The results were obtained both in adult plants of *Actinidia chinensis* cv. Hort16A four years after inoculation with Psa::*gfp* at low inoculum dose, and in *Actinidia deliciosa* cv. Hayward plants obtained from micropropagated shoots similarly inoculated more than three years before. Both types of plant were *in toto* analyzed to re-isolate Psa::*gfp* on selective media, and to check its ability to induce disease symptoms in host plants and hypersensitivity reaction in tobacco plants. PCR analysis was carried out to confirm the identity of the re-isolate Psa::*gfp*. On the fourth year following inoculation, the data confirmed that Psa can colonize plants for a long time staying latent at very low concentrations. In the pathosystem *Actinidia*-Psa the pathogen appears to have achieved a high degree of pathoadaptation which is witnessed in a pluriannual latency period. The implications of what reported are especially important for its impact on the nursery industry, and control strategies in kiwifruit orchards to prevent the spread of the pathogen present in asymptomatic plants.

ISOLATION AND CHARACTERIZATION OF AN ENDOPHYTE FROM *ACTINIDIA* SP. SHOWING A STRONG ANTAGONISTIC ACTIVITY AGAINST *PSEUDOMONAS SYRINGAE* PV. *ACTINIDIAE*. R. Tontou[1], F. Gaggia[2], L. Baffoni[2], V. Venturi[3], E. Stefani[1]. *[1]Dipartimento di Scienze della Vita, Università di Modena & Reggio Emilia, Viale A. Allegri 9, 42121, Reggio Emilia, Italy. [2]Dipartimento di Scienze Agrarie, Viale Fanin 44, 40127, Bologna, Italy. [3] Group of Becteriology & Plant Bacteriology, International Centre for Genetic Engineering and Biotechnology, Padriciano 99, 34149, Trieste, Italy. E-mail: emilio.stefani@unimore.it*

The bacterial canker of kiwifruit is the most destructive disease of cultivated *Actinidia* spp. The causal agent is the Gram

negative bacterium *Pseudomonas syringae* pv. *actinidiae* (*Psa*). The pathogen grows inside the trunk, vines and leaves of its host plants, causing wilting and formation of cankers, with subsequent death of the plant. During the past three years, samples have been collected from various parts of asymptomatic *Actinidia* spp. within infected kiwi orchards and many endophytic bacteria have been isolated. Among them, a few isolates were identified and proved to be able to strongly inhibit *Psa* namely an isolate of *Pantoea agglomerans*, two pseudomonads belonging to the *fluorescens/putida* group, and one novel *Pseudomonas* sp., which proved to be highly effective in inhibiting, *in vitro*, several important phytopathogenic bacteria. This last isolate was chosen for further studies. Concentrated supernatant of its liquid culture in LB medium could inhibit *Psa*, indicating that the active compound produced by the antagonist is excreted from the cell into the environment. The nature of that biotoxin is not proteic, and its size is smaller than 3 kDa, as a result of its partial purification. Moreover, a bank of mutants deficient in their antagonistic activity has been constructed by triparental mating, in order to search and identify genes involved in antagonism against *Psa*. Fifty six prospective mutants have been already obtained: twenty two of them were subject to Southern blotting to verify a single transposon insertion. Gene identification is under way.

PEPTIDES AS INHIBITORS OF TYPE THREE SECRETION SYSTEM FOR ECOFRIENDLY CONTROL OF BACTERIAL DISEASES OF PLANTS. **C. Biancalani[1], M. Cerboneschi[1], S. Macconi[1], M.R. Moncelli[2], S. Smeazzetto[2], S. Biricolti[1], P. Bogani[3], S. Tegli[1].** *[1]Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Università degli Studi di Firenze, Laboratorio di Patologia Vegetale Molecolare, Via della Lastruccia 10, 50019 Sesto Fiorentino, Firenze, Italy. [2]Dipartimento di Chimica, Università degli Studi di Firenze, Via della Lastruccia 13, 50019 Sesto Fiorentino,Firenze, Italy. [3]Dipartimento di Biologia, Università degli Studi di Firenze, Via Madonna del Piano 6, 50019 Sesto Fiorentino,Firenze, Italy. E-mail:carola.biancalani@unifi.it*

The control and management of bacterial diseases of plants still rely mainly on applications of copper and antibiotics. The use of antibiotics in plant protection is not allowed in EU State Members, while copper is among the very few chemicals still authorised in organic agriculture as well, although its use was strictly regulated within EU for its ecotoxicological negative effects (Directive 2009/37/EC; Regulation (EC) 1107/2009; Commission Regulation EU 284/2013). Furthermore, repeated copper treatments were demonstrated to cause a dramatic increase of antibiotic-resistant bacteria into agroecosystems, due to a cross-selection mechanism, with risks for human and animal health. While some promising alternatives were already proposed for fungicides, no sustainable options have ever been investigated for the control of plant pathogenic bacteria for reducing/replacing copper. In this work we propose an innovative strategy, targeting the translocation of bacterial pathogenicity and virulence factors instead of bacterial viability, in order to theoretically avoid or slow the development of any resistance. The pathogenicity of a broad spectrum of Gram-negative bacteria, both of plants and of mammalian hosts including humans, relies on Type Three Secretion System (T3SS). T3SS injects pathogenicity and virulence effectors (T3Es) directly into host cells. This makes T3SS an attractive and ideal target for novel antimicrobial drugs, inhibiting T3SS assembly and/or activity. Several oligopeptides were designed, targeting the main component of T3SS pilus of *Pseudomonas savastanoi* pv. *nerii* (*Psn*23). Preliminary experiments demonstrated these peptides compromising *Psn23* pathogenicity on Oleander and HR on Tobacco.

IN VINEYARD GENETIC VARIABILITY OF '*CANDIDATUS* PHYTOPLASMA SOLANI'. S. Murolo, V. Mancini, G. Romanazzi. *Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University Via Brecce Bianche, 60131 Ancona. E-mail g.romanazzi@univpm.it*

'Candidatus Phytoplasma solani' is a phytoplasma of the stolbur group (subgroup 16SrXII-A), associated with Bois noir (BN), recently responsible of outbreaks in several European countries, and particularly in the Mediterranean area. It is transmitted by the polyphagous cixiid planthopper *Hyalesthes obsoletus* to a wide range of wild plants, which represent potential inoculum sources, while grapevine is only occasionally infected and can be considered a dead-end host for the phytoplasma. The multiple interactions with wild and cultivated annual and perennial host plants and insect vectors in different ecosystems might be responsible for generating genetic diversity of '*Ca.* P. solani'. Aim of this study was to evaluate *'Ca.* P. solani' genetic diversity of isolates infecting a vineyard, using multilocus sequence typing analysis for the *vmp*1, *stamp*, and *secY* genes. Several haplotypes per gene were detected, showing high genetic diversity even in a restricted area such as the vineyard. The high genetic variability, recorded in particular in genes encoding the membrane proteins, represents an adaptation strategy common to living microrganisms, particularly useful for generalist pathogens, which colonized different environments (host plant and vector tissues).

MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF PHYTOPLASMAS IN CULTURE FROM PLANTS SOURCED IN THE FIELD. N. Contaldo[1], E. Satta[1], Y. Zambon[1], A. Canel[1], S. Paltrinieri[1], A. Bertaccini[1]. *[1]Dipartimento di Scienze Agrarie, Alma Mater Studiorum, Università degli Studi di Bologna, Viale Fanin 42– 40127- Bologna, Italy. E-mail: nicoletta.contaldo2@unibo.it*

Recently the proof that phytoplasmas can be grown on laboratory media was provided employing specific commercially available media and using as a source micropropagated phytoplasma infected periwinkle shoots from the collection established more than twenty years ago. Following these results, further work was carried out from field collected phytoplasma-infected samples. Shoots from trees showing typical symptoms of phytoplasma infection, together with asymptomatic ones of the same species, were employed. After phytoplasma identification by PCR/RFLP analyses, midribs stripped from fresh leaves were selected for phytoplasma cultivation. From each sample two midribs were surface sterilized for 1 min in 1% NaClO, ends were then discarded and two half midribs per sample were used for tube inoculation. Uninoculated tubes and tubes inoculated with midribs from healthy shoots were also processed under the same conditions. Phytoplasma colonies were obtained in 24 to 72 hours only from tubes inoculated with symptomatic plant material and purified by filtering. Several purified colonies were separately collected from three plates per strain, and subjected to nucleic acid extraction by commercial kits. At the same time nucleic acid was also extracted from the corresponding tubes containing cultures by a phenol/chloroform based method. Phytoplasma identification was carried out by PCR assays on phytoplasma 16S rDNA gene with general and group specific primers. Identification of detected phytoplasmas was done using RFLP analyses with appropriate restriction enzymes and/ or sequencing; the real and/ or virtual profiles obtained from liquid cultures and from purified colonies were identical to the ones of the original strains.