



## Abstracts of presentations at the XXIV Congress of the Italian Phytopathological Society

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### Structure and dynamics of airborne fungal populations in organic and conventional vineyards

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Recent studies have demonstrated the presence of a huge number of microorganisms in air samples. However, the relationship between the plant microbiome and the fungi present in the air surrounding the plant has not been intensively investigated. The structure and dynamics of airborne fungal communities associated to grapevine in organic and conventional vineyards were characterized in this study. Seven phyla, including Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota and Glomeromycota, were identified. Overall, 638 fungal genera were detected with *Alternaria*, *Mycosphaerella* and *Stemphylium* representing about 70% of the total fungal community. Data analysis indicated that management practices had a significant impact on the structure of fungal community. However, population dynamics in organic and conventional farms showed the same trend. It appeared to be related prevalently to the biology of different taxa. Results of this study confirm the important role of circulating air in the spread and distribution of microorganisms. Epidemiological implications of population dynamics of important grapevine pathogens and useful fungal microorganisms, such as yeasts, over the growth season are discussed.

### A new real-time loop-mediated isothermal amplification (LAMP) assay to rapidly detect *Phytophthora ramorum* and *P. lateralis* invasive plant pathogens

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An effective framework for early warning and rapid response is a crucial element to mitigate or prevent the ecological and economic impacts of invasive plant pathogens. Molecular detection of these pathogens is now preferred for its greater sensitivity and specificity respect to classical tools. These analyses are generally PCR-based and require a well-equipped lab. Significant advantages in terms of prompt response to threats might be obtained by using an on-site detection tool. A real-time monitoring LAMP-based method has been optimized for a rapid and sensitive detection of *P. ramorum* and *P. lateralis*, two quarantine pathogens that cause mortality on forest trees and ornamental plants in Europe and North America, by using the Genie<sup>®</sup> II system. The assay was able to recognize each pathogen with a high level of specificity and sensitivity also in plant tissues (to 4 pg  $\mu\text{L}^{-1}$  of DNA) in only 30 minutes. Great simplicity, sensitivity, specificity, and minimum required equipment make the LAMP assay ideal for application in the field and for routine plant testing at ports of entry. The use of portable and handled instruments allows a fast analysis of the collected sample reducing the diagnosis time and may have implications for disease management and for the control of *P. lateralis* and *P. ramorum*.

### Environmental genomics reveals the diversity of *Phytophthora* populations in phytocoenoses of “Complesso Speleologico Villasmundo-S. Alfio” Natural Reserve in Sicily

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The “Complesso Speleologico Villasmundo-S. Alfio” (Melilli, Siracusa) was established as a Natural Integral Reserve in 1998. It sits on a karstic calcareous subsoil and includes two deep river valleys delimiting a plateau. Because of its naturalistic and environmental features it has been recognized as a *Site of Community Importance - SCI - (92/43/EEC)*. As part of a broader study aimed at investigating the causes of the decline of native vegetation in Natural Reserves in Sicily, the diversity of *Phytophthora* populations in six different phytocoenoses in this Natural Integral Reserve has been studied, including the riparian wood *Platanus-Salicetum pedicellate*, the termophylic evergreen wood *Pistacio-Quercetum ilicis*, the Mediterranean maquis *Myrto communis-*

plants. Based on *16S rRNA* gene amplification and nucleotide sequence analyses, ‘*Candidatus Phytoplasma solani*’ was detected in 82% of symptomatic tomato plants and in bindweed plants. Molecular characterization based on *tufB* and *stamp* gene nucleotide sequence analyses revealed the presence of two ‘*Ca. P. solani*’ strain types: the type *tufB*-b/St5, identified respectively in 83% and 100% of tomato and bindweed infected plants, and the type *tufB*-a/St18 identified in 17% of tomato infected plants. These results suggest that ‘*Ca. P. solani*’ ecology in the examined agroecosystem is associated mainly with the bindweed-related host system. Based on such evidences, it should be interesting to evaluate the incidence dynamics of the disease and to investigate its epidemiology by tracing the movements of ‘*Ca. P. solani*’ throughout neighbouring fields.

## ***Fusarium graminearum* cerato-platanin proteins weaken cellulosic materials and enhance cellulase activity in an expansin-like manner**

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Cerato-platanin proteins (CPPs) belong to a family of small secreted non-catalytic fungal proteins with phytotoxic activity. CPPs have been recently classified as expansin-like proteins because of structural and functional features related to plant expansins, small secreted proteins able to loosen and disrupt the non-covalent bonding networks of plant cell wall polysaccharides without enzymatic activity. The genome of *Fusarium graminearum*, the causal agent of *Fusarium* head blight disease of wheat and other cereal grains, contains two genes putatively encoding for CPPs (FgCPPs). To characterize their role, the two proteins have been heterologously expressed in yeast. Enzymatic assays have shown the ability of the recombinant FgCPPs to reduce the viscosity of a cellulose soluble derivative (carboxymethyl cellulose, CMC) mainly with a non-enzymatic activity. Indeed, differently from other fungal CPPs and similarly to expansins, FgCPPs seem trapped by cellulose and not by chitin, thus suggesting that they could interact with cellulose. The incubation of CMC with a cellulase in presence or absence of the two recombinant proteins has shown that the FgCPPs enhance cellulase activity. A double knock-out mutant deleted of both FgCPPs encoding genes produces higher cellulase activity when grown on CMC, thus suggesting that the absence of FgCPPs forces the fungus to produce more cellulase activity to compensate for the lack of expansin-like activity. Finally, the preliminary demonstration that the FgCPPs act also loosening filter paper, a natural insoluble cellulose, could suggest a possible future biotechnological application in second-generation biofuels production from agricultural lignocellulosic biomasses rich in cellulose.

## **Use of MLVA as a promising tool for the identification and tracing of *Pseudomonas savastanoi* populations**

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A multilocus variable-number tandem repeat analysis (MLVA) approach was developed to assess the genetic diversity between and within *Pseudomonas savastanoi* pathovars. *Ps. savastanoi* is a bacterial species split into four pathovars that are responsible for knot disease on olive and oleander, and bacterial canker on ash. Twenty Tandem Repeat (TR) loci were selected, by analysing the genome of *P. savastanoi* pv. *savastanoi* strain NCPPB 3335 (accession No. CP008742), matching primers were designed and used for the amplification of genomic DNA from 60 strains belonging to the pathovars *savastanoi*, *neri*, and *fraxini*. Then capillary electrophoresis was used to estimate exactly the dimension of the amplicons and, consequently, the TR numbers per each locus in each strain. Fifteen loci were used for the final MLVA analysis, due to amplification failure of five loci. Genetic relationship among the strains was assessed according to pathovar classification, the geographic and time of origin of the individuals. This assay represents a promising tool for the identification and tracking of *P. savastanoi* populations.

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## **Effectiveness of essential oils used under a hypobaric environment to control postharvest gray mold of strawberries**

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Strawberry fruit are highly perishable after harvest, as they are sensitive to desiccation and to postharvest decay. Among these, the most important is gray mold, which is caused by *Botrytis cinerea*, followed by Rhizopus rot, caused by *Rhizopus stolonifer*, and other minor rots (e.g., Mucor rot, blue mold, anthracnose, induced by Mucor spp., *Penicillium expansum*, and *Colletotrichum gloeosporioides*, respectively). The use of essential oils fits perfectly within the strategies aimed at the control of postharvest decay through the application of alternatives to synthetic fungicides. When applied under hypobaric conditions, the vaporization of the essential oil increases, compared to application at room pressure. Therefore, strawberry fruit were exposed for 16 h to the vapors of *Citrus bergamia* (bergamot), *Mentha piperita* (mint) and *Rosmarinum officinalis* (rosemary) at 0.5 atm. Postharvest decay was recorded for the strawberry fruit during later exposure at room temperature (20 °C). The first trials showed a good effectiveness of *M. piperita* and *R. officinalis* essential oils, which more than halved disease incidence, compared to the control. Further trials are needed to determine consumer acceptance of strawberries exposed to essential oil vapors.

## **Influence of chitosan on the antioxidant status of tomato plants infected by cucumber mosaic virus**

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Fruit quantity and quality loss represents a huge problem for farmers and more generally for human nutritional needs. Viruses are dangerous phytopathogens, that cannot be faced using agrochemicals. Hence, they are responsible for severe crop and quality losses. In such a scenario, chitosan is an interesting eco-friendly solution. The strong decrease of the load of cucumber mosaic virus strain Fny (CMV-Fny) in *Solanum lycopersicum* plants, treated with the combination of *Trichoderma harzianum* T-22 and chitosan, has been previously reported. The present research was aimed to investigate the influence of chitosan on the antioxidant status of CMV-inoculated tomato plants. The transcript amounts of the genes encoding peroxidase, phenylalanine ammonia lyase (PAL) and phytoene synthase 2 (PSY2), were analyzed in leaves. Lutein, lycopene,  $\beta$ -carotene, vitamin C and polyphenols as rutin and naringenin, were determined in ripe fruits. Results show that chitosan treatment, both before CMV and alone, down-regulated peroxidase- and PSY2-related transcripts, while upregulated PAL-related transcripts, compared to only infected plants. Fruits harvested from plants treated with chitosan alone had the highest lutein, lycopene and  $\beta$ -carotene contents. The same fruits also contained more caffeoyl glucoside and less naringenin chalcone, compared to the fruits of control plants. Furthermore, the fruits of control plants had the lowest vitamin C level. In conclusion, chitosan was able to enrich fruits with nutraceuticals, as carotenoids, and it controlled CMV infection likely through phenylalanine-derived products, such as salicylic acid, by affecting the plant antioxidant status.

## Synthetic elicitors affect *Hyalesthes obsoletus* behavioral responses

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*Hyalesthes obsoletus* Signoret (Hemiptera: Cixiidae) is considered to be the principal vector of 'Candidatus Phytoplasma solani', which is the causal agent of the grapevine yellows "Bois noir (BN)" in European vineyards. This planthopper is a polyphagous species living mainly on wild and cultivated herbaceous plants often hosting the phytoplasma. Among alternatives to synthetic pesticides, a good interest was gained by the application of resistance inducers to control plant pathogens and insect herbivores. In a recent study, the activity of some elicitors in inducing recovery of BN infected grapevines was evaluated, and encouraging results were provided by benzothiadiazole (BTH) and by two different formulations containing glutathione plus oligosaccharides (GO1 and GO2). In this study, Y-tube olfactometer bioassays were designed to investigate *H. obsoletus* behavioral responses to volatile emitted by grapevine shoots sprayed with these three commercial formulations containing BTH, GO1 and GO2. Each diluted formulation was applied at three different times (0, 2 and 7 days before bioassays). The number of planthoppers tested was 32 for every treatment (total n=288 specimens). Our study showed that BTH-treated grapevine shoots were significantly repellent for *H. obsoletus* when applied one week before insect bioassays. Moreover, planthoppers were significantly attracted to volatiles emitted by grapevine shoots sprayed with GO2 formulation the same day of bioassays, when compared to the control. No behavioral responses have been elicited by treatments with GO1. The results of this study could be used as part of an integrated pest management strategy to control *H. obsoletus*.

## Clues on the epidemiology of 16SrV-C phytoplasma on *Spartium junceum* in Sicily

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Epidemic of the syndrome 'spartium witches broom' (SWB) on spanish broom (*Spartium junceum*) began in Sicily in 2010. Based on phylogenetic analysis of the 16S rRNA gene, the presence, on symptomatic plants, of phytoplasmas 16SrV-C either alone or in association with 16SrX-D has been ascertained. The role played by insects as vectors of SWB has been investigated in the last four years in different areas of the region. Although 18 species of Homoptera have been collected, only one psyllid, belonging to genus *Livilla*, was found infected by both phytoplasmas. The present study was focused on the assessment of the involvement of this psyllid in the transmission of both phytoplasmas. Psyllids were collected by net directly on spartium plants showing symptoms of SWB. Total genomic DNA was extracted from whole individual psyllids and processed by direct and nested PCR with universal and specific primers for 16SrV and X groups. Moreover, head and trunk of some adults were dissected and processed separately in order to preliminarily ascertain the presence of phytoplasma in salivary glands. Thirty-five % of the analyzed insects were positive to both 16SrV and -X, in single or mixed infections, with a prevalence of 16SrV. Preliminary results of insect heads evidenced only the presence of 16SrV. Both findings, together with the absence of other phytoplasma infected insect species, allow us to hypothesize a major role of this psyllid in the epidemic of SWB.

## Eliciting, antimicrobial and film-forming properties of chitosan applied on fresh fruit and vegetables

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Chitosan is a natural biopolymer obtained from arthropod exoskeletons or fungi by incomplete deacetylation of chitin. It is known for its biocompatibility and bioactivity and it is used in slimming diets or as stabilizer in tablets. When applied on fruit and vegetables, chitosan produces a coating on the treated surface, with chemical and physical properties depending on the acid used to dissolve it. Since '90 of last century, a wide literature is available on the effectiveness of chitosan in the control of postharvest decay of fruit and vegetables, obtained following postharvest dipping or preharvest spraying. The biological activity of chitosan depends on its eliciting, antimicrobial and film-forming properties, which based on literature and our experience, can account for 30-40%, 35-45%, and 20-30%, respectively, depending on the rate of application, coated fruit or vegetable and infecting pathogen. A standard application rate for the control of postharvest decay is at 1%, except for the control of *Penicillia*, where higher concentrations may be needed to provide a good