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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[®] 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 μL^{-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 thermophilic evergreen wood *Pistacio-Quercetum ilicis*, the Mediterranean maquis *Myrto communis*

The use of trees to preserve, improve or restore the structure and the functioning of soils contaminated by heavy metals represents a promising strategy (dendroremediation). In this context, fast-growing and high biomass-producing species showed interesting potentialities. This study was aimed at investigating the physiopathological effects of different heavy metals on 11 poplar and 8 willow clones. Furthermore, their tolerance, phytoextraction potential and the accumulation pattern were also assessed. Two-month-old sprouting-cuttings were cultivated in hydroponic system and treated with one of following salts: cadmium sulphate, lead nitrate, copper sulfate pentahydrate and zinc sulfate heptahydrate. Treatments were carried out in four replicates and untreated cuttings were included as controls. Biomass production variables were measured and phytotoxicity symptoms were assessed. At the end of the trial, the heavy metal accumulation was quantified in leaves, stems and roots. Clones were ranked and clustered based on biomass-production, phytotoxicity symptoms, pathogens and pests resistance, phytoextraction potential and accumulation pattern. Treated clones did not show substantial phytotoxic effects. However, the phytoextraction efficiency and the accumulation patterns displayed high variability depending on the heavy metal. While zinc was the most accumulated metal, cadmium, copper and lead were absorbed with lower concentrations. Although some heavy metals were more efficiently sequestered by leaves, some clones achieved good performances in the absorption at stem level. A first screening of the best scoring clones resulted in the selection of potential candidates for dendroremediation purposes, with special emphasis on clones with high accumulation of heavy metals in the woody tissues.

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Assessing the seasonal patterns of spore deposition of *Gnomoniopsis castaneae* in some chestnut orchards in northern Italy

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Gnomoniopsis castaneae Tamietti is an emerging fungal pathogen of sweet chestnut causing symptoms ranging from nut rot to leaves necrosis, up to bark cankers. To date, little is known about the epidemiology of this pathogen. The aims of this work were i) to detect and quantify the airborne inoculum of *G. castaneae*, ii) to assess the effect of seasonality on spore deposition, and iii) to study the correlation between spore deposition and climatic conditions. Samplings were performed between 2013 and 2015 in three orchards in the northwest of Italy using a novel and optimized spore trapping method combined with a real-time PCR approach. In each orchard, one permanent transect was established by installing 14 spore traps at approximately 15 m intervals. The airborne inoculum was sampled continuously every two weeks. The disease incidence was also assessed by performing fungal isolations from 40 ripe nuts collected from chestnut crowns in each orchard. Climatic data were obtained by installing thermopluviometric stations. Airborne inoculum of *G. castaneae* was detected in all sampling sites. The spore deposition showed different patterns depending on site and climatic conditions; however, similar patterns were recorded in the two years of samplings. On average, disease incidence ranged from 10% to 42.5% in 2013, from 5% to 57.5% in

2014, and from 0% to 40% in 2015. This study provides key information that may be used for the development of epidemiological models.

Monitoring the presence of mycotoxigenic fungi and mycotoxins on Italian rice during the growing season

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Rice is an important crop for Italian agriculture, covering 230,000 ha and represents about 50% of the European production. Climate change is creating several problems in rice cultivation areas because of conditions prone to mycotoxin producing fungi. This could lead rice to have mycotoxin contamination higher than fixed levels by European Commission (Regulaments CE 1881/2006, UE 165/2010, UE 488/2014 and UE 1006/2015). For this reason, a study was planned taking into account nine different rice varieties cultivated in three different areas with three different sowing densities. Rice samples were collected four times along the growing season from milk stage to harvest. An additional sampling time was considered taking into account rice overstay in field for other 15 days after harvest time. Rice samples were analysed for fungal incidence, in particular for *Fusarium* spp. and *Aspergillus* spp., and for AFB₁, DON and sterigmatocystin (STC), a mycotoxin recently signalled in rice, using, respectively, HPLC-FLD, GC-MS and LC-MS/MS. *Fusarium* spp. were the most represented fungal species found in rice (mean incidence from 0 to 19%) and were found mainly at harvest. *Aspergillus* spp. were found with low incidence, in particular *A. section Flavi* (found mainly at milk stage), *A. section Nigri* (found mainly at harvest) and *A. versicolor* (found from milk stage to harvest and even able to increase in samples left in field after harvest time). Mycotoxins tested resulted always below legal limits, however STC resulted >0.1 µg/kg in some samples. No differences were among the three sowing densities considered.

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Assessing the variability of pathogenicity within a group of Italian isolates of *Fusarium verticillioides*, pathogen of *Zea mays*, differing in unique genes of virulence

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The *Fusarium fujikuroi* species complex (FFSC) is one of the largest *Fusarium* complexes. Phylogenetic and molecular analyses show a close relationship within the FFSC species; they may have distinct

phenotypic traits, like mycotoxin production, host-specificity and supernumerary chromosomes (SCs) in addition to species-specific core chromosomes that may even differ among isolates in presence/absence, length and gene-abundance. SCs are important in the biology of pathogenic fungi. In a previous study, adopting a bioinformatic approach, we ascertained the presence of “extra” genomic regions (EGRs), a putative SC, in the genome of an Italian *F. verticillioides* isolate ITEM10027, which lacked in the *Fv.* reference genome Fv7600. For assessing the putative peculiarity of this EGRs within the Italian fungal strains, we collected *Fv* samples from maize kernels sampled in the whole Po valley, Northern Italy. To select a subset of strains, we analyzed the EGRs presence by a PCR approach (presence/absence). The genomes of 24 strains were sequenced by Illumina MiSeq. A bioinformatic pipeline able of highlighting *inter* and *intra* specific differences within the EGRs among the 24 samples was designed. An interesting set of genes with a Gene Ontology differing among the 24 *Fv* strains were found. *In vivo* pathogenicity assays on *Zea mays* kernels indicated significant difference among the 24 samples. We can argue that some of the unique and specific genes found in the 24 strains may provide a sort of “gain of function” in the pathogenicity toolkit that can possibly explain the difference emerged in the pathogenicity assays *in planta*.

Leafhoppers in the vineyard, preliminary data about their possible implication in grapevine leaf mottling and deformation epidemiology

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Grapevine Pinot gris virus (GPGV), a pathogen causing grapevine leaf mottling and deformation (GLMD), has a worldwide distribution on different grapevine cultivars. GPGV can be spread by the mite *Colomerus vitis*, which was demonstrated to be able to acquire and transmit this virus. Since 2017, this virus was described also on many other host plants. As *C. vitis* is monophagous on grapevine, the presence of other vectors of GPGV was hypothesized. The aim of this study was to investigate whether other arthropods can transmit GPGV from host to host. Therefore, hemipteran populations were sampled in infected vineyards in Trentino and Veneto regions from April to November 2017. The collected leafhoppers were classified based on morphological characters and then RNA and DNA were isolated by the TRIzol reagent. Each specimen was tested by RT and qRT-PCR for GPGV detection. Moreover, the morphological species identification was confirmed by sequencing the mitochondrial DNA barcode region. In Trentino region, GPGV was detected from June in *Psammotettix* sp., *Macrostelus* sp., *Agallia* sp., *Jassargus* sp., *Laodelphax striatellus* and in other leafhopper species. On the other hand, preliminary results obtained in Veneto region revealed the occurrence of GPGV in rare individuals of *Aphrodes makarovi* and *Empoasca decipiens*. The comparative analysis with GPGV isolates deposited in the GenBank showed high identity with GPGV. These results revealed that different hemipteran species present in infected vineyards can acquire GPGV, thus opening new possibilities for epidemiological studies as well as for developing control strategies for the disease.

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Preliminary detection of necrotrophic fungal species associated with local caraway and cumin seeds in the Cap Bon region of Tunisia

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This study was performed in order to determine the importance of seed-borne fungi associated with local seeds of caraway (meridian fennel) and cumin. Six samples of caraway and five of cumin were collected from farmers in the Cap Bon region during 2017 to be analyzed by the blotter method to isolate the associated seed-borne fungi. Based on morphological criteria, seven species of necrotrophic fungi were isolated and identified in caraway and cumin seeds, namely *Rhizoctonia* sp., *Alternaria alternata*, *Stemphylium botryosum*, *Cladosporium* sp., *Chaetomium globosum*, *Ulocladium* sp. and *Aspergillus* sp. The main species associated with caraway seeds were *Rhizoctonia* sp., *Alternaria alternata* and *Stemphylium botryosum* with an incidence of 28.5%, 21.8% and 18.6%, respectively. The most frequent fungi in cumin seeds were *Rhizoctonia* sp. followed by *Chaetomium globosum*, with an incidence of 19.7% and 6.25%, respectively. These preliminary results could lead to the carrying out of studies about the implication of such fungal species in seedling mortality in the field, and on the presence of harmful mycotoxins into grains harmful for consumers.

Preliminary investigations on ‘*Candidatus Phytoplasma solani*’ genotypes associated with grapevine Bois noir in Iran

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Bois noir (BN) is induced by ‘*Candidatus Phytoplasma solani*’ (‘*Ca. P. solani*’), and represents an important grapevine yellows disease that causes severe damage to vineyards in a number of countries. In Iran, BN was recorded recently, but there is no information available on the molecular types and their epidemiology. Investigations were carried out from 2015 to 2017 from July to September in the main grapevine-growing regions of Iran. Red and white berry cultivars were observed during the surveys, and leaf samples were collected from vines showing reddening or yellowing of the leaves, leaf rolling, flower drying, shriveled bunches, and/or small black pustules along the canes. After total DNA extraction, amplification of 16S rDNA was carried out. Most of the symptomatic samples were positive using the universal primer R16F2n/R2. Infection from ‘*Ca. P. solani*’ was confirmed using the specific primers for stolbur detection, fStol/rStol. Amplification of the *tuf* and *vmp1* genes was carried out, followed by restriction fragment length polymorphism analysis, to gain more detailed insight into the molecular diversity of these ‘*Ca. P. solani*’ strains associated with grapevines. All of the tested samples belonged to *tuf*-type b. The restriction fragment length polymorphism analysis of *vmp1* allowed five different *vmp1*-types to be defined, with V10 as the most prevalent. These molecular data will contribute to better understanding of the epidemiology of grapevine BN in Iran.