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Microbiome experimental evolution for post-harvest disease management

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The microbial community that develops inside fruit wounds during post-harvest is crucial in fostering or preventing fruit diseases development. However, we still know little about the mechanisms behind the assembly of the wound microbiome, and whether we can steer this process to assemble a beneficial microbiome. Here, we used an experimental evolution approach to select a beneficial wound microbiome able to prevent the development of fruit pathogens. Using apples as a model, we inoculated fruit wounds with five different initial microbiomes (sourced from wild apples, *Pyrus spinosa*, oranges, quinces, and lichens), testing both the ability of fruits to drive convergent selection of wound microbiome composition, and the potential of different sources to generate beneficial microbiomes. The experimental evolution was carried over 10 fruit re-inoculation cycles, and at each cycle we used the wounds from the previous cycle as inoculum. At each generation we estimated the size of the microbial community by plating a portion of the crushed wound, and inferred changes in the diversity, structure, and composition of the wound microbiome using amplicon metagenomics (16S and ITS). After the final cycle, we tested whether the experimentally evolved microbial communities were able to contrast the development of a fruit pathogen, by co-inoculating the crushed wound with conidia of *Botrytis cinerea*. Results show that experimentally evolved communities stabilized after a few cycles of selection and varied in their ability to reduce the

development of *B. cinerea*, suggesting that this approach can be successfully used to generate microbiome-based solutions to post-harvest problems.

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Application of commercial formulations and plant extracts to improve yield, quality, and storability of hydroponic lettuce

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The urgent need to increase the sustainability of crop production has pushed the agricultural sector towards the use of biostimulants based on natural products. The current work aimed to determine whether the preharvest application of two commercial formulations, based on a Fabaceae enzymatic hydrolysate or a blend of nitrogen sources including fulvic acids, and two lab-made aqueous extracts from *Moringa oleifera* leaves (MLEs), could improve yield, quality,

and storability of lettuce grown in a hydroponic system, as compared to untreated control. Lettuce plants treated with the MLEs showed significantly improved quality parameters (leaf number, area, and color), total phenolic content and antioxidant activity, and resistance against the fungal pathogen *Botrytis cinerea*, comparable to that obtained with commercial formulæ, particularly those based on the protein hydrolysate. A difference between the *M. oleifera* extracts was observed, probably due to the different compositions. Although further large-scale trials are needed, the tested MLEs seem a promising safe and effective preharvest means to improve lettuce agronomic and quality parameters and decrease susceptibility to rots.

Testing the efficacy of the Sanodyna® product for the control of *Gnomoniopsis castaneae*

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Gnomoniopsis castaneae (syn. *Gnomoniopsis smitholgyvi*) is an emerging fungal disease causing nut rot of sweet chestnut (*Castanea sativa*), currently affecting chestnut fruits production in several Italian regions. There is a lack of effective management and control strategies against this fungus due to the little knowledge about its epidemiology and its endophytic lifestyle. To date, post-harvest treatments, like the “curatura”, which consists of submerging the fruits in hot water (50 °C) for 45–50 min and cooling them in a water bath at 15–18 °C for an equal time, are the only methods applied to control and manage the disease, also because in pre-harvest, i.e., in the chestnut groves, the application of fungicides is strictly limited by law. This study aimed at testing the efficacy of Sanodyna®, an environmentally-friendly product commercially sold as a sanitizer, to control *G. castaneae* in post-harvest treatments. Three concentrations, minimum (15 ppm), medium (110 ppm) and maximum (520 ppm), were tested for their ability to inhibit *G. castaneae*. The test was first carried out *in vitro*, by using different strains of *G. castaneae* and adding Sanodyna® into the culture-media. Then, the product was tested on chestnut nuts. A reduction in growth was observed *in vitro* compared to the control, but the treatment did not eradicate the disease

from chestnut fruits. Indeed, the product was effective in sanitizing the external surface of the fruit, without being able to reduce significantly the frequency of the fungus. Results obtained in this work are a preliminary step towards implementing a control method against *G. castaneae* using Sanodyna®.

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Development of a new LAMP assay for the fast diagnosis of *Elsinoë fawcettii*

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The ascomycete fungus *Elsinoë fawcettii* Bitancourt and Jenkins (Myriangiaceae, Elsinoaceae) is a quarantined organism in the EU as it can cause important diseases (citrus scab, sour orange rind and common rind) on *Citrus* species, cultivars and hybrids. As the disease is still restricted to Georgia in the EPPO region, the possibility to rely on accurate and solid diagnostic protocols to unequivocally identify *E. fawcettii*, even at its latent stage, would be of great importance in order to prevent its possible introduction in uncontaminated areas and to control its impact. Traditional diagnosis of this fungus can be troublesome and time consuming, requiring well-furnished laboratories and expert operators with skills in mycology. DNA-based detection could therefore enable to overcome these drawbacks and allow a rapid, sensitive and accurate diagnosis of the pathogen. Recently, PCR and qPCR-based assays have been developed, able to simultaneously detect different pathogens affecting *Citrus* species, including *E. fawcettii*. To further

improve detection efficiency, in this work a species-specific assay based on Loop mediated isothermal AMPLification (LAMP) reaction was developed. Results proved the high specificity of the protocol, able to detect as low as 1.00 pg/ μ L of pathogen DNA in less than 30 minutes. The accuracy of the assay and its compatibility with a user-friendly, portable LAMP device, make this real-time monitoring method a tool of choice for routine phytosanitary control of plant material, which could profitably assist in the diagnosis, surveillance and control of *E. fawcettii*.

Stem and root rot of *Cycas revoluta*, a new disease caused by *Phytophthora pseudocryptogea*, and first report of *P. nicotianae* and *P. multivora* on *C. revoluta* worldwide

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Stunting, leaf yellowing and blight, root and basal stem rot were observed in pot-grown 3-year-old sago palm (*Cycas revoluta*) plants in commercial nurseries of ornamentals in Eastern Sicily (Italy). The objective of this study was to investigate the disease etiology. Three *Phytophthora* species (*P. multivora*, *P. nicotianae*, and *P. pseudocryptogea*) were recovered from rhizosphere soil of symptomatic plants, using leaf baiting and a selective medium. However, *P. pseudocryptogea* was the sole species isolated directly from rotten roots and stems. The isolates were identified based on both morphological features and phylogenetic analysis of multiple DNA regions (ITS, β -tub, and COI). The pathogenicity of the three *Phytophthora* species was assessed on potted one-year-old plants using both stem inoculation by wounding and root inoculation through infested soil. *Phytophthora nicotianae* and *P. pseudocryptogea* reproduced all the symptoms shown by plants with natural infections, but the latter was more virulent. Conversely, *P. multivora* induced only mild symptoms of root rot. *Phytophthora pseudocryptogea* was identified as the

pathogen responsible for the new disease observed in Sicilian nurseries, as it was reisolated from artificially inoculated plants, thus fulfilling Koch's postulates. It is the first report of these three *Phytophthora* species associated with *C. revoluta* worldwide.

Into the deep of biological control agents against main grapevine diseases: results of quantitative and network meta-analysis

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The Farm to Fork and Biodiversity Strategies have recently set new goals for the reduction of pesticide usage and substitution of more hazardous active substances. Among alternatives, biological control agents (BCAs) are widely considered thanks to their low impact on human health and the environment. However, studies to evaluate the effectiveness of BCAs in suppressing plant pathogens often report inconsistent results. This research aims to provide a comprehensive overview of the performed studies and achievements for the biocontrol of major grapevine fungal diseases. In particular, a systematic literature review was conducted to summarize the studies on BCAs against downy and powdery mildews, black and sour rots, gray and black molds, and grapevine trunk diseases. The quantitative synthesis of published information showed a positive trend in the number of studies on biocontrol since the '90s. Overall, the most investigated genera were *Bacillus*, *Pseudomonas*, and *Trichoderma*. BCA efficacy depended on BCA-pathogen interactions, and the experimental conditions, with greater variability observed in field. To deepen our knowledge on BCAs efficacies under field conditions, network meta-analyses were applied to collected data on powdery mildew and grapevine trunk diseases. Black mold was also used as meta-analysis case study to provide new insights on the biocontrol of grape ochratoxigenic fungi.

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Isolation and characterization of disease-associated fungal colonies of *Buxus sempervirens* in northern Italy

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Buxus sempervirens (boxwood) is a representative shrub, grown as an ornamental plant in nurseries and mainly used in gardens and parks. Boxwood plants exposed to environmental stress conditions are more prone to subsequent infections, especially after pruning. In a historic garden, located in the province of Verona, prestigious century-old plants of *Buxus sempervirens* are planted. In July 2022 and May 2023, damage such as defoliation, wood reddening, total desiccation and reddened spots on leaves and branches were observed in the boxwood plants. Therefore, the present research aimed to isolate and characterize at the phylogenetic level, based on sequencing of ITS partial regions (rDNA) and TEF-1 α , the fungal colonies causing the lesions. In both years, 2022 and 2023, *Diaporthe stictica* was isolated mainly from branch desiccation and vascular necrosis, while FTSC14 of the *Fusarium tricinctum* species complex (FTSC) was obtained from leaf yellowing; both species have already been reported on boxwood in Italy, France, Germany, and East Asia. In 2023, symptoms of leaf and branch reddening were most frequently observed, and isolates obtained from these symptoms showed morphological aspects attributable to the *Nectriaceae* family. Phylogenetic analysis revealed that these isolates belonged to the FTSC complex; however, they formed a separate group and clustered with none of the *Fusarium* species described so far. Further characterization, such as morphological description and whole genome sequencing, will be necessary to determine whether a new species is involved. Pathogenicity tests will be conducted to confirm their virulence on boxwood seedlings.

Influence of attacks of *Marssonina brunnea* on the transpiration of poplar leaves

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In order to evaluate the effect of *Marssonina brunnea* infection on poplar leaf transpirations, many studies were carried

out in different years on leaves of six-month-old plants (clone "I-214") grown in a thermo-hydro-conditioned greenhouse. Leaves were inoculated with the pathogen by placing the plants for 24 hours under diseased adult plants during prolonged infectious rains and compared with not inoculated ones. Leaf transpiration ($\text{gr/m}^2/10'$) was evaluated using a high-pressure plant transpiration meter. The transpiration observed in leaves with more than 5 necrotic spots per cm^2 of leaf surface was significantly higher than that of healthy leaves. The transpiration peak was observed in the hours 1-5 pm, on leaves of 30-40 days old, inoculated to their 4th-5th day of age, with the highest attack intensity, equal to 15 necrotic spots per cm^2 . On these latter, the transpiration observed 35 days after inoculation during an entire day (24 hours) with 27 °C (average) was about 490 g/m^2 while in the healthy leaves was 438 g/m^2 , showing an increase of over 12% due to the pathogen. During the hour between 3 and 4 pm, with temperatures of 32 °C, the transpiration of the diseased leaves was about 32 g/m^2 against 26 g/m^2 on the healthy ones, with 23% increase. The transpiration increase could be associated to the direct loss of water through the necrotic spots caused by the fungus, uncontrolled by the stomata. Considerations were conducted on the possibility of interaction of increased transpiration with summer drought, increasingly frequent due to climate change.

Biological control of wilting disease of snapdragon (*Antirrhinum majus*) caused by *Phytophthora cryptogea*

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Phytophthora diseases are a severe problem in nurseries and some species, such as *P. cryptogea*, have a broad host range. *Phytophthora cryptogea* is known to cause root and crown rot in more than 140 genera of 50 plant families and has been reported on more than 40 species of ornamental plants. This pathogen is one of the most common species threatening annuals and herbaceous perennials, particularly those belonging to the orders Asterales and Lamiales. The common snapdragon (*Antirrhinum majus*; order Lamiales) is native to the Mediterranean region, and it is a very popular garden perennial. In the European Union, biological control is preferred to conventional chemical fungicides,

due to their side effects on human health and the environment. In this study the efficacy of *Trichoderma hamatum* strain TA against *P. cryptogea* *in vitro* and on snapdragon seedlings was evaluated. *Trichoderma hamatum* significantly reduced pathogen growth *in vitro* and disease severity *in vivo* compared to control. Biological control activity was enhanced when treatments were combined with the use of biochar as a component of the growing soil. The present study provides preliminary information that can be used for the development of integrated control strategies for *Phytophthora* spp.

Preliminary studies on the resistance of Italian *Pyrenophora teres* isolates to fungicides with different modes of action

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Pyrenophora teres f. *teres* (*Ptt*) and *P. teres* f. *maculata* (*Ptm*) are respectively the causal agents of net and spot forms of barley net blotch (NB), an economically important fungal foliar disease. Fungicides play a crucial role in NB management, along with cultivar choice and cultural practices. However, the emergence of fungicide resistance in *Ptt* and *Ptm* populations, reported in several worldwide areas, has become of great concern. For this reason, this study aimed to assess the sensitivity of an Italian *P. teres* population to the main fungicide modes of action (MoA) commonly used during barley cultivation (tebuconazole for demethylation inhibitors, DMI; azoxystrobin for quinone-oxidoreductase inhibitors, QoIs; fluxapyroxad for succinate dehydrogenase inhibitors, SDHIs). A total of 67 isolates were tested *in vitro* using fungicide discriminatory concentrations to identify potentially resistant isolates. Molecular analysis was performed to evaluate specific mutations linked with fungicide resistance. Mitochondrial cytochrome b aminoacidic substitutions, mutations in three subunits of SDH gene (B, C, D) and in the promoter and coding sequence of Cyp51A and Cyp51B were investigated. Finally, for the different fungicides, the dose-response relationship and EC₅₀ were calculated. Mutations associated with resistance to all tested MoAs were detected in 20 *P. teres* isolates and a significant

reduction in the *in vitro* fungicide sensitivity was observed. Further research will be realized increasing the number of isolates and active ingredients to obtain more representative information. Studies on fungicide resistance in *Ptt* and *Ptm* populations are essential to promote a correct and effective management of barley NB.

Antimicrobial and antimycotoxigenic proprieties of *Trametes versicolor* extract against *Fusarium* spp. and *Phytophthora infestans*

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Maize, wheat and potato are among the four most cultivated crops worldwide and affected by fungal and oomycete diseases leading to significant yield losses. *Fusarium* spp. cause head blight in wheat and maize, causing cereal contamination with mycotoxins, e.g. fumonisins and trichothecenes, with a notable impact on human and animal health. *Phytophthora infestans*, the causal agent of potato late blight, affects leaves and tubers. In view of promoting the use of bio-products capable of preserving efficacy and low toxicity, natural fungal products represent a promising source to control phytopathogens and mycotoxins. Here, we propose the use of a cultural filtrate (CF), rich in peptides and polysaccharides, derived from the culture of asexual mycelia of *Trametes versicolor*, a ligninolytic fungus known for its antimicrobial and antioxidant properties. Moreover, an active principle present in the CF was also tested, that is Tramesan, an exopolysaccharide known for its capacity to activate the plant defenses and control the aflatoxin production in *Aspergillus flavus*. Several *in vitro* tests were chosen to mimic cereal infection and mycotoxin production of *F. verticillioides*, *F. graminearum*, and *F. langsethiae*, and to monitor growth of *P. infestans*. CF and Tramesan have different effects on mycelial growth of the pathogens and revealed a selective inhibition mechanism on *Fusarium* toxins. These valuable insights suggest the need for specific screening of mycotoxin biosynthesis pathways before the use of natural compounds, especially for CF. Therefore, the discovery and application of new fungal metabolites represent a sustainable method for controlling pathogen growth and mycotoxin accumulation.

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Features and comparative genomics between three strains of *Colletotrichum graminicola* using chromosome level genome sequences

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Colletotrichum graminicola is a filamentous Ascomycete fungus and a pathogen of maize. Recently, we presented the updated version of the genome sequence of strain M1.001 (CGRA01V4, from USA). Now, we include the genome sequences of two more strains of *C. graminicola*. We used PacBio, ONT and Illumina sequencing technologies to obtain the genome sequence of CROI41 (from Croatia), while for ARG5133-7 (from Argentina) we used ONT and DNBSeg sequencing platforms. First, we filtered the reads by length and quality. Then, we prepared a draft assembly using minimap2-miniasm. To improve these assemblies, we used one round of polishing with Pilon, and second round of polishing with Racon. To get the final assembly, we did a scaffolding using longstitch. Finally, we manually verified the assembly using dotplots to the reference genome and manually checking the alignments of the long reads. The assemblies are chromosome-level with CROI41 having 12 sequences and a total length of 57.9 Mb, while ARG5133-7 presented a total of 13 contigs and a total length of 56.4 Mb. The largest contig presented a size from 8 and 8.3 Mb. Additionally, we observed different numbers of contigs with a size less than 2 Kb. CGRA01V4 had three minichromosomes, while CROI41 exhibited two contigs, and ARG5133-7, three contigs with similarity to the minichromosomes. Our results also revealed the presence of several structural rearrangements (SR) among the three strains. In conclusion, our comparative genomic analysis has provided some preliminary insights about the genomic features and structural rearrangements between CROI41, ARG5133-7 and CGRA01V4.

Wood Vinegar: an eco-friendly and sustainable solution for plant health from Circular Economy

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Pesticides and fertilizers are widely used worldwide to protect plants' health and to support their growth and development. However, these chemicals could negatively affect the environment, for their toxicity on terrestrial and aquatic ecosystems. Consequently, policies aiming at a “Green” and Circular Economy are increasingly introduced by many governments worldwide. The objective is to use by-products, waste and residues as secondary raw materials to generate high-value and useful products. In this frame, Wood Vinegar (WV) could be a feasible alternative to traditional pesticides and fertilizers, and it is perfectly integrated in the context of Circular Economy. Indeed, it is a by-product of woody-biomass pyrolysis, and it is composed of 80-90% of water, and 10-20% of organic compounds, mainly acetic acid, phenols and ester. According to its richness in acetic acid and tannins, it has been often reported as an exceptional booster of plant defences, as well as of root system development, strengthening the plant and making nutrient assimilation more efficient. However, scientific evidence about the biological mechanisms involved in its bioactivities is still lacking. Here, data are reported about its effects as a biostimulant, especially on seed germination and on root development by using *Nicotiana tabacum* and other important food crops (e.g., tomato, lettuce or bean) as model plants. In addition, gene expression analyses were conducted to successfully reveal the defence pathways triggered in WV-treated plants, supporting its activity as biostimulant and defence inducer. Lastly, its antimicrobial activity against Gram-positive and Gram-negative phytopathogenic bacteria has been also assessed.

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Phytophthora citrus diseases: current situation and management strategies in Tunisia

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In the Mediterranean areas, citrus is an important agricultural crop, with a production of about 10 million tons/year. Citrus fruits constitute a significant portion of the agricultural outputs in Tunisia. In 2019/2020, a land area of 29 thousand hectares was occupied by citrus. However, production in this region is affected by *Phytophthora* diseases. In spring 2023, a survey was conducted in Tunisian citrus orchards, to study the citrus farms characteristics (type of management, citrus varieties, geographic data), farmers' knowledge of the pathogens and management strategies applied to control *Phytophthora* spp. The results showed that the majority of the farmers were male, middle-aged adults and had a secondary education. Most of the citrus orchards were of medium area (59%) and the most common densities were 350, 360 and 400 ha. *Citrus sinensis* L. Osbeck and *C. limon* varieties planted on *C. aurantium* rootstock were the most used. Symptoms of *Phytophthora* citrus diseases consisted mainly in gummosis, dieback, wilting, and damping-off. Such information should be taken into consideration for a best management strategy of *Phytophthora* citrus diseases.

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Endophytic, canker-inducing Botryosphaeriaceae causing flowering ash (*Fraxinus ornus*) dieback in central Italy

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A widespread dieback of flowering ash (*Fraxinus ornus* L.), with high mortality of young regeneration, was observed in some woody areas of central Italy (Tuscany). Symptoms appeared at the beginning of the growing season as typical sunken, light-brown cankers on the stem of young trees, which died as soon as the cankers girdled the stems. Many plants reacted vigorously to infection trying to callus over the lesions. Starting from 2018, a study was undertaken to clarify the etiology of the disease. Three botryosphaeriaceous fungi were isolated with high frequency from symptomatic plants: *Botryosphaeria dothidea*, *Diplodia fraxini* and *Neofusicoccum parvum*; a *Fusarium* sp. was also sometimes isolated. Artificial infection tests were carried out in July 2022 on two-year-old *Fraxinus ornus* seedlings with the four fungal taxa. All the botryosphaeriaceous fungi produced cankered lesions, with *B. dothidea* which reproduced the typical elongated cankers observed in the field. It is hypothesized that environmental stress was the main factor weakening trees and predisposing them to aggressive attack and colonization by pathogenic Botryosphaeriaceae. In fact, the areas with higher disease incidence and severity were those characterized by harsh environmental conditions, with low moisture, prolonged drought, especially on poor and gravelly soils.

First report of *Phytophthora megasperma* and *P. pseudocryptogea* on *Rosmarinus officinalis* in Tuscany, Italy

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In January 2023, a severe dieback of *Rosmarinus officinalis* L. plants was observed in a rosemary plantation at Casole d'Elsa, Siena (Italy). Symptoms, including root rot, browning of the collar with discoloration of the internal tissue, leaf blight and stunting, closely resembled the typical root and crown rot syndrome induced by *Phytophthora* species. The lateral flow immunoassay test specific for *Phytophthora* species applied *in situ* on *R. officinalis* on symptomatic tissue samples gave positive results. In order to identify the associated *Phytophthora* taxa, roots, stem and soil samples were collected and taken to the laboratory. The baiting method was used in the laboratory for priming oomycetes. Only one of the two

cultured morphotypes, produced gametangia and oospores, confirming to be homothallic. The other one was heterothallic. DNA-based identification of these isolates revealed complete (100%) homology with respectively *Phytophthora megasperma* and *P. pseudocryptogea* for both rDNA-ITS and CoxI gene regions. This is the first report of *P. megasperma* and *P. pseudocryptogea* causing disease to *R. officinalis* in Italy. The pathogenic occurrence of these two harmful oomycetes on *R. officinalis*, could represent a new problem to the cultivation of this economically important aromatic plant species in the Mediterranean area.

Microbiome innovations to support the ecological transition in agriculture

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Plant microbiomes are key components for ecosystem health in all terrestrial ecosystems including agriculture. The plant microbiota, which consist of bacteria, archaea, protists and fungi, is vertically transmitted by seeds and replenished horizontally from soil. All plants are holobionts and form a functional unit with its microbiome. Plant diversification and co-evolution shaped the plant microbiome and designed their specific composition and functional interplay including natural biocontrol of pathogens. Human activities in the Anthropocene, and especially intense agriculture, are linked to a significant shift of diversity and evenness of the plant microbiota. This shift is characterized by a decrease of host specificity and symbionts, and an increase of r-strategic microbes, pathogens, and hypermutators. Findings from plant microbiome research over the past 20 years clearly call for management of the microbiome and joint attention to the crop holobiont. Plant microbiomes can be managed either directly by applying (i) microbiome transplants, (ii) microbes with beneficial properties, or (iii) microbiota-active metabolites, or indirectly by changing environmental conditions in a way that microbiomes also shift their structure and function from dysbiosis into a healthy state. Examples for the different strategies for plant protection will be presented, and risk associated with the technology will be discussed. Beyond, the plant microbiome is connected across systems and crucial for human and planetary health

issues as well. This will be discussed in frame of the biodiversity crisis and the planetary boundary concept.

Evaluation of soil quality and differences in microbial community structure in asymptomatic and symptomatic kiwifruit orchards

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Actinidia chinensis has been affected in Italy since 2012 by the Kiwifruit Vine Decline Syndrome (KVDS), which causes many plant and production losses. This study aims to characterize the aetiology of KVDS, using both traditional and molecular approaches, and to assess the impact of the disease on soil health and biodiversity. Root and rhizosphere soil samples were collected in 2022 from 5 kiwifruit orchards in Lazio, both from asymptomatic and symptomatic trees (n = 4 replicates), in two different seasons: spring and autumn. Isolations from symptomatic roots and baiting technique from rhizosphere samples confirmed the presence of potential kiwi pathogens, i.e.: *Cylindrocarpon* spp., in particular *Ilyonectria liriodendri*, *I. robusta*, *Dactylonectria pauciseptata*, *D. ecuadoriensis*, and oomycetes, *Phytophthora vexans*, *P. chamaeophyon*, *P. litorale*, *P. heliocoides*, *Pythium dissotocum*, *Phytophthora plurivora*, and *P. acerina*. Pathogenicity tests of the most representative isolates are in progress at CREA-DC of Rome. Physico-chemical characteristics (pH, electrical conductivity, total nitrogen, total organic carbon, elemental contents, water-soluble carbon), enzyme activities (urease, phosphatase, and β -glucosidase), microbial activity (basal soil respiration), biomass and structure of the microbiome using phospholipid fatty acid (PLFAs) extraction were analyzed on soil collected from the rhizosphere of healthy and diseased kiwi plants to understand possible correlations with KVDS. Preliminary results showed well-defined diversity between

asymptomatic and symptomatic rhizosphere in terms of soil physicochemical parameters and microbial living biomass. Pathogenicity test will shed light on the role of the different fungal and oomycetes species in the development of the diseases.

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Transmission evidence by *Aculops lycopersici* (Acari: Eriophyidae) of tomato fruit blotch virus (ToFBV)

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Tomato fruit blotch virus (ToFBV) is a blunavirus (*Kitaviridae* family) recently identified in several tomato cultivation areas worldwide. Although ToFBV infectivity and vectors have not yet been well clarified, a correlation has been observed between ToFBV infection in tomato plants and infestation of the tomato russet mite *Aculops lycopersici* (family Eriophyidae), suggesting that the latter may play a role in virus transmission. The present study aimed to ascertain the function of *A. lycopersici* in ToFBV transmission, evaluating the virus' presence in mites collected from infected tomato plants, and studying the mites' transmission ability to healthy tomato plants. To confirm ToFBV infection in both infected tomato samples and russet mites collected from the same samples, a conventional RT-PCR assay with specific primers was performed. Therefore, using a stereomicroscope, 15 russet mites were transferred on each healthy tomato plant, for a total of 30 plants, and the mite propagation area was surrounded with insect adhesive barrier to confine the mites to the selected leaves. The infested plants were grown within mite-proof cages and moved to growth chamber. The experiments were performed in duplicate, using a healthy tomato plant as negative control for each test. For individual repetition, new shoots from each plant were collected at 3, 7 and 10 days post inoculation (dpi) and tested by conventional RT-PCR. Results showed that russet mites successfully transmitted ToFBV to healthy tomato plants under laboratory conditions as early as 3 dpi, highlighting the potential epidemiological role of *A. lycopersici* in spreading ToFBV in the field.

Sustainable products to control the strawberry angular leafspot

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Within the scope of the EU Regulation proposal on the sustainable use of pesticides to reduce dependence on chemicals until reaching Zero-tolerance by 2030, reliable and convenient alternatives to control the plant bacteriosis are investigated. Unfortunately, sustainable biocontrol agents are not always available or their economic impact on crop production is penalizing compared to the conventional products (e.g., heavy metals and antibiotics). In this study, the essential oils-based product Microsap[®] BIO 458 (458), the foliar fertilizer Probaction[®] and the resistance inducer Bion[®] (unregistered on strawberry) were tested *in vitro* against *Xanthomonas fragariae* (Xf, DISTAL 12191 strain), the causal agent of strawberry angular leafspot (ALS). Two experiments on young strawberry plants were carried out under controlled conditions by using 458 at 0.15% and 1%, Probaction[®] at 0.4% and Bion[®] treated once at 150 ppm and twice at 100 ppm against ALS. *In vitro*, all the tested products, except Bion[®], resulted bactericidal. *In vivo*, all products reduced the ALS severity on strawberry plants, in particular, 458 at 0.15% and 1% gave a relative protection (RP) of about 40% and 34%, respectively; Probaction[®] gave 67% RP, while Bion[®] treated once and twice provided approx. 38% and 74% RP, respectively. Streptomycin sulphate (100 ppm) used as positive control gave RP of about 65% in the first experiment and of 44% in the second. All tested products showed their ability in reducing ALS severity and thus they offer a valid alternative to chemicals in the disease management to reduce the inoculum sources.

A novel promising bioproduct to control bacterial plant diseases

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The control of bacterial diseases is nowadays still limited to copper compounds, whose use is regulated by European Union since 2020 for its environmental impact, or to few biological control agents (BCAs). In this study, the efficacy of the foliar fertilizer Probaction® at 0.3% and 0.4% was tested *in vitro* by macrodilution method against strains of *Xanthomonas vesicatoria* (Xv), *X. fragariae* (Xf), *Pseudomonas savastanoi* subsp. *savastanoi* (Pss) and *Erwinia amylovora* (Ea). Probaction® at 0.3% was also tested *in planta* under climatic chamber conditions against bacterial leaf spot of tomato (BLST) caused by Xv. In particular, tomato plants cv. VF10 were treated with the product at the leaves or at the root apparatus; after 24 h, the plants were experimentally inoculated by spraying the Xv suspension (*ca.* 10⁷ CFU/mL) at the leaves. Streptomycin sulphate (100 ppm) and sterile distilled water (SDW) were used as positive and negative controls, respectively. *In vitro*, Probaction® inhibited the growth of Xf, but it was not effective against Xv, Pss and Ea. *In planta*, the tomato plants treated at leaves or at roots showed a lower BLST severity (*ca.* 18 and 14 spots/leaf, respectively), in comparison to that on negative control plants (SDW, *ca.* 27 spots/leaf). The efficacy of Probaction® in reducing BLST severity, associated to its inability in inhibiting Xv growth *in vitro*, may suggest its role in triggering the plant immune response.

Effects of waterlogging and drought stresses on pathogens infection in pomegranate

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Pomegranate (*Punica granatum* L.) in Italy is threatened by several biotic and abiotic stresses. Their impact on pomegranate varies according to the intensity of the individual stresses and the simultaneous presence of different stressors. Pomegranate cultivation is currently limited by the emerging pathogens *Coniella granati* and *Phytophthora palmivora*. It is ignored if they benefit from previous stress caused in pomegranate by periods of heavy rainfall or intense drought. Therefore, in this work we evaluated the effect of drought or waterlogging on the aggressiveness of the two pathogens. With

this purpose, two-year-old plants were subjected to regular watering (C), drought (D) and waterlogging (W) conditions and, afterwards, stem-inoculated, individually or simultaneously, with *C. granati* and *P. palmivora* or with a PDA plug as control. Lesion length in the stem was measured and used as a proxy of plant susceptibility. W significantly increased *C. granati* lesion length (140%), and D significantly decreased *P. palmivora* lesion length (60%), as compared to C-infected plants. *C. granati* lesion length significantly increased when plants were co-inoculated by *P. palmivora* and this was dependent on the watering condition (C, D or W) experienced by plants before infection. Lesion length caused by *P. palmivora* was not altered when plants were co-inoculated with *C. granati* irrespective of the watering conditions experienced by plants before infection.

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Selection of endophytes isolated from Italian rice cultivars as biocontrol agents against *Fusarium fujikuroi*

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Rice is among the most important staple foods worldwide. Italy is the biggest rice producer in Europe. The main seed-borne disease affecting rice in Italy is bakanae disease, caused by *Fusarium fujikuroi*. Typical symptoms include seedling damping off and internode elongation. Bakanae disease has become a concern for seed companies and farmers as fludioxonil, the only conventional active ingredient allowed for *F. fujikuroi* control, is a candidate for substitution in the EU. The main disadvantage of biocontrol agents is their reduced efficacy in field conditions. Endophytes, on the other hand, due to their close relationship with the host, are theoretically less susceptible to abiotic stressors. The aim of the work was to select potential biocontrol agents for preventive seed dressing against *F. fujikuroi* among a collection of rice endophytes isolated from 24 Italian cultivars. *In planta* screenings were

performed using a total amount of 135 isolates in order to select endophytes capable of reducing bakanae symptoms similarly to fludioxonil under controlled conditions. The selected strains were molecularly identified and tested for their potential to inhibit *F. fujikuroi* *in vitro*. A second *in vivo* biocontrol efficacy assay allowed to confirm the selection of 6 endophytes, 4 bacterial and 2 fungal strains, due to their ability to reduce bakanae disease severity up to 50%. Further investigation on biocontrol mode of action and plant growth promotion features of the endophytic strains will be performed through gene expression analyses to select candidates for field trials and to develop downstream stabilization and formulation.

Selection of beneficial bacteria with potential application as seed treatment agents for eco-friendly approach in sustainable agriculture

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Seed-biopriming is an emerging attractive technique based on the application of beneficial microorganisms on the seed surface to stimulate seed germination, plant growth, and protect the seed from biotic and abiotic stress. In this study the impact of seed treatment with bacteria previously isolated from the seed endosphere on seed germination, plant growth and black rot disease caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*) was evaluated. More than 100 bacterial endophytes were isolated from seed of *Brassica* genotypes. Based on 16S rRNA gene sequence analysis they belonged to different genera within *Firmicutes*, *Proteobacteria* and *Actinobacteria*. Ten shortlisted isolates were chosen based on antagonistic activity against relevant pathogens of Brassicas and plant growth promoting traits. To optimize a biopriming protocol, different priming times and bacterial concentrations were tested. Viable cell count and germination ratio were determined. Exposure time and endophyte concentration did not affect seed germination although influenced the number of viable cells adhering to the seeds surface. The optimized protocol was used to treat surface-sterilized broccoli seeds cv. Calabrese Natalino and trials were conducted in pots under greenhouses conditions. At the stage of 3–4 true leaves, the plants were challenged with *Xcc* by leaf clipping and disease monitored up to 21 days.

Some bacterial endophytes effectively enhanced initial step of broccoli development by increasing seed germination and/or reducing incidence and severity of black rot disease. Further study will be carried out to develop microbial consortia and to evaluate their collective performance on plants to select those potentially adoptable in sustainable agriculture.

Phylogeny and morphology of the oak pathogen *Phytophthora quercina* isolates from Europe and North Africa, reveal a new closely related species

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Phytophthora quercina is a fine root pathogen associated with oak decline in Europe. More recently this oak-specific pathogen has spread across Mediterranean basin, including Northern African countries, mainly associated with chronic decline on *Quercus cerris*, *Q. faginea*, *Q. ilex*, *Q. petraea*, *Q. pubescens* and *Q. robur* and, more recently, *Q. suber*. Since several distinct morphological phenotypes were detected amongst a European population of *P. quercina* isolates, morphological and phylogenetical analyses were conducted to assess the intraspecific variability. Overall, 64 different *P. quercina* isolates from several European and Northern African countries, previously identified based on ITS sequences analyses, were studied. Colony morphology, growth rate at different temperatures and morphological characteristics of the reproductive structures were assessed. In addition, three nuclear (ITS, HSP90 and *βtub*) and the two mitochondrial (NADH and *cox1*) gene regions were investigated by phylogenetical analyses. The results showed a high variability within the *P. quercina* isolates, in terms of colony morphology, size of the main reproductive structures and growth rates. Phylogenetical analyses (Bayesian Inference and Maximum Likelihood) discriminated 12 different lineages, with clear association between host and geographic provenance. In addition, a putative new species, closely related to *P. quercina* was isolated from *Chamaecyparis lawsoniana* var. *globosa* nursery plants in Croatia, is here described.

First report of *Fusarium* isolate belonging to the *tricinctum* species complex (FSTC) causing olive fruit rot in Italy

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Olive (*Olea europaea* L.) is affected by different fungal pathogens causing fruit rot, which may influence both oil yield and quality. In Autumn 2022, rotting fruits bearing red-yellow mycelium and ochre sporodochia were observed in different olive batches in the Viterbo province, Italy. Colonies producing abundant fluffy white aerial mycelium with yellow to red pigmentation from the bottom were obtained from sections plated on PDA. Macroconidia were slender, slightly curved, from 5 to 7 septate, while microconidia were sporadic, unicellular or with a single septum, and measured $8.5\text{--}13.5 \times 3.5\text{--}5.0 \mu\text{m}$. Pathogenicity tests were performed using fungal pure cultures inoculated on healthy sterilized olives and the fungus re-isolated and morphologically and molecularly characterized, fulfilling the Koch's postulates. This is the first report of olive rot caused by *F. arthrosporioides/avenaceum* in Italy and abroad, suggesting that different species belonging to the *Fusarium* genus can cause fruit rotting and that their specific role remains to be investigated.

Molecular and morphological characterization of *Colletotrichum fioriniae* (*Colletotrichum acutatum* species complex), a new emerging pathogen of olive fruits in central Italy

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Olive anthracnose causes dramatic losses of fruit yield and oil quality worldwide. Twelve *Colletotrichum* species belonging to *C. acutatum*, *C. boninense*, and *C. gloeosporioides* complexes are associated with this disease, with *C. godetiae* being the dominant species in Spain, Italy, Greece, and Tunisia, *C. nymphaeae* in Portugal and *C. fioriniae* in California. *C. fioriniae* was reported for the first time in Italy in 2021. During

a survey in different municipalities of Viterbo province in Latium, central Italy, in 2022, 210 symptomatic drupes, representing variable rates of infected olives in the different orchards (from 2.5 to 12.8%) were collected. Fungal colonies showing morphological traits of *Colletotrichum* spp. were isolated from all the drupes and presented a greyish cottony mycelium, pale orange with dark flecking in reverse, hyaline smooth-walled aseptate conidia, narrowly elliptical, measuring $7\text{--}14 \mu\text{m} \times 3\text{--}5.25 \mu\text{m}$. A molecular analysis using different marker genes (ITS, TUB2, ACT, CHS-1, HIS3 and GAPDH) was performed on a randomly selected pool of 50 isolates. Unexpectedly, 48 isolates perfectly matched the deposited sequences of *C. fioriniae*, whilst the two-remaining to *C. acutatum*. Pathogenicity tests on punctured and non-punctured fruits for a selection of *C. fioriniae* strains showed a level of pathogenicity at least comparable with *C. acutatum*. These results suggest that *C. fioriniae* might be a new emerging *Colletotrichum* species causing olive anthracnose in central Italy.

Genome mining approaches reveal secondary metabolites in *Streptomyces* sp. related to biocontrol activity

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Streptomyces are a large and valuable resource of biocontrol agents, bioactive and complex secondary metabolites with a wide range of applications. Genomic information can critically guide functional studies to understand phenotypic characters and to propose valid hypotheses on the related mechanisms. Phylogenetic and functional genome analysis was carried out to explain the endophytic lifestyle, the biocontrol activity of *Streptomyces* sp. DEF48 in reducing *Fusarium graminearum* infection and deoxynivalenol contamination in wheat. The genome of DEF48 was sequenced using Illumina technology. The complete genome of the strain DEF48 consists of 9,147,966 bp (71.26 mol% GC content). Phylogenetic analyses revealed that *Streptomyces* sp. DEF48 was closely related to *Streptomyces griseoviridis*. Interestingly, functional annotation highlighted the presence of genes involved in plant growth promotion and biocontrol activity. The presented approach aids to explain the capability of DEF48 to directly antagonize *Fusarium graminearum*, confirming the effectiveness of genomics studies.

Ozone treatments: environmentally friendly and effective techniques for sanitation of seed-borne pathogens on maize and corn salad

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Maize sprouted seeds and corn salad microgreens are popular ready-to-eat food of high nutritional value. The use of pathogen-free seeds is crucial to ensure high seed quality and prevent potential microbial contamination. In this study, we developed a lab-scale process with dry gaseous ozone (O₃) and ozonated water (W-O₃) as a green technology for disinfecting the seed-borne pathogens *Pantoea stewartii* (*Ps*) on maize and *Acidovorax valerianellae* (*Av*) on corn salad and to enhance seed germination. Maize and corn salad seeds were experimentally infected with *Ps* and *Av*, respectively (approximately 10² CFU seed⁻¹). Then, by using an ozone generator (Kleffis Groups, s.r.l., Italy), infected seeds were treated with O₃ and W-O₃, respectively. Seed testing confirmed the sanitation of both *Ps* and *Av* in maize and corn salad and, besides, a remarkable reduction of culturable saprophytes (≥90%) on all seeds. Furthermore, germination tests highlighted a significant ($p < 0.05$) increase of the germination performance for both O₃ and W-O₃ treated maize samples, as compared to control. For corn salad seeds, no significant differences of germination were recorded between treated and untreated seeds (>96% for all samples). Thus, the implemented ozone treatment is able to sanitize infected seeds and improve their germination rate. Further studies are currently ongoing to confirm the disinfection efficacy of the same protocol on clinical bacteria, such as *Salmonella* spp. and *Escherichia coli*, which are reported to be present associated to seeds.

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Pathogenicity tests on 'Golden Delicious' leaves indicate *Colletotrichum chrysophilum* as a key pathogen compared to *Alternaria* species

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The increase of *Colletotrichum gloeosporioides* species complex (CGSC) is generating considerable concern in Europe, particularly the species *Colletotrichum chrysophilum*, which has been reported in Spain, Trentino-Alto Adige and Emilia-Romagna regions (northern Italy) to cause Glomerella leaf spot (GLS) and Apple bitter rot (ABR). During surveillance and microbiological isolations of leaves from apple orchards in Emilia-Romagna with symptoms attributable to GLS, colonies belonging to CGSC and high concentration of *Alternaria* sp. were obtained. The genus *Alternaria* is associated with *Alternaria* leaf blotch on apple leaves, a disease that has remarkable symptom similarities with GLS, such as chlorosis, severe leaf necrosis and early defoliation, leading producers to mismanage the disease. Therefore, a pathogenicity assay was conducted with molecularly characterized leaf isolates M932 of *C. chrysophilum* and P864/22 of *Alternaria* sp., in order to compare their virulence on apple leaves tissues. Clean surface of wounded and unwounded detached 'Golden Delicious' leaves were inoculated in three infection points with 20 µl of spore suspension (10⁵ spores ml⁻¹) and incubated in moisture chamber at 25 °C with a 16/8-h photoperiod. After 12 days, only M932 was able to produce necrosis and chlorosis on leaves, whereas both P864/22 and the control with sterile water remained asymptomatic. Based on these preliminary results, further pathogenicity tests will be conducted by inoculating *C. chrysophilum* in combination with *Alternaria* sp. on leaves, fruits and two-year-old apple plants to assess whether *Alternaria* sp. acts as a secondary colonizer promoting disease severity.

Ecological studies on *Diaporthe eres* and predictive modelling for hazelnut defects: insights to support effective crop protection

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The browning of hazelnut kernels visible upon half-cutting and surface discoloration were recently attributed to *Diaporthe eres*. A comprehensive understanding of its ecology and epidemiology is requested for effectively support crop protection. Over the last decade, efforts have been dedicated to collect all information available and to produce data necessary to define the growth stage of crop susceptibility and to develop a prediction model for defect incidence. A comprehensive ecological study was performed to describe and model the effect of different abiotic factors such as

temperature (T, 5–35 °C) and water activity (a_w 0.83–0.99) regimes on *D. eres* mycelial growth, pycnidial conidiomata development and asexual spore production. Crop susceptibility was defined based on artificial inoculation managed in greenhouse. Building upon these insights, a mechanistic model was developed, encompassing *Diaporthe*'s infection cycle and ecological requirements. This model leverages hourly meteorological data to deliver a disease index, in two hazelnut growth stages, BBCH81 and BBCH 89, compared to hazelnut defects incidence at harvest to estimate the risk of hazelnut defect occurrence at BBCH89. The validation of the model employed georeferenced data on hazelnut defect occurrences, supported by meteorological data, collected from the Caucasus region and Turkey between 2014 and 2019 (480 records). The incidence of defective hazelnut was correctly predicted in 71 % and 72% using respectively model output at BBCH81 and BBCH 89. While further data acquisition and research efforts are imperative, this study represents a pivotal tool for stakeholders involved in the hazelnut value chain.

The pathogenic role of soil-rhizosphere in Fusarium foot rot of durum wheat

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This investigation examined *Fusarium* populations from soil rhizosphere of durum wheat plants affected by *Fusarium* foot rot (FFR). Plants were harvested at tillering growth phase in 8 cereal farms in the most representative areas of durum wheat production in Sicily. Seventeen *Fusarium* species were identified based on their morphology and molecular traits. Thirty pure cultures of the most frequent and representative *Fusarium* spp. (i.e., *Fusarium acuminatum*, *F. compactum*, *F. equiseti* and *F. culmorum*) were assayed *in vitro* to evaluate their effect on seed germination and seedling development. In comparison to the (non-inoculated) controls, *F. culmorum* showed the greatest inhibition of all vegetative parameters followed by *F. acuminatum*, which decreased seed germination, shoot height, root length and vegetative vigour by 80.2, 79.6, 90.8 and 97.4%, respectively. In contrast, *F. compactum* and *F. equiseti* increased seed germination rate, albeit without reaching statistical significance. Instead, *F. compactum* significantly increased both root length (+ 39.0 %) and vegetative vigour (+ 17.0 %), whereas *F. equiseti* improved both coleoptile growth (+ 34.0 %) and vegetative vigour (+ 18.0 %). These results corroborate the pathogenicity of *F.*

acuminatum and *F. culmorum* in terms of reduced seed germination and seedling growth of durum wheat. On the contrary, *F. compactum* and *F. equiseti* seem uninfluential on seed germination. In particular, *F. equiseti* increased shoot growth while both species (*F. compactum* and *F. equiseti*) increased root development and vegetative vigor, prompting speculation as to the actual role of these two species in the pathogenic process of FFR complex of durum wheat.

Bacterial strains as potential biological control agents against Fusarium species: a promising approach for organic farming

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Fusarium is a widespread mycotoxigenic plant pathogen, posing a significant challenge in organic farming due to limited use of chemical pesticides for its control. To address this issue, exploring alternative solutions is crucial, particularly by exploring the potential of beneficial bacteria. In this study, five bacterial strains from the *Lactobacillus* and *Bacillus* genera were evaluated for their efficacy against nine different *Fusarium* species. In overlay assay, lactic acid bacteria (LAB) strains exhibited a halo of inhibition >10 mm against *F. graminearum*, *F. proliferatum*, and *F. fujikuroi*. Percentage inhibition in radial growth in dual culture was measured after 7 dpi, revealing that *B. amyloliquefaciens* strains FLN13 and MA8 were effective against all tested *Fusarium* strains. CFSs obtained after 72 h of bacterial fermentation showed significant fungicidal activity (>3 mm) in agar well diffusion against *F. verticillioides*. UHPLC-Q-TOF-MS analysis revealed the production of surfactin and fengycin by *Bacillus* species, while benzoic acid, hydrocinnamic acid, phenyllactic acid, and lactic acid (>28.6 g/L) were detected in the LAB strains. Furthermore, CFSs of FLN13, DAN39, and MA8 strains significantly reduced the *in vitro* production of FUM B1 by *F. proliferatum* and *F. verticillioides*. In addition, these strains were utilized in seed priming tests of durum wheat, along with artificial inoculation of *F. culmorum* strain F1126, demonstrating their potential in reducing disease index. These findings highlight the potential of bacterial strains as biological control agents against *Fusarium* species. *In vivo* assays are necessary to explore the potential use of these strains in organic farming.

A green novel compound to control soilborne pathogens on kiwifruit plants

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The application of novel and green compounds to control different plant pathogens is a trend that has increased during the latest years. From the perspective of reducing the input of agrochemicals within 2030 as set by the European Green Deal, a green and circular approach is required. Chitosan is a natural, safe, and cheap product widely used to stimulate plant growth, as an antimicrobial agent, and to promote the activation of systemic acquired resistance (SAR). Lignin represents a highly valuable bioresource and its peculiar polyphenolic structure is of high interest to the generation of novel sustainable pesticides. Thanks to its natural origin, biocompatible and biodegradable, and its antioxidant and antimicrobial properties, lignin represents a valid biopolymer useful for green plant protection strategies. In this study, the application of a chitosan and lignin-based compound at rhizosphere level of kiwifruit plants was evaluated by physiological and molecular analyses. In particular, the chlorophyll content and the total phenolic acids content of the roots were evaluated with a fluorometric assay and spectrophotometric method respectively. The expression level of six genes involved in the induction of SAR was also investigated. Furthermore, the effect of this compound on the control of *Fusarium solani* and *Fusarium oxysporum* - two noted soilborne pathogens isolated from damaged kiwifruit roots - was tested to check the efficacy of the control of root diseases.

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A metagenomic approach to investigate bacterial and fungal rhizosphere communities: are there new players involved in the development of Kiwifruit Vine Decline Syndrome in Italy?

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During the last decade, the Kiwifruit Vine Decline Syndrome (KVDS) had already caused significant economic losses in the Italian kiwifruit production. Traditional methods for identifying the causal agent of KVDS have been unsuccessful, leading to the hypothesis that the disease may be caused by a complex interaction between multiple factors, including abiotic and biotic stressors. In recent years, metagenomic approaches have emerged as a promising tool for identifying microbial communities associated with plant diseases. The aim of this study is to describe the fungal and bacterial communities of the rhizosphere with a metagenomic approach. A field trial has been carried out in an experimental kiwifruit orchard in Lazio region showing KVDS symptoms since 2019. Roots and soil from plants showing KVDS symptoms, together with healthy-looking plants, were chosen for sampling at different time points during the vegetative season. A metabarcoding approach targeting both fungal ITS and bacterial 16S was performed within an Illumina platform. Interestingly, the results show different microbiome compositions between KVDS and asymptomatic kiwifruit plants in association with an evolution during the plant phenological stage and seasonal state, including climate condition as well. Soon, a transcriptomic and a metabolomic analysis will shed light on the plant defense mechanism against KVDS, narrowing down the circle of main factors involved, helping to design more specific KVDS control strategies.

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'Candidatus Phytoplasma solani': from insect transmission to complete genome sequencing of several strains inducing different symptoms in tomato

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Phytoplasmas are obligate, phloem-limited prokaryotes, leading to severe economic losses. The current unavailability of an axenic culture method and the low complexity of their genomes are obstacles for the assembly of complete chromosomes. Bois noir (BN), associated with 'Candidatus Phytoplasma solani', is one of the most important grapevine yellows disease in Europe, regularly found in Italian vineyards. Nine local strains were

transmitted from the field to the model plant tomato cv. Micro-Tom. The strains were derived from two ecological pathosystems involving the natural host plants stinging-nettle and bindweed, associated with different *tuf*-types of the pathogen, *tuf*-type a and *tuf*-type b, respectively. The study aimed to investigate strain's: (i) biological and molecular characteristics, (ii) *in planta* colonization patterns and (iii) genomic diversity. It was possible to distinguish the strains into three main clusters and five distinct lineages based on the analyses of *tuf*, *secY*, *vmp1* and *stamp* gene sequences and to link *tuf*-type to specific symptom development. Additionally, complete chromosome sequences of four strains were obtained. Comparative genome analysis revealed remarkable diversities. Chromosome's length ranged between 751.188 and 973.640 bp with annotated CDS varying between 725 and 1064. Mining of the four genomes resulted in the prediction of 13–34 candidate effector genes. Comparisons with already known phytoplasma effector proteins evidenced strain-specific sets of effectors that may explain the different symptoms induced on tomato. The fully assembled genome sequences are the first complete representatives of '*Ca. P. solani*' species and contribute to provide the basis for studying pathogenicity-mechanisms of phytoplasmas.

Efficacy evaluation of different seed disinfection chemical treatments against tomato brown rugose fruit virus

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Tomato brown rugose fruit virus (ToBRFV) is a tobamovirus that is causing important losses in tomato production. ToBRFV is a seed-borne virus, and spreads rapidly in the cultivation area; for these reasons, growers are encouraged to take preventive measures to manage this highly contagious virus. In this study, we evaluated the antiviral activity of three different chemical-based treatments to inactivate ToBRFV virions on seeds according to the following protocol submerging 100 seeds for each treatment in: (a) 10% trisodium phosphate solution for 180 min, and two disinfectants provided by CID Lines N.V.; (b) "Virocid" (0.25%) for 5 min; (c) "D50" (2.5%) for 5 min. After each treatment, seeds were washed three times for 5 min with sterile water and dried. Fifty seeds were analyzed by RT-qPCR, while 50 seeds were pre-germinated to evaluate the germination rate. Each obtained plantlet was analyzed by RT-qPCR after 30 days to verify the ToBRFV presence. Results showed that for all treatments, seeds resulted positive, with a Ct value of 29.37, 20.81 and 18.74 for treatments (a),

(b) and (c), respectively. Moreover, with treatment (a) the seed germination percentage reached 100% after 2 weeks, and only one plantlet gave positive result via RT-qPCR; treatment (b) showed 100% seed germination after 14 days, and all plantlets gave negative results; treatment (c) reached 98% after 8 days and all plantlets gave negative results, indicating that the virus is detectable but not infectious after treatments. This work offers additional alternatives to inactivate ToBRFV transmission processes through seeds in tomato production.

Olive leaf yellowing associated virus dispersion assessment in Sicily using a real-time RT-LAMP

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Olive (*Olea europea* L.) cultivation is one of the most valuable crops in the Mediterranean basin. However, many viruses, such as olive leaf yellowing-associated virus (OLYaV) (family *Closteroviridae*), threaten olive production. OLYaV lead to symptoms in some olive cultivars, while remaining latent in others; however, the current European legislation requires the absence of this virus from propagation material. In this work, a survey in different olive production sites located in four Sicilian provinces (Agrigento, Trapani, Messina, Palermo) was conducted during spring 2023 to evaluate the OLYaV dispersion. Eight-hundred symptomatic/asymptomatic olive samples belonging to "Giarraffa" and "Nocellara del Belice" cultivars were collected; in detail, 100 plants of each cultivar were sampled for each province. Each plant was georeferenced; total RNA was extracted using a commercial kit, quantified, and adjusted to ≈ 50 ng/ μ L. To evaluate the incidence a real time RT-LAMP, developed by Caruso and co-workers (2023), was used. Out of 800 samples analyzed, 110 and 89 samples of "Giarraffa" and "Nocellara del Belice" cvs resulted positive to OLYaV, with a total incidence percentage of 27.5% and 22.25%, respectively. Moreover, eight and five samples resulted positive were collected from asymptomatic olive plants. Regarding "Giarraffa" cv, the higher incidence was recorded in Trapani province (34%), followed by Messina, Agrigento and Palermo provinces, while for "Nocellara del Belice" cv, an incidence of 33% was observed in Trapani province, followed by Agrigento, Palermo and Messina provinces. In conclusion, OLYaV was found in olive orchards independently of the cultivar, crop management or geographical location in Sicily.

Development of the membrane-based extraction method for the detection of *Xylella fastidiosa* by Real-Time LAMP

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Xylella fastidiosa, a xylem-limited and vector-transmitted bacterium, is responsible for several diseases affecting a number of plant species, primarily grapevine, citrus, almond, and olive. For the latter, it causes the devastating quick decline of olive trees in Apulia region, Italy. A rapid, sensitive, and onsite-based detection method is necessary to monitor the pathogen at the borders, in the nurseries and in the framework of surveillance programmes. The aim of this study is to develop a membrane-based extraction method for detecting *Xylella fastidiosa* by real-time LAMP, avoiding the movement of infected plant material. Stem sections of Xf-infected olive and oleander were printed onto nitrocellulose membranes. Discs of printed membranes were subjected to rapid nucleic acid extraction and subsequent isothermal amplification using the commercial ICGene PLUS ONE system with the LAMP kit for the detection of *Xylella fastidiosa* (Enbiotech Srl, Italy). All samples were analyzed simultaneously by DTBIA and Real-Time PCR. Real-Time LAMP results were equivalent to those obtained with Real-Time PCR, whereas DTBIA proved to be less sensitive, with some false-negative results. The presence of an endogenous internal control allowed to verify the efficacy of the extraction and amplification of the samples within the same test tube. The developed method proved to be faster than Real-Time PCR and printed membranes can be easily stored and shipped for analyses, thus avoiding the risk of movement of infected plant material.

Potato tuber rots caused by *Fusarium brachygibbosum*

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Fusarium brachygibbosum is a fungal plant pathogen that satisfies all the criteria to be regarded as a potential Union

quarantine pest, as reported by the Plant Health EFSA Panel. In the EU, the pathogen has been reported in Italy in soil/marine sediments, quinoa, and durum wheat seeds, and recently on potato tubers in Emilia Romagna (North Italy). The present study aimed to assess, in a three-year trial conducted in Central Italy, the effects of a sustainable and circular cultural model consisting of two conservative tillage techniques (subsoiling, and spading) versus plowing and organic fertilization by compost versus mineral on the severity of tuber dry rot and fungal species involved. A high percentage of dry-rotted tubers was recorded in the cropping season (2022), characterized by abnormally high temperatures. In this year, spading combined with compost fertilization has led to a significant decrease in dry rot. *F. brachygibbosum* was the predominant and most virulent pathogen isolated from symptomatic tubers. Fertilization with compost, particularly when combined with subsoiling, was reflected by a significant decrease in the frequency of *F. brachygibbosum*. The report of this potential pathogen also in Central Italy suggests a risk of spreading to other crops in Italy and the EU. Besides quarantine measures, sustainable and circular agriculture management systems represent a model for maintaining or restoring soil fertility through conservative tillering and organic fertilization and minimizing overtime losses due to diseases such as dry rot of potato tubers.

Temperature plays a decisive role in the ability of *Pseudomonas syringae* to trigger ETI and drives in planta bacterial growth dynamics

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The *Pseudomonas syringae* species complex has a very important economic impact, causing diseases on many plant species. Considerable research effort is being invested to understand how effector repertoires could determine the host-range of a given strain. Recently, we showed that the incapacity of *P. syringae* pv. *actinidiae* to induce the hypersensitive response (HR) in *Arabidopsis thaliana* is due to its inability to inject effectors rather than the absence of a recognized effector. In this context, we compared several *P. syringae* strains carrying the same plasmid-borne avirulence gene for their ability to induce an HR in *A. thaliana* Col-0, at different temperatures. *Pto* DC3000 and *Pma* M6 consistently triggered a strong HR while other strains induced it at

different intensities significantly depending on temperature. Moreover, the strains were also compared for their growth dynamics at different temperatures in about ten plant species from various botanical families. Although temperature influenced bacterial growth dynamics *in planta*, the differences observed among the strains were partially correlated to their ability to activate their type-three secretion system. Together, these results indicate that (i) Pto DC3000 is a reliable model strain but not representative of the *P. syringae* complex, (ii) temperature plays a crucial role in bacterial fitness, and (iii) deployment of molecular virulence factors may be influential but not sufficient to predict the outcome of plant-bacteria interactions.

Aureobasidium spp. strain UC14 mitigates patulin production by *Penicillium expansum* Link. on apple

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Penicillium expansum is the causal agent of apple blue mold and one of the causes of the production of patulin. The study displayed the efficacy of a potential biocontrol agent (BCA), *Aureobasidium* spp. (UC14), isolated from cold environment, in controlling fungal growth and patulin production. The efficacy of UC14 was tested by *in vitro* antibiosis experiments. Volatile and non-volatile metabolites reduced *P. expansum* on average by 50%. To verify UC14 efficacy on apples, different concentrations of its cellular suspension were assayed. The concentration 1×10^8 cells mL⁻¹ was the most efficient, completely inhibiting apple blue mold symptoms during the shelf-life week. The biological antagonism of UC14 was further demonstrated by qPCR analysis, with an almost total reduction in the pathogen's abundance. During the cold storage, the BCA displayed a good persistence on fruits showing a reduction of disease severity. Subsequently, apples were sampled and analyzed for patulin content. Results confirmed the activity of the strain on the reduction of the mycotoxin on 'Golden delicious' and 'Fuji' apples by 98.1% and 96.2% with respect to the control. The strain, displaying a promising effectiveness in controlling blue mold of apples and patulin, showed a potential as BCA to involve during the postharvest phase.

Utilizing microbial biodiversity to reduce environmental risks of chemical agro-products

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In natural and anthropogenic ecosystems, microorganisms play a crucial role in preservation of the natural resources and consumer well-being; their interactions with soil and crops are important for agricultural production. Numerous *Bacillus* species are rhizobacteria, able to provide benefits to plants due to their ability to function in soil nutrient availability, provide crop protection to biotic and abiotic stresses, and stimulate plant growth, potentially acting as natural fertilizers and pesticides. Lignin is one of the main underperforming materials of different industrial processing, with numerous potential uses as botanical compound, being rich in valuable functional molecules as polyphenols. Copper (Cu) has long been employed as pesticide for disease control, but its prolonged and frequent application has negative impacts on soil biota, groundwater, and human health. With limitations in the use of Cu-based products imposed by the European Union, research for sustainable alternative solutions became an imperative. The aim of this work was to develop an innovative formulation, including *Bacillus* sp., lignin, and Cu at reduced concentrations, able to control different plant pathogens. Three bacterial isolates were biologically characterized, and their ability to grow on Cu- and lignin-enriched media was evaluated. The resistance of *Bacillus* to Cu was assessed using different concentrations of metal (up to 500 ppm). The compatibility between bacteria with organic-inorganic hybrids, consisting of combinations with UPM Solargo® (lignin) and 40 ppm of Cu²⁺, was also evaluated. Among the three *Bacillus* sp. under consideration, *B. vallismortis* resulted the best candidate, showing the highest compatibility with the tested materials.

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In vitro activity of a bio-stimulant formulation against oomycete and fungal pathogens of grapevine

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In the past the use of pesticides had been generally considered a simple and inexpensive solution against plant pathogens; however, their inappropriate and overuse application had negatively impacted on soil, water, and agricultural biodiversity. Understanding the urgency of changing this trend, has prompted researchers to make significant efforts to select alternative solutions. Naturally based products have attracted special attention and a huge number of plants have been studied to identify and characterize new components with antimicrobial activity. Pine extracts had been shown to have a great potential as a source of antimicrobial bioactive compounds for food-borne, human and plant pathogens. In this study the *in vitro* activity of Agrusever (Savory Sun VA LLC), a bio-stimulant formulated with bark pine and seaweed extracts, was tested. The preventive effect of the product on *Plasmopara viticola* was carried out on grape leaf disks, whereas the direct antimicrobial activity against mycelial growth of *Fomitiporia mediterranea* and *Botrytis cinerea* was assessed by poisoned food method. The assay was carried out with pine or seaweed extracts alone and in complete formulation at three doses, including those recommended by manufacturers. The development of downy mildew sporangiophores measured by visual scoring and the mycelial growth of both fungi was inhibited only by pine extract and Agrusever with a dose-dependent effect. Although the results clearly highlight the bioactivity of pine bark extracts against some grape pathogens, field trials will be necessary to unravel full potential of the product in mitigation abiotic and biotic stresses of grapevine.

A new means of controlling lemon mal secco disease: SAP-Cu, a superabsorbent polymer for a slow, dose-reduced release of copper

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Mal secco of lemon is a highly destructive vascular disease caused by the mitosporic fungus *Plenodomus tracheiphilus*. Current management strategies involve pruning symptomatic branches and sanitizing them through spraying of Cu²⁺-based fungicides. However, this approach leads to the dispersion of Cu²⁺ in the environment, which has harmful impacts on soil microbiomes, living organisms and food-safety. To address this issue, a study was conducted to investigate the effectiveness of a super absorbent polymer (SAP) containing reduced amounts of copper (SAP-Cu) as an alternative means for controlling mal secco. SAPs possess technical properties that make possible the controlled release of metal ions from aqueous solutions. This feature ensures a longer-lasting effectiveness of the treatment while preventing excessive soil and groundwater contamination caused by the leaching of the metal. Chemical-physical properties of SAP-Cu were characterized by AAS, UV-VIS spectroscopy, and ToF-SIMS. The inhibitory effects of SAP-Cu against *P. tracheiphilus* were determined by *in vitro* tests on both PDA cultures and naturally infected lemon cuttings. Results highlighted that SAP adsorbed up to 30 times its weight of Cu²⁺ solution at 236 mM. Additionally, when SAP-Cu was in contact with lemon twigs, it transferred selectively Cu²⁺ ions along the xylem vessels. Furthermore, SAP-Cu significantly inhibited the viability of *P. tracheiphilus* both in PDA medium and in naturally infected lemon twigs. This study evidenced that SAP could be a suitable carrier for Cu²⁺ or other antifungal compounds. This approach has the potential to reduce environmental contamination and provide an effective treatment for mal secco.

Anthraxnose of pepper caused by *Colletotrichum scovillei*: first report in Europe

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Pepper (*Capsicum annuum* L.) is one of the most important vegetables cultivated in Kosovo, as in several countries in the Mediterranean. In September 2022, 40% of pepper fruits, in two commercial fields in the municipalities of Peja and Rahovec (Kosovo), showed typical symptoms of anthracnose. Dark lesions developed on fruits, which later appeared sunken, necrotic and surrounded by brown haloes. After isolation onto PDA, morphological characteristics of colonies and single spores of both isolates revealed to be consistent with the description of *Colletotrichum scovillei*. Phylotyping of ITS, GAPDH and TUB2 sequences of the two isolates was performed. To confirm Koch's postulates, a conidial suspension for both isolates was inoculated into healthy pepper fruits and incubated in humid chamber. Ten days after pathogen's inoculation, typical anthracnose symptoms developed, from which the fungus was successfully reisolated. No symptom was ever observed in fruits inoculated with sterile water. To the best of our knowledge, this is the first report of anthracnose caused by *C. scovillei* in Europe. Given the economically importance of the crop and the significant quality and yield losses observed, measures are urgently needed aiming to pathogen eradication or, alternatively, to ensure an efficient disease control to avoid pathogen's spread to other geographical areas. Finally, two microorganisms from the UNIMORE collection were tested *in vitro* against *C. scovillei*: preliminary data showed their effectiveness in reducing mycelium growth. Studies are currently ongoing to test such antagonists *in planta* under controlled conditions, to search for possible alternatives to the extensive use of fungicides.

Antifungal activity of an innovative chitosan derived from insects

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Reduction of agri-food wastes, as well as extending the shelf-life and protection of perishable fruits by alternative and eco-friendly solutions, in a circular economy context, are pivotal global challenges. In this study, the *in vitro* antifungal activity of two different chitosans (unbleached, called PEND, and bleached, named PEDEC) derived from an innovative

and highly sustainable source, that is the exoskeletons of pupal exuviae of the bioconverter insect *Hermetia illucens* (L.) (Diptera: Stratiomyidae), against *Botrytis cinerea* and *Fusarium* spp. was investigated. Concentrations of 0, 0.06, 0.125, 0.25, 0.5, and 1 mg mL⁻¹ of each chitosan were used in MIC (minimum inhibitory concentration) assays. PEND at 0.5 mg mL⁻¹ (determined from MIC assay) significantly reduced mycelial growth and changed morphology (e.g., larger vesicles in the mycelium, cellular disorganization, hyphal coiling) and ultrastructural damages of fungi when observed by scanning electron microscopy of *B. cinerea*, *F. sporotrichioides* and *F. oxysporum* f. sp. *lycopersici*, compared with negative control. Chitosans were then tested as protective treatments for postharvest storage of strawberry and grape, PEND resulting in a significant protection against artificially inoculated *B. cinerea* at doses of 0.5 and 1 mg mL⁻¹. Commercial chitosan (K) was used as a control in all trials. The results open the door to the employment of *H. illucens* as an innovative, sustainable, natural source and potentially alternative to crustaceans to obtain chitosan to be used for plant protection.

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CRISPR/CAS9 mediated gene knockout in the brown rot fungus *Monilinia fructicola*

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M. fructicola is one of the most important causal agents of brown rot causing blossom and twig blight, as well as pre- and post-harvest fruit rot with severe yield losses, especially on stone fruit. In this work, a simply and efficient method to generate targeted gene disruption mediated by a CRISPR/Cas9 ribonucleoprotein complex coupled with PEG-mediated transformation was applied to the reference strain Mfrc123 of *M. fructicola*. Based on genomic

and transcriptomic data, the endo- β -1,4-xylanase (*xynIIA*-like) gene predicted as an effector candidate and the polypeptide synthase (*pks*) in a gene cluster putatively involved in chloromonilicin biosynthesis were selected as genes of interest. Chloromonilicin has been described as a non-phytotoxic compound showing a strong antimicrobial activity. The recombinant Cas9 protein was extracted and purified from *Escherichia coli* RosettaTM (DE3) strain transformed with the pHis-parallel1-NLSH2BCas9 plasmid, and single guide RNAs (sgRNAs) designed and synthesized for the two genes. The ability of the purified Cas9 RNPs complex to efficiently cleave at the specified target sites was firstly confirmed in *in vitro* assays. No mutants for the putative effector gene of *M. fructicola* were obtained, while eight mutants were generated by targeting the *pks* gene. For all of them, PCR-based molecular analysis confirmed single, double or multiple insertions of the hygromycin cassette used as selective marker at the target site. Further molecular and phenotypic characterization of the obtained mutants is in progress. This will provide new knowledge of the functional role of a BGC potentially involved in the chloromonilicin biosynthesis in *M. fructicola*.

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Shedding light on the genetic diversity and evolutionary dynamics of geographic populations of Wisteria vein mosaic virus: a case study for the spread of emerging potyviruses in Europe?

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Wisteria vein mosaic virus (WVMV) is a member of the genus *Potyvirus* associated with Wisteria mosaic disease (WMD), the most serious disease affecting *Wisteria* spp. In 2022, severe symptoms of WMD were observed on the leaves of a Chinese wisteria (*W. sinensis*) tree growing in an urban area in Apulia (Italy). The presence of

WVMV was ascertained by RT-PCR analysis. Although the occurrence of WVMV in Italy had been posited in the late 1960s, no molecular information had been reported for any Italian isolate prior to this study. Subsequent phylogenetic analyses based on NIB and CP genes placed the WVMV Italian isolate within a large clade identified in the genus *Potyvirus* as the BCMV supergroup. Based on the increasing number of reports of the virus worldwide, we attempted an exploratory analysis of its genetic diversity and possible mechanisms that may have shaped its geographic population structure. Relying on the N-terminus of the CP, available for twenty WVMV isolates from Europe, Asia, and Oceania, sixteen different haplotypes were identified. A high haplotype diversity was found, particularly relevant in the European population. The measured dN/dS ratio led to the assumption that the target region is under purifying selection. Tests evaluating the neutrality of nucleotide variability showed different results for the European and Asian groups. The estimation of inter-population genetic differentiation showed a high level of gene flow between the two populations. Overall, our results provide a possible approach to understanding the mechanisms of WVMV emergence in Europe and draw attention to its further spread and the increasing threat of this and other neglected potyvirus species to the ornamental nursery sector.

Identification of plant pathogenic bacteria using Raman microspectroscopy coupled with a dielectrophoretic device

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Phytopathogenic bacteria induce devastating effects on crops and their specific detection and identification are of utmost importance. Current diagnosis of bacteria is mainly based on serological and molecular methods, which are time-consuming and expensive. Rapid, accurate, reliable, and cheap diagnostic procedures for plant diseases and

correct identification of etiological agents are needed, in order to guarantee high quality and quantity of agricultural products and food. Among these innovative technologies, Raman spectroscopy (RS) is gathering considerable attention. RS provides a direct and non-destructive analysis of the biochemical components present in a matrix, including bacterial cells. In this work, a dielectrophoretic (DEP) device was used to increase bacterial concentration and amplify the intensity of the Raman spectroscopic signal. Using dedicated Raman-DEP platform, a dataset of spectra from different isolates of harmful phytopathogenic bacteria belonging to the genera *Pseudomonas*, *Xanthomonas*, and *Erwinia* was obtained. With regards to *Pseudomonas*, successful differentiation of three different species and five different pathovars within the *P. syringae* species was possible. Machine learning approaches allowed to identify and discriminate isolates at the genus, species, and pathovar level, reaching in the latter case accuracies greater than 85%. This Raman-DEP strategy offers interesting advances in the identification of microorganisms and the evaluation of susceptibility to antibacterial substances. Moreover, the technique could be readily extended to environmental and food diagnostics.

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Split to multiply: essential oil emulsions against *Pseudomonas syringae* pv. *actinidiae*

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Crop pathogen management is a priority for sustainable agriculture development. Indeed, the large use of pesticides threatens both human and environmental health, while simultaneously pushing antimicrobial resistance. The development of effective, durable, and environmentally neutral solutions is not trivial and requires pushing research towards new and innovative strategies. Essential oils (EOs) are naturally occurring compounds displaying a potent antimicrobial activity with

multiple targets among molecular and structural components of microbial cells. However, their high extraction cost, low stability in the environment and poor water solubility slow down their potential application in crop protection. Modern approaches like nanotechnologies can provide solutions to the previous issues and, at the same time, can outperform the antimicrobial activity of essential oils. We thus produced and tested different emulsions of essential oils for their antimicrobial activity against *Pseudomonas syringae* pv. *actinidiae* (Psa), the causal agent of the kiwifruit bacterial canker disease. The encapsulation of EOs in a polymeric matrix proved to be an interesting solution which led to an increase in antimicrobial activity and stability compared to bulk EOs. Moreover, a possible synergic effect among different emulsions to target simultaneously multiple aspects of Psa vitality and virulence is under evaluation. Finally, the emulsions were able to protect kiwifruit plantlets from artificial inoculation of Psa in *in-vitro* experiments which supports the feasibility of their application in field trials.

***Botryosphaeriaceae* species, *Paraconiothyrium brasiliense*, *Seimatosporium vitis* and *Truncatella angustata* associated with grapevine dieback in Piedmont: characterization and pathogenicity**

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In recent years, reports of diseases caused by fungi colonizing grapevine wood (Grapevine Trunk Diseases, GTDs) increased worldwide. GTDs cause major economic losses due to reduced yields and productivity, as well as young plant death and a shorter vineyard life, especially in Mediterranean countries. During 2021–2022, a survey was conducted in Piedmont to investigate the species

diversity and distribution of GTDs pathogens. Morphological and multi-locus phylogenetic analyses allowed the identification of different fungal species within the family of Botryosphaeriaceae such as *Botryosphaeria dothidea*, *Diplodia seriata*, *Diplodia mutila* and *Neofusicoccum parvum*. Other pathogens, commonly associated with other GTDs, such as *Eutypa lata*, *Fomitiporia mediterranea* and *Phaeomoniella chlamydospora* were found. Less-common species such as *Neocucurbitaria juglandicola*, *Paraconiothyrium brasiliense*, *Seimatosporium vitis* and *Truncatella angustata* were also identified in the symptomatic grapevines. Pathogenicity trials were performed using representative strains on 1-year-old potted grapevine cuttings of ‘Barbera’. All strains caused brown wood lesions and necrotic vascular discoloration and were successfully re-isolated, except for *N. juglandicola*. The most severe lesions were produced by *Neofusicoccum parvum*. Moreover, *Paraconiothyrium brasiliense* and *Seimatosporium vitis* showed high aggressiveness compared to other common GTDs pathogens. The effect of the temperature on mycelial radial growth was also investigated showing the optimum growth temperatures of the tested isolates ranged between 20 and 25 °C. *Diplodia mutila*, *Paraconiothyrium brasiliense* and *Neocucurbitaria juglandicola* were reported for the first time in association with symptomatic grapevine and as pathogenic in Italy.

Preliminary study on the microbiota associated with *cimiciato* hazelnuts

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Cimiciato hazelnuts are inedible and unmarketable. This defect is caused by the feeding activity of various species of pentatomids. The *cimiciato*-defect in hazelnuts is characterized by the presence of dry and necrotic tissues on the kernel, alterations in the lipid composition profile,

and an increased susceptibility to lipid oxidation. These factors affect the organoleptic parameters and shelf-life of the product. Insect biting into hazelnuts determines the release of enzymes associated to saliva that induce biotic responses in plant tissues and metabolism alterations in kernels. Moreover, microbial inoculation during the feeding process is a well-documented phenomenon, as in the case of *Eremothecium coryli* (Peglion) Kurtzman, the causal agent of kernel dry rot in hazelnuts. Our study aims to explore the microbiota associated with the *cimiciato* defect occurring in insect-bitten hazelnuts of the San Giovanni cultivar harvested in Teano (CE), Campania region. Fruits were externally sterilised, shelled and disinfected. *Cimiciato* and healthy hazelnuts were then placed onto potato dextrose agar (PDA) or PDA + 1% lactic acid and transferred to a growth chamber. Fungal and bacterial colonies were observed and counted, to assess their presence or absence in each tested fruit. Noteworthy, *cimiciato* hazelnuts showed a higher presence of fungi compared to healthy hazelnuts. In contrast to this, no significant differences were found for bacteria. Further studies will be required for the molecular and phenotypic characterization of *cimiciato*-associated microbes together with metabolomic analysis, to determine how they contribute to development of this defect.

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Widespread dieback and mortality of wild olive trees associated with *Phytophthora* species in Sardinia, Italy

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Wild olive (*Olea europaea* var. *sylvestris*) is a drought-tolerant and disease-resistant tree, often used as rootstock for grafting cultivated olive varieties. However, since 2022 an extensive dieback and mortality of wild olive trees have been reported in central Sardinia (Italy). Infected trees showed severe leaf chlorosis, wilting or defoliation often associated with root rot and necrotic lesions on the feeder roots. In the investigated sites, estimated tree mortality and disease incidence ranged between 60 and 80%. Rhizosphere soil samples from more than a hundred symptomatic and healthy trees were collected and subjected to the baiting

technique, using fresh *Ceratonia siliqua* leaves as baits and SMA as *Phytophthora* selective medium. The isolates obtained were identified by analyses of their morphological traits and sequences of ITS and *cox1* genes. Eight *Phytophthora* species from Clades 2, 6, 7 and 8 were isolated from symptomatic trees. Pathogenicity of the most frequently isolated species was tested using 6-month-old wild olive seedlings and the soil infestation method. Disease outbreak has progressed rapidly over 2.000 ha in only one year, with more than 6.000 trees already dead. Investigations are currently ongoing to better understand the epidemiology of the disease and other possible factors involved in the outbreak. Apart from the ecological impact, a consequence of this damaging new disease is that further use of wild olive to produce propagation material by grafting olive cultivars could be limited. Possible control strategies will be also discussed.

Fungal community dynamics in grapevine leaves during esca symptom progression and in different symptom phenotypes

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Leaves in grapevines affected by esca may occasionally manifest a symptom called ‘esca leaf stripe symptom’. The triggering factors that lead to symptom onset and progression are still under debate, but they seem to originate from an advanced fungal infection in perennial tissues. The esca leaf stripe symptom starts as chlorotic spots in interveinal tissue, it is followed in time by scorching, and development of pigmentation between scorched and healthy tissue. Such pigmentation is typically of tonalities of red, however, purple and no pigmentation are occasionally observed. Working on cultivars Cabernet Sauvignon

and Touriga Nacional, we used DNA metabarcoding (i) to explore the microbial dynamics during symptoms progression in esca-affected leaves, and (ii) to reveal the fungal diversity in different symptom phenotypes, along with their anthocyanins profile. The mycobiome profiling revealed a large fungal richness (260 taxa), and a beta diversity influenced by cultivar ($p < 0.01$) and vintage ($p = 0.001$). We observed significant differences in beta diversity between leaves affected by chlorotic spots and asymptomatic ones ($p < 0.05$), revealing an important shift in fungal community composition during early stages of symptoms progression. Comparing asymptomatic and different symptom phenotypes, we detected cultivar and vintage-dependent alterations in alpha and beta diversity, as well as in individual taxa abundance (e.g., *Botrytis caroliniana* in red leaves). In Touriga Nacional, and to a lesser extent in Cabernet Sauvignon, purple leaves had significantly lower abundances of tri-hydroxylated anthocyanins and acyl-derivatives, when compared to red leaves. This suggests a correlation between fungal endophytes and anthocyanins profile, which requires further evaluation.

Involvement of Botryosphaeriaceae fungi in holm oak (*Quercus ilex*) decline in the Salento area of southern Italy

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Oak decline is a widespread phenomenon affecting forests around the world and can be caused by a combination of biotic and abiotic factors. These include various biotic agents such as fungi, bacteria and insects, coupled with abiotic stressors such as drought, soil nutrient imbalances, pollution and climate change impacts, that can severely compromise affected forests. Among the fungal pathogens, species from the Botryosphaeriaceae family have been identified as significant contributors to holm oak decline in many regions. These fungi can infect trees through wounds or natural openings leading to symptoms such as canker and

dieback of branches, leaf discoloration, defoliation, and ultimately tree death. Since 2016, a sudden decline of holm oak plants has been observed in the Salento Peninsula, Apulia, Southern Italy. Following inspections and surveys in different woods, fungal colonies were isolated from symptomatic plants and representative isolates were morphologically and molecularly characterized to investigate the membership of the isolates to the Botryosphaeriaceae family. Strains identified as *Diplodia* spp. and *Neofusicoccum* sp. were object of further investigations on their role in pathogenicity, confirming their ability to cause symptoms on artificially inoculated holm oak plants. In addition, analysis of their genetic relationship and evolutionary patterns allowed us to classify the isolated strains into their respective clades. However, there is further need to clarify the evolutionary relationships within the clades of *D. corticola* and *D. gal-lae*, which remain unclear.

***In vitro* and *in vivo* collateral effects of organic fertilizers against phytopathogenic bacteria and fungi**

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In response to the significant growth of the organic agricultural sector, new trends in plant protection are focusing on the selection and characterization of new sustainable products with zero residues and low environmental impact, capable of counteracting major plant pathogens commonly found in agricultural crops. In this scenario, organic fertilizers/amendments can play an important role in controlling plant pathogens. Besides providing essential nutrients, some of these products can promote the natural plant defence mechanisms, stimulate the growth of beneficial microorganisms, or even directly inhibit phytopathogens, thereby providing multiple benefits for the plant/soil ecosystem and for food safety. In this study, a selection of mineral and/or organic fertilizers were tested for their *in vitro* activity against two important bacterial phytopathogens, *Pseudomonas syringae* pv. *tomato* (*Pst*), and *Xanthomonas campestris* pv. *campestris* (*Xcc*), as well as

two important phytopathogenic fungi, *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) and *Botrytis cinerea* (*Bc*), and *in vivo* on tomato plant inoculated with *Pst* and *Fol*. The results show that some of the tested products have a high inhibitory activity *in vitro* against these plant pathogens. Furthermore, they are also able to significantly reduce the disease severity on tomato plants inoculated with *Pst* or *Fol*. These products, which are already available on the market as fertilizers and/or biostimulants, could potentially be utilized to mitigate the negative effects caused by plant pathogens. This research reveals new low impact solutions for sustainable agriculture and meets the need to improve disease management in organic and integrated agriculture.

Investigation on seedborne microflora in vegetable landraces: a perpetual game of balance between pathogenic fungi and beneficial saprophytes

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Landraces are valuable resources for modern agriculture as well as the cultural heritage of specific communities. The cultivation of those local varieties is mostly performed by using traditional methods, based on the seed reproduction by the farmers and the use of minimal chemicals to maintain the crops. In this scenario, seed pathology not only provides critical information on seed health by identifying potential pathogens, but it can also lead to new knowledge on resident beneficial saprophytes. Multidisciplinary approaches, such as morphological and molecular studies, phylogenetic analyses, pathogenicity tests and host range experiments were used in this study to identify novel seedborne pathogens that threaten crop success. *Apiospora arundinis* were discovered in common bean for the first time while *Sclerotinia sclerotiorum* and *Stemphylium vesicarium* were detected in faba bean, as new report in the area of origin. Moreover, new Botryosphaeriaceae strains were detected in legumes highlighting the effects of climatic changes on pathogenic microflora composition. These novel pathogens compromise germination and, in a wide host range, seedlings development. On the contrary,

some examined seeds appeared to be healthy and colonized only by saprophytic fungi, mostly *Chaetomium* spp. These fungi have demonstrated the ability to replace the pathogens and aid in seed germination *in vitro*. Furthermore, preliminary studies showed antagonistic effects against legumes seedborne pathogens. Overall, these findings shed light on the critical role of seed pathology not only in early detection of novel outbreaks, but also in the development of novel strategies to control seedborne diseases starting from the resident microflora.

Comparative metabolomic profiling of the brown rot fungi *Monilinia fructicola*, *Monilinia fructigena* and *Monilinia laxa*

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Brown rot, caused by *Monilinia* species, is a devastating disease of stone and pome fruits leading to yield losses in fruit orchards and even more in postharvest. This study investigated differences in the production of secondary metabolites among *M. fructicola*, *M. fructigena*, and *M. laxa* through genomic and metabolomic analyses. Genomic analysis using the antiSMASH prediction tool revealed varying numbers of biosynthetic gene clusters (BGCs) in the three species. Sixty-three orthologous BGCs were identified, with *M. fructigena* showing greater differences in the shared clusters. Nine clusters associated with siderophores, melanin, chloromonilinic acid, botcinic acid, solanapyrone, and retinal production were highlighted through annotation and literature review. Metabolomic analysis was conducted on five strains of each species to establish molecular fingerprints and identify differences in metabolomes. Two strains per each species were cultivated on various media to study BGC expression, linking metabolites with their corresponding gene clusters. Furthermore, *M. fructicola* was inoculated on nectarine fruits to explore secondary metabolite expression patterns during early stages of infection. Metabolomic analysis using UHPLC-MS/MS and bioinformatics tools (e.g. GNPS) identified several molecules associated with *Monilinia* species or other phytopathogenic fungi. Notable compounds included retinal, scytalone, solanapyrone,

botcinic acid derivatives, and chloromonilinic acid, in line with the identified BGCs. Differences were observed in the presence of selected molecules among the three *Monilinia* species, with *M. fructigena* exhibiting the most significant variations. This research enhances our understanding of brown rot and provides knowledge on metabolites in *Monilinia* spp. and new potential targets to define effective disease management strategies.

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Volatile organic compounds (VOCs) as potential biomarkers of *Cadophora luteo-olivacea* presence on kiwifruits

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Cadophora luteo-olivacea is the causal agent of the skin-pitting disease of kiwifruit, a syndrome that appear after 4–5 months of cold storage. However, it is assumed that the infection takes place in the field during fruit development. The present work takes into consideration the production of volatile compounds of *Actinidia deliciosa* cv. 'Hayward' at different phenological phases as potential *C. luteo-olivacea* infection biomarkers. *In vitro* assays were conducted to gain knowledge on the effect of kiwifruit volatile organic compounds (VOCs) on the pathogen conidial germination and mycelial growth. By SPME-GC-MS analysis, VOCs produced by kiwifruit, at different phenological phases, inoculated or not with *C. luteo-olivacea*, were analyzed. Ethanol, o-xylene, D-limonene, and acetic acid showed a significant increase in presence of fungal inoculation. Ethanol and D-limonene were also detected as volatile metabolite of the pathogen. The effect of each compound was tested on the fungal conidial germination at different concentrations, showing a growth stimulation at lower aliquots. These results showed how the production of some VOCs can contribute on the knowledge of fruit-pathogen interaction in the field with the aim to develop future tools for an early disease detection and a consequent effective control.

Bacterial genome sequencing and plant transcriptional analysis shed lights on the biocontrol activity of *Pseudomonas mediterranea*

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Pseudomonas mediterranea strain PVCT 3C was subjected to whole-genome shotgun sequencing to investigate the genetic determinants of its biocontrol activity. Comparative genomics showed that it possesses a smaller genome compared to other *Ps. mediterranea* strains. Numerous gene clusters encoding for secondary metabolites, including siderophores, alginate, cyclic lipopeptides (CLPs) and volatile organic compounds (VOCs), were predicted. The marked antagonistic activity against the fungus *Plenodomus tracheiphilus* (*Pt*), causal agent of citrus Mal secco disease (MSD), encouraged us to perform studies *in planta*. Sour orange and lemon 'Femminello siracusano 2kr' plants were treated by leaf-spraying with a PVCT 3C suspension before leaf-inoculation with *Pt*. In all trials, the bacterial pre-treatment significantly reduced the disease incidence and index and the area under the progress curve (AUDPC) compared with non-treated but *Pt*-inoculated control plants, mostly during the early disease stage. Lemon leaf transcriptome was analysed with an NGS and *de novo* assembly RNA-seq approach in the latent phase 7 days after *Pt* inoculation. Results showed that PVCT 3C reduced leaf transcriptome remodelling and left unchanged the expression of several metabolic pathways (e.g. photosynthesis) otherwise compromised by *Pt* infection. On the whole, these results suggest that the presence of the bacterium near the inoculation sites, and probably inside them, affected conidia germination and fungal growth, at least during the asymptomatic phase of the disease, as also confirmed by quantitative real-time PCR. By the fact, the bacterial pre-treatment drastically slowed down disease onset, probably by competing with *Pt* for space and nutrients due to its secondary metabolites.

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Stemphylium vesicarium, a new fungus associated with hazelnut kernel rot in Italy

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Hazelnut is one of the most cultivated nut crops worldwide, with Turkey (665,000 tons) and Italy (140,560 tons) representing the leading countries in the global production, with a market portion in constant growth. During summer 2022, a survey was carried out to collect fungi associated with damaged/diseased kernels in different commercial orchards in Latium region in Italy. The newly recorded specie was isolated from symptomatic nuts using standard techniques in 17.91% of the samples analyzed. Morphological characteristics of pure cultures corresponded to those of *Stemphylium* spp. Genomic DNA of representative isolates was extracted and the internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and calmodulin (CMD) partial genes were amplified and sequenced. The nBLAST analysis of the sequences revealed 100% identity with deposited sequences of *S. vesicarium* strains confirming the identity of the isolates from hazelnut as *S. vesicarium*. Pathogenicity tests were carried out and the inoculated hazelnut kernels showed necrosis and rotting seven to 10 days after inoculation, whereas the controls remained healthy. To fulfill Koch's postulates, *S. vesicarium* was reisolated from the symptomatic kernels, and the identity was confirmed. To our knowledge this is the first report of *S. vesicarium* on hazelnut in Italy. These findings suggest that *S. vesicarium* could severely affect hazelnut production in Italy.

This research was carried out in the frame of the "Econocciola" project.

Development and validation of a quantitative PCR assay for the detection of *Piggotia coryli*, the causal agent of hazelnut anthracnose

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A novel real-time PCR assay was developed for the detection of *Piggotia coryli*, the causal agent of hazelnut anthracnose. A molecular tool for sensitive detection and quantification of

P. coryli in symptomatic and asymptomatic host tissues was required to better understand *P. coryli* biology and epidemiology. Species-specific primers for *P. coryli* were designed based on the sequence of the β -tubulin gene. The amplification efficiency of pure target DNA was 93.2 %, the limit of detection (LOD) of the assay was *in silico* calculated at approximately 1.5 pg/PCR reaction and the limit of quantification (LOQ) at 15 pg/PCR reaction. The pathogen was steadily detected in artificial mixtures of plant and pathogen DNAs with the same estimated LOD as pure fungal DNA. When wet lab tested the LOD was slightly higher than predicted value and was prudentially fixed at 8 pg/PCR reaction. In naturally infected samples, the assay consistently revealed the presence of the pathogen in all symptomatic specimens, as well as in some asymptomatic tissues from samples collected in infected orchards. This molecular tool will be of substantial aid in detecting and quantifying *P. coryli*, even in latent state and in different hazelnut tissues.

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Exploiting shrimp shell extracts for the sustainable management of plant diseases

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Pre- and post-harvest diseases caused by plant pathogenic fungi are a serious threat for agricultural production. In the last few years, conforming to the objectives of European Green Deal, the search of nontoxic and environmentally safe products for a sustainable management of these diseases has been intensified and there is a growing interest in all forms

of reuse and valorization of wastes of industrial processes. Wastes of the shrimp industry, constituted prevalently by shell residues, being rich in proteins, chitin, calcium, phosphorus, and other substances, are a good candidate to this purpose. In this study we tested the antifungal activity of four extracts (water-extract, EtOAc-extract, MetOH-extract and nitric-extract) obtained by minimal processing from shrimp waste. The activity of extracts was tested against fungi and oomycetes of the genera *Alternaria*, *Colletotrichum*, *Fusarium*, *Penicillium*, *Plenodomus*, and *Phytophthora*. *In vitro* tests showed that the nitric-extract exerted a significant inhibitory activity on the mycelium growth of all tested pathogens, with values of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) ranging from 2 to 3.5%. In *in vivo* tests on citrus and apple fruits artificially inoculated with *Penicillium digitatum* and *P. expansum*, respectively, the nitric-extract was effective in preventing molds. HPLC-ESI-MS-TOF analysis identified 35 diverse substances in the nitric extract, including phenolic compounds such as p-coumaric (4-hydroxycinnamic acid), acid 1-2-dihydroxybenzene (catechol), 3-(4-hydroxy-3-methoxyphenyl) propionic acid and ellagic acid. Results are promising and encourage to extend the tests to other pathosystems and to develop suitable formulations for application of shrimp waste extract in real conditions.

Whole genome sequencing of *Fusarium nodosum*, a new producer of type B trichothecenes

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Fusarium nodosum (L. Lombard & Crous), previously identified as *F. sporotrichioides*, was recently recognized as a new species. It belongs to the *F. sambucinum* species complex (which comprises some of the most destructive agricultural pathogens). According to its phylogeny, it is closely related to T-2 toxin-producing species, like *F. sporotrichioides*, and previous studies report its ability to produce diacetoxyscirpenol and Fusarium Head Blight symptoms on *Triticum aestivum*. However, a systematic understanding of its pathogenicity, mycotoxigenic

potential, and genomic features are still lacking. In this study, the whole genome sequencing of *F. nodosum* strain T8 and *F. sporotrichioides* strain I101 was performed, with the aim to identify similarities/differences between the two *Fusarium* species by revealing the orthologous genes. The assembly comes from the combination of long reads sequencing and short reads sequencing, and an automatic annotation was carried out. In addition, artificial inoculation on *Triticum turgidum* subsp. *durum* (cv. Svevo) spikes was performed and mycotoxin production (TCTB, TCTA, beauvericin, enniatins, zearalenone) was analyzed at grain maturity. Mycotoxins production using rice kernels as substrate was investigated too. *F. nodosum* showed weak pathogenicity on wheat spikes and it is able to produce only beauvericin using rice kernels as substrate, while in the spikes it produces abundant nivalenol among the TCTB, and traces of beauvericin and diacetoxyscirpenol. This is the first insight of TCTB production by *F. nodosum*. Further research is required to confirm these findings, considering more than one strain, and understand the potential risk to food safety represented by this new species.

This study was supported by the project “Valorizzazione Multifunzionale di Frumenti Pigmentati per la realizzazione di Alimenti Funzionali e di NanoAgrofarmaci Green per la Protezione del Frumento” acronym MULTIFRU funded by POR FESR Lazio 2014-2020 action 1.2.1 and by University of Tuscia, Dipartimento di Scienze Agrarie e Forestali (DAFNE).

High polyphenolic wheat genotypes: a study on Fusarium Head Blight (FHB) resistance

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Polyphenols are secondary metabolites well known to act as antioxidants and to provide a protective effect against biotic and abiotic stresses. Pigmented wheat lines, rich in anthocyanins (a class of polyphenols) in the aleurone layer (blue genotypes) or pericarp (purple genotypes), constitute interesting materials for the dual purpose of identifying resistant wheat and understanding

the molecular mechanisms of resistance. This research examines the interaction between five different pigmented wheat cultivars (Purendo, Skorpion, Rosso, Vanilnoir and Indigo) and the fungal pathogen *Fusarium graminearum*, the causal agent of Fusarium Head Blight. The results (severity at 21 days post infection, fusarium damaged kernels, area under disease progress curve, mycotoxin accumulation) showed that Skorpion and Rosso were very susceptible, Purendo and Indigo tolerant, while Vanilnoir was FHB-resistant, but pigmentation and resistance seemed to be not correlated. Interestingly, fungal biomass quantification (by RT-qPCR) suggested the involvement of a type II resistance mechanism in Vanilnoir and revealed that the most susceptible genotype (Skorpion) had a low fungal diffusion in the spike. Additionally, the differential expression of genes involved in polyphenol biosynthesis showed a differential activation pattern for the production of the secondary metabolites in Vanilnoir. A multi-omic approach (transcriptomics and metabolomics) was finally carried out with the aim to understand the molecular mechanisms of FHB resistance detected in Vanilnoir. RNAseq was used to identify differences in gene expression between a purple resistant genotype (Vanilnoir) and a purple susceptible genotype (Rosso), while metabolomics was used to identify resistance/susceptibility biomarkers among all the considered genotypes.

This study was supported by the project Valorizzazione Multifunzionale di Frumenti Pigmentati per la realizzazione di Alimenti Funzionali e di NanoAgrofarmaci Green per la Protezione del Frumento acronym MULTIFRU funded by POR FESR Lazio 2014-2020 action 1.2.1 and by University of Tuscia, Dipartimento di Scienze Agrarie e Forestali (DAFNE).

Botryosphaeriaceae and Phytophthora species associated with emerging diseases of avocado (Persea americana) in Italy

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Within the wide panorama of exotic crops rapidly expanding in Italy, avocado (*Persea americana* Mill.) attracted the

attention for the profitability of cultivation and the nutritional value of fruits. In this new exotic crop paradigm, some already known diseases and new ones are emerging as a major factor limiting the productivity of avocado plants. In particular, severe and unusual leaf, branch, stem and root diseases on young and mature trees have recently been observed in several orchards in Sardinia and Sicily (Italy). Since there is little information about the aetiology of these new pathosystems and given the high economic value of these orchards, an in-depth study was carried out to identify the main causal agents. Since 2022, 218 symptomatic samples from trees showing leaf spot, shoot blight, branch canker and dieback as well as collar and root rot with inner bark lesions extending up from below ground level were collected in 6 orchards. Based on morphology, colony appearance and DNA sequence data, 12 species belonging to the genera *Botryosphaeria*, *Diplodia*, *Dothiorella*, *Lasiodiplodia*, *Macrophomina*, *Neofusicoccum* and *Phytophthora* were isolated and identified. These included the polyphagous and exotic pathogens *Neofusicoccum australe* and *Phytophthora cinnamomi*. The results obtained provide new insight into the emerging avocado diseases in Italy and highlight that the diversity of Botryosphaeriaceae and *Phytophthora* species with both soil and aerial life style is greater than previously recognised. Further studies are necessary to ascertain a possible synergistic interaction among these pathogens and to develop suitable management strategies.

Farina di Basalto® - based formulations act as multi-site agrochemicals against three impacting phytopathogens while displaying biocompatibility on wheat, olive and grapevine plants

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Farina di Basalto®-based formulations are corroborants admitted in organic agriculture and already demonstrated fortifying properties on several crops. In this study, we evaluated two formulations: Farina di Basalto® XF, a micronized flour of volcanic origin rich in silicon, potassium, iron, calcium, and magnesium, and Effetto Scudo, containing a consortium of beneficial microorganisms; they restore nutrients for soil fertility and can also potentially protect plants against pathogens, thanks to their fortifying and nutritional properties. We tested *in vitro* and *in vivo* the antimicrobial potentiality of Farina di

Basalto® XF and Effetto Scudo against three impacting phytopathogen: *Fusarium graminearum* (Fg) causing Fusarium head blight (FHB) on wheat, *Botrytis cinerea* (Bc) causing grey mould on grapevine, and *Pseudomonas savastanoi* pv. *savastanoi* (Psav) causing knot on olive tree. *In vitro* assays demonstrated that Farina di Basalto® XF acts as a multi-site agrochemical, since it showed both direct growth inhibition and indirect interactions with conidia and bacterial cells, suggesting its ability to block the first step of the infection stages by favouring cells flocculation and decreasing their adhesion on the host surface. Effetto Scudo inhibited Bc by producing soluble antimicrobial compounds. On plants, both formulations demonstrated biocompatibility on wheat, olive and grapevine plants, since the Nitrogen Balance Index (NBI) was not negatively affected by the treatments. Ongoing and future experiments will be focused on evaluating the diseases severity on plants by quantifying the fungal and bacterial biomass by Real-Time qPCR, in order to assess the ability of the two formulations to control the diseases spread.

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Prevalence of new and invasive geminiviruses and beta-satellites in plants: an extensive survey in Southern Italy on vegetables in the frame of GeMed, a research project involving five Mediterranean countries

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Viruses represent serious threats to crop plants. They are responsible for a considerable decrease in yield, quality, and safety of crops and food security, with social repercussions all over the world. The main objective of the GeMed project is the prevention and control of new and invasive geminiviruses (NIGs) infecting vegetable crops in the Mediterranean area. Mono- or bipartite geminiviruses, are among the most worrying viruses due to their economic impact and their high propensity to recombine. A serious NIG, spread in the Mediterranean area, is tomato leaf curl New Delhi virus (ToLCNDV, *Begomovirus*), that, after its first detection in 2012 in Spain, progressively extended its geographic range to Tunisia, Italy and Morocco. Likewise, other geminiviruses, so far restricted to some Mediterranean areas, are expected to spread further: among the most dangerous, chickpea chlorotic dwarf virus (CpCDV, *Mastrevirus*), up to now reported only in North Africa, and squash leaf curl virus (SqLCV, *Begomovirus*) and watermelon chlorotic stunt virus (WmCSV, *Begomovirus*) restricted to eastern Mediterranean areas. Moreover, the presence of satellite molecules needs to be monitored as satellites can dramatically increase virulence. PRIMA project GeMed involves Italy, France, Jordan, Morocco and Tunisia. In Italy, surveys have been performed in two eco-agrosystems, Sicily and Campania during the 2020, 2021 and 2022 growing seasons. Symptomatic plants have been sampled in eighty-five different fields. Total nucleic acids have been extracted and specific PCR for each of the NIGs here considered and for beta-satellites were performed. The results of this survey will be presented.

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Biocontrol of potato late blight by *Trametes versicolor* fungal extract with antimicrobial properties

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Potato late blight is a devastating disease affecting cultivated potato (*Solanum tuberosum*) and caused by the oomycete *Phytophthora infestans*. The use of biocontrol agents and plant resistance inducers are sustainable and alternative ways to fungicides to contrast the disease even at field level. In this study, we tested the effect against potato late blight of the cultural filtrate (CF) derived from the liquid

culture of the lignin degrading basidiomycete *Trametes versicolor*, that we have already employed for its antioxidant and antimicrobial properties. CF is composed of exopolysaccharides (specifically, Tramesan), lipids, peptides, etc.; the mode of action of the singular components is currently undergoing studies. To evaluate if CF can be used to counteract *P. infestans* in potato, we conducted *in vitro* and *in planta* assays, by growing the pathogen in presence of the extract and analyzing plant defense responses (via HPLC-MS/MS and RT-qPCR). Interestingly we show that the CF of *T. versicolor* can affect the growth of *P. infestans*, inhibiting mycelium development and sporangia germination. Instead, when used as a biostimulant in potatoes, it did not enhance significant defense responses prior the infection, although it did not show any phytotoxic effect. Further studies in a potato-*P. infestans* pathosystem will help to confirm whether CF can be used as natural antimicrobial more than a priming agent and be implemented in field trials for sustainable potato disease management.

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Physical mode of action of a chitosan-based product against *Plasmopara viticola*

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The EU Commission is progressively limiting the use of hazardous active ingredients. Natural compounds may represent an alternative for crop protection. The aim of this study was to explore a component of the Physical Mode of Action (PMoA) of a chitosan-based product (extracted from *Aspergillus niger*) against *Plasmopara viticola*, the causal agent of grapevine downy mildew (DM). Specifically, the preventative activity of the product was evaluated in potted vines (cv. Barbera) in comparison with copper and an untreated control. Treatments were applied at different plant growth stages for two seasons (2022 and 2023), at label dose. After each treatment, leaves were collected at different Days After Treatments (DAT), exactly at 1, 3, 5, 7, 10 and 15 DAT, artificially inoculated with a

sporangial suspension and incubated under optimal conditions for infection. At disease onset, disease severity was assessed, and treatment efficacy evaluated in comparison to the untreated control. The copper-based product showed a preventative efficacy > 80% up to 7 DAT, and then decreased. At pre-flowering, the chitosan-based treatment showed 70% efficacy at 1 DAT, which decreased to 50% at 7 DAT. At later growth stages, its efficacy was approximately 50% till 5 DAT and then quickly decreased. These preliminary findings confirmed the importance of PMoA studies for better understanding plant protection products' potential and best application timings. These studies also provide additional keys for better understanding the role and activity of each plant protection product in a fungicide schedule in which various products are used in alternation or mixture.

Mycotoxigenic potential of *Penicillium* species isolated from fresh chestnuts and derived products

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Species of genus *Penicillium* produce a great variety of secondary metabolites, including mycotoxins. This characteristic is associated with the presence of biosynthetic enzymes organized in biosynthetic gene clusters (BGCs). In the present work, the genomes of ten *Penicillium* species isolated from fresh chestnuts and derived products were sequenced and annotated. Predicted protein sequences were pooled with those of previously annotated *Penicillium* spp. and outgroup species in the Eurotiales order to generate phylomes. Phylome data was parsed using the protein sequence of enzymes experimentally associated with the production of target mycotoxins (patulin, andrastin A, mycophenolic acid, penitrem A, meleagrins and verrucosidin) to identify

putative homologues in the new strains' proteome. Production of associated mycotoxins was verified by both HPLC-DAD and HPLC-MS/MS on whole mycelium extracts. Results of bioinformatic analyses showed the presence of a complete andrastin A cluster in *Penicillium bialowiezense*, *Penicillium crustosum*, *Penicillium glandicola* and *Penicillium taurinense*; a complete mycophenolic acid cluster in *P. bialowiezense*; a complete penitrem A cluster in *P. crustosum* and *P. glandicola*; a shortened, biosynthetically active roquefortine C cluster in *P. crustosum* and *P. taurinense*, as well as a complete meleagrins cluster in *P. glandicola*. In addition, putative partial andrastin A clusters from *Penicillium palitans* and *Penicillium discolor* were subsequently identified as an atlantinone A cluster. For all of them, chemical analyses confirmed production of the associated metabolite. These results elucidate the genetic bases of mycotoxin production and permit the evaluation of the evolution of BGCs in different species of *Penicillium* spp.

On understanding the arsenal of the EU quarantine plant pathogen *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*: role of a hypothetical serine protease

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Phytopathogenic bacteria have evolved a sophisticated arsenal to successfully colonize their host plants and establish the infection. Recently, the involvement in the pathogenicity processes of several phytopathogenic bacteria of some serine proteases has been demonstrated. The plant pathogenic bacterium *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*), which is the causal agent of bean bacterial wilt and soybean tan spot, harbors a hypothetical pathogenicity island (PAI), encoding for three serine proteases highly homologous to some pathogenicity factors found in *Clavibacter michiganensis*. The *Cff* PAI is absent in saprophytic and soil-borne *Curtobacterium* spp. Moreover, *Cff* strains are unequivocally identified by several PCR-based tests targeting one of these proteases,

named S1 serine protease (AN: QIH95653.1). Hence, the hypothesis is that S1 serine protease may contribute to *Cff* virulence as well as to its transition from a saprophytic to a pathogenic lifestyle. Here for the first time, a protocol was developed for the mutagenesis of the gram-positive quarantine bacterium for Europe *Cff*. The knock-out mutants of serine protease S1, obtained by marker exchange mutagenesis, showed a role of this protein in *Cff* virulence on artificially infected bean plants. Furthermore, a significant reduction (50%) of proteolytic activity was demonstrated on mutants by an *in vitro* test, performed by using skim milk medium. Accordingly, S1 serine protease has been expressed by using the vector pMAL-p5X and *Escherichia coli* Rosetta strains as recipients. However, purified *Cff* S1 showed no HR in host and non-host plants, raising new questions about its actual mechanism as an effector of the *Cff* arsenal.

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Natural stilbenes and their derivatives as antifungals against rice blast

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Among the fungal plant pathogens, *Pyricularia oryzae* poses a serious threat to sustainable agricultural production causing serious damage on rice and other crop plants. In rice, blast disease by *P. oryzae* causes annually 10–30% of harvest losses. Overreliance on fungicides, especially those with single-site mode of action, monoculture cropping, and use of blast-susceptible rice varieties, have been favoring the development of resistance in pathogen populations increasing thus food insecurity. This in turn creates a strong demand for the development of novel fungicides for rice blast management with emphasis on their low environmental impact and circular economy. Here, we investigated the antifungal activity of natural stilbenes and their use as scaffolds for the development of novel biofungicides. Monomeric and dimeric stilbenoids were tested against wild-type (WT) and strobilurin-resistant (RES) strains of *Pyricularia oryzae* and several compounds showed inhibition higher than 40%, and in

particular deoxyrhapontigenin inhibited mycelial growth of both WT and RES strains by 60–80%. Furthermore, a set of molecules based on methoxyacrylate stilbene (MOAS) were synthesized, merging a nature derived stilbene fragment with the β -methoxyacrylate moiety of strobilurin fungicides. We identified two molecules with activity comparable to the reference commercial fungicide azoxystrobin. However, low mycelium growth inhibition of resistant strains indicates that these compounds most likely retain the strobilurin-like mechanism of action.

A biodiversity journey around Italian beech forests through metabarcoding analysis

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The World Heritage Forests composed mainly by beech forests (*Fagus sylvatica*) located in the Mediterranean Basin are considered a hot spot of biodiversity, today harshly threatened by global warming, deforestation and tree decay, which would lead to habitat loss or even extinction. Recent scientific findings acknowledge that plant fitness is linked, among other factors, to the composition and abundance of the endophyte microbiome. Thus, we aimed to describe the endophytic communities of two unmanaged ancient beech forests recognized by UNESCO World Heritage and located in central Italy “Faggeta Vetusta del Monte Cimino” (UNESCO site) in Soriano Nel Cimino (VT), “Faggeta Vetusta di Monte Raschio” (UNESCO site) in Oriolo Romano (VT), together with a third beech forest located in Monte Terminillo (RI). In each site, seeds and leaves were collected from five different trees at two different sampling areas. The microbial biodiversity was then investigated by metabarcoding analysis of both fungal ITS and bacterial 16S amplicons sequenced in Illumina platform. Our analysis aimed to identify those endophytes constantly associated with beech, despite their location and status (core microbiome), those involved in plant resilience to challenging conditions (resiliome), as well as those associated with tree decline or stress (weakness pathobiome). Since the influence of these organisms in shaping the adaptability of the species is more than a hypothesis, these outcomes will represent a basis for the understanding of the endophytic community under specific conditions and a pioneering natural approach to the preservation and conservation of European beech forests.

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Unravelling the microbiota composition of heterogeneous agricultural soils in Lazio through metabarcoding analysis with PacBio long reads sequencing

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Soil microorganisms act as crucial ecological agents within terrestrial environments, regulating organic matter cycling, nutrient transfer across trophic levels, and exerting profound effects on plant growth and overall soil health. The aim of this study is to analyze the fungal biodiversity within the diverse agricultural soils of the Lazio region, with particular interest in phytopathogenic species. In recent years, advances in next-generation sequencing platforms have further facilitated the study of complex soil and plant microbiota. Metabarcoding, a widely used molecular analysis technique using specific genetic markers followed by sequencing, has emerged as the primary approach to assess microbiota biodiversity. However, the diverse chemical and physical properties of soils make extracting environmental DNA (eDNA) challenging. We developed an eDNA extraction protocol that could be applied to the wide range of soil types sampled for further molecular analysis. To achieve a comprehensive understanding of complex microbiota, we sequenced a wider ITS region (ITS9-ITS4) using PacBio long-read sequencing, which provides longer sequences and allows for greater fungal species taxonomy resolution compared to short-read sequencing. Moreover, we analyzed the microbiota to characterize the ecological functions of each sample by incorporating knowledge from the field of phytopathology into soil microbiota research. However, the availability of a comprehensive species sequence database is still a critical bottleneck to achieve an accurate analysis. This work highlighted the remarkable diversity of the sampled soils and the need for specific molecular and bioinformatic protocols suited for different agricultural soils, to ensure accurate and standardized characterization of microbiota in environmental studies.

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Characterization of the causal agents, epidemiology and biological control of white haze on apple

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White haze, caused by an extensive fungal colonization of the apple surface, is an emerging issue in several European apple production areas. It results in compromised quality and decreased marketability of the fruits, leading to economic losses. In this study, fungal strains were isolated from apple in Northern Italy and the species diversity was assessed using molecular and phylogenetic tools. Moreover, the ability of the isolated species to reproduce symptoms on healthy apples artificially inoculated was tested. Six species belonging to different basidiomycetous genera (*Entyloma*, *Golubevia*, *Tilletiopsis*) were identified. The most frequently isolated strains were inoculated on healthy apple fruit, showing to be able to reproduce symptoms on red-skin apples, fulfilling Koch’s postulates. Metabarcoding was used to characterize both epiphytic and endophytic microbial communities of apple fruit across six time points from early fruit development up to the end of shelf life. *Golubevia* was the most abundant epiphyte (2–4%) from BBCH 81 (1–7% relative abundance) to the end of shelf life. Moreover, two *Aureobasidium pullulans* strains were tested against white haze of apple. An efficacy comparable to the chemical control treatment was demonstrated at the end of shelf-life. This work provides new insights to increase knowledge about aetiology and epidemiology of white haze on apple. In addition, biocontrol of white haze with antagonists was tested for the first time.

Surface adhesion is regulated by HAM-7 and required for full virulence in *Fusarium oxysporum*

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HAM-7, a conserved cell-wall-anchored protein in filamentous ascomycetes, is hypothesized to form a sensor complex for the activation of the cell wall integrity mitogen-activated protein kinase pathway MPK1. In *Neurospora crassa*, HAM-7 is involved in cell-to-cell fusion and sexual development, however its biological role in fungal plant pathogens is largely unknown. BlastP searches using the *N. crassa* HAM-7 protein as a bait identified a single 233-amino-acid long orthologue in the *Fusarium oxysporum* (*Fol*) genome. Here, to dissect the contribution of the *Fol ham-7* gene in plant pathogenesis, we used a reverse genetic approach. Similarly, to *N. crassa*, *ham-7Δ* mutants in *Fol* were impaired in vegetative hyphal fusion, but not in the penetration of cellophane membranes or growth under stress conditions. Additionally, microscopic analysis showed that *ham-7Δ* mutants are unable to undergo mycelial agglutination, a process regulated by both cell-to-cell fusion and adhesion. To understand if HAM-7 plays a role in cell-to-surface adhesion, we tested the ability of *ham-7Δ* mutants to remain attached to an artificial substrate or a host tissue after a washing step. Importantly, a higher proportion of wt but not of *ham-7Δ* cells remained firm on both tested surfaces, suggesting that HAM-7 could play a major role during plant infection. In line with this, plant virulence assays showed that *ham-7Δ* mutants had a slower progression of the infectious process compared to the wt strain. Collectively, our results reveal a new role for HAM-7 in mycelial network formation, host surface attachment and virulence in the pathogen *F. oxysporum*.

Cell compression drives host penetration in *Fusarium oxysporum*

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The fungal cell wall, a dynamic structure providing cell type-specific morphology, protects cells from stresses, and functions as a physio-chemical rheostat for the transmission of extracellular signals through a large set of cell-wall-anchored proteins. *Fusarium oxysporum* f. sp. *lycopersici* has been

previously reported to gain access into the host plant and to colonize root tissues by exploiting both apoplastic and symplastic routes. In both cases hyphae are supposed to readapt their shape and size to be able to fit into the sub-micrometric spaces that the fungus encounters along its way to the xylem. Which external signals are involved in *F. oxysporum* sensing of host tissue cells and how they modulate hyphal penetration is largely unknown. Here, to gain fundamental insights on the physical and mechanical cues driving fungal tropic behaviours and pathogenicity of plant roots we used reverse genetics approaches, fluorescence microscopy and microfabrication of biomimetic plant platforms. By using chemically inert patterns, we found that *F. oxysporum* hyphae are able to both follow ridges and to adapt to small-sized channels through thigmomodification of extremely thin filaments that resemble those growing in between host plant epidermal cells. We further show that fungal hyphae can penetrate and grow for several microns into sub-micrometric pores as those normally found in the plant apoplast. Importantly, the compressive stress generated by forced growth in nanoenvironments leads *F. oxysporum* surface penetration suggesting that compression itself and mechanosensing might drive the colonization process in the soil pathogenic fungus *F. oxysporum*.

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Biodiversity of *Fusarium* species and DON contamination in two crucial stages of wheat growing cycle in Tunisia

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The most destructive diseases in wheat cultivated areas are Fusarium Foot Rot (FFR), in the early growing stages, and Fusarium Head Blight (FHB), from flowering to harvesting. Although a broad range of *Fusarium* species can be isolated from stem and kernels, *Fusarium culmorum* and *Fusarium graminearum* are the prevailing species, worldwide. This study aimed to investigate both the *Fusarium* species distribution and the Deoxynivalenol (DON) accumulation in stem and grains, in Tunisia. Sixty-four wheat fields from five different regions, during two crop-seasons (2020–2021, 2021–2022) were investigated. Based on morphological traits, 313 representative *Fusarium* strains isolated from crown and grains were molecularly identified by *TEF* gene sequencing. Deoxynivalenol contamination was determined by HPLC/UV after Mycosep® 227 column (crown samples) or immunoaffinity column extracts (grain samples) clean-up. *Fusarium culmorum*, in both two years, was the most common species on kernels (78.8 and 95%) as well as in the crown, in the first year (66%). Several *Fusarium* species were isolated, with a greater biodiversity observed in the stem more than kernels, since members of 8 different phylogenetic Species Complex were detected. DON contamination in crowns ranged from 0.1 to 51 ppm in 2021 and from 0.1 to 4.2 ppm in 2022. DON level in grains was under 0.4 ppm in both growing seasons. We report for the first time the occurrence of *F. anthophilum*, *F. nygamai*, *F. algeriense*, *F. redolens* and *F. brachygybosum* in Tunisian wheat. Moreover, the detected amount of DON provides important information on the toxicological risk of wheat in Tunisia.

Screening barley varieties for resistance to *Drechslera graminea* and *Drechslera teres*

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Barley diseases caused by fungi of the genus *Drechslera* spp. can cause serious yield losses for growers in all barley producing countries. This genus can cause three different types of disease on barley: barley leaf stripe caused by *Drechslera graminea* and net blotch caused by *Drechslera teres*. Net blotch exists in two different forms: “net”, caused by *D. teres* f.s. *teres* and “spot”, caused by *D. teres* f.s. *maculata*. *Drechslera graminea* is a seed-borne pathogen, unable to perform secondary infections, unlike *D. teres* that can infect other plants starting from conidia present on the infected leaves.

Resistance genes for *D. graminea* have been introduced in a common barley genetic background by gene pyramiding. The aim of this work was to screen 21 barley lines for resistance to one isolate each of *D. graminea* and *D. teres* for functional analysis of the introgressed genes. Pathogenicity tests of *D. graminea* are based on the so-called “sandwich method” that is to grow barley seeds between two layers of *D. graminea* mycelium grown on Potato Dextrose Agar (PDA). A Real-Time method has been set up to identify the presence of the fungus just after the “sandwich” to simplify and shorten *D. graminea* pathogenicity tests. For *D. teres*, the pathogenicity test has been performed both on detached leaves and on young seedlings using the homogenized mycelium of a selected isolate. Symptoms have been observed after 48 hours post inoculation for detached leaf-based assay and after 8–10 days post inoculation for seedling inoculation test.

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Eremothecium coryli transmission by *Halyomorpha halys* in hazelnut

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The “dry rot” of hazelnuts is a disease caused by *Eremothecium coryli*, a particularly harmful yeast, which also affects other plants (e.g., tomato, soybean, citrus, etc.). *E. coryli* has recently been suspected to be involved in the worsening and spread of the phenomenon of hazelnuts rot in hazel orchards cultivated in Piedmont region and other regions of Italy. In fact, it is well known that many other insects with a stinging-sucking mouth apparatus (such as *Nezara viridula*, *Anasa tristis*, *Philaenus spumarius*) can transmit severe phytopathogenic organisms. The goal of this work was to assess the involvement of *H. halys* in *E. coryli* transmission to hazelnuts, through laboratory tests. Transmission tests of *E. coryli* were performed as follow: adult *H. halys* specimens were fed with artificial pathogen-infected hazelnuts, subsequently the same bugs were fed with healthy hazelnuts, in order to verify uptake of *E. coryli* from hazelnut to bug and transmission from bug

to hazelnut, respectively. Bugs and hazelnuts of transmission tests were analyzed for the presence of *E. coryli* by using a specific diagnostic method we set up, based on real time PCR. Tests carried out over 2 years of experimentation show that bugs fed with artificially infected hazelnuts turns out to be positive for the presence of the yeast, in the second phase these bugs containing the yeast in their rostrum were able to transmit it into healthy hazelnuts as confirmed by the presence of *E. coryli* in these hazelnuts after contact with the insects. This result confirms that *H. halys* represents a vector of *E. coryli*.

This work was supported by HCo Ferrero Company through the research project NOCEREHAL “Possibile trasmissione ed associazione del fungo Eremothecium coryli agente causale del dry rot della nocciola con la cimice asiatica (Halyomorpha halys)”

Assessing disease suppression on temperate terra preta soils: the role of microbiome and biochar ageing

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Charcoal pits (kilns) are ancient structures used for charcoal production. These patches of charred ground represent a unique opportunity to study long-term effects of their biochar content. The aim of this study was to characterize the charcoal in the soil from these kilns and investigate its disease suppression capacity related to microbiota composition and diversity. Kilns from two mountain sites Gelbison and Vesole, located in Southern Italy within the Cilento National Park and Vallo di Diano, were studied to compare their parent rock and soil chemistry. Interestingly, *in vitro* biological suppression tests were conducted using three phytopathogens, i.e., *Sclerotium rolfsii*, *Sclerotinia minor*, and *Pythium ultimum*. Standard chemical analyses i.e., pH, organic carbon, salinity, macro- and micro-nutrients, and next-generation sequencing techniques were employed. Furthermore, imaging through scanning electron microscopy (SEM) combined with energy dispersive X-ray spectroscopy (EDS) was performed to characterize the topological and

elemental composition of biochar particles. The SEM-EDS analyses revealed the presence of heterogeneous silicates mixture of calcium-aluminium, limestone, and the presence of a microbial organic coating over aged biochar particles. Sterilisation of the kiln soils resulted in higher disease incidence compared to non-sterile conditions suggesting a microbial-based disease suppression. Analysis of the microbiota revealed greater bacterial diversity outside of the kiln sites in the Vesole mountain, while both bacterial and fungal composition showed greater variety between the two mountains. Overall, this study underscores the long-lasting effects of biochar accumulation and its role in modulating soil microbiota and disease suppression.

Pushing the limits: Land use intensity impacts soil microbiome and the emergence of plant pathogens

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Soil microbiome plays a crucial role in key soil processes, such as litter decomposition, nutrient cycling, and plant productivity. In this study, we investigated soil chemical variability, microbial diversity, and composition in seven ecosystems in Southern Italy, i.e., *Fagus sylvatica* and *Quercus ilex* forests, a Mediterranean grassland and shrubland, a vineyard, and horticultural cultivation in both open field and under greenhouse, representing a range of land-use intensities. We examined soil chemistry parameters and microbiota was characterized using high-throughput sequencing. Greenhouse soils had the highest pH, while *Fagus* soils had the lowest. Horticultural, greenhouse, and grassland soils exhibited lower levels of organic matter (OM) and total nitrogen (N), while shrubland soils had higher levels of OM, N, and C/N ratio. *Fagus* soils displayed the highest levels of OM, N, and C/N ratio. Furthermore, Horticultural soils had the lowest bacterial diversity, while shrubland soils had the highest fungal diversity. *Pseudomonadota* dominated in *Fagus*, grassland, and vineyard soils, while *Actinomycetota* dominated in *Quercus*, shrubland, and greenhouse soils. *Cyanobacteria* were predominant in horticultural soils. *Ascomycota* were the dominant fungal group in most ecosystems, except for *Quercus* soils, where Basidiomycota prevailed. Bipartite network analysis revealed shared bacterial species across ecosystems, indicating a common community, while

fungal species showed habitat specificity. Agricultural ecosystems, especially greenhouses, harboured a higher abundance of fungal pathogens, including *Alternaria*, *Fusarium*, *Gibellulopsis*, *Stemphylium*, *Acremonium*, and *Curvularia*. This emphasizes the importance of effective management in agricultural systems while preserving natural ecosystems due to the greater presence of plant pathogens in agricultural environments.

Onfoods: insight on emerging mycotoxigenic fungi and their sustainable control

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The agri-food product quality and safety could greatly be compromised by fungi able to produce toxic metabolites as well as by the residues of the conventional means used for their control. Several pathogenic fungi can produce different metabolites of a given mycotoxin, and in some cases more than one chemical type is produced. Moreover, climate change has increased the occurrence of toxigenic genera, as well as the food-processing systems contributed rising the risk of contamination by emerging and re-emerging metabolites. Reducing the biological/chemical risk and guarantee food safety (*i.e.*, reduction of contamination by pesticides residues, mycotoxin, etc.) is of paramount importance, as well as finding novel sustainable control strategies that might fit the postharvest handling and transformation phases. Within this framework, ONFOODS aims at applying omics techniques to greatly strengthen the knowledge and better understand the possible hazards of eating food contaminated by well-known toxigenic genera (*Aspergillus* or *Penicillium*), still poorly studied genera (*e. g.* *Alternaria*), or not yet recognized genera (as *Monilinia*). Next-generation sequencing methods coupled with metabolomic approaches will be used to rapidly generate huge quantity of data useful to develop highly specific detection assays, to identify new/emerging pathogens and metabolites, and to understand clearly/better the mechanisms underlying the production/accumulation of different mycotoxins. At the same time, the project will investigate and apply multiple mitigation solutions to the postharvest and retailing

phase offering new strategies/approaches according to the “multiple-hurdle” concept.

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Trends and future perspectives in plant pathogens detection techniques

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Europe is highly exposed to the invasion of numerous new/emerging pests and pathogens that cause extensive damages and economic losses to agriculture, with serious repercussions on farmers’ incomes and livelihoods, food security and safety, the environment, national economies, and international trade. Therefore, quick and accurate detection and identification of pathogens is of utmost importance to reduce the agricultural losses associated with them, enabling the application of effective disease management strategies, with highly specific preventive/control approaches and less pesticide use, thus contributing to more sustainable agriculture production. Besides to be highly specific, sensitive, accurate and reliable, plant pathogen detection techniques should be fast, easy to use, inexpensive, capable of detecting pathogens in complex matrices and, if possible, be employable directly in situ. Current and emerging trends in plant pathogen detection will be reported, from more conventional methods, such as serological and nucleic acid-based assays, to more advanced ones, such as biosensors and high-throughput sequencing techniques. The main advantages and disadvantages of the proposed techniques will be discussed, thus enabling researchers and stakeholders to easily compare the different options available and select the one best suited to their specific case, pointing some relevant examples of how each technique has been successfully used for the detection of plant pathogens.

Characterization of Volatile Organic Compounds of *Xylella fastidiosa* and *Tilletia indica* for the training of two detection dogs

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Within the framework of two National Projects, the researchers of CREA-DC are training two detection dogs for the early detection of *Xylella fastidiosa* subs. *pauca* and subs. *multiplex* causal agents of several disease in plants, and of *Tilletia indica*, agent of Karnal bunt of wheat, through the characterization of Volatile Organic Compounds (VOCs) emitted by both pathogens. The analysis of VOCs profiles has been already successfully used in the detection and diagnosis of phytopathogenic bacteria and fungi. The odour of both *Xylella* spp. and *Tilletia* spp. was successfully transferred to inert adsorbent supports, by using a scent transfer unit (STU-100), thus allowing the training of the dogs in the open air. For the rapid VOCs characterization, a portable e-nose (Smell Inspector, SmartNanotubes Technologies), was used, bearing four different multi-channel gas detector chips able to measure a specific pattern of gas mixture associated with the specific smell of each pathogen. The raw data have been analyzed through the development of artificial intelligence algorithms. Preliminary results showed that VOCs of *T. indica* clustered separately from other *Tilletia* spp. not of quarantine concern, and from healthy durum and soft wheat samples. VOCs analyses of *Xylella* spp. from naturally infested tissues (stems, leaves) are in progress. The results obtained with the portable e-nose will be compared and validated with those obtained by the Proton Transfer Reaction - Time of Flight - Mass Spectrometry. Combining digital electronic nose and trained detection dogs are a potentially useful way to improve the diagnostic ability of quarantine pests.

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The main fungal diseases of wheat in Italy in the last decades

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Fungal pathogens pose a significant threat to wheat production as they can lead to compromised yields. Moreover, these pathogens have negative effects on the qualitative, hygienic, and sanitary characteristic of wheat. To address these issues, CREA has been conducting extensive phytopathological monitoring in the main cereal growing regions of Italy, through the National networks of variety trials of common and durum wheat. The objectives of this monitoring program are to evaluate the incidence and spread of plant diseases, identify different pathogens, and determine their distribution across space and time. Additionally, the program aims to assess the resistance or susceptibility of wheat varieties. The trials were carried out in 10 m² plots in 20 to 45 locations, utilizing a randomized block design with three replicates. In this study, we have analysed phytopathological data collected over a span of 30 years. This dataset has provided valuable insights on the spread of the fungal diseases and yearly epidemics in the Italian environments, and on the resistance levels of the wheat varieties towards the main fungal diseases. Notably, the data highlights a reduction of powdery mildew attacks, on both common and durum wheat species, which can be attributed in part to the availability of resistant cultivars developed through breeding. However, there has been an increase in the incidence of the septoria disease complex. Rusts have been observed through decades in both common and durum wheats, and fusarium head blight infections has been prevalent on these cereals in the northern regions of Italy.

Durum wheat kernels phytosanitary status and rhizosphere microbiome composition in a sustainable production process

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Conventional agricultural practices are now known to impact the environment by inducing soil degradation, pollution, loss of water resources, natural habitats, and biodiversity. Innovative agricultural processes and technologies are crucial for developing a sustainable agricultural system. The aim of the study was to monitor the phytosanitary status of durum wheat kernels and examine the microbial rhizosphere community of wheat in a three-year trial (2020–2022) located in Viterbo, Lazio, consisting of conservative tillage (subsoiling and spading compared to plowing) and fertilization (compost compared to mineral) in rotation with potato. Wheat kernels are susceptible to different diseases caused by various soil-borne microorganisms, mostly mycotoxigenic fungi, such as *Alternaria* and *Fusarium* species. Soil samples were collected for the metagenomic analysis. Fungal species were recovered from kernels by deep-freezing blot test and examined by morphological and molecular analyses for species identification. According to the results obtained from soil samples in the first year of trial, significantly higher biodiversity of fungal species in plots treated with compost was observed. The greatest frequency had the order *Hypocreales*, represented by *Fusarium* species, more abundant in plots treated with mineral fertilizer. On the other hand, the kernels analyses showed the occurrence of both *Alternaria* and *Fusarium* species in plots treated with compost, which, however, was recorded at a lower rate. By moving from an agroecosystem to a natural ecosystem, the conditions can become less favorable for the pathogen development fertilization by compost may act as a limiting factor for disease development.

Antifungal activity of *Trichoderma*-bioactive extracts for the *in vitro* and *in vivo* inhibition of mycotoxigenic fungi

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Fungal contamination and mycotoxin production are significant concerns in the agriculture industry. *Trichoderma* species are well-known biocontrol agents that can inhibit the growth and development of various plant pathogenic fungi. In this study, we investigated the *in vitro* inhibitory activity of bioactive metabolites extracted from *Trichoderma asperellum* and *Trichoderma atroviride* against several fungal and oomycete plant pathogens. Additionally, we evaluated the effective dose of these extracts in inhibiting the *in vivo* contamination by mycotoxigenic fungi and related mycotoxins in different plant matrices, such as tomato fruits, wheat, and maize. *In vitro* tests highlighted that extracts from both *Trichoderma* species had significant cytotoxic effects against several phytopathogenic fungi, including *Penicillium*, *Aspergillus*, *Fusarium* and *Phytophthora* species, with minimum fungicidal concentration ranging 0.19–6.25 mg/mL. In the *in vivo* tests, extracts from both *Trichoderma* species affected the viability of mycotoxigenic pathogens in all tested plant matrices. The extract from *T. atroviride* was the most effective in reducing mycotoxin content, showing a marked dose-dependent reduction of aflatoxins by *Aspergillus flavus* and ochratoxin A by *Penicillium verrucosum* in wheat and maize, respectively. In contrast, the extract from *T. asperellum* only showed a reduction of mycotoxins at the highest tested concentration. These findings suggest that *Trichoderma* species and their bioactive metabolites are means with great potentialities for both control of plant pathogens and for the management of contamination by mycotoxins in food and feed.

Evaluation of Italian flora biodiversity as a source of metabolites for plant protection

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Currently, in Europe, plant bacterial disease management is mainly based on the use of copper-based products. However, several concerns have recently raised regarding their

application due to the emergence of bacterial resistance, phytotoxicity and persistence in the environment. There is thus a crucial need for sustainable alternatives for plant protection towards bacterial diseases. A promising approach consists in inhibiting bacterial virulence, such as the type III secretion system (T3SS), an important virulence mechanism of bacterial phytopathogens, which allows effector translocation to suppress host immunity and construct a favorable niche for successful infection. It thus represents a key target for weakening bacterial pathogens. In this context, with the aim of valorizing Italian flora biodiversity, the scope of the project is the high-throughput screening of extracts from around 600 plant species, sampled all over Italy, for their ability to inhibit bacterial T3SS. To investigate extract efficacy, a reporter system combining the blue fluorescent protein (BFP)-encoding gene with the promoter of *hrpA1* gene, encoding one of the main components of the T3SS pilus, has been used in *P. syringae* pv. *actinidiae*, the causal of kiwifruit bacterial canker. The first results, obtained from the ongoing screening on a subset of extracts, already provided promising outcomes, with 4 extracts showing a significant inhibitory activity. The combined metabolomic analyses will allow to identify the compounds, single or as phytocomplexes, responsible for virulence suppression and further studies will be performed to decipher their mode of action at molecular level.

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Biostimulants to reduce nitrogen fertilizers, improve lettuce and wall-rocket production and quality in sustainable agriculture

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Nitrogen (N) fertilization increases crop production in agriculture but can cause risks to human health due to the accumulation of nitrates in foods such as leafy vegetables, or in the environment. Sustainable strategies to reduce

N inputs include the use of plant biostimulants, containing combinations of beneficial microorganisms capable of improving plant productivity and decreasing fertilizer applications. Furthermore, effectiveness of bioformulations can be increased by adding bioactive compounds derived from plant extracts. This study investigated the effects of nitrogen fertilization (N_0 , $N_{0.25}$, $N_{0.5}$ and N_1 , corresponding to doses of 0, 30, 60 and 120 kg N ha⁻¹ respectively), and four biostimulants (B) on the growth, yield and nutritional quality of lettuce and wall-rocket. The bio-treatments included known beneficial microorganisms as *Trichoderma virens* GV41 or *Azospirillum brasilense*, a commercial lignin extract (SolargoTM), and a combination of *T. virens* GV41 and SolargoTM. GV41+SolargoTM formulation applied under $N_{0.25}$ fertilization produced the highest lettuce yields in terms of fresh and dry weight, significantly greater than the $N_{0.25}$ control (+74% and +50% respectively), the N_1 control (+33% and +14% respectively), and all other biostimulant x nitrogen doses. A similar result was obtained with wild-rocket when the same bio-formulations were applied under $N_{0.5}$ fertilization regime. Moreover, the nutritional value of phytochemicals present (i.e., ascorbic acid, polyphenols and glucosinolates) in treated lettuce and rocket leaves was enhanced. This demonstrates that beneficial microorganisms and lignin extracts can positively contribute to an eco-friendly strategy of crop production and improve food health quality.

Plant-derived volatile organic compounds induce grapevine systemic resistance and direct defence against downy mildew by transcriptional changes

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Plant defence responses are based on numerous mechanisms, and volatile organic compounds (VOCs) play a crucial role in the activation of defence processes against biotic stresses, such as grapevine downy mildew caused by *Plasmopara viticola*. Previous studies revealed that the amount of some VOCs was higher in downy mildew-resistant compared with susceptible grapevine genotypes upon *P. viticola* inoculation.

These VOCs reduced downy mildew severity in susceptible genotypes, indicating the possible involvement of grapevine VOCs in defence mechanisms against *P. viticola*. However, the activation of transcriptional responses to VOC treatment has not yet been elucidated in grapevine. This study aims at identifying the molecular mechanisms triggered by plant-derived VOCs against downy mildew by investigating the transcriptional response of grapevine leaves to VOC treatments. Susceptible grapevine leaf disks were treated with one isoprenoid and one benzenoid compound, or with water, and they were inoculated with *P. viticola* or water. Samples were collected at one and six days post inoculation (dpi) for transcriptomic analysis. VOCs treatments generated a transcriptional reprogramming that included early and late defence responses against downy mildew. Particularly, treatment with the volatile isoprenoid up-regulated the expression of defence-related genes at 1 dpi in mock-inoculated and *P. viticola*-inoculated samples, suggesting an early activation of defence processes. These results provide a better understanding of the mechanisms involved in plant-plant communication mediated by VOCs and in plant defence mechanisms activated by VOCs.

Biocontrol of Citrus Mal secco: advances on effectiveness of BCAs based-formulations against *Plenodomus tracheiphilus* and deeply understanding of their population dynamics

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Mal secco, caused by *Plenodomus tracheiphilus*, is an economically important fungal vascular disease in citrus-growing countries of the Mediterranean basin. Limited control measures and further restriction on the use of copper led to investigate on alternative sustainable management solutions. The effectiveness of commercial products based on different strain of *Bacillus amyloliquefaciens*, *Trichoderma* spp. and *Pythium oligandrum* was evaluated through *in vitro* and *in vivo* experiments against *Plenodomus tracheiphilus* compared to copper and fludioxonil compounds. Commercial formulations based on *Bacillus amyloliquefaciens* and *Trichoderma* spp. provided the best performances in reducing disease incidence and symptoms severity on *Citrus volkameriana* seedlings maintained in growth chamber, suggesting their potential application within an integrated

management strategy. Understanding the survival and plant colonization ability of antagonist microorganisms is a crucial step to assess their effectiveness and scheduling application in the field. In detail, *Trichoderma* spp. and *Bacillus* sp. from internal wood and leaf tissue of treated citrus plants were detected and quantified using both agar dilution method, which is based on the assessment of colony forming units (CFU), and qPCR assay for the estimation of DNA by using primers/probe set for *Trichoderma* and *Bacillus* strains. In the further studies, BCAs showing the best performance under growth chamber will be selected to assess their effectiveness in field conditions.

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Characterization of *Trichoderma* species pathogenic to *Pleurotus* cultivation

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Nine isolates of *Trichoderma* were isolated from various processing phases during commercial production of oyster mushroom varieties (*Pleurotus* spp.). Pathogenic strains of *Trichoderma* spp. were morphologically and genetically described. The typical characteristic of green mold disease is the formation of dark green spores on the developing mushrooms and compost, that in cases of acute outbreaks results in a total loss of production. Molecular fingerprinting indicated that most isolates belonged to *T. pleuroticola* and *T. harzianum*. To identify potential control measures, physical factors affecting *Trichoderma* spp. growth and development were manipulated, such as temperature and pH, and effects on the growth of various *Pleurotus*

isolates were also determined. Moreover, we tested singly two approved fungicides to determine the application doses most effective on *Trichoderma* without producing undesirable consequences on *Pleurotus*. Optimal growth temperatures were 25 °C for *Trichoderma* and 28 °C for *Pleurotus* isolates, with *Trichoderma* always growing faster than *Pleurotus*. Pathogenic *Trichoderma* strains developed well over a wide range of pH, with best growth between pH 5 to 7, whereas *Pleurotus* preferred more alkaline conditions (pH 8 to 9). Prochloraz and metrafenone were found to control the germination and sporulation of the pathogenic *Trichoderma* isolates, with different dose responses, and did not affect the growth of the *Pleurotus* varieties.

Carpospheric microbiome composition of different citrus accessions and impact of field treatments on community structure

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Citrus is one of the major fruit crops globally cultivated, whose carposphere microbiome composition and function has received limited investigation. Moreover, since pesticides applications are essential in crop management, understanding their implication on citrus beneficial microbiome is crucial. Here we report a comprehensive analysis of the structural composition of citrus carposphere microbiome of three citrus accessions ('Tarocco Scirè' and 'Tarocco Tapi' oranges, 'Femminello Siracusano 2KR' lemon) and its modification accordingly to different phytosanitary treatments (biological and copper-antimicrobials) applied in the field, using both amplicon sequencing and culture-dependent techniques. Results revealed that the microbiota in the three citrus accessions had similar taxonomical composition but varied in relative abundance, with *Proteobacteria*, *Firmicutes*, *Ascomycota* and *Basidiomycota* phyla being dominant in all the samples. The core microbiome comprised 51 prokaryotic and 27 fungal Amplicon Sequence Variants (ASVs), encompassing *Bacillus*, *Pseudomonas*, *Massilia*, *Sphingomonas* and *Aureobasidium* genera widely reported as plant beneficial microbes. Within the same accessions, data were analysed comparing different applied treatments (chitosan, sweet orange essential oil, their mixture and copper-based products) on citrus fruits. While the cultural-method did not show relatable changes in microbial community, the cultural-independent method revealed substantial differences in the microbial composition and

abundance profiles of the bacterial community when copper-based treatments were applied, whereas alternative products induced minimal or no differences. The fungal community structure appeared less sensitive to phytosanitary products applications. The present study represents an advancement of the current knowledge of citrus fruit microbiome and confirms that pesticides can cause measurable shifts in microbial communities.

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Preliminary observation on "resilient" olive trees infected by *Xylella fastidiosa* in the Salento area (Apulia, Italy)

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Xylella fastidiosa subsp. *pauca* is causing serious economic losses both to olive industries and to the Salento landscape. In the infected area, many hectares of olive groves have completely withered. Recently, a resprouting of pollons, suckers and shoots from severely symptomatic plants of the susceptible cultivars Ogliarola salentina and Cellina di Nardò has been observed in this area. In some cases, in recent years, some trees show a fully restored crown without any visible symptoms of decline (i.e., twig and branch dieback). Such trees that have overcome the Olive Quick Decline Syndrome (OQDS) are referred to as "resilient". Preliminary investigations in olive orchards, located in Lecce province, previously severely attached by *X. fastidiosa* subspecies *pauca* (*Xfp*), were carried out by evaluating the bacterial concentration of several "resilient" plants. Bacterial concentration was assessed by real-time quantitative PCR by sampling these trees in May-June of 2022 and 2023. The results showed that the average density of *Xfp* bacterial cells varied from medium values (about 10⁴ cfu/ml) to high values (10⁷ cfu/ml), in the olive groves. Further studies are in progress to in depth evaluate the fitness of *X. fastidiosa* in resilient plants, to elucidate the epidemiological facets and the fate of these trees in the long term period.

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Beneficial fungal microbes as novel eco-sustainable tools for forage crops

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Traditional plant protection approaches are based on the use of chemicals. Nevertheless, these products are costly, contaminate the environment and are harmful to animals and humans. Their reduction or elimination is needed, and the application of biostimulants and biological control agents could be an efficient eco-sustainable alternative. In this study, formulations based on two *Trichoderma* strains, *T. harzianum* M10 and *T. afroharzianum* T22, and a solution of 6PP (6-pentyl- α -pyrone isolated from *T. atroviride*) were tested *in vitro* and *in vivo* (field trial) on oat. A significant plant growth promotion was observed with 6PP after 96 h compared to control. All treatment significantly improved oat stem length at 120 h, while only treatment with 6PP and T22 were significant at 192 h compared to control. Moreover, a metabolomic approach was used to evaluate the effects of treatments on plants physiology. Several metabolites were detected, whose abundance was related to the specific treatment. It was possible to identify molecules belonging to different class of compounds, such as fatty acids, terpenes, organic acids, phenol derivatives and sterols. These compounds are used by the plants to limit pathogen, enhance antimicrobial synthesis, and improve crop protection. Black spots symptoms associated to five fungi, two *Alternaria* species, *Cladosporium cladosporioides*, *Epicoccum nigrum* and *Fusarium verticilloides* (morphological and

molecular characterization), were detected. Preliminary evaluation of data did not show significant differences of disease symptoms between treated and untreated plants. The application of beneficial fungi and their metabolites could be considered a valid alternative to chemicals for sustainable forage production.

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Antifungal activity of rosemary and oregano essential oils in combination

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Plants are susceptible to a wide variety of pathogens limiting the quality and yield of crops. *Botrytis cinerea* Pers. Fr., is an airborne plant pathogen with a necrotrophic lifestyle attacking over 200 crop hosts worldwide, causing grey mould on strawberry fruits. Alternatives to conventional pesticides can consist of plant extracts containing bioactive molecules as essential oils (EOs). Essential oils obtained by steam distillation from rosemary and oregano, were tested *in vitro* against *Botrytis cinerea*, to determine minimal inhibitory concentration (M.I.C.). For rosemary essential oil (R), the following concentrations were tested: 2000 mg/L, 1500 mg/L, 1000 mg/L and 500 mg/L. The M.I.C. was fixed at 2000 mg/L. For oregano essential oil (O), the following concentrations were tested: 2000 mg/L, 1000 mg/L and 500 mg/L. The M.I.C. was fixed at 1000 mg/L. The two essential oils were mixed at the following concentrations to study their activity *in vitro* against *Botrytis cinerea*: R 1000 mg/L+O 750 mg/L, R 750 mg/L+O 750 mg/L and R 750 mg/L+O 500 mg/L. The best results were achieved with R 1000 mg/L+O 750 mg/L and R 750 mg/L+O 750 mg/L that inhibited fungal growth and showed fungistatic nature at their respective M.I.C. The antifungal efficacy of the EOs was to be greater than that of standard fungicides (boscalid+pyraclostrobin).

The rules behind the assembly of plant pathobiomes

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The study of plant microbiomes is increasingly attracting a large interest worldwide, generating groundbreaking knowledge on the composition, function, and origin of the plant-associated microbial communities. However, we are only beginning to understand the rules behind the assembly of plant microbiomes, and we still lack key information about the drivers and dynamics behind the assembly of detrimental microbiomes. In this study, we leverage on in-house and publicly available data, collected and analyzed under a common framework. Using a variety of metrics and approaches, we tested several questions, including: (i) which are the major factors contributing to the assembly of plant microbiomes/pathobiomes? (ii) do different factors (biotic/abiotic stressors) generate unique plant microbiomes/pathobiomes? (iii) does plant phylogeny reconcile with the diversity/structure of plant microbiomes/pathobiomes? Our results contribute to a more general understanding of plant-microbiome interactions, in particular those generating detrimental effects on plant health, which is key towards achieving an eco-sustainable plant protection.

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Application of hyperspectral imaging to monitor downy mildew (*Plasmopara viticola*) on *Vitis vinifera* L.

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Downy mildew caused by the oomycete *Plasmopara viticola* is one of the most serious diseases of grapevine (*Vitis vinifera* L.); invading leaves, shoots, and young berries under warm-moist conditions, using plant nutrients to produce sporangia, which cause secondary infections impacting on photosynthesis and grape growth. In 2023, continuous rains in May and June, and subsequently a very hot and humid meteorological trend favored the uncontrollable pathogen development. Even when phytosanitary treatments were conducted, they were unable to control the disease, which definitively compromised the consistency and state of health of developing grape clusters. Spectral mapping, associated to visual assessment of disease severity, was conducted on 12 plots (100 m²) in a 1.5 ha field located at Mirabella Eclano, AV (Campania Region, Italy) to identify bands useful in describing disease progression and monitor the general field conditions. The assessments were carried out one month after the first symptoms were observed. The disease severity index was comprised between 0 and 90%. Hyperspectral images were acquired by using the SPECIM IQ camera working in the range of 400–1.000 nm. Downy mildew determined a heavy reduction of reflectance between 520–600 and 700–1.000 nm, respectively. Among 46 different vegetation indices analyzed, DVI, PVI, RGRcn, RVSI and TVI, related to chlorophyll content and vegetation, were able to discriminate different disease severity levels, accounting for more than 90% of the observed variability. These vegetation indices are promising candidates for tracking the effectiveness of control strategies in open field trials.

Utilization of bacteria and fungi to increase phosphorus solubilization

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Phosphorus (P) is one of the essential macronutrients for plants and its deficiency affects different biochemical

processes, thus limiting plant growth. Inorganic raw material fertilizers, such as phosphorite and apatite, are non-renewable sources of P which is not directly available to plant. The utilization of phosphate-solubilizing microorganisms (PSMs) can improve P solubilization in fertilizer production, being able to produce organic acids that release available forms of P to plants. PSMs include species of bacteria (*Bacillus*, *Pseudomonas* and *Rhizobium*) and fungi (*Penicillium*, *Aspergillus* and *Trichoderma*). In this work, we evaluated the effects of different PSMs in improving the solubilization of phosphates from phosphorite. Different *Bacillus* species (*B. subtilis*, *B. megaterium*, *B. pumilus*, *B. amyloliquefaciens*) and *Trichoderma* strains (*T. afroharzianum* T22, *T. virens* GV41 and *T. asperellum* T25), either applied alone or in combination, were tested. Compatibility between PSMs and phosphorite was evaluated *in vitro* at different concentrations. All *Trichoderma* strains were able to grow on phosphorite-amended substrate up to 20 g/L, while *Bacillus* species showed different growth rate compared to control in presence of 10 g/L phosphorite. Specifically, *B. pumilus* and *B. megaterium* resulted the best performing species in terms of colony forming units (CFUs). Production of organic acids by *Bacillus* spp. was also evaluated, resulting in a pH decrease from 7.5 to 5.8 at 5 days post inoculation (dpi). Combinations of fungal and bacterial species were also tested. These results may help the formulation of fungal and bacterial isolates for improving P solubilization from inorganic fertilizers.

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Association of different ‘*Candidatus Phytoplasma solani*’ strains with bois noir symptom severity and evolution in grapevine cv. Chardonnay in northeastern Italy

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Bois noir (BN) is one of the most important and widespread grapevine yellows (GY) diseases in Europe and is associated with ‘*Candidatus Phytoplasma (Ca. P.) solani*’ (16SrXII-A). This study aims to investigate a possible influence of

different ‘*Ca. P. solani*’ strains on BN symptom severity, including disease outcome (symptom persistence, plant death, or recovery). We performed our study in two Chardonnay vineyards in northeastern Italy (Cormons, Friuli Venezia Giulia), which have been monitored for the presence and incidence of BN since 2007 and 2012, respectively. Molecular typing of ‘*Ca. P. solani*’ strains was conducted in 2022, revealing that the most prevalent genotypes were tuf-a/secY26/Vm15/St9, tuf-b/secY1/Vm76/St1 and tuf-b/secY1/Vm43/St10. Statistical analyses were performed by Fisher’s Exact Test to determine whether the tuf-type of ‘*Ca. P. solani*’ significantly influences the severity and evolution of BN symptoms. In 2022, the percentage of grapevines belonging to the class of most severe symptoms (third class: absent production) was significantly higher for those infected with ‘*Ca. P. solani*’ tuf-a strains versus those infected with tuf-b strains (52.0% vs 25.6%; $p=0.0377$). Furthermore, the mapping over the years of symptomatic plants in the two vineyards revealed that the percentage of dead grapevines was significantly higher in cases of infection with ‘*Ca. P. solani*’ tuf-a strains (47.9% vs 20.4%; $p=0.0012$), whereas the percentage of recovered plants was significantly higher in cases of infection with ‘*Ca. P. solani*’ tuf-b strains (38.8% vs 14.6%; $p=0.0016$). Therefore, all these results suggest a higher virulence of ‘*Ca. P. solani*’ tuf-a strains in grapevine.

Characterization of fungal pathogens associated with fungal trunk diseases of hazelnut in Northern Italy

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Hazelnut (*Corylus avellana*) represents one of the most economically important nut crops across the world. Italy is the second largest hazelnut producer worldwide with 84,526 ha cultivated and 98,666 tonnes of fruit harvested in 2022. The regions with the highest productivity are Piedmont, Latium, Campania and Sicily. In Piedmont, an expansion of hazelnut cultivation and production was registered during the last decade. However, the changing climatic condition characterized by water deficiency, late-spring frosts, and hot summer temperatures, along with the high incidence of *Cytospora*

cankers, known also as ‘Mal dello stacco’, and twig blight represent a serious threat to this crop. Considering the severe impact of fungal trunk diseases (FTD) on this crop and lack of studies conducted so far to investigate the respective causal agents in Piedmont, surveys were conducted in seven hazelnut orchards during 2021–2022. Eight fungal species were identified: *Anthostoma decipiens*, *Botryosphaeria dothidea*, *Diaporthe eres*, *Diaporthe rudis*, *Diplodia seriata*, *Diplodia subglobosa*, *Dothiorella parva* and *Nothophoma brennandiae*. Species identification was achieved through morphology assessment and multilocus phylogeny. The fungal species were confirmed as pathogenic when inoculated on healthy hazelnut plants of the cv. ‘Tonda Gentile’. The present study is the first report of *B. dothidea*, *Dia. eres*, *Dia. rudis*, *Dip. subglobosa* and *N. brennandiae* as causal agents of FTD on hazelnut in Italy. Moreover, the provided clarification of the fungal pathogens associated with FTD on this crop in Piedmont is crucial for further investigations on disease epidemiology and management strategies.

***Alternaria* species: a "From Farm to Fork" phytopathological and toxicological global concern**

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The ubiquitous *Alternaria* genus, colonizing several agri-food crops, represents a serious problem both in field conditions and in post-harvest. For a long time, it has been considered only a phytopathological issue, but the recent interest to food safety and consumer health pone this genus among the most important mycotoxigenic fungi. Indeed, *Alternaria* species produce more than 70 metabolites, including alternariol, alternariol monomethyl ether, tenuazonic acid, altenuen mycotoxins. In 2011, EFSA published the first scientific opinion on the toxicological risk due to the presence of *Alternaria* toxins in food, suggesting to pose more attention to *Alternaria* contamination. Based on phylogeny, the taxonomy of *Alternaria* has been revised grouping all species in 26 phylogenetic Sections. In the last years, we studied the occurrence of *Alternaria* species on different crops and their mycotoxin profile. About 1000 strains isolated from wheat, tomato, potato, date palm, the halophyte *Cackile maritima* collected in Italy, Lebanon, Iran, Tunisia, Algeria, Albania, and Argentina were studied. Phylogenetic analyses

revealed that the most frequent species occurring on wheat kernels belonged to *Alternaria* and *Infectoriae*. Sections and the majority of strains isolated from the other hosts belonged only to *Alternaria* Section. Few strains grouped in *Ulocladiodes*, *Chalastospora* and *Pseudoalternaria* Sections. More than 90% of strains included in *Alternaria* Section produced multiple mycotoxins, with variable amount. Our in-depth investigations demonstrate that geographical origin and host plants did not influence *Alternaria* species distribution and genetic diversity. Finally, the plasticity of this genus represents a great pathological and toxicological risk at worldwide level.

Unusual incidence of hazelnut defects in 2023 vegetative season

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Hazelnut (*Corylus avellana* L.) plays a key role in the agricultural economy of Italy, the second largest world producer of this fruit, especially in some areas of Italy. In the framework of a 3-year monitoring of defects and rotting of nuts in Central Italy, during the 2023 campaign, a peculiar defect has been abundantly and unexpectedly observed. Externally, the nuts showed an evident browning of the basal portion, in contrast with the still green apical part of the nut. In several cases, the browning was flanked by a more or less evident shrinking of the nut. Internal tissues were completely degraded, brown, spongy, and wet. As the defect appeared on the nuts, they stop their growth. Consequently, the symptom was observable in nuts at different development stages. Representative samples were collected from orchards scattered all over Viterbo province. After surface sterilization, fragments from different parts of the nuts were plated on different culture media: PDA supplemented with streptomycin for fungal isolation, MEA, and King’s B for bacterial isolation. Surprisingly, it was not possible to isolate fungi or bacteria from the husk or bracts or internal tissues, suggesting the occurrence of a physiological disorder (as for the “brown stain” defect). However, looking at the very basal part of the nut and the insertion of the petiole in the nut, necrotic tissue can be observed. Isolation from these tissues led to the growth of a few microorganisms. Further experiments are ongoing to shed light on the origin of this hazelnut defect.

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Efficacy of a multiplex qPCR assay in detecting different *Phytophthora* species

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Holm oak (*Quercus ilex*) and cork oak (*Quercus suber*) are the two dominant evergreen oak species of the Mediterranean basin, usually occurring in pure and mixed forests or agroforests. These species are threatened by Alien Invasive Forest *Phytophthora*'s (AIFPs), whose spread intensification along climatic gradients is putting at risk evergreen oak forests in Southern Europe. Among the AIFPs, *Phytophthora multivora*, *P. cambivora* and *P. cinnamomi* require attention, especially the latter for being the most destructive one, responsible of root and stem cankers, biodiversity loss, extensive decline, and mortality of broadleaves species. In the framework of the LIFE FAGESOS project, a multiplex qPCR assay able to test and quantify the presence of the different AIFPs using probes specific for each of the above-mentioned species and one probe specific for the genus, is under evaluation. It will be firstly validated using DNA extracted from pure cultures and from seedlings inoculated with AIFPs in a controlled environment, periodically monitored to evaluate the disease severity. Once the qPCR assay will be well-established and validated, it will be applied to evaluate the efficacy of different bioproducts having resistance induction activity (chitosan and silicon), antagonist activity (*Trichoderma* spp. complex.), or using plant growth-promoting microbes (PGPM) known to increase the availability of soil nutrients and the resistance against pathogens, in reducing AIFPs in mesocosm to overcome the potassium phosphonate (K-phosphonate), banned in a recent European regulation.

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Can whole genome sequencing approach resolve taxonomic ambiguities in fungi? The *Colletotrichum* case study

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The genus *Colletotrichum* encompasses more than 250 species grouped into 15 complexes; among these we find destructive plant pathogens and important model systems. Accurate species delimitation is crucial for managing plant diseases caused by *Colletotrichum* fungi. Identification methods face challenges due to variation in morphology, overlapping traits, intraspecific variability and hybridization events. Moreover, no standard molecular approach is available as different lineages of *Colletotrichum* may need different loci for a correct characterization. This study aims to evaluate a whole genome sequencing (WGS) approach for *Colletotrichum* characterization and species delimitation. The research goals are: 1) to develop a comprehensive genomic platform that cover the diversity of the genus; 2) to propose a genomic approach for genetic characterization, to study genetic diversity and evolutionary relationships within the genus; 3) to evaluate the impact of WGS on species boundaries. The methodology involves high-throughput genome sequencing using Illumina technologies, assemblies and comparative genomics to identify and analyze high resolution single-copy genes. Selected loci have been aligned and analyzed to evaluate their resolution and genealogical concordance. Results obtained will be compared to those collected using a standard MLST approach based on a limited number of loci. The capability of the WGS approach to study genetic diversity, species boundaries, evolutionary

relationships and to identify natural hybrids within the *Colletotrichum* genus will be presented.

A biofumigation approach for the control of oomycetes involved in kiwifruit vine decline syndrome

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Since its appearance in 2012, kiwifruit vine decline syndrome (KVDS) has affected most of the kiwifruit vocated areas in Italy. Oomycetes belonging to *Phytophthora* sp. and *Phytophytium* sp. are considered among the most relevant pathogens associated with this dysbiosis, whose activity is enhanced by flooding events. In order to properly manage KVDS, an integrated framework is needed. The use of resistant rootstocks is a promising tool for new plantings, while preventive and control measures are needed for existing and not yet compromised orchards. Reshaping the microbiome in KVDS infected soils by biofumigation and enhancing the presence of beneficial microorganisms could be an interesting and sustainable approach. We evaluated leaf extracts from 7 rocket (*Eruca vesicaria* subsp. *sativa*) cultivars for their *in vitro* effects against *Phytophytium vexans*, *Phytophytium chamaeaphon*, and *Phytophthora citrophthora*. Moreover, to scale the *in vivo* evaluation of the extracts, we used potted kiwifruit cv. Hayward plants grown for two months in KVDS inducing soil and added with rocket extracts before the flooding events adopted to promote disease onset. The *in vitro* experiment evidenced the activity of rocket extracts against the oomycetes, with an average mycelia inhibition of about 70% (considering all cv. and pathogens). Indeed, the application of rocket extracts on potted plants significantly reduced the typical KVDS root symptoms (e.g., rat-tail and necrosis of fine roots) by about 60% on average. These results suggest a possible role of biofumigation practices as part of a multifactorial approach to reduce the impact of KVDS in productive kiwifruit orchards.

Novel organic methods for preventing Fusarium Head Blight (FHB)

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Wheat holds its place among the most consumed cereals as it contributes to the daily caloric uptake. Apart from genetic improvement, it is necessary to formulate revolutionary approaches to control major diseases. Synthetic fungicides are used but are also often insufficient. For this reason, nanotechnologies can help by improving coverage and reducing chemical dispersion. Among them, one sustainable and effective solution is cellulose nanocrystals (CNC). This study investigates the role of amylose-enriched starch in FHB resistance of three different bread wheat genotypes with different amylose content (Sumai3, Cadenza and Cadenza High Amylose) treated with a nanotechnology bio-based agrochemical. Phytopathological experiments were performed to validate the efficacy of the bio-based agrochemical compound in FHB management compared to a reference fungicide, and a biochemical study was conducted to investigate the effect of such treatments on final yield and grain quality. Transcriptomics (RNAseq) was performed, at 7 days after treatments, to compare the putative effects of FHB infection and of bio-based agrochemical compound on the transcriptional response amongst genotypes with different amylose content. Furthermore, metabolomics was performed with the aim of both identifying secondary metabolites, with a major role in the interaction between plants and pathogens, and determine the qualitative and quantitative mycotoxins content within the spike at harvest stage. Root systems contribute substantially to plant productivity and environmental adaptability playing an essential role in water and nutrient uptake. In order to investigate the effect of nanotechnology bio-based agrochemical on root systems, 2-D rhizoboxes and in soil-filled rhizotrons placed vertically at an angle of 30° are being used to evaluate root architecture and their spatial distribution within the soil profile and to quantify root and shoot biomass.

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The role of *Trichoderma* for the bioremediation of copper-contaminated matrices

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Copper (Cu)-based products are widely used as antimicrobial compounds for the defense of agricultural crops against phytopathogens. However, their frequent applications can cause Cu accumulation in soil and water sources, creating a risk for consumer health and negatively impacting biodiversity. The development of effective and economic solutions which can contribute in reduce the pollutant release and accumulation in the agroecosystem is needed. Bioremediation is a sustainable technique that involves natural ability of organisms to absorb, accumulate and degrade pollutants from contaminated environmental matrices. The present research was aimed at testing the bioremediation capacity of 4 *Trichoderma* isolates to remove Cu from growth substrates enriched with a copper-based fungicide increasing concentrations of fungicide (CuSO₄, Cupravit Bioadvance®, Bayer), ranging from 0.18 to 1.8 g/L. Cu content in supernatant and fungal biomass were analyzed at the beginning of the experiment (T0) and after 192 hours of growth by inductively coupled ion plasma mass spectrometry (ICP-MS). Among the selected strains *Trichoderma asperellum* T25 showed the greatest bioremediation capacity reducing up to 40% from the media containing 0,12 g/L of Cu. *Trichoderma harzianum* E45 shows an high accumulation capacity uptaking over 35,000 mg/kg of Cu in its biomass. The data suggest that Cu tolerant strains of *Trichoderma* can be used as an effective method for the bioremediation of severely copper-contaminated matrices. Further research is needed in order to understand the Cu-removal mechanisms employed by *Trichoderma*.

Chemical composition and antifungal activity of rosemary essential oils

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Novel strategies to reduce the use of synthetic pesticides for plant disease management are required by the European Green Deal where it was reported to reduce to half the application of pesticides by 2030. During the last decades, many efforts to minimize the use of pesticides have been realized. Plant extracts as essential oils (EOs) can be a useful alternative to conventional “pesticides”. They can differ in chemical composition regarding on different factors as the harvest period. The variation in the chemical composition of essential oils obtained from *Rosmarinus officinalis* (L.), harvested in summer and autumn and distilled with steam distillation method, was performed. The EOs were different in chemical composition analyzed by gas chromatography-mass spectrometry. The summer-harvested rosemary essential oil was composed by α -pinene (28.3%), verbenone (12.3%), 1,8-cineole (12.1%), camphor (7.8%) and borneol (6.0%) as main components. The autumn-harvested rosemary essential oil had the same main components with a different ratio: α -pinene (19.8%), 1,8-cineole (11.4%) and verbenone (10.5%) decreased while increased content’s borneol (12.4%) and camphor (10.9%). Moreover, two EOs were tested, *in vitro*, against *Botrytis cinerea* to investigate the antifungal activity, at concentrations of 20, 200 and 2000 mg/L. The best result was achieved with essential oil of rosemary at 2000 mg/L, harvested in autumn, which inhibited completely fungal growth showing greater efficacy than standard fungicides (boscalid+pyraclostrobin). The other thesis did not provide protection against the target pathogen. The essential oil obtained from autumn harvesting was chosen as candidate for future trials for insights into antifungal activity.

Preliminary study on bacteria isolated from bleeding cankers of *Aesculus hippocastanum* in Emilia Romagna Region

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Since 2017, *Aesculus hippocastanum* is listed as vulnerable in the European Red List of Trees because its extinction is likely in the near future, given current knowledge about its population trends, and recent threats. In particular, among biotic stresses, horse-chestnut declines is caused by the leaf-miner moth, *Cameraria ohridella*, and by *Pseudomonas syringae* pv. *aesuli* (Psae), the causal agent of bleeding cankers across Europe, at present never reported in Italy. In this study, from horse-chestnut tree showing typical bleeding canker symptoms of Psae, several bacterial colonies were isolated (both from bark trunk and fresh liquid oozing) on different substrates (KB, NSA and EMB) amended with cycloheximide (100 µg/ml) and subsequently purified by sub-culturing to obtain axenic isolates. On Psae and *Brenneria* spp. like colonies were carried out: 1) *in planta* assays to test their ability to induce hypersensitive reaction (HR) on leaves of tobacco plant; 2) carbon source utilization screening by using the Biolog GenIII™ system; 3) quantitative real-time PCR analyses to identify Psae with primers AM-Aes1F/AM-Aes1R and Psa2F/Psa2R; 4) PCR analyses to identify both *Brenneria nigrifluens* using the primers F1/C3 and *B. rubrifaciens* with primers BR1/BR3; 5) 16S rDNA gene sequence analysis. From these preliminary analyses, no isolates were identified as Psae and also attempts to isolate *Phytophthora* spp. on PAR selective substrate were unsuccessful. Pathobiomechanisms involved in bacterial diversity of symptomatic tissue that harboured opportunistic or animal pathogens were discussed.

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Reduction of copper inputs in the management of key diseases of grapevine, olive and tomato by an innovative Zeo-biopesticide

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The use of copper in agriculture and its accumulation in soil are no longer sustainable in modern agro-ecosystems. Our research aimed to evaluate the efficacy of an innovative Zeo-biopesticide as an alternative to copper-based products, in line with the European Green Deal. We developed a bio-formulation, based on the association between a zeolite carrier

and one bacterial antagonist (*Pseudomonas synxantha*, strain DLS65). *P. synxantha* was checked *in vitro* and *in planta* against the following pathogens: *Pseudomonas syringae* pv. *tomato* (Pst), *Xanthomonas vesicatoria* (Xv), *Pseudomonas savastanoi* pv. *savastanoi* (Pss), *Plasmopara viticola* (Pv), and *Spilopodia oleaginea* (So). *In vitro*, *P. synxantha* showed a strong antagonistic activity by significantly reducing the growth of pathogenic bacteria up to 20%, whereas the growth of *So* mycelium was reduced by 30%. The zeolite-*P. synxantha* formulation allowed the survival and bio-activity of the bacterial antagonist for longer than 6 months. Therefore, the industrial formulation of the Zeo-biopesticide was evaluated *in planta*. In potted 2-years plants, the bio-formulation was able to significantly reduce the severity of downy mildew of grape, whereas on olive experiments are still ongoing. Trials in commercial tomato fields, vineyards, and olive groves are currently ongoing in Italy, Croatia, and Spain, to assess the bio-formulation efficacy in different geographical areas. The first field evaluation of this innovative Zeo-biopesticide, in comparison with copper-based products, suggest that our formulation is a prospective biopesticide, but its dosage should be adapted to crop and area.

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Ecological processes driving microbiome assembly in gnotobiotic plants

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Plant microbial assembly is influenced by external factors (e.g., the environment, stresses) as well as the plant itself. Despite these previous findings, little is known about the mechanism behind the plant microbiome assembly and whether it is governed by stochastic or deterministic processes. In this study, we investigate this aspect by using gnotobiotic lettuce plants (plants with a limited diversity of associated microorganisms) germinated in sterile isolated microcosms, and each inoculated with different soil inocula. After one week, we characterize the bacterial and fungal microbial communities from shoots, roots, and each soil inocula using amplicon metagenomics. We found that root microbial assembly was mainly driven by stochastic processes and influenced by soil inocula, whereas the shoot microbiome was influenced by deterministic processes

through a selection from the root microbiome. Our results provide novel information for future research focusing on clarifying the biological, ecological, and evolutionary consequences behind these microbiome assembly processes.

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Trichoderma induction of plant defence, a concept beyond the ISR and SAR dichotomy

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Among all the biological control agents (BCAs) used in crop protection, *Trichoderma* is possibly the most studied and best known, and in plant interactions has been demonstrated to be an excellent growth promoter and inducer of plant defence against biotic and abiotic stresses. The ability to induce resistance in plants to pathogens and insect pests is likely to be registered and marketed as an indirect BCA (via the plant). It is commonly accepted that plants defend themselves against biotrophic pathogens by means of salicylic acid (SA) dependent resistance known as SAR, whereas their defence against necrotrophic pathogens and insect herbivores occurs by means of the jasmonic acid (JA) dependent pathway, known as induced systemic resistance (ISR). Pioneering work demonstrated that cucumber roots colonized by *T. asperellum* accumulated substantial levels of JA and ethylene (ET) within 24 h, supporting the notion that processes activated by beneficial microorganisms conform to an ISR response. However, subsequent evidence suggests that *Trichoderma*-triggered plant responses to pathogenic fungi, even viruses and nematodes, were modulated by both JA/ET and SA. Here, we present how *Trichoderma*-induced defence in plants is dynamic, and depending upon the moment in which the interaction is studied will reveal whether an ISR- or SAR-type systemic pathway will be activated. The ISR and SAR dichotomy has been very useful in understanding how plants and beneficial microorganisms interact with one another. However, we must bear in mind that the *Trichoderma*-plant dialogue is long-lasting, and that

studies should have to consider different time points during the interaction.

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Analysis of the soil microbial communities in lemon groves affected by Mal secco disease

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Soil biodiversity plays a central role in providing large variety of nutrients, especially for shaping the microbial functions of rhizosphere, which is a fundamental compartment for plant growth and disease defence. In this study, soil bacterial and fungal communities of lemon groves located in Syracuse (Sicily, Italy) were analysed. They differed each other for plant age, farming and rootstock (*Citrus aurantium* or *Citrus volkameriana*). Citrus Mal Secco disease, caused by the xylematic fungus *Plenodomus tracheiphilus*, was recorded in the groves. Five plots with uniform characteristics, in which the presence of *P. tracheiphilus* in the soil was ascertained, were considered for sampling. Soil total genomic DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen). The microbial community profiling was based on high-throughput amplicon sequencing using the Illumina NovaSeq 6000 instrument. Beta diversity analysis showed that each grove harbour different bacterial and fungal communities. Relative abundances of the most abundant bacterial phyla, namely Proteobacteria, Actinobacteriota, Acidobacteriota and Planctomycetota change in the groves. Regarding fungal community, an overall fluctuating but consistent abundance of Ascomycota, the most abundant phylum, was observed along with Basidiomycota, Mortierellomycota and Chytridiomycota. The microbial core community, present in at least 75% of the total samples, is

represented by bacterial genera with putative plant beneficial activity such as *Pseudomonas*, *Streptomyces*, *Pedospaera*, *Nitrospira* and *Gemmata*. *Penicillium*, *Mortierella*, *Rhizopus* and *Aspergillus* represent part of the core mycobiome. A predictive study of functional profiles will further delineate the potential role of beneficial microbiota in citrus plants facing biotic stressors.

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Real-time PCR assay for early detection and quantification of *Tilletia laevis* and *Fusarium culmorum* in durum and bread wheat

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Tilletia laevis and *Fusarium culmorum* represent the most common seed-borne diseases affecting the yield and grain quality of durum (*Triticum durum* Desf.) and common wheat (*T. aestivum* L.) in southern Italy. In order to screen wheat germplasm and identify tolerant/resistant genotypes, a rapid method based on SYBR Green RT-PCR was developed to quantify *T. laevis* and *F. culmorum* at early growth stages. Different DNA extraction protocols were firstly tested, allowing us to select the CTAB method as optimized protocol to obtain high-quality DNA. Then, two specific targeting sequence characterized amplified regions (SCAR) were selected and specific primers were tested for both pathogens to assess their specificity and sensitivity. Amplifications on DNA samples obtained following artificial inoculation with both fungi revealed high specificity, as also confirmed by melting temperature analysis. Both fungi were identified at early growth stages, including roots and coleoptiles, confirming the robustness of the method. The limit of detection (LOD) was 60 fg/μl for *T. laevis*, and 68 fg/μl for *F. culmorum*. Moreover, the primers of *F. culmorum* have been tested on various strains of different origin, confirming the specificity of the molecular assay. In conclusion, we described a robust real-time PCR method for the early detection of *T. laevis* and *F. culmorum* providing a rapid test for screening

of wheat germplasm and developing new tolerant/resistant wheat varieties.

This research was funded by Ministry of Agriculture, Food Sovereignty and Forests (MASAAF) within the project ‘CERES-BIO’ (*Cereali resistenti a malattie fungine trasmesse da seme per l'agricoltura biologica*).

Scope of organic nanocompounds in controlling the threat of *Xylella fastidiosa* – The case of ANCOSIX Project

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Xylella fastidiosa (*Xf*) is a gram-negative bacterium that causes severe diseases in economically important crops such as olives, almonds, grapes, citrus, and others. The Olive Quick Decline Syndrome, caused by *Xylella fastidiosa* subsp. *pauca* and emerged in Italy in 2013, has had a shattering impact on the historical olive landscape in Italy and other EU countries. Although several methods have been proposed to counteract the bacterium, only a few have proven effective, including controlling insect vectors, monitoring wild hosts, implementing good agronomic practices, employing rapid detection methods, screening for tolerant and resistant genotypes, and eradicating diseased plants. However, researchers are exploring novel approaches with potential field applications, such as RNA interference, satellite imagery, phages, and the use of nanomaterials. Building on our previous successful findings, where the hydroalcoholic extract of pomegranate peels at 0.02 g/mL (patent no. TO2013A001085) and cellulose nanocrystals at 0.01 g/mL demonstrated the ability to reduce the *in vitro* growth of planktonic cells and the synthesis of biofilms in two *Xf* strains (NCPB4605 and CFBP8402), we embarked on an experimental project to formulate a novel nanocompound using these two substances. The ANCOSIX project, funded by the Italian Ministry for Agriculture (MASAF), aims to discover sustainable methods to directly deliver the proposed nanocompounds to diseased plants in the field and investigate its effects on *Xf* infection. By leveraging the potential of the developed nanocompound, the project seeks to provide a promising and sustainable solution to

check the spread of *Xf* and mitigate its devastating impact on agricultural crops.

Monitoring the occurrence of *Plasmopara viticola* populations resistant to single-site chemical fungicides in Trentino Alto Adige

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The control of *Plasmopara viticola* represents one of the most relevant challenges in viticulture worldwide, as *P. viticola* might cause severe qualitative and quantitative losses. Single-site chemical fungicides play a crucial role in disease management thanks to their efficacy and their systemic or cytotoxic activity. However, *P. viticola* populations resistant to single-site chemical fungicides have been reported worldwide, impairing the efficacy of fungicide applications. Thus, monitoring the selection of *P. viticola* resistant populations is crucial to guarantee an efficient disease management. In the present study, infected leaves were collected during 2021 and 2022 from 18 vineyards in Trentino Alto Adige, where *P. viticola* was controlled by integrated and organic disease management strategies. Leaf discs were treated with eight active ingredients, namely ametoctradin, azoxystrobin, cymoxanil, dimetomorph, fluopicolide, mandipropamid, oxathiapiprolin and zoxamide, at 100%, 10%, 3.3%, 1% and 0.1% of the maximum field dose. Subsequently, leaf discs were inoculated with *P. viticola* sporangia collected from the 18 vineyards. After seven days, the EC₅₀ was calculated and compared with reference values in literature. Notably, many populations were not inhibited completely by 100% of fungicide field dose, suggesting that *P. viticola* tolerant and/or resistant populations are present in the region. The EC₅₀ of several *P. viticola* populations was above the threshold for a relevant number of the active ingredients, regardless of the disease management strategy. However, the outcome was not repeated in both growing seasons, with few exceptions. Overall,

these results confirm that a constant monitoring is essential to preserve disease management success.

The seed-associated microbiome: relationship with seedling development in two rainfed rice cultivars from Cameroon

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Rice (*Oryza sativa* L.) is the most important food crop in large parts of the world, and its cultivation extends over five continents. Rice seedlings' growth depends on several factors, including the abundance and diversity of seed microbial endophytes. Two Cameroonian rice rainfed varieties were used for investigating the seed-associated microbiome using the Illumina-based 16S rRNA gene. Significant differences were observed in terms of richness index between normal and abnormal seedlings developed from sprouting seeds under axenic conditions. PCoA revealed that microbial communities formed two distinct clusters in normal and abnormal seedling phenotypes. Furthermore, the core microbiome was defined, and the differential abundance of 28 bacteria genera was assessed. The major genera that were increasingly detected in normal seedlings were *Sphingomonas*, *Luteibacter*, *Microbacterium* and *Streptomyces*, which, notoriously, may include beneficial species. Conversely, in abnormal seedlings, we observed an increased abundance of a few bacteria (*Kosakonia*) that might have controversial aspects (beneficial or pathogenic), together with the presence of *Clostridium sensu stricto*, commonly correlated to sick plants. Six bacterial endophytes isolated from rice normal seedlings confirmed their ability to solubilize inorganic phosphorus, synthesize siderophores, produce ammonia and have antagonistic activities against fungal phytopathogens. Data presented in this study may help the scientific community in understanding the importance of the seed microbiome for driving a correct development of rice plants at the early stages and to identify possible beneficial bacteria for technological applications.

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Overcoming of some resistant loci of grapevine varieties by an Italian strain of *Plasmopara viticola*

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Vitis vinifera L. varieties are highly susceptible to pathogen infections, leading to serious quantitative and qualitative yield reductions in viticulture. Grape downy mildew is one of the most serious diseases of grapevine. The use of disease-resistant grapevine varieties is a long-term but promising solution to reduce pest control treatments in vineyards. Several *Vitis* species showing variable levels of resistance to downy mildew and mechanisms of disease control have been identified. To date, 27 quantitative trait loci (QTL) associated with *P. viticola* resistance (Rpv) have been identified. The aim of the study was to evaluate the defense response to *P. viticola* strain collected in Emilia-Romagna in genotypes carrying different Rpv loci (Rpv3, Rpv10 and Rpv12). The study was performed by detached leaves test in order to characterize a set of resistant varieties (Bronner, Cabernet-Volos, Fleurtaï, Merlot-Khantus, Merlot-Khorus, Solaris) compared to a susceptible one (Chardonnay). Our results showed that the sporulation was present on the varieties carrying Rpv3 and Rpv12 loci. Varieties carrying Rpv 10 locus demonstrated the capacity to inhibit pathogen growth. Further studies are ongoing in order to genetically characterize the *P. viticola* strain to better understand the host-pathogen interaction.

Evaluation of the effects of *Trichoderma* spp. and the role of beneficial microorganism in sustainable potato cultivation

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The complex network of interactions established by plants with the rhizosphere microbiota greatly affects crop health and fitness. The demonstrated benefits include stimulation

of plant growth, enhanced pathogen control, improved abiotic stress tolerance, increased nutrient availability and uptake, higher yield, and better product quality. The goal of the present study was to evaluate the effects of beneficial microorganisms in the sustainable management for potato cultivation. In the seasons 2022 and 2023 three experimental fields located in Cervinara (AV), Maddaloni (CE) and Avezano (AQ), were cultivated. The fields were divided in three treatments: Control, Mycosat (CCS Aosta srl) and a new formulate made with *Trichoderma* species selected at IPSP-CNR (SOS-TATA). To test the effects of the treatments five plants from each thesis with respective tubers were sampled in order to assess: (i) plant fresh weight, (ii) number of potatoes, (iii) potatoes fresh weight, and (iv) plant dry weight. Preliminary data for the season 2022 showed significant differences in Cervinara field for potatoes fresh weight and potato number in the SOS-TATA treatment. In particular, potatoes fresh weight of SOS-TATA was 110% and 75% higher than the Control and Mycosat treatments, respectively. SOS-TATA treatment showed also 128% more of potato tuber compared with the control. For the Avezano experimental field, only the plant fresh weight of the SOS-TATA treatment was significantly higher compared to Mycosat treatment. Same analysis will be assessed for the season 2023 to confirm the effectiveness of beneficial *Trichoderma* strains treatments.

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Fungi are an evolutionary hub of viroid-like RNAs that may modulate fungal pathogenicity

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Viroids and viroid-like satellite RNAs, canonically considered plant-pathogens, are infectious, circular, non-protein coding RNAs, and some contain self-cleaving ribozymes. Prior to

2022, about fifty viroids and viroid-like satellite RNAs had been identified. To discover more of these infectious agents we performed an ultra-high-throughput search strategy, designed to identify ribozyme-bearing circular RNAs from metagenomic/metatranscriptomic data. Screening 198,000 publicly available metagenomes and metatranscriptomes, we reported the existence of more than 20,000 potential new viroids and viroid-like RNAs across global geographic areas and ecological niches. The genome of these agents (with a size ranging from ~200 to ~6000 nucleotides) shows a remarkable tapestry of diverse organizations, such as small non-protein coding RNAs, short open reading frames (ORFs), longer ORFs encoding *bona-fide* proteins. For some high-complex viroid-like genomes, we provided solid experimental evidence supporting their existence in fungi and replication through a symmetric rolling circle mechanism. Relevant among these novel infectious agents are ambiviruses and mitoviruses, which are here shown to be hybrid infectious agents joining the features typical of both viroid-like RNAs (circular RNA genomes containing ribozymes) and viruses (encode a replicase protein). The observation that ambivirus infection may reduce the virulence of the plant pathogenic fungus *Chryphonectria parasitica* opens interesting perspectives to the possible exploitation of these infectious entities as biocontrol agents. These data show the existence of a new layer of microbial biodiversity based on circular self-cleaving RNA genomes (ribozycirculome) associated with fungi and likely with other organisms that must be considered in plant metatranscriptomics analysis and when holobionts are characterized.

Effect of the introduction of synthetic consortia of biocontrol bacteria in the resident microbial communities of tomato rhizosphere

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Three bacterial consortia were designed using 10 bacterial isolates, belonging to seven genera, that showed biocontrol activity *in vitro* and *in vivo* as well as plant growth promoting traits. Individual isolates grown overnight in LB medium were mixed in equal proportions to prepare the bacterial consortia that were applied as water suspension by soil drenching immediately after transplanting near to the root zone of

tomato plants cv. ‘Proxy’. All the bacterial consortia showed clear beneficial effects on multiple plant growth parameters as compared to the control treated with water. Tomato rhizosphere samples were collected at different time points to evaluate the effect on resident microbial communities. Total genomic DNA was extracted from the samples with the DNeasy PowerSoil Pro Kit (Qiagen). Microbial communities were profiled by amplicon sequencing using the NovaSeq 6000 system (Illumina). The differences in community diversity, structure and complexity were analysed with the Amplicon Sequence Variant (ASV) method. Despite the limited taxonomic resolution of amplicon sequencing, we could identify by sequence alignment some of the bacterial ASVs putatively matching with the introduced bacterial isolates. Differential abundance analysis at the ASV level at each time point revealed that *Bacillus* and *Glutamicibacter* ASVs, included in the three consortia, were significantly enriched in all treated samples compared to control plants. Overall, the microbial communities’ analysis suggests that the plant growth promoting effects could be better explained by changes in the resident community diversity and composition, instead of introduction of plant-beneficial traits into the existing community along with consortia.

Chemical composition and antifungal activity of oregano essential oils

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Plant pathogens, the causal agents of plant diseases, affect crop performance by compromising quality and reduction yield. Recently, there is a growing need to find safer alternatives to conventional “pesticides”, harmful to both human health and environment. Plants are a source of bioactive compounds and, essential oils (EOs), are well known for their medicinal properties. EOs can differ in chemical composition regarding on various factors as distillation techniques. Two different distillation techniques, hydrodistillation and steam distillation, were used to obtain EOs from oregano. The two distilled EOs have different chemical composition. They were analyzed by gas chromatography-mass spectrometry. Using hydrodistillation, the essential oil composition detected showed *trans*-sabinene hydrate and carvacrol as most abundant components (25.3% and 21.1%, respectively) while with steam distillation, terpinen-4-ol and γ -terpinene were the most abundant (27.3% and 26.9%,

respectively). Moreover, the two EOs were tested, *in vitro*, against *Botrytis cinerea* to investigate the antifungal activity, at concentrations of 20, 200 and 2000 mg/L. The best results were achieved with EOs at 2000 mg/L that inhibited completely fungal growth and they were more effective than standard fungicides (boscalid+pyraclostrobin). The EOs, at 200 and 20 mg/L did not show antifungal activity. The essential oil obtained from steam distillation exhibited a strong antifungal activity, even at the lowest concentrations tested, allowing us to choose it as a candidate to study in depth its antifungal activity. Furthermore, its chemical composition was matched by Principal Component Analysis and cluster analysis with Italian oregano EOs published in the literature.

Transcriptome dynamics underlying AtSEOR2-mediated defense responses of Arabidopsis to phytoplasmas

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Phytoplasmas are bacterial pathogens that inflict significant economic losses on a global scale. Residing in the sieve elements of their host plants, phytoplasmas impair the immune system, altering the development, *via* secretion of effector proteins. Many studies focused on the identification of the effector-mediated mechanisms altering plant physiology following phytoplasma infection. However, the mechanisms underlying the activation of the immune response by the host plant in response to phytoplasmas remain largely unknown. In Arabidopsis, a sieve-element occlusion-related (SEOR) protein, AtSEOR2, is an “effector hub” involved in plant immunity. Arabidopsis mutants expressing unbound AtSEOR2 display reduced phytoplasma titer compared to Col-0 wild type (wt) plants in early infection stage. The present study sought to elucidate the gene expression profiles associated with the AtSEOR2 protein of infected plants. RNA was extracted from the leaves of plants expressing unbound AtSEOR2 protein followed by RNA-seq

in an Illumina platform. Comparative transcriptome analysis revealed 1036 differentially expressed genes (DEGs) (893 up- and 143 down-regulated) plants expressing unbound AtSEOR2. DEGs were significantly enriched in two pathways associated with plant immunity. Plants expressing unbound AtSEOR2 exhibited significant upregulation of key genes involved in plant defense, in contrast to wt plants. The observed decrease in phytoplasma titers in plants expressing unbound AtSEOR2 can thus be attributed to a heightened immune response, indicating a stronger immune reaction compared to wt plants. The development of an interaction network between up- and down-regulated DEGs and AtSEOR2 highlights the main metabolic pathways involved in AtSEOR2-mediated defense processes.

Principle for the evaluation of the health risk of mycotoxins

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Risk assessment in food toxicology is of utmost importance to ensure consumer safety. It is performed on the basis of data derived mainly from studies carried out on animals for daily consumption over a lifetime. Dangers are often identified retrospectively, following mass poisoning. What's more, it is very difficult to correlate an increase in the incidence of a disease with the population's exposure to a toxicant through food consumption. A distinction must be made between substances and foodstuffs subject to authorization and natural food contaminants such as mycotoxins. In the first case, an application for authorization for use is required, regulated either at European or national levels, based on toxicological studies carried out in accordance with OECD guidelines. In the case of natural food contaminants, the situation can be very critical insofar as there are not always sufficient high-quality toxicological studies available to identify a no-effect dose. The Tolerable Daily Intake (TDI) is then proposed on toxicological data. Consumer exposure is assessed as accurately as possible, and compared to the TDI. Risk characterization then makes it possible to determine whether or not consumers are being put at risk by voluntary exposure to these substances. The risk of exposure to genotoxic food contaminants must be taken into account, while genotoxic substances subject to authorization will be systematically excluded. New approaches to risk assessment, such as the toxicity threshold (TTC), the margin of safety, the margin of exposure, the mode of action, read-across and mode of action, could also be used when toxicological data are limited.

Research support for biocontrol of *Aspergillus flavus* use in Europe

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AF-X1 is an aflatoxin biocontrol product containing the non-aflatoxigenic strain of *Aspergillus flavus* MUCL54911, endemic to Italy as active ingredient. The strain belongs to the vegetative compatibility group (VCG) IT006. The aim of this study was: I) to evaluate the persistence of VCG IT006 in the soil after AF-X1 deployment in the field, based on soil samples collected in 2020 and 2021 from seven areas placed in North Italy; II) to characterize southern European population of *A. flavus* and the distribution of the IT006 in maize grain samples collected from different areas across Serbia, Spain and Greece. The use of AF-X1 has a residual effect that improves the structure of the *A. flavus* population resident in both the treated fields and in the neighbouring untreated fields, so that the AF-active ingredient is more common and the frequency of the aflatoxin producers is reduced. The application of AF-X1 promotes the creation of these safer *Aspergillus* populations, with no significant effects on the total fungal communities; nevertheless, yearly distribution is still suggested. The analysis of *A. flavus* population revealed high diversity within and among countries and dozens of haplotypes shared among countries. Linkage disequilibrium analysis indicate asexual reproduction and clonal evolution of *A. flavus* resident in Europe. Moreover, haplotypes closely related to MUCL54911 were found to belong to the same VCG IT006, relatively common and endemic to all three countries. The outcome of this research work will open new perspective to use the biocontrol product AF-X1 in south Europe for aflatoxin mitigation.

Biocontrol activity of four non- and low-fermenting yeast strains against *Aspergillus ochraceus* and *A. westerdijkiae* on maize products

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The contamination of agricultural commodities by mycotoxin-producing fungi is an important threat to human and animal health. Ongoing climate changes will have a significant impact on the spread of toxigenic fungi and it is estimated that an increase in temperature of 2 °C in 2050 will increase the risk of mycotoxin contamination in the Mediterranean area by more than 50%. It appears urgent to develop new strategies to mitigate the risk of contamination by mycotoxins based on the containment of toxigenic fungi during the conservation phases. Different biological control strategies were developed to control mycotoxin contamination: exploiting the antimicrobial activity of yeast-derived volatile organic compounds (VOCs) with antimicrobial activity is receiving increasing attention. The efficacy of VOCs produced by two non-fermenting (*Cyberlindnera jadinii* 273 and *Candida friedrichii* 778) and two low-fermenting (*Candida intermedia* 235 and *Lachancea thermotolerans* 751) yeast strains to control *Aspergillus ochraceus* and *A. westerdijkiae* on maize kernels and maize flour has been tested *in vitro*. Among the tested yeast isolates, *L. thermotolerans* 751 prevented sporulation and vegetative growth by the two pathogens in both matrixes. The analysis of ochratoxin A (OTA) contamination in the tested matrices is in progress. The biological control approach is proposed as an efficient preventative strategy to reduce OTA contamination in maize grain during the postharvest phases. Selected antagonists are being characterized to determine their VOCs blend composition, aiming to develop innovative storage systems allowing the controlled release of VOCs with antimicrobial action.

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Trichoderma-Bacillus-Pseudomonas based microbial consortium to biocontrol *Sclerotinia* soft rot on wild rocket (*Diplotaxis tenuifolia*)

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A microbial consortium for the biocontrol of plant diseases consists of individuals capable of synergizing and overlapping their range of action, succeeding in adapting to different environmental and host-pathogen ecological conditions. The present work is aimed to enhance shelf life, safety, and sustainability of wild rocket (*Diplotaxis tenuifolia*) baby-leaf crop with the application of a consortium of microbial antagonists, to gain improved effectiveness of disease management means alternative to synthetic fungicides. Stepwise assembling consortium included a first investigation of a set of ten bacterial strains belonging to *Bacillus subtilis*, *B. methylotrophicus*, *B. amyloliquefaciens* and *Pseudomonas fluorescens* and two fungal strains belonging to *Trichoderma longibrachiatum* and *T. atroviride*, respectively. Plate challenging tests showed the compatible combinations among individuals and allowed final selection of three strains: *B. amyloliquefaciens* 17S, *Pseudomonas fluorescens* CREA16 and *Trichoderma longibrachiatum* TL35. Biocontrol assays on plant showed the highest effectiveness of the three components to counteract disease severity with +50% biocontrol, on average, in comparison with the single or pair strain(s) inoculation. On the parallel, biostimulation properties of the microorganisms/consortium on the plant target was also considered. The selected consortium based on biodiversity and multitrophic mechanisms can ably support wild rocket cropping system. Trials of formulating microbes with co-formulants are ongoing.

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Non-destructive early detection of wild rocket tracheofusariosis caused by *Fusarium oxysporum* f. sp. *raphani* using infrared imaging

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Fusarium oxysporum f. sp. *raphani* is a key soil-borne pathogen of wild rocket (*Diplotaxis tenuifolia*), responsible for a marked stunting of growth already during emerging seedlings stage, widespread yellowing, wilting, and collapse. The

disease control strategies must increase their effectiveness. Imaging technologies that can detect outbreaks at an early stage could provide significant support in this regard. Thermal imaging is based on the acquisition of signals returned by plants in the infrared spectral region in function of the thermographic temperature, the distribution of which can be modulated by stress(es). Here, thermography was applied to wild rocket plants that developed tracheofusariosis after artificial inoculation, either by dipping the roots after trimming tips and transplanting or by flooding the basal layer of the pots with the pathogen conidia suspension. Sterile distilled water was used for the control plants. Plants were incubated at 25 °C and, during pathogenesis, infrared emissions were captured by FLIR X6580 sc mid-waveinfrared (MWIR) camera. Passive analysis showed, over the next eleven days, consistently higher average leaf temperatures in infected plants than in the control in relation to inoculum concentration. The maximum ΔT recorded reached 0.6–0.9 °C in the interval 5–6 days after inoculation. Active analyses, carried-out by examining the leaves after thermal stimulation with a halogen lamp (500 W, 5 sec), revealed reduced thermal inertia under pathogenic attack immediately after 48 h. Thermography anticipates the clear visualisation of symptoms by at least four days, highlighting early attacks that can be confirmed by a specific but destructive molecular analysis.

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Carpospheric microbial populations of tomato: diversity in relation to environmental conditions and applications for an ecofriendly management of postharvest grey mould

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The beneficial microbiota naturally present in fresh vegetables plays an important role in maintaining food quality, by preventing pathogen development. In this work, interactions between carpospheric microbial populations, territory

of origin and chemical composition of tomato fruits were evaluated, and carposphere-competent biocontrol agents were selected for the control of grey mould caused by *B. cinerea*. Microbial populations were investigated on eight tomato cultivars collected from twenty greenhouses located in Ragusa, Syracuse and Catania provinces (south-east of Sicily, Italy). Principal Component Analysis (PCA) showed that microbial populations clustered into groups according to the geographical area of origin of the tomatoes and the elemental profile detected on tomato pulp by X-Ray Fluorescence (XRF) spectroscopy. Approximately 200 representative bacterial strains were tested in *in vitro* assays against *B. cinerea* and a high percentage of them showed strong antagonistic activity. Analysis of the 16S rRNA gene sequences revealed a predominance of antagonistic bacteria in *Bacillus*, *Pseudomonas*, *Citrobacter* and *Enterobacter* genera. The effectiveness of *B. velezensis*, *B. cereus*, *B. thuringiensis* and *B. licheniformis* strains was confirmed in growth chamber and in greenhouse conditions. Their efficacy was also compared with that of three commercial products. All the tested strains significantly reduced disease incidence and severity, protecting fruits from the development of grey mould during storage when applied three and ten days before tomato harvesting. Results suggest the existence of correlations between microbial populations, environmental conditions and tomato chemical composition. Moreover, this study provides the basis for developing carposphere-competent bio-inoculants to be used in preharvest for an ecofriendly management of postharvest grey mould.

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Peptides derived from a *Trichoderma* peptaibol as an alternative method to control fungal diseases of wheat

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Wheat is one the primary source of staple food throughout the planet, with a global food demand projected to

increase by 1.3% per year over the coming decade. The necessity to increase agricultural production is closely linked to the control of fungal plant diseases, such as the Fusarium Head Blight (FHB), caused by the mycotoxigenic fungus *Fusarium graminearum*, or powdery mildew caused by *Erysiphe graminis*, which can lead to huge yield and grain quality losses. Nowadays, the control of these fungal disease mainly relies on chemical fungicides. However, the negative effects on human health and the environment and the risk of emergence of resistant strains have stimulated the researchers to develop new eco-friendly alternatives. Our research group has recently developed antimicrobial peptides analogs of the natural peptaibol *Trichogin GA IV* produced by *Trichoderma longibrachiatum*. Peptaibols are short non-ribosomal synthetic peptides with non-canonical amino acids and a high stable helical structure. These synthetic water-soluble peptaibol analogs were tested by *in vitro* assays against *F. graminearum*. Microscopy analyses highlighted that the treated spores presented morphological changes such as cytoplasmic agglutination and were not able to germinate. The most effective peptaibols *in vitro* were used to treat *Triticum aestivum* and *Triticum durum* spikes and leaves before inoculation with spores of *F. graminearum* and *E. graminis*. Symptom development and DON contamination demonstrate that these peptides can be promising molecules for the future development of effective biopesticides able to control wheat diseases.

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Tomato leaf curl New Delhi virus in field dispersion analysis in Sicily (Italy) using ready-to-use LAMP detection kit

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Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus (family *Geminiviridae*) that affects cucurbitaceous and solanaceous species, causing economic losses in severe cases. To investigate ToLCNDV dispersion in Sicily, a real-time LAMP assay developed by Caruso and co-workers (2023) was used. During 2022, to assess the ToLCNDV

dispersion in the main horticultural production Sicilian areas, a total of 500 samples (250 tomato and 250 zucchini squash samples) were collected from five Sicilian provinces (Agrigento, Trapani, Siracusa, Ragusa and Palermo), and processed using a rapid extraction method that could be employed in field, to avoid the use of commercial DNA extraction kit. Real-time LAMP tests were performed directly in field by using a portable 12V battery-powered bCUBE® thermal cycler (Hyris Ltd) and a ready-to-use LAMP detection kit, previously prepared in laboratory. The results showed that 74 and 46 of the zucchini and tomato samples, respectively, resulted positive to ToLCNDV, most prevalently identified in Trapani province for both species, followed by Ragusa province; the incidence percentage was 29.6% for zucchini squash and 18.4% for tomato, with a 24% of total incidence. Thanks to the data obtained in this work, a negative trend of ToLCNDV dispersion was observed, compared to the last five years; in fact, the incidence percentage has decreased from 65% in 2017 to 24% in 2022. The real-time LAMP technique used in this work, associated with the rapid sample extraction method, represent an efficient tool for a rapid on-site ToLCNDV detection and the study of its dispersion.

Antimicrobial activity of different compounds against *Xanthomonas campestris* pv. *campestris* and *Pseudomonas syringae* pv. *tomato*

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Plants are a valuable source of bioactive compounds, including terpenes, alkaloids, phenolic compounds, essential oils (EOs), that can have effect on plant and human health. EOs are volatile extracts obtained mostly from aromatic and medicinal plants by distillation and have demonstrated antibacterial, insecticidal, fungicidal, nematocidal, herbicidal, antioxidant, and anti-inflammatory activities. Moreover, fungal secondary metabolites (SMs) play a pivotal role in the antagonistic activities of some biocontrol species of *Trichoderma* resulting in the suppression of plant pathogens. The lack of effective control measures against plant bacterial diseases has gained the attention to alternative natural compounds to be used for crop protection. The objective of this work was to evaluate the effects of different EOs and the SM 6-n-pentyl-6H-pyran-2-one (6PP): i) on the growth of the plant pathogenic bacteria *Xanthomonas campestris*

pv. *campestris* (Xcc) and *Pseudomonas syringae* pv. *tomato* (Pst); ii) on the severity of black rot disease of cabbage and tomato bacterial spot, respectively. The biological activity of EOs from cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum* L.), and 6PP was tested *in vitro*. Each compound was tested at different concentrations using a double layer agar assay. Complete growth inhibition of both pathogens by cinnamon and clove EOs was observed at 0.025% (v/v) and with 10⁻⁵M 6PP. Preliminary *in planta* results showed a 70% reduction of disease severity on tomato plants infected with Pst and treated with 6PP (10⁻⁵M), compared with control. Evaluation of the effects of EOs on disease severity are currently in progress.

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Stressed plants produce root exudates affecting fungal-plant interactions

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Plants live in close association with microorganisms that populate the soil, and their interaction is essential for plant growth and health. Bioactive compounds released by the roots into soil rhizosphere act as chemical signals influencing the biological activity, and consequently the composition of the associated microbiota. In this work, tomato plants were exposed for 24 h to various abiotic and biotic stress (wounding; foliar pathogen *Botrytis cinerea*; insect pest *Spodoptera littoralis* -larvae stage-; aphids *Macrosiphum euphorbiae*). The root exudates (RE) were collected, then used in a chemotropic assay to test their “attractiveness” to biological control agents (BCAs) *Trichoderma* (BCA to pathogens) or *Beauveria bassiana* (BCA to insects), and the vascular fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici*. RE obtained from tomato plants exposed to the biotic stress were able to stimulate fungal growth and act as chemoattractants for the two BCAs. In contrast, most of these same RE acted as chemorepellents to *F. oxysporum*, in particular those obtained after wounding or from interactions of the plant with insects. This suggests a differential response of non-pathogenic versus pathogenic microbes to RE that may be specific to the plant

depending upon its exposure to its interactions with stress factors. RE were characterized by GC-MS: volatile compounds produced in tested conditions were diverse, whereby the chemical profiles were more similar among RE produced by plants treated with H₂O (control), wounding or *Botrytis*, in comparison to those from aphid and larvae treatments. This suggests that plants modulate their microbiota by chemical cross-talk in the rhizosphere.

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Ligninic biomass from second generation bioethanol production combined with *Trichoderma* spp. as a promising tool for plant disease management

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Ligninic biomass, by-products from second-generation bioethanol production, are promising resources for agriculture. Lignin has been tested in applications as plant biostimulant and in suppression of soil-borne plant pathogens. To date, this by-product is mainly burnt to produce energy. Potential use of recycled organic products in agriculture could contribute to beneficial restoration of carbon stock in soils and is in line with the latest European policies on circular economy in agroecosystem. Many fungi belonging to genus *Trichoderma* are part of soil-plant microbiota and recognized for their beneficial crop protection and growth promotion capabilities. Here, we investigated the effect of the ligninic materials and combinations with *Trichoderma* spp. to control the soil-borne plant pathogen, *Sclerotium rolfsii* (Southern blight disease) that attacks a wide variety of plants. Ligninic product at concentrations from 0 to 80% (w/v) of artificial substrate were tested *in vitro*. Inhibition in the mycelium growth was noted, that positively corresponded to increasing doses of material used, reaching 100% inhibition at concentrations 40 and 80% (w/v), six days after inoculation; sclerotia reduction was also noted. No inhibition in the growth of some *Trichoderma* strains was

observed; and *in vitro* assays revealed that only *T. afroharzi-anum* and *T. atroviride* combined with the material inhibited the growth and parasitized *S. rolfsii*. In conclusion, ligninic biomass together with *Trichoderma* could be a potential strategy for sustainable management of *S. rolfsii*.

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Rapid detection of plant pathogenic fungi using a smartphone-based fluorescent array sensor

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The prompt detection of plant pathogenic fungi is crucial for ensuring food safety and human health. However, routine diagnosis of a large number of samples requires fast and easy-to handle detection techniques. The recent development of array technology can help solve this problem. To this aim, we designed and implemented a fluorescent array-based sensor featuring multiple organic receptors and using a smartphone as detector. The array sensor was specifically tailored and validated for the selective detection of the post-harvest fruit pathogens *Penicillium italicum*, *Alternaria alternata*, and *Fusarium sacchari*. The detection limit of this new device for *P. italicum* in a water suspension was of around 2×10^3 conidia ml⁻¹, which is by far higher than the detection limit of quantitative PCR (qPCR), regarded as the most sensitive presently available detection method. However, the array-based method does not require destructive sampling and is very fast and extremely easy to handle also by not specifically trained personnel. Initially, this diagnostic device was conceived as a proof-of-concept. In light of these preliminary but

encouraging results we are testing receptors capable of detecting selectively several other fungal pathogens and even mycotoxins in plant products, processed food and environment samples.

Observations of xylem occlusions and metabolic profiles of olive cultivars for the selection of germplasm resistant to *Pseudomonas savastanoi* pv. *savastanoi*

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Olive knot disease, caused by *Pseudomonas savastanoi* pv. *savastanoi*, is one of the most damaging diseases to olive trees. Penetration of the bacterium into the plant generates hypertrophy and hyperplasia in the surrounding tissue, resulting in the formation of bacterial aggregates, microcolonies and biofilms. To evaluate the influence of *P. savastanoi* pv. *savastanoi* on the rate of xylem vessel occlusions, semi-thin cross sections of healthy and infected 2-year-old branches of some olive tree cultivars (Coratina, FS17, Leccino, and Leccio del Corno) were observed. Particularly, studies were conducted on healthy 30-year-old plants in the open field, healthy 2-year-old olive trees, and artificially inoculated olive trees. Specifically, studies were conducted on healthy 30-year-old plants in open field, healthy 2-year-old olive trees and artificially inoculated olive trees. Investigation of the occurrence of vessel occlusion provided insight into the plant response to bacterial colonization of the xylem, and it is hypothesized that it may be an effective parameter for varietal characterization and selection. Initial results suggested that each variety may be characterized by a differentiated frequency of occlusions. In fact, xylem of young plants exhibits a higher percentage of occlusions than in branches of older plants of the same cultivar as a sign of reactivity of the tissue. Furthermore, analysis of the ¹H NMR detected metabolic profile, associated to leaves extracts of the studied cultivars, suggests correlation with occlusions occurrence.

Although this study is currently in progress, the observed xylem occlusions, combined with the metabolic profile, appear to be a valid tool for the selection of cultivars resistant to *P. savastanoi* pv. *savastanoi*.

Natural co-infection of turnip yellow virus and citrus exocortis viroid in *Phytolacca americana* in southern Italy

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Phytolacca americana L., commonly known as “American pokeweed”, is an invasive neophyte species native to North America, cultivated in southern Europe as a garden plant and today largely naturalized and a weed throughout Italy. In spring 2021, a pokeweed plant showing viral disease-like symptoms, consisting in yellowing evolving in necrotic yellowing, was collected in the Gussone park of the Royal Palace in Portici (Napoli) and a leaf sample was subjected to high-throughput sequencing (HTS) analysis. The Illumina TruSeq Stranded mRNA Library Preparation protocol was followed to generate cDNA libraries. HTS was performed using the Illumina NovaSeq 6,000 platform by Macrogen (Seoul, South Korea). The HTS data were analyzed using the “trim reads” and “map reads to reference” tools built into CLC Genomics Workbench software (Qiagen Bioinformatics, Hilden, Germany). Viral and viroid genome sequences were assembled by mapping the reads to the Reference Viral and Viroid DataBase. All the virus-related sequence reads were mapped to turnip yellow virus (TuYV) and citrus exocortis viroid (CEVd). Two isolates of TuYV and one isolate of CEVd were definitely assembled. Reverse transcription PCR was used for further validation using primer pairs based on the previously assembled sequences of TuYV and CEVd. Amplicons obtained were sequenced and aligned with the assembled sequences. Results confirmed the co-infection in pokeweed plant of two divergent isolates of TuYV with CEVd. This is the first report of TuYV in Italy and first discovery of pokeweed as a host for both TuYV and CEVd.

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Small things matter: the contribution of the microbiome to induced systemic resistance along the root-shoot axis

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In nature, plants harbor myriads of microbes. Microbes like *Pseudomonas simiae* WCS417 (WCS417) are beneficial as they enhance plant growth and defense. Upon colonization of Arabidopsis roots, WCS417 activates an induced systemic resistance (ISR) in different plant compartments including the shoot. Key components of WCS417-induced ISR, MYB72 and BGLU42, are also required for production and exudation of coumarins in the rhizosphere. Coumarins can shape the root microbiome, e.g., by favoring beneficial and repelling detrimental microbes. Ongoing work in our group suggests that root colonization by WCS417 induces coumarin accumulation not only in roots but also in leaves where ISR is expressed. Therefore, we hypothesize that coumarins can have a role in ISR by regulating the phyllosphere microbiome. To study the contribution of the plant microbiome on ISR, we grew Arabidopsis in natural soil with its resident microbiome and tested for ISR against the leaf pathogen *Pseudomonas syringae*. Our findings revealed that root colonization by WCS417 significantly affects microbiome assembly along the root-shoot axis. To what extent changes in the phyllosphere microbiome contribute to ISR is under investigation. Furthermore, to understand the role of coumarins in microbiome assembly on roots and shoots of ISR-expressing plants, we currently explore microbiome assembly on roots and shoots of WCS417-colonized Arabidopsis mutants *myb72*, *flh1*, and the overexpressor line ox-BGLU42.

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Physiochemical responses of ancient Tuscan varieties ‘Mora’ and ‘Ruggine’ to apple chlorotic leaf spot virus

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Although Tuscan autochthonous apples (*Malus domestica*) have large diffusion and genetic interesting features, they were poorly investigated from a virological point of view. To address this gap, a commercial orchard composed by the autochthonous apple cultivars ‘Chitignano’, ‘Mora’ and ‘Ruggine’ was selected in Arezzo district (Tuscany), and the presence of a number of apple viruses was assayed (i.e., apple chlorotic ringspot virus, ACLSV; apple mosaic virus, ApMV; apple stem grooving virus, ASGV; and apple stem pitting virus, ASPV; prune dwarf virus). Molecular analysis carried out on leaves revealed a large presence of ACLSV in ‘Mora’ and ‘Ruggine’ (65% of tested samples). Thus, ‘Mora’ and ‘Ruggine’ physiochemical responses to ACLSV were further investigated at both leaf and fruit levels. Interestingly, ACLSV infection induced a decrease of photosynthesis, mostly due to a reduction of chlorophyll content and to alterations of the xanthophyll cycle, and this detrimental effect resulted harsher in ‘Mora’ than in ‘Ruggine’. Moreover, a reduction of organic acid contents (i.e., malic, quinic, succinic acids) was detected in ‘Mora’ leaves, while an increase in sugar contents (i.e., glucose, sucrose) was observed in ‘Ruggine’ ones. Interestingly, ‘Mora’ fruits resulted smaller than ‘Ruggine’, but only the latter showed an ACLSV-induced reduction of both organic acid (i.e., citric, malic, succinic acids) and sugar (i.e., fructose, glucose, sucrose) levels. Overall, the results here presented represent an important step to fill knowledge gaps about the effects of ACLSV in autochthonous Tuscan apples.

Effects of biological and chemical treatments against *Heterobasidion* species present in Europe on stump microbial communities

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Application of treatments on freshly cut stumps acts as a preventative measure targeting airborne infections of the fungal forest pathogens *Heterobasidion* spp. In this study, we assessed the short-term impact of biological (Proradix[®], cell-free filtrate of *Pseudomonas protegens* [strain DSMZ 13134], conidial suspension of *Phlebiopsis gigantea* [strain MUT 6212], Rotstop[®]) and chemical (urea) treatments against the three native *Heterobasidion* species and the invasive *H. irregulare* on both fungal and bacterial communities inhabiting stump surfaces. Investigations were conducted in forests of *Abies alba*, *Picea abies*, *Pinus pinea*, and *P. sylvestris*, each infested by their own host-associated *Heterobasidion* species. ITS and 16S amplicon metagenomic

sequencing was used to characterise microbial communities of 15 stumps per each treatment and controls 4–8 months after treatment application. We observed a strong decrease in both diversity and richness of fungal communities of *P. pinea* stumps treated with Rotstop® and urea compared to other treatments and controls. Impact of treatments on microbial communities inhabiting *A. alba*, *P. abies* and *P. sylvestris* was less evident as diversity and richness metrics of their communities were never significantly different from those of controls. However, correspondence analysis indicated that microbial community composition was always shaped by treatments. The application of urea on stumps of *A. alba* and *P. pinea* led to distinct microbial communities compared to those of biological treatments. This work provides additional and new insights on the short-term impact of state-of-the-art and newly developed biological treatments against *Heterobasidion* spp. on non-target microbes.

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Multitrophic interactions among metaorganisms and bioinspired plant protection

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Reduction of pesticide use in agriculture requires the sustainable exploitation of ecosystem services provided by functional biodiversity, and an increasing availability of low-impact tools and strategies of pest control. Current research being carried out seeks to develop novel control strategies and highly specific biopesticides based on knowledge obtained from the understanding of the molecular mechanisms underlying multitrophic associations among plants, pests, and their natural antagonists, taking into account the important role played by the associated microbiota. The balance of energy flow in natural food webs is modulated by interkingdom competitions which span across different trophic levels, with a continuum of interactions between the soil and the aboveground communities. An in-depth understanding of the mechanisms underlying these multitrophic interactions allows not only the development of bioinspired pest control tools but also the definition of protection strategies for beneficial organisms and the ecosystem services they provide.

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Foot rot pathogens of durum wheat: *in vitro* antagonistic activity of *Bacillus velezensis*

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Durum wheat is one of the most important field crops in Sicily, exceeding 260,000 ha. Among diseases affecting wheat crops, Fusarium foot rot (FFR) is the most widespread and harmful to wheat in the Mediterranean area. Accordingly, with the aim of pursuing effective strategies for sustainable disease management programs, the microbial preparation Cilus® Plus, containing *Bacillus velezensis* (IT 45) microorganisms, was evaluated *in vitro* for the control of *Fusarium* spp. isolated from durum wheat plants affected by FFR complex. Antagonistic activity of *B. velezensis* was performed by dual culture technique on potato dextrose agar medium. We measured radial mycelial growth until the fungal growth in the control reached the edge of the plate and, calculated the percentage of mycelia growth inhibition (MGI), according to the following formula: $MGI = [(D1-D2) / D1] \times 100$, (where D1 = growth of the pathogen in the absence of antagonist and D2 = growth of the pathogen in the presence of antagonist). Compared to control, mycelial growth of 31 *Fusarium* isolates was significantly ($p \leq 0.01$) reduced by *B. velezensis*. The greatest mycelial growth inhibition was observed for *F. crookwellense* with an average decrease of 60.2 %, followed by *F. graminearum* (59.9 %) and *F. culmorum* (57.8 %). Mycelial growth reduction was also found for *F. verticillioides*, *F. avenaceum*, *F. equiseti*, and *F. compactum*, with average inhibitions of 48.4 %, 45.2 %, 53.8 % and 41.1 %, respectively. Furthermore, compared to controls we observed, for *F. culmorum*, abnormal hyphal tips and swollen chlamydospore-like cells. No diffusible inhibitory volatile substances were detected neither on PDA nor on King's B media.

Role of *Fusarium graminearum* killer proteins (FgKP4L) in the competitive interactions against other *Fusarium* species involved in FHB of wheat

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Fusarium Head Blight (FHB) is a complex disease affecting cereals caused by several species included within the Fusarium Head Blight Species Complex (FHBSC), with *F. graminearum* (*Fg*) as the most virulent and widespread in Central Europe. Killer Proteins (KPs) - reported in several yeasts and fungi - are toxic secreted compounds that interfere with biological pathways in targeted organisms. In *F. graminearum* four genes have been shown to encode for KP Like 4 family (the clustered *Fgkpl-1*, *-2*, *-3* genes and *Fgkpl-4*). Since their regulation has been already demonstrated in competitive interactions with the beneficial isolate *Trichoderma gamsii* T6085, this work aims to investigate whether *FgKPL4* can support *F. graminearum* competition occurring with other *Fusarium* species, i.e., *F. sporotrichioides*, *F. langsethiae* – belonging to FHBSC – and *F. verticillioides* (a competitor for wheat cultural debris, here used as outgroup). Modulation of the expression of the four *Fgkpl* genes was investigated both in dual cultures that on wheat spikes where all the four pathogens were co-inoculated. Results showed a different regulation of the four genes depending on the competitive species both *in vitro* that on spikes. Interestingly, while during the interaction with *T. gamsii* T6085 *Fgkpl-4* resulted to be the most involved, when in presence of the other *Fusarium* species the clustered *Fgkpl-1*, *-2*, *-3* genes seemed to be highly activated. Results here collected supports a possible involvement of *FgKPL4* in the competitive interactions occurring among pathogenic *Fusarium* spp., particularly those involved in FHB.

Selection and application of cold-tolerant *Trichoderma* hybrids for biocontrol and plant growth promotion activities

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Beneficial microbes such as *Trichoderma* spp. offer novel opportunities to reduce the use of pesticides in agriculture and promote sustainable crop cultivation. In this work we used protoplast fusion (PF) to produce hybrid cultures from

different *Trichoderma* species aiming to select those strains with improved properties compared to parentals in terms of biocontrol and/or plant growth promotion (PGP) activities. Moreover, the tolerance to cold temperatures was assessed to test their efficacy on winter crops. Thirteen hybrids from PF of two different hybrid mixtures (MG: *T. harzianum* strain M10 + *T. virens* strain GV41; TK: *T. harzianum* strain T22 and *T. asperellum* strain KV906) were isolated from the rhizosphere of inoculated tomato plants showing PGP or biocontrol activity against *Rhizoctonia solani*. Molecular characterization of the hybrid cultures, using the primers ITS, TEF and RPB, showed that in each PF, there was a predominant parent: in the MG mixture, 6 out of the 8 hybrids were *virens*, while for TK mixture, the hybrids were homologous to the *asperellum* species. In addition, *in vitro* assays were carried out to evaluate the cold tolerance of *Trichoderma* hybrids. After 5 days at 15° C, hybrids named A3 and B2 (from PF MG), and hybrids named C1, C2, C4, C5 (from PF TK) increased about 30% mycelium growth compared to the parental strains. Moreover, hybrids A3 and C2 improved significantly the germination of spinach (*Spinacia oleracea* cv. Donkey) seeds compared to controls. Further analyses are ongoing to evaluate the activity of *Trichoderma* hybrids, both on different crops and at different temperatures.

Wood anatomical changes behind the rapid effectiveness of *Verticillium dahliae*, a promising biological control agent candidate to counteract *Ailanthus altissima*

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Ailanthus altissima is one of the most invasive alien plant species worldwide, able to colonize archaeological structures, ruderal and paved areas causing cracks and damages to masonry and foundations. In Italy, the bioherbicide candidate based on *Verticillium dahliae* strain VdGL16, isolated in Tuscany, represents the most promising solution to counteract *A. altissima*. In late spring 2017, four *A. altissima* trees located along the medieval walls of Pisa, were artificially inoculated at breast height with VdGL16. Development of foliar symptoms was monitored monthly during the

vegetative season over three years. In 2020, trees (apparently dead) were felled and stem cross sections were sampled at different heights to analyse their wood anatomy. For each annual ring, transversal and longitudinal microsections (15 µm thick, produced using a microtome) were coloured with safranin:astrablü (1:1) and scanned to have high quality images. Fungi were isolated from remaining tissues. Results revealed that the morphology of vessels changed after inoculation, showing a decrease in lumen area and a concomitant increase width (-35 and +50% in 2020 compared to 2017, respectively). Vessels occlusions were observed at 1 m (reaching +75% of the total vessels in 2020) and 4 m height (annual average 15%) attributable to mycelium, tyloses or gums. Fungal structures were observed both longitudinally and across the annual rings meaning a successful colonization of VdGL16, re-isolated *in vitro*. No resprout from the stumps colonized by VdGL16 was observed, so demonstrating the potential of using VdGL16 for a long-term control of *A. altissima*.

PLAVIT: from a collecting culture to a collection culture

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Virus collections contribute to the knowledge and maintenance of viral biodiversity, consisting of expertly preserved, authenticated isolates and derived products made available to the scientific community. All the collections are developed, managed and maintained in accordance with internationally recognised quality standards. They are in continuous expansion to include new accessions in the collection. Moreover, the aim of viral collections is to promote the collaboration and exchange of knowledge, ideas and information among virologists. The Italian Collection of Plant Viruses PLAVIT (Plant Virus Italy) is based at the Institute for Sustainable Plant Protection (CNR) in Torino and Bari, Italy. It consists of more than 1000 isolates of plant viruses and viroids, originating from several countries and hosts, collected over the last 50 years. PLAVIT is planning to develop a parallel collection of virus-derived products, like antisera and cloned full-length virus or subviral molecules. PLAVIT is partner of the Italian node of the Microbial Resource Research Infrastructure (MIRRI-IT) which preserves microbial collections throughout the Italian territory, and of SUS-MIRRI-

IT (Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy), a PNRR project focusing on new opportunities that microbiology offers to cope with human well-being, food quality, climate change, and environmental pollution. PLAVIT is also a core partner of EVA-GLOBAL, a Horizon 2020 project encompassing several EU and non-EU institutions with expertise in animal, human and plant virology, with the aim to collect and distribute authenticated viruses, viroids and derived products through an online catalogue providing easy access to the end-users.

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Unveiling degradation capabilities and phenotypical traits of white-rot agents from grapevine affected by Esca Complex of Diseases in different geographical areas

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The presence of Esca Complex of Diseases (ECD), which includes wood white rot, poses a rising threat to global viticulture, with the severity varying according to geographical region. Some authors suggest that wood degradation byproducts and metabolite production could play a role in the development of foliar symptoms. Recently, the importance of wood-degrading white-rot agents has been greatly reconsidered as techniques like trunk surgery on decayed wood by *Fomitiporia mediterranea* (Fmed) showed to be effective in reducing foliar symptoms development. Therefore, this study aims to explore the differences among ECD-associated white rot agents in different grape-growing areas in the world such as Fmed, *F. capensis*, *F. langloisii*, *F. polymorpha*, *F. australiensis*, *Stereum hirsutum*, *Inonotus vitis*, and *Tropicoporus texanus*. The degradation capabilities and phenotypical characteristics of representative strains were investigated. Production of enzymes involved in wood

degradation such as laccases, and Class II peroxidases have been assessed. Following recent research on Fmed, the ability to adopt the major steps of the non-enzymatic radical-generating pathway was assessed. This includes the production of Fe^{3+} reducing low molecular weight compounds, and hydroxy radical generation through redox cycling. Phenotypical characteristics were assessed by measuring biomass production, growth rate, and *in vitro* wood degradation. Further investigation into the matter could lead to a better understanding of the mechanisms and the role of white-rot degradation in ECD.

Hyperspectral detection of temporal and spatial development of wheat blast disease

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First reported in Brazil in 1985, wheat blast (WB) disease, caused by the fungus *Magnaporthe oryzae* pathotype (pt) *Triticum*, represents an escalating threat to wheat production. Although spike symptoms are distinct, leaf symptoms are usually inconspicuous. As WB development of leaves is not well understood, this study aimed to elucidate the capability of full-range (400–2400 nm) reflectance spectroscopy to detect the temporal (0–5 days) and spatial (old-young leaves) development of WB on leaves. Plants were inoculated at two stages (Feekes 6 and 10) with a *M. oryzae* pt *Lolium*, used as a pt *Triticum* surrogate, at 0, 1,000, 20,000, and 10,000 spores ml^{-1} concentrations. At Feekes 6, leaf spectral profiles of leaves were statistically different (PERMANOVA, $P < 0.05$) for the ‘inoculum concentration \times day’ combinations. At Feekes 10, a significant ‘inoculum concentration \times leaf \times day’ effect was even observed, and older leaves infected by the highest spore concentration were mostly discriminated already at three days from inoculation (PLS-DA, accuracy: 0.85), even in the absence of symptoms. These differences were confirmed by physiochemical alterations displayed by spectral indices or traits predicted from spectra. This study highlights the potential of optical sensors-based technologies to detect plant/pathogen interactions by means

of altered amounts of reflected light, allowing the possibility to discriminate plant health conditions. Nevertheless, further efforts are needed to build an applicative tool that can be easier exploited by farmers to manage plant diseases.

Tomato brown rugose fruit virus can be vectored by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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In the last few years, tomato (*Solanum lycopersicum* L.) production was undermined by tomato brown rugose fruit virus (ToBRFV), an emerging tobamovirus (*Virgaviridae*). ToBRFV is a highly infectious virus that quickly spreads once established in field. It is mostly spread by contact between infected plants and surfaces; only one mechanical transmission case by arthropods (*Bombus terrestris* L.) is reported. In this study, *Tuta absoluta*, one of the most invasive tomato insect pests, was evaluated as a possible ToBRFV vector under laboratory conditions. The experiments were carried out using tomato seedlings and *T. absoluta* reared within insect-proof cages. Healthy tomato plants were mechanically inoculated with ToB-SIC01/19 isolate. Thereafter, uninfected adults of *T. absoluta* were released and left ovipositing on infected plants. The obtained pest progeny resulted positive to ToBRFV in the emerging adults by RT-qPCR. Subsequently, *T. absoluta* ToBRFV-infected adults were released on healthy plants, to evaluate their ability to transmit ToBRFV; this hypothesis was confirmed. Lastly, pupae obtained from larvae fed on infected plants have been disinfected and compared with not-disinfected pupae. Moreover, adults obtained from disinfected and not-disinfected pupae were analysed and compared. The ToBRFV presence was confirmed in both adults and pupae of disinfected and not-disinfected *T. absoluta*, revealing that the ToBRFV virions are located within the insect body. In this study, we demonstrate that *T. absoluta* can acquire and transmit ToBRFV in laboratory conditions. Further studies will be conducted

to identify the ToBRFV localization in the insect body and to investigate spreading dynamic in the field.

An epiphytic strain of *Stenotrophomonas rhizophila* inhibits growth of *Botrytis cinerea* through the emission of volatile compounds and restricts leaf infection by priming defense genes

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The bacterium *Stenotrophomonas rhizophila* has been frequently isolated from the rhizosphere of crops and reported to be beneficial for plants. In the present work, we isolated an epiphytic strain of *S. rhizophila* that we named Ep2.2 from the phyllosphere of an ornamental plant (*Hibiscus syriacus*) and investigated its possible application in crop protection. The strain was characterized biochemically and metabolically, and compared to *S. maltophilia* LMG 958, a well-known plant beneficial species also known as opportunistic human pathogen. *S. rhizophila* Ep2.2 showed distinctive features as compared to *S. maltophilia*, such as different motility, a generally reduced capacity to use carbon sources, a greater sensitivity to fusidic acid and potassium tellurite, and the inability to grow at the human body temperature. *S. rhizophila* Ep2.2 was able to inhibit *in vitro* growth of the plant pathogenic fungi *Alternaria alternata* and *Botrytis cinerea* through the sole emission of volatile compounds. Simultaneous PTR-MS and GC-MS analyses performed on *S. rhizophila* Ep2.2 grown *in vitro* revealed the emission of volatile organic compounds (VOCs) with well-documented antifungal activity, such as furans, sulphur-containing compounds and terpenes. When sprayed on tomato leaves, *S. rhizophila* Ep2.2 was able to restrict *B. cinerea* infection and to prime the expression of *Pti5*, *GluA* and *PR1* plant defense genes.

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Field isolates of *Beauveria bassiana* display biocontrol heterogeneity

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Beauveria bassiana (*Bb*) is a cosmopolitan fungal pathogen of insects playing multiple roles in natural agroecosystems including endophytism, plant growth promotion and disease control. Here, we characterized two types of interactions (*i.e.*, with insects and soil-borne fungal pathogens) that naturally occurring *Bb* isolates might face throughout their life cycle. Our analysis revealed that *Bb* is characterized by a high level of inter-isolate heterogeneity in terms of nutritional requirements, establishment of intra- or inter-kingdom interactions and types of metabolites produced. Indeed, some *Bb* isolates grew and sporulated preferentially on poor nutrient media suggesting their adaptation to an endophytic rather than a saprophytic lifestyle. Moreover, different *Bb* isolates showed varying killing speeds of *Spodoptera littoralis* larvae when a contact infection assay but not an intrahaemocoel injection assay was performed, suggesting a different level of adaptation to the insect host. Contrarily to this, all *Bb* isolates showed a similar biocontrol activity against the fungal plant pathogen *Fusarium oxysporum* and efficiently protected tomato plants from wilting. Interestingly, metabolomic analysis of *Bb* showed that all isolates produce a large variety of organic- and fatty acids. While some have proven antifungal, antibacterial and insecticidal properties, others (*e.g.* oxalic acid) may be involved in softening the insect cuticle and lowering environmental pH, a mechanism already described to inhibit fungal pathogen penetration in plant root tissues. Further experiments will be required to investigate the biological role that the different classes of metabolites produced by *Bb* play in plant-interaction and biocontrol.

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Control of cortical necrosis of grapevine in a wet season: product efficacy for protection in organic viticulture

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Cortical necrosis of grapevine (often known by the incorrect name of “excoriose”, which should be used only for *Neofusicoccum parvum* cortical necrosis), caused by *Diaporthes* species, mainly *D. ampelina*, is one of the diseases that were considered minor problems but are evolving as a hidden enemy that is often not detected by growers, but is still able to cause considerable damage along the growth season. Not only it is the cause of significant damage but it also contributes to wood infection of pruning wounds resulting in cankers, indistinguishable from wood necrosis caused by other agents, like Botryosphaeriaceae and Esca complex agents. Traditional protocols dedicated to cortical necrosis control place treatments in the very first stage of the growing season, with one or two applications after bud burst. The disease is particularly difficult to control in organic agriculture. Therefore a trial was set up in a Cabernet Sauvignon vineyard in the Chianti area, Tuscany, to compare different protocols and products that are allowed in organic agriculture. The trial showed different efficacy of the chemicals used and the relevance of extending the time for treatments in very rainy seasons, like the spring of 2023. Infections, which usually, even in the same vineyard, are concentrated in the first three–four internodes, in this wet season reached even the 10th internode with variable severity. *In vitro* trials supported the results obtained in the field. The importance of detecting the presence of the disease and selecting more efficacious products that can control powdery or downy mildew but also cortical necrosis is underlined.

Puzzling out the metabolic reprogramming of winter wheat plants under FHB infections and biocontrol strain inoculation in field

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Fusarium head blight (FHB) is a major fungal disease affecting winter wheat (*Triticum aestivum*) worldwide, caused by species belonging to the *Fusarium* genus, so being responsible for huge yield losses and mycotoxin contamination of grains. Through an untargeted metabolomic approach, this study aimed to characterize the metabolic responses of two cultivars (Bingo and Rebelde, more and less susceptible to FHB, respectively) to *F. graminearum* infection after being treated with a systemic agrochemical or *Trichoderma gamsii* T6085 (a well-known biocontrol agent of FHB on wheat), in open field conditions. Metabolomic signatures of spikes were characterized through an UHPLC/QTOF-MS untargeted analysis. Results showed a hierarchically prevalent effect of the “cultivar”, followed by the “FHB” and “treatment” levels. Supervised multivariate statistics (OPLS-DA) highlighted the main metabolomic differences among plants from different cultivars, FHB infection and treatment conditions (control, T6085 inoculation and agrochemical treatment), with good correlations and prediction ability ($0.995 < R^2 Y < 0.992$ and $0.678 < Q^2 Y < 0.517$), and adequate validation parameters (CV-ANOVA $P < 0.01$). Pathway analysis from differential metabolites showed that nitrogen-containing compounds, terpene, phytoalexins and hormones synthesis were mostly affected compared with control conditions in Bingo plants inoculated with T6085 and then with FHB, while in those inoculated only with FHB these metabolic pathways show a down-modulation. Conversely, Rebelde plants showed a relevant up-regulation of all secondary metabolisms, and the plants previously inoculated with T6085 turned out to be more performing than the non-inoculated ones, also following the FHB infection, suggesting that the metabolic performance of Rebelde is involved in its lower disease susceptibility.

A VEM (Vector Enabled Metagenomic) approach to investigate the presence of potentially invasive geminiviruses in Southern Italy in the frame of GeMed, a research project involving five Mediterranean countries

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Geminiviruses are monopartite or bipartite circular single-stranded DNA viruses, infecting major vegetable crops in the Mediterranean area, i.e., tomato and cucurbits. They are considered among the most worrying plant viruses due to their economic impact, the frequent introduction of exotic species, and their high propensity to recombine, continuously generating potentially invasive and resistance-breaking strains. Moreover, geminiviruses can be associated to different satellite molecules, i.e. betasatellites, further increasing their pathogenic potential. To prevent and control the spread of new and invasive geminiviruses (NIGs), and of betasatellites, a vector-enabled metagenomics (VEM) approach has been used to identify the population of NIGs and satellites circulating in the South of Italy. Insects present in the surveyed areas (mainly *Bemisia* spp., leafhopper spp., aphids spp., and mirids spp.) have been collected in Campania and Sicily regions, considered as two different agroecosystems, from 2020 to 2022. DNA was extracted from 15 different pools of insects, and circular DNA molecules were amplified by Rolling Circle Amplification (RCA). The RCA products were subjected to high-throughput generation sequencing (Illumina) and data were analysed using *ad hoc* bioinformatic pipelines, focusing on NIGs and betasatellites. To confirm the presence of virus/betasatellite in insects, validations by specific PCR were carried out. Due to its potential to detect new geminiviruses at an early invasion stage, this approach will help to develop prompt prevention and control measures. In the frame of the GeMed PRIMA Project, analogous VEM approaches have been carried out also in Tunisia and Morocco.

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The binomial nomenclature for virus species: an update on plant virus taxonomy

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The International Committee on Taxonomy of Viruses (ICTV) is responsible for developing and maintaining an internationally agreed system of hierarchical classification of viruses and naming of taxa. The advancements in virus taxonomy are made available to the scientific community through a website (<https://ictv.global/>) and published reports and papers. To reflect current knowledge on the evolution of global virosphere, taxonomic ranks have been expanded beyond orders up to realms, provided that taxa in this 15-rank taxonomy are monophyletic. At present, virus taxonomy consists of 6 realms, 10 kingdoms, 17 phyla, 2 subphyla, 40 classes, 72 orders, 8 suborders, 264 families, 182 subfamilies, 2818 genera, 84 subgenera and 11273 species. The expansion to 15 ranks aligns virus taxonomy to other biological taxonomies, which adopt a Linnaean Latinized binomial format (i.e., binomial nomenclature), consisting of two italicized words indicating the genus (“genus name”) and the species (“specific name/species epithet”), respectively. To reach a standardized nomenclature, in 2021 the ICTV ratified the adoption of a binomial nomenclature for virus species. Based on this, a virus species name will be composed by two italicized words, the first one being the genus name and the second one consisting of a “free-form” species epithet. In this framework, Linnaean-style, Latinized virus species are permitted, but not mandated. This change in nomenclature must be completed by 2023 and it will apply to virus species only. Virus common names will remain unchanged. Following open debates, the Plant Viruses Subcommittee Study Groups have developed a binomial nomenclature format for virus species.

Dothiorella sarmentorum associated with grapevine trunk diseases in Algeria: study on the production of secondary metabolites

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A large number of species in the family Botryosphaeriaceae (Dothideomycetes, Botryosphaeriales) have been associated with canker and dieback of grapevines. Grapevine trunk diseases (GTDs) are among the prime causes of serious damages on vineyards and significant economic losses worldwide. In the wide spectrum of GTDs, species belonging to *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* are most commonly reported as causative agents of botryosphaeria dieback. Main symptoms of disease include cankers, wood necrosis, dead arms, canes and shoots, and bleached canes. In the last decades, many studies related *Dothiorella* species to botryosphaeria dieback in grapevine growing areas; however, even if the observed symptoms could be attributed to the production of phytotoxins by the pathogen, there is a lack of information in this respect. Therefore, a strain of *D. sarmentorum*, isolated from grapevine showing botryosphaeria dieback symptomatology in Algeria, was investigated for its ability to produce secondary metabolites *in vitro*. Five compounds were purified from the crude extract of culture filtrate by chromatography whose structures were elucidated by NMR spectroscopy, mass spectrometry and optical rotation. Among them, four 6-methylpyridione analogues were identified: fruit-rot-toxin (FRT-A), isoflavipucine, (8*R*,9*S*)- and (8*S*,9*S*)-dihydroisoflavipucines and (3*R*,4*R*)-4-hydroxymellein. The presence of these secondary metabolites in the crude extract was also confirmed by the HPLC-ESI-HRMS analysis in addition to six unidentified compounds. To the best of our knowledge, this is the first work leading to the isolation and identification of 6-methylpyridione analogues from *Dothiorella* species.

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Characterization of new *Trichoderma* species potentially useful for biocontrol applications

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Plant fungal diseases represent a global problem usually treated with pesticides, an effective but dangerous method

not only for human health but also for the environment. More sustainable and eco-friendly solutions are required such as the application of biocontrol strategies. At *Trichoderma* genus belong fungi widely used in agriculture both as biocontrol agents and plant growth promoters with important differences among species in terms of activity. To find new strains of potentially biocontrol activity nine *Trichoderma* species were isolated from different site of Campania Region and were characterized both morphologically and molecularly by PCR using different gene markers. Blast of the obtained sequences in NCBI database allowed us to identify four *T. asperellum*, one *T. atroviride*, three *T. harzianum*, and one *T. pyramidale*. Subsequently mycoparasitic activity of the nine different species of *Trichoderma* against four different plant pathogenic fungi (*Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Aspergillus* spp., and *Botrytis cinerea*) was evaluated. Dual plate assays were conducted and significant differences among the strains were observed, in terms of growth and antagonistic activity. *Trichoderma atroviride* AGR2 showed the greatest antagonistic activity against each one of the pathogenic fungi as well as the most rapid growth rate on plate. Further studies such as the evaluation of the enzymatic activity of the *Trichoderma* strains and their resistance against several fungicides are planned to have an overview of the biocontrol potential of each microorganism thus allowing their future employment as biocontrol agents.

Exploring the role of tomato yellow leaf curl Sardinia virus infection and viral C4 protein in priming tomato plants against drought stress and powdery mildew infection

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Abiotic and biotic stresses, such as drought and viral infections, can occur simultaneously in plants. It has been suggested that viruses can compensate host plants by assisting them to withstand stresses. This theory supports the presence of shared molecular networks governing the reaction of plants to stimuli induced by biotic and abiotic pressures. Begomoviruses have recently been proven to increase tolerance to heat and drought in *Arabidopsis thaliana*, possibly mediated by the C4 protein. We investigated how the infection of the begomovirus tomato yellow leaf curl Sardinia virus (TYLCSV) and the overexpression of its C4 protein

regulate physiological and molecular processes in tomato plants subjected to drought stress. In addition, we also investigated if TYLCSV C4 could improve resistance to infections of *Oidium neolycopersici*, causing powdery mildew. We observed reduction of powdery mildew symptoms and changes in the expression of genes involved in pathogenesis and phytohormone production. Understanding how TYLCSV, its C4 protein, and the defence signalling pathways interact at the molecular level could help scientists create new approaches for priming plant resistance to different stresses. In conclusion, research on the function of TYLCSV and its C4 protein in protecting tomato plants from drought stress and fungal infection brings up new perspectives on the intricate relationships between viral pathogens and plant stress responses.

Nitrogen sources influence extracellular pH and biocontrol activity in *Trichoderma* spp.

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Trichoderma spp. represent a class of filamentous ascomycetes fungi widely used in agriculture because their ability to control plant pathogen populations, boost plant growth and produce multiple secondary metabolites with antimicrobial or plant growth promoting activities. Despite much research has been performed on the strategies used by *Trichoderma* spp. to control plant pathogens, little is known about their ability to modify the environmental pH and how this process is related to antibiosis. Here we used the *Trichoderma harzianum* isolate M10, a renowned producer of bioactive metabolites [e.g., harzianic acid (HA)], and *Trichoderma asperellum* a powerful biocontrol agent to understand how different nitrogen sources (nitrate and ammonia) influence extracellular pH (pHex) modification in these fungi. We found that in liquid culture both *Trichoderma* species strongly acidified the extracellular milieu when grown on ammonia-supplemented media, a well-known phenomenon related to disease suppression in the rhizosphere. Reversely, poor N sources (nitrate) induced extracellular alkalization which led to a more abundant production of conidia. To

assess the relationship between nitrogen sources, pHex, and biocontrol activity both *Trichoderma* species were grown in plate confrontation assays together with the tomato plant pathogen *Fusarium oxysporum*. Our results suggest that ammonium-mediated pHex acidification increases the biocontrol activity of both *Trichoderma* tested isolate against the tomato plant pathogen *F. oxysporum* *in vitro*. Further experiments are necessary to better understand the correlation between nutrient sources and biocontrol activity of *Trichoderma* *in vivo*.

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Characterization of *Fusarium* species diversity causing crown and stalk rot on maize in Northern Italy

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The genus *Fusarium* includes many agronomically important species and toxin-producers strains, that can cause a wide range of diseases of many horticultural, cereals, ornamental, and forest crops. *Fusarium* spp. are worldwide distributed and reported as the main causal agents of crown and stalk rots and grain infections on corn. Crown and stalk rot development is usually developing during germination, but the disease may affect the later phases of plant growth, causing a premature senescence and lodging of the plants. In Italy, corn is affected by several species of *Fusarium*, so the purpose of this study was to investigate the diversity and the pathogenicity of 41 strains isolated during springs 2019 and 2020, from symptomatic seedlings collected in fields located in the provinces of Vercelli (Piedmont) and Vicenza (Veneto), and from seeds with five different geographical origins. The pathogenicity was tested, and a multi-locus phylogeny analysis, based on four genomic loci (*tef1-α*, *rpb2*, *calm* and *tub2*), was performed for 23 representative isolates. *Fusarium* representative strains were identified as species belonging to three species complexes. *Fusarium verticillioides* and *F. annulatum* of the *Fusarium fujikuroi* species complex. *Fusarium commune* was identified in the *Fusarium nisikadoi* species complex, and three different lineages in the

Fusarium oxysporum species complex. Pathogenicity was confirmed for the 23 representative isolates, causing rotting on maize seedlings. This study widens our knowledge on *Fusarium* species on maize, and it reports *F. annulatum*, and two lineages of the *Fusarium oxysporum* species complex as corn pathogens for the first time in Italy.

Decipher the communication mediated by lipids entities within the *Xylella fastidiosa* subsp. *pauca* endocrine quorum sensing and paracrin communication with the plant

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The pathogens virulence is tuned by different kind of molecules, among these lipid entities might serve as signal in several process, e.g. in cell-to-cell communication, as autocrine or paracrine signalling with the host and/or the vector. Free Fatty Acids (FFA) and oxylipins entities of *X. fastidiosa* subsp. *pauca* (Xfp) associated to the the Olive Quick Decline Syndrome (OQDS) was disclosed. A targeted mass spectrometry approach was used to unveil the lipid profile of: i) Xfp in pure culture; ii) *Nicotiana tabacum* Petite Havana SR1 artificially infected with Xfp; iii) *Olea europaea* cultivar Ogliarola salentina and Leccino uninfected (healthy), naturally infected with Xfp, treated and untreated with Dentamet[®]; iv) *Olea europaea* trees that overcame the OQDS. The analysis of FFAs and oxylipins combined with chemometry and supervised machine learning can discriminate diseased, healthy, Dentamet[®]-treated and restored plants. The thesis predictors are various FFA or oxylipins e.g., C16:1, C18:1, C18:2, C18:3, the LOX-derived oxylipins 9- and 13-HPOD/TrE. These entities are going to be exploited as robust markers of olive trees infected by Xfp. The analysis of transcriptomic profile of *Olea europaea* Xfp positive and Xfp negative, corroborate the lipidomic frame highlighting phospholipases and lipoxygenase genes whose expression is altered in trees that show OQDS symptoms. The lipid entities individuated were also tested in vitro

to evaluate their ability to modulate Xfp quorum sensing (oxylipins dependent quorum sensing: ODS).

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Innovative bioformulation with lignin extracts and *Trichoderma virens* for plant growth promotion in *Allium cepa* var. Tropea

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An innovative and eco-sustainable strategy to increase agricultural production and reduce chemical fertilizers inputs is the use of plant biostimulants, biological formulations that include beneficial microorganisms and natural substances (i.e. plant extracts), able to stimulate plant development, yield, and nutritional quality. This study aimed to investigate the effects of new *Trichoderma*-based formulation, containing *Trichoderma virens* GV41, known as biological control agent and growth promoter, in combination with commercial lignin extract (SolargoTM) on *Allium cepa* (var. Tropea) grown. For this purpose, *T. virens* GV41 and SolargoTM were administrated singularly or in combination and tested *in vivo* assays in greenhouse under controlled conditions. The effect of the treatments on seeds germination, plant growth (height, leaf number, fresh and dry weight), and bulb characters (diameter and fresh and dry weight) was analyzed. SolargoTM-*Trichoderma* formulation increased seed germination percentage of +16% compared to untreated control and +17% and +23% compared to single constituents (SolargoTM and *T. virens* GV41, respectively). In addition, SolargoTM-*Trichoderma* formulation was the most effective in terms

of growth promotion, significantly increasing the root length and stem fresh weight both compared to the control (43% and 33%, respectively) and compared to the components applied individually. Furthermore, the Solargo™ + *T. virens* GV41 effect on bulb growth promotion in terms of fresh and dry weight resulted statistically different compared to the control (+25% and +60% respectively) and single components. These results suggest that beneficial microorganisms in combination with natural bioactive substances can contribute to increase productions in a sustainable manner.

Narrowing down suspects: oomycete species are the best candidate causal agents of Kiwifruit Vine Decline Syndrome

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The Kiwifruit Vine Decline Syndrome (KVDS) causes a progressive decay of kiwifruit vines roots and the consequent collapse of the entire plant. The agent(s) and mechanisms behind KVDS are not yet fully understood, although spatial occurrence and symptoms suggest that this syndrome has a biotic origin and that oomycetes might play a primary role. Here, we focused on clarifying the causes behind KVDS by analysing the microbiome of soil and roots of symptomatic and asymptomatic kiwifruit plants using amplicon metagenomics targeting bacteria, fungi, and oomycetes. Results show marginal differences in the diversity and structure of microbial communities, regardless of the presence of KVDS symptoms. However, amplicon sequence variants (ASVs) identified as *Phytophthora vexans* were enriched in both soil and root samples collected from symptomatic plants. These findings were confirmed by the isolation of the oomycete community using baiting. Although several other oomycetes were isolated (including *Phytophthora litorale*, *Phytophthora cryptogea*, *Globisporangium* sp., and *Pythium* sp.), *P. vexans* was the most frequently isolated. Taken together, our results support the role of oomycetes as causal agents of KVDS, with *P. vexans* likely playing a major role. Further work will elucidate the mechanisms leading to the induction of KVDS, clarifying the specific contribution of *P. vexans* and the other

oomycetes in the wider context of the plant-microbiome-environment interactions.

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Studying the diversity among *Xanthomonas euvesicatoria* strains collected from pepper plants in Southern Italy

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Xanthomonas euvesicatoria (Xe) is one of the main relevant pathogens responsible for bacterial spot disease on tomato and pepper. Building resistance in plants and designing sustainable strategies to protect these crops is hardly challenging in the context of climate change. Moreover, very little data are available on the pathogenic behavior of Xe in Italy. The core aim of this work was to study the molecular diversity among several Xe strains isolated from pepper plants in Basilicata and Campania regions. Twenty-five strains were isolated from pepper leaves and seeds and molecularly identified by a Real-Time PCR assay, and then they were processed to extract the genomic DNA. The whole nucleic acid was quantified and sequenced on an Illumina NextSeq 2000. *De novo* assembled core genomes were compared to reference ones to create a phylogenetic tree. Effectors were also analyzed to define the races. Collected strains were tested for copper and streptomycin resistance eventually. Core genome analysis showed the presence of four clades, close to several reference genomes from all over the world. Effectors analysis revealed the presence of six different races among the strains, while no resistance to copper or streptomycin were noticed. The results highlight the huge genetic diversity in the studied population of Xe, despite the narrow geographic area they came from, suggesting different origins or introduction moments in Italy. This leads to the conclusion that the biology and the evolutionary patterns of these populations must be deepened to better understand how to manage future interactions with pepper plants.

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Inorganic salts as antifungal treatments against postharvest pathogens of strawberries and tomatoes

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Due to the delicate nature of their tissues, tomato and strawberry fruits cannot be subjected to many of the common treatments after harvest; in fact, dipping or spraying such fruits before storage could have the opposite side effect of increasing their susceptibility. Therefore, active packaging would be fundamental to prolong their shelf-life, avoiding wastes. To select active elements for this purpose, some soluble salts of bioactive metal ions (Cu²⁺ or Zn²⁺) and GRAS compounds (sodium and potassium bicarbonate) were tested at different concentrations against a selection of the main postharvest pathogens for the commodities, such as *Botrytis cinerea*, *Alternaria alternata*, *Rhizopus stolonifer*, *Colletotrichum acutatum*, and *Penicillium expansum*. A multi-well spectrophotometric assay was built up. Scalar concentrations of the compounds were added to the liquid growth medium (Potato Dextrose Broth), which was then inoculated with conidial suspensions of the selected pathogens. The fungal growth was monitored daily for 4 days by assessing the turbidity of the broth. In parallel, the test was conducted on the solid growth medium (PDA) amended with the salts at the same concentrations to confirm data and assess other macroscopic features of the colony. The purpose was to establish the Minimum Inhibitory Concentration (MIC) and the most effective concentration, which will be further tested *in vitro* and *in vivo*.

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Diversity of *Phytophthora* species in Mediterranean nurseries and best biosecurity management strategies

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The oomycete genus *Phytophthora* comprises several species that can cause high economic losses in crops and natural ecosystems worldwide. Trade of plants for planting is considered the main pathway of introduction and geographic distribution of many *Phytophthora* species. Infected nursery plants represent a serious concern for outbreaks of root disease in orchards, gardens, parks and natural ecosystems. Under the European Green Deal, the EU biodiversity strategy for 2030 commits to planting at least 3 billion additional trees in the EU in order to provide wellbeing, health benefits and climate change mitigation. Therefore, in the upcoming years, an increase in the requests of plant material for restoration programs of trees and urban forestation represents a challenge to the Italian forest nursery sector, such as the production of a conspicuous number of healthy plants. This work investigated the diversity of *Phytophthora* species in 13 Sardinian public nurseries and provides an example of the application of best biosecurity management practices to produce plants free of *Phytophthora*. Samplings were carried out on seedling productions, irrigation water, substrate piles and discarded plants. *Phytophthora* species were detected through the sensitive baiting method and subsequently identified by DNA sequencing. Over 20 different *Phytophthora* species were detected in all investigated nurseries from 52 different hosts, substrate piles, irrigation water and discarded plants. The implementation of best biosecurity management practices in seedlings production showed that *Phytophthora* contamination can be significantly reduced, if not eliminated.

Effectiveness evaluation of plasma decontamination treatments against *Fusarium proliferatum* and fumonisin accumulation

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The control of phytopathogenic fungi responsible for the accumulation of mycotoxins in cereals is a global concern. In recent years, environmentally sustainable tools alternative to pesticides are strongly required. Among the new strategies proposed, the use of "plasma technologies" for the control of microorganisms responsible for the deterioration of agri-food products seems to provide encouraging results. In order to evaluate the most effective technology, maize seeds, artificially inoculated on the surface with *Fusarium proliferatum* spores, were subjected to various plasma chemical treatments. A first investigation was carried out to test the effects of the direct treatment of the plasma generated with 2 different inert gases (air in atmospheric conditions and argon), in the absence and in the presence of aqueous aerosol. Treatment with plasma generated from air in the presence of aqueous aerosol was more active in inhibiting the fungal growth (80%). Further investigations used a planar Dielectric Barrier Discharge reactor to produce plasma-treated liquids containing reactive oxygen and nitrogen species. Direct and indirect plasma chemical processes were applied to treat seeds artificially contaminated with conidial suspensions of *F. proliferatum* ITEM 12072 strain or with the mycotoxin fumonisin B1. Direct treatment with nitrite-rich plasma was active in both reducing fungal growth and degrading fumonisin B1. Furthermore, indirect plasma treatment with air showed a clear fungal inhibition effect (over 90%), with a high degree of reproducibility. The data generated on plasma treatments with exhaust gases assume particular importance to obtain drastic reduction

of both fungal and mycotoxins contamination with an eco-friendly approach.

Vineyard brotherhood: isolation, identification and characterization of potential beneficial microorganisms from vineyards in Verona province

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Grapevine is one of the most relevant crops in Italy, in particular in the Veneto region. However, viticulture, despite many efforts, is still largely based on the extensive use of fungicides and chemical fertilizers to improve yields. Following the recent reform of the Pesticide Regulation by the European Commission, Italy should halve the use of pesticides by 2030; therefore, alternative approaches, sustainable and eco-friendly, should be investigated. The aim of this study is to identify and characterize microbial strains, isolated from local vineyards, to be applied as alternatives to chemical products to improve the resilience of vineyards to biotic and abiotic stresses. Out of around 110 bacterial strains, isolated from the rhizosphere of three different vineyards located in the Verona province, 15 isolates were selected from a first qualitative screening for their potential PGP capacity and further characterized at quantitative level. All isolates were also screened for their biocontrol potential against the pathogen *B. cinerea*, and the most effective were evaluated for their enzymatic activities (protease, cellulase and chitinase). Besides, 17 *Trichoderma* strains were isolated from the same vineyards and evaluated *in vitro* for their biocontrol against important local pathogens and for the secretion of extracellular enzymes (protease, cellulase and chitinase). These results indicate that vineyard biodiversity represents an important source of endogenous microbe-based potential alternatives to chemicals to improve viticulture sustainability.

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Application of OSMaC strategy to modulate the metabolism of beneficial microbes

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Most of the novel eco-sustainable approaches employed for plant protection and growth promotion involve the use of beneficial microbes. Their effects are frequently regulated by effector metabolites produced during the interaction with plants and other microbes. Nevertheless, many of these compounds are still unknown since the biosynthetic pathways are not activated or up-regulated under standard laboratory conditions. Several strategies were developed to overcome this limitation, including the variation of culture conditions, named “One Strain Many Compounds” (OSMaC) strategy, which is considered the most effective tool to modulate metabolic profiles. OSMaC strategy was used with *Trichoderma harzianum* M10 by cultivation in different media (potato dextrose broth – PDB, 1/10 diluted PDB, yeast peptone glucose - YPD, minimal medium – MM - and MM + 1 mM of FeCl₃) and under different conditions (light or dark, static or shaking - 140 rpm - for 15 days at 25 °C). The production of secondary metabolites was evaluated through untargeted metabolomic analysis of culture filtrates performed using mass spectrometry coupled to liquid chromatography (LC-MS). Among the statistically different molecules detected it was possible to identify siderophores (i.e. ferricrocin and coprogen B), harzianic acid (and its derivatives) and butenolides. Furthermore, a specific set of conditions induced the production of a novel metabolite never isolated from *T. harzianum* M10. The compound, a methoxychromone derivative, was characterized by spectrometric and spectroscopic techniques. These results demonstrate how the OSMaC strategy represents a valuable approach to modulate the biosynthesis of microbial metabolites in laboratory conditions.

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Innovative tools for the sustainable control of virus disease in grapevine: from traditional approaches to emerging biotechnologies

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Grapevine (*Vitis vinifera*) is one of the world’s most significant fruit crops, standing out among cultivated plant species for its paramount socio-economic relevance. Viruses represent a major threat to viticulture and the wine industry, drastically affecting plant health and survival and causing heavy losses and significant reductions in yield and fruit quality, which often require costly approaches to mitigate damage. Environmental changes, and global warming are promoting the emergence of unknown viruses, whose spread is favored by the trade in plant products. Their management is challenging and mostly relies on prophylactic measures that are intended to prevent the introduction of novel viruses into vineyards. Besides the use of virus-free planting material, the employment of agrochemicals is a major strategy to prevent the spread of insect vectors. Given the impending drastic reduction in the use of chemical pesticides (at least 50% by 2030, according to the Green Deal objectives of EU policy), implementation of the innovative biotechnological tools that have been developed so far represents the only promising strategy for the sustainable control of viral disease in vineyards. Our work presents a set of innovative tools that have been developed to induce virus resistance in plants, including modern transgenesis, the still-debated genome editing technologies, and RNAi-based strategies, discussing numerous illustrative studies that highlight the effectiveness of these promising strategies for the management of viral infections in grapevine. Finally, the development of viral vectors from grapevine viruses is described, revealing their positive and unconventional roles, from targets to tools, in emerging biotechnologies.

Multiple host infection methods prove *Fusarium musae* infects plants and animals

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Fusarium musae is an emerging potential cross-kingdom fungal pathogen. It causes crown rot in banana fruits and nail and keratitis infection as well as systemic disease in immunocompromised patients. To study *F. musae* pathogenicity we collected 18 strains isolated worldwide from bananas and human patients. Results showed that all strains can grow at 24 °C as well as 37 °C *in vitro* supporting the hypothesis of cross-kingdom pathogenicity of the species. We next aimed to collect experimental proof of multiple host infection to investigate the potential cross-kingdom pathogenicity of *F. musae*. We developed two *in vivo* infection models. As plant model we chose banana fruits and infected them by skewering the fruits with toothpicks dipped into spore suspension. We built a specific scale to score disease progression by the presence of brown spots surrounding the point of toothpick insertion. As animal model we chose the “human proxy” *Galleria mellonella*. Disease progression was assessed by a scoring system based on increased melanisation, loss of activity and cocoon formation. All strains caused consistent disease in both models providing the first experimental proof of the ability of *F. musae* to invade both plant and animal pathosystems. Our animal and plant models showed comparable level of infection confirming *F. musae* can be considered as cross-kingdom pathogen. Furthermore, we established animal and plant *F. musae* infection models with accurate disease indexes for quantifying virulence degrees in the two hosts, posing the bases for the investigation of the host-pathogen interactions of *F. musae* with its hosts.

Occurrence of *Fusarium* species and their mycotoxins in barley grains harvested in Italy

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Fusarium head blight (FHB), a complex disease caused by several *Fusarium* species, can affect also barley, compromising grain yield and quality due to mycotoxin contamination. In the present study, the presence of eight *Fusarium* species (*F. graminearum*, *F. avenaceum*, *F. culmorum*, *F. poae*, *F. proliferatum*, *F. sporotrichioides*, *F. tricinctum*

and *F. langsethiae*), was assessed by quantitative real-time PCR on 54 barley grain samples collected in northern and central Italy in 2019. In addition, fungal secondary metabolites, including the main *Fusarium* mycotoxins, were quantified by LC-MS/MS. Considering all samples, *F. poae*, *F. avenaceum* and *F. graminearum* showed the highest incidence (65%, 61% and 50% of positive samples, respectively), while, regarding fungal biomass levels, *F. graminearum*, *F. culmorum* and *F. poae* showed the highest amounts. In detail, *F. culmorum* and *F. poae* were the most abundant species in the grains harvested in central Italy, while *F. graminearum* and *F. poae* were prevalent in those from northern Italy. These last two species showed a significant higher biomass amount in the samples collected in the northern area in comparison to the central one. Concerning mycotoxin contaminations, enniatins were found in all the analysed grain samples, with the highest levels found in northern areas (average level of 99.8 µg/kg). Deoxynivalenol (DON) was mainly present in the samples from northern Italy (93%; average level of 493 µg/kg) with respect to those from central Italy (41%; average level of 62.2 µg/kg). In three of them, the DON amount was higher than the EU legal limits.

Cadophora luteo-olivacea: pathogenicity and control strategies on apple and kiwifruit

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Cadophora luteo-olivacea is known as the causal agent of trunk disease of grapevine and cordon dieback of kiwifruit but has also been linked with postharvest diseases of kiwifruit, pear, and apple. In Friuli Venezia Giulia and South Tyrol regions, *C. luteo-olivacea* has been isolated from rotten kiwifruit and apple fruit under cold storage. Five fungal isolates were selected to assess their pathogenicity on different kiwifruit and apple cultivars and to determine their sensitivity to different fungicides, commonly used in integrated and organic management systems for both crops. The pathogenicity test was firstly performed on organic fruits of two kiwifruit cultivars ('Hayward' and 'Jintao') and three apple cultivars ('Royal Gala', 'Golden Delicious', and 'Fuji'). The fruits were artificially wounded and inoculated with conidial

suspensions of each isolate, subsequently incubated at room temperature and cold storage for one and four months, respectively. Disease incidence and severity were assessed. Furthermore, the pathogenicity of the isolates was tested on dormant wood branches. The inhibitory effect of different concentrations of ten commercial fungicides on spore germination was evaluated for each isolate and the relative EC50 values were calculated. Due to the ongoing climate change, more studies, like the present one, are required to deepen the biological characteristics of *C. luteo-olivacea* in order to understand the epidemiology and to define appropriate control strategies for this emerging pathogen.

Monitoring of tomato brown rugose fruit virus (ToBRFV) in Campania region (2019-ongoing): results and prospects

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Tomato brown rugose fruit virus (ToBRFV) is a tobamovirus infecting tomato and pepper and causing serious economic damage mostly in worldwide greenhouse tomato production. Starting from 2019, ToBRFV has been introduced in the monitoring plan of the URCOFi project (Unit for coordination and development of surveillance, research, monitoring and training activities for phytosanitary purposes) in order to prevent and/or contain the spread in the Campania region (Southern Italy). Extensive monitoring in greenhouse and open field crops, plant nursery, supermarkets and tomatoes retailers was conducted, including sampling and molecular identification, through official methods provided by the European and Mediterranean Plant Protection Organization (EPPO), in leaves, fruits (RT-PCR) and seeds (qRT-PCR). The first reports of ToBRFV in the region concerned supermarket tomatoes imported from Sicily, showing browning and roughness. ToBRFV was also found in 2022 in a rootstock seed lot and recently in different tomato greenhouse crops in the provinces of Naples, Benevento and Salerno. Due to its stability and easy mechanical transmission, it is difficult to contain the spread of ToBRFV. For this reason, in 2023 phytosanitary controls have been increased in order to contain the spread of ToBRFV.

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Source of resistance to cucurbit aphid-borne yellows virus in *Cucurbita moschata*

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Since the first report of tomato leaf curl New Delhi virus (ToLCNDV) on zucchini squash in the Campania region in 2015, extensive monitoring in greenhouse and open field cucurbit crops throughout the region was performed to limit its spread. However, ToLCNDV must not be considered the only dangerous virus for cucurbits, due to other occurring emerging viruses such as poleroviruses, including cucurbit aphid-borne yellows virus (CABYV). In the last two years, CABYV was found with a very high incidence in several cucurbit crops, almost comparable to that of ToLCNDV (very often consisting in 100% of infected plants), causing widespread yellowing in addition to symptoms induced by ToLCNDV. During a field screening of *Cucurbita moschata* and *C. pepo* germplasm for resistance to ToLCNDV, we also looked for the presence of CABYV by RT-PCR with universal primers for poleroviruses. Amplicons of the corresponding size was obtained in all the germplasm tested with the exception of three *C. moschata* lines. Considering the high inoculum pressure in the field and the consequent high incidence of CABYV in the plants, field results suggest that these lines could be very promising as a source of resistance to CABYV in breeding programme.

Whole genome sequencing and comparative genomics of *Colletotrichum fioriniae* and *Colletotrichum acutatum* isolated from olive fruits in central Italy

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Anthraco-nose is one of the most important olive diseases caused by several species of the *Colletotrichum* genus, belonging to different *Colletotrichum* species complexes. During a survey conducted in 2022 in the Viterbo province mills, we observed wrinkled, rotten drupes with mummified tissue showing reddish spots and covered by orange fungal conidia. These symptoms lead to yield losses, olive oil of low quality and drupes not suitable for human consumption. Both *C. acutatum* and *C. fioriniae* were isolated from the infected drupes. To better understand the genetics behind the convergence

of different *Colletotrichum* species into the same host, we sequenced their genomes combining the advantages of the short Illumina and the long Nanopore reads, through a hybrid assembly approach. Their coding sequences were annotated and compared with other *Colletotrichum* genomes, isolated either from olives or from other plant species. The identification of specific host-related traits, together with common virulence features, could give insights into the infection mechanism of these variegated pathogens. These premises would define the foundation for future analysis focused on the relationship within the *Colletotrichum* genus, the development of customized control strategies.

Metagenomic and culture-based approaches to untangle the hazelnut buds microbiome infested by *Phytoptus avellanae* mite

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Corylus avellana L. is an economically important shrub species cultivated for its nuts and Italy is the second largest world hazelnut producer. Despite its acknowledged resilience to adverse environmental conditions, hazelnut is constantly facing aggression by a plethora of biotic entities. Among those, the mite pest *Phytoptus avellanae* strongly demands attention, given the still poorly understood biology, epidemiology, and its effect on hazelnut production. By invading the generative and vegetative buds in early spring, this pest makes them swollen (hereby defined galls), reddish, and unable to further develop. Infestation levels are commonly assessed by winter inspection, evaluating, in randomly selected branches, the ratio between the number of galls and the number of buds per branch. In the Latium region, treatments are allowed if 15–20% of buds are affected by the mite. According to the current literature, the gall formation may be driven by the synergistic effect of different players: the mite, but also bacteria and fungi, even if no precise description is available yet. Thus, we collected both healthy buds and galls from different hazelnut cultivars and investigated the microbiome biodiversity by bacterial and fungal culture-based isolation from both galls and buds. Since most of the species may be unculturable, total genomic DNA extraction and 16S and ITS metabarcoding was also performed in parallel.

Knowing the biology and the infestation mechanisms of these agents can ensure accurate monitoring and the formulation of proper control strategies.

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Quantification of airborne spores of *Colletotrichum* spp. in olive grove using volumetric spore trap and quantitative PCR

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Olive (*Olea europaea* L.) anthracnose is one of the most serious and widespread fungal diseases caused by various species of *Colletotrichum*. In many olive-growing areas of Italy, anthracnose has become endemic, leading to frequent and severe epidemic outbreaks. It is currently considered a re-emerging disease. This study aimed to develop and validate a method for the detection and quantification of *Colletotrichum* airborne spores using volumetric spore traps and quantitative real-time PCR (qPCR) assay. An efficient DNA extraction protocol from the spore trap tapes was set up and a duplex qPCR was used to quantify the target DNA, using the universal eukaryotic 18S locus as endogenous control. The qPCR assay showed suitable values for quantitative analysis, including high efficiency (95%–110%) and sensitivity (10 fg/reaction). The method was able to detect up to 100 *Colletotrichum* spp. spores per tape. Aerobiological monitoring was carried out in two Italian olive groves, in Lazio (2019–2022) and Calabria (2020–2023) regions, by weekly sampling. The results showed, generally, a low spore density during the growing seasons, confirmed by absence of pathogen isolation from drupes. However, two peaks of increased spore density were observed in two specific site/year combinations, corresponding to flowers and fruits developmental stages. These data, in agreement with the literature, show the greatest sporulation in relation to the inflorescence and fruit ripening. Furthermore, a correlation analysis of qPCR data with meteorological available data are in progress. These results could be effectively used for epidemiological studies and to optimize control strategy in relation to major fungal sporulation periods.

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Biocontrol agents and mycotoxins: how do they interact?

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Fusarium mycotoxins contaminating agricultural products are a serious threat to both animal and human health. Deoxynivalenol (DON) is probably the most common contaminant of cereal grains worldwide, while enniatin B (ENB) is among the most frequently detected emerging mycotoxins. In this work we analysed the dynamics of interaction that occur between these two mycotoxins and the promising *Streptomyces* sp. DEF39 strain, selected previously for its biocontrol potential and the ability to decrease the accumulation of DON in wheat. The DEF39 sensitivity to ENB and DON, alone and in mixture, was assessed using both microplate and disk diffusion assays. ENB showed no negative effects on the bacterial growth even at the highest dose of 100 µg/ml. DON effects on DEF39 growth can be observed starting from the concentration of 2 µg/L in liquid culture, and 100 µg/L in solid culture. The DON-ENB co-exposure revealed similar results of DON alone, suggesting that it has the major toxic effect. For what concerns the influence of ENB on DEF39, using a biosensor-based microplate test to assess trichothecene pathway repression by *Streptomyces* secondary metabolites, we observed a modulation of DEF39 repression ability when it was exposed to ENB. Therefore, despite the lack of ENB toxicity on DEF39 growth, we hypothesize that ENB modulates the pathway of microbial secondary metabolites decreasing the DEF39 effect on DON suppression. This work suggests that the dynamics of interaction between microbiota and mycotoxins are tricky and need to be investigated also in the framework of development of effective biocontrol agents.

Effect of antagonistic microorganisms and biostimulants for managing Fusarium wilt of tomato under greenhouse conditions

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Fusarium wilt of tomato, also known as Fusarium wilt disease, is a fungal infection caused by *Fusarium oxysporum* f. sp. *lycopersici* (Fol) and represents one of the most destructive diseases affecting tomato plants worldwide. The use of biocontrol agents, physical methods, genetic resistance and grafting are the most applied tools for managing Fol. On the other hand, biostimulants, primarily used to promote plant health and productivity, have also shown potential in managing soilborne plant diseases. Seven trials have been carried out on potted tomato plants cv Cuor di Bue in greenhouse with the aim to evaluate the efficacy of antagonistic strains of *Fusarium* spp., *Trichoderma* spp. and *Pseudomonas* sp., obtained from suppressive soils, substrates and compost, and of microbial and non microbial biostimulants to control Fol. Biocontrol agents and biostimulants were applied with different methods, in nursery and at transplanting, and were compared to a commercial formulation of *Trichoderma* spp. and to thiophanate-methyl. Tomato plants were grown using a commercial peat substrate infested with 1 g/L of Fol race 1 fungal biomass. Four replicates of 6-10 plants each were considered for each treatment. Several antagonistic Fusaria strains, particularly 233/1 RB, MSA35, and FC21, a microbial biostimulant containing mycorrhiza and two plant-based biostimulants significantly reduced by 40-70% Fusarium wilt severity. The results of this study suggest the potential effectiveness of antagonistic microorganisms and biostimulants in controlling Fol. Furthermore, they confirm the importance of optimizing their application methods to ensure effective management of Fusarium wilt.

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Characterization of the olive endophytic community in genotypes displaying a contrasting response to *Xylella fastidiosa*

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Endophytes mediate the interactions between plants and other microorganisms, and the functional aspects of interactions between endophytes and their host that support plant-growth promotion and tolerance to stresses signify the ecological relevance of the endosphere microbiome. In this work, we studied the bacterial and fungal endophytic communities of olive tree (*Olea europaea* L.) asymptomatic or paucisymptomatic genotypes sampled in groves heavily compromised

by *Xylella fastidiosa* subsp. *pauca*, aiming to characterize microbiota in genotypes displaying differential response to the pathogen. The relationships between bacterial and fungal genera were analyzed both separately and together, in order to investigate the intricate correlations between the identified Operational Taxonomic Units (OTUs). Results suggested a dominant role of the fungal endophytic community compared to the bacterial one, and highlighted specific microbial taxa only associated with asymptomatic or paucisymptomatic genotypes. In addition, they indicated the occurrence of well-adapted genetic resources surviving after years of pathogen pressure in association with microorganisms genera such as *Burkholderia*, *Quambalaria*, *Phaffia* and *Rhodotorula*. Findings represent a crucial starting point in understanding plant defense strategies against *X. fastidiosa* subsp. *pauca* in the infected areas.

Development of an early detection protocol of *Erwinia amylovora* from corbicular pollen to monitor its spread in apple orchards

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It is now widely accepted that honeybees mediate the spread of *Erwinia amylovora*, the causal agent of fire blight a disease threatening apple production worldwide. Based on this body of knowledge, a molecular detection protocol was developed for monitoring *E. amylovora* spread in apple orchards. To do that, uninfected pollen was inoculated with a spontaneous rifampicin-resistant Ea strain. Pollen not inoculated with *E. amylovora* cells was used as untreated control. One gram of spiked samples was serially diluted and plated on Nutrient Agar amended with rifampicin. At the same time, spiked samples were homogenized in an extraction solution (NaCl 0.85%, TWEEN[®]80 0.001%) and incubated on ice. After one hour, samples were centrifuged, and the supernatants were collected and centrifuged again. The resulting pellets were suspended in Tris-HCl (pH 8) buffer and exposed to thermal shock. Total DNA was extracted with DNeasy mericon Food (QIAGEN) and amplified in qPCR according to EPPO standard PM 7/20. Once validated, the robustness of this protocol was tested on corbicular

pollen collected in two Trentino valleys. In 2023, 200 samples of corbicular pollen collected in Valsugana and Val di Non were analysed in real time. Results showed a consistency between results achieved with the molecular detection protocol and the occurrence of fire blight infections on apple plants. In future, the protocol developed in this study might provide an important predictive tool for a prompt warning of potential *E. amylovora* infection in apple orchards.

Molecular and metabolomic characterization of a dimethylallyl-tryptophan synthase deletion mutant of *Trichoderma arundinaceum* IBT 40837

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Trichoderma arundinaceum IBT 40837 (Ta37) can be considered a possible super-producer of secondary metabolites (SMs) among *Trichoderma* species, as characterized by a wide arsenal of SMs backbone genes. The genome of Ta37 encodes a putative ergot alkaloid biosynthetic cluster enclosing a dimethylallyl-tryptophan synthase (*dmats*), both subunits of the lysergyl peptide synthase complex (*lps1* and *lps2*) and nine homologs of genes coding for tailoring enzymes involved in ergot biosynthesis. Time-course gene expression analysis of *dmats*, *lps1* and *lps2* revealed gene co-expression, thus suggesting the ability of Ta37 to synthesize lysergic acid derivatives. LC-MS qTOF analysis of Ta37 culture filtrates revealed the presence of several putatively identified metabolites including dimethylallyl tryptophan, the substrate opening the ergot alkaloid biosynthetic pathway, and the amide derivative of lysergic acid. This suggests the presence of a functional ergot alkaloid biosynthetic pathway in Ta37. In order to generate a *dmats* knockout mutant ($\Delta dmats$) of Ta37, the *dmats* flanking regions were amplified and fused to the hygromycin-resistance cassette by two rounds of PCR, for the implementation of the "split-marker" deletion strategy. The final construct was used for protoplast transformation, and after three rounds of selection, *dmats* deletion and

cassette integration was validated by PCR and sequencing. The $\Delta dmats$ strain displayed phenotypical differences compared to the wild type (wt), as it showed faster mycelial growth rate on PDA and less sporulation ability. Gene expression analysis of *lps1* and *lps2* and comparative metabolomics against the wt are ongoing and will shed some light into the regulation of this putative pathway.

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***Trichoderma arundinaceum* ITB 40837 is a prolific source of nitrogen-containing secondary metabolites**

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Trichoderma species are characterized by a wide and plastic secondary metabolite (SM) gene arsenal that facilitates niche colonization, stress adaptation and fungal interactions. Horizontal gene transfer events between Pezizomycotina species have contributed to SM gene diversification in *Trichoderma* species. The genome of *Trichoderma arundinaceum* IBT40837 (Ta37) encodes a putative ergot alkaloid biosynthetic cluster of 71.7 Kb enclosing a dimethylallyl-tryptophan synthase (*dmats*), both subunits of the lysergyl peptide synthase complex (*lps1* and *lps2*) and other nine homologs of genes coding for tailoring enzymes involved in ergot biosynthesis. Time-course *dmats*, *lps1* and *lps2* gene expression analysis at 24, 72, 96, 120 and 144 h revealed gene co-expression, thus suggesting the ability of Ta37 to synthesize lysergic acid derivatives. Metabolomic analyses were conducted on liquid cultures of Ta37 grown in Potato Dextrose Broth at different incubation times, both in shaking (4 and 12 days) and in stationary conditions (30 and 60 days). Gas Chromatography-Mass Spectrometry (GC-MS) analysis of Ta37 filtrates extracted with ethyl acetate enabled

the identification of 32 nitrogen-containing compounds, including ten 2,5-diketopiperazines and one indole alkaloid. Analysis of Ta37 organic extracts by Liquid Chromatography-Mass Spectrometry (LC-MS) allowed the putative identification of dimethylallyl tryptophan (DMAT), the starting metabolite for the ergot alkaloid biosynthetic pathway, and the D-lysergic acid amide. These results suggest the presence of a functional ergot alkaloid biosynthetic pathway in Ta37, and represent the first report of DMAT-derived compounds in *Trichoderma* species.

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The Phenospex 3D multispectral scanning platform revealed high-performing *Trichoderma* antagonistic strains of *Fusarium oxysporum* f. sp. *lycopersici* and *Sclerotium rolfsii* on tomato plants

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The fungal genus *Trichoderma* (Ascomycota) includes members of particular interest in enhancing the sustainability of agriculture, as biological control agents of several plant diseases and effectors of multiple plant benefits. The availability of new multitrophic and multifunctional *Trichoderma* strains, capable of meeting the current challenges of climate change and the rationalization of pesticide use, is a crucial goal of the research in the field. This work aims to screen a wide collection of *Trichoderma* wild-type isolates, belonging to different species. The isolates were applied to tomato plants (*Solanum lycopersicum*) as seed coating followed by drenching, and both plant-growth promotion activity and biocontrol of *Fusarium oxysporum* f. sp. *lycopersici* and *Sclerotium rolfsii* were evaluated. These pathogens are responsible of two remarkable tomato diseases: tracheofusariosis and southern blight, respectively. All *Trichoderma* isolates antagonized *in vitro* the two pathogens showing different mechanisms of action, such as antibiosis, overgrowth, and secondary metabolites production. Furthermore, biocontrol trials performed on tomato plants (n=792) were assessed for disease severity by using a 0-3 symptom degree scale and phenotyped by 3D multispectral scanning to automatically measure several

morphological, biometric, and spectral parameters. The best performing isolates reduced up to 30 and 70% severity vascular wilt and southern blight disease, respectively. Moreover, analysis of the Normalized Difference Vegetation Index (NDVI) and the Plant Senescence Reflectance Index (PSRI), related to health and stress plant status respectively, was also performed. The results provided the most promising *Trichoderma* strains for sustainable disease management of tomato.

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Monica: a dedicated tool for metabarcoding third generation sequencing data

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16S ribosomal RNA and ITS analysis is widely accepted to characterize microbial communities. Short-read 16S rRNA has been widely used although can achieve resolution at genus level. However, long-read sequencing (e.g. PacBio and Oxford Nanopore) of 16S rRNA and ITS allowed a better classification. Nevertheless, the current tools are not specifically designed to handle high-error and long read. In this work, Monica was developed to classify amplicons of 16S and ITS of microbial communities sequenced via MinION and compared to tools like Emu, NanoCluster, Kraken2 and CCMetagen. First, an *in silico* dataset of 16S rRNA, 18S rRNA and ITS gene regions have been created using a tool called DeepSimulator. Using these datasets, we found that Monica and Emu outperformed that other tool. Furthermore, Monica classified a higher percentage of reads compared Emu. Then, an *in vitro* fungal mock community made of 16 fungal strains was used to test the performance after Oxford Nanopore sequencing. At first, we amplified each individual fungal DNA with ITS1-4 and pooled the PCR fragments. After sequencing, Monica and Emu identified all species in the mock community. Next, we mixed the fungal DNA and we performed PCR amplification using ITS1-4. The results

showed that Monica could call two samples at species level. Surprisingly, Emu called *Escherichia coli* in the fungal mock. In conclusion, Monica is a reliable tool for taxonomy classification of long reads metabarcoding, with solid statistic compared to other already available tools and an excellent sensitivity in calling taxonomy even at sub-species level.

Desensitization of α -Ste2 signalling in *Fusarium oxysporum* is partially Bar1-independent

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Fusarium oxysporum (*Fo*) is one of the most destructive plant pathogens affecting a wide variety of crops used for both human food and animal feed worldwide. Its growth and development are regulated by the interaction between peptide pheromones, A and α , and their cognate receptors, Ste3 and Ste2. This interaction modulates different aspects of fungal physiology and pathology, including germination, chemotropism and quorum sensing. To retain cell responsiveness in overcrowded environments, fine-tuning of pheromone-receptor desensitization is critically required to localize and discriminate among different signalling compounds. In the model fungus *Saccharomyces cerevisiae*, the secreted aspartyl protease Bar1 acts as a "barrier" and antagonist of α -Ste2 signalling. Here we performed an LC-MS q-TOF-MS analysis of synthetic *Fo* α -pheromone (WCTWRGQPCW) either pre-incubated with wild-type or Δ Bar1 fungal germlings or their exudates. Notably, α -pheromone treatment with either wt or Δ Bar1 fungal exudates produced two complementary peptides generated by cleavage between the Cys² and Thr³ residues. On the contrary, direct incubation of α -pheromone with wt but not Δ Bar1 fungal germling released two complementary sub-products deriving from pheromone cleavage between Thr³ and Trp⁴ residues, suggesting that Bar1 activity is associated with fungal cells in *Fo*. In line with this, bioinformatic analysis identified a C-terminal localized transmembrane domain in *Fo* BAR1 protein, differently from other fungal counterparts (i.e., *Candida albicans* and *S. cerevisiae*). Altogether, our results suggest the existence of a yet unknown Bar1-independent α -Ste2 desensitization mechanism in *Fo*.

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Coat protein is responsible for tomato leaf curl New Delhi virus pathogenicity in tomato

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Tomato leaf curl New Delhi virus (ToLCNDV), a bipartite begomovirus belonging to the family *Geminiviridae*, causes severe damage to many economically important crops worldwide. In the present study, pathogenicity of Asian (ToLCNDV-In from Pakistan) and Mediterranean isolates (ToLCNDV-ES from Italy) were examined using infectious clones in tomato plants. Only ToLCNDV-In could infect the three tomato cultivars, whereas ToLCNDV-ES could not. Genome-exchange of the two ToLCNDVs revealed the ToLCNDV DNA-A segment as the main factor for ToLCNDV infectivity in tomato. In addition, serial clones with chimeric ToLCNDV-In A and ToLCNDV-ES A genome segments were generated to identify the region determining viral infectivity in tomatoes. A chimeric clone carrying the ToLCNDV-In coat protein (CP) exhibited pathogenic adaptation in tomatoes, indicating that the CP of ToLCNDV is essential for its infectivity. To better understand the molecular basis whereby CP function in pathogenicity, a yeast two-hybrid screen of a tomato cDNA library was performed using CPs as bait. The hybrid results showed different interactions between the two CPs and Ring finger protein 44-like in the tomato genome. The relative expression levels of upstream and downstream genes and Ring finger 44-like genes were measured using quantitative reverse transcription PCR (RT-qPCR) and compared to those of control plants. This is the first study to compare the biological features of

the two ToLCNDV strains related to viral pathogenicity in the same host plant. Our results provide a foundation for elucidating the molecular mechanisms underlying ToLCNDV infection in tomatoes.

Aerated and not-aerated compost teas produced from a walnut chain residues-based compost for disease management and biostimulation of wild rocket

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(CT) is a fermented water-extract of quality compost containing useful microorganisms and bioactive molecules to be applied to crops for both disease management and biostimulation purposes. According to the production method regarding the active oxygenation of the liquid mass, aerated (ACT) and not-aerated compost tea (NCT) can be supplied. CT is proposed to increase sustainability of agricultural production. Wild rocket (*Diplotaxis tenuifolia*) baby leaf crop is devoted to the packaging of high convenience food preparations and need to improve quality and shelf-life through proper organic cultivation protocols. In this work, two CTs, Aerated-CT3 and Not-aerated-CT3, obtained by fermentation of composted walnuts residues (60% dry weight) + wood and giant reed chips (40%), were characterized for the main physicochemical and microbiological properties, and assessed for biostimulation and biocontrol activities on wild rocket plantlets, to assess the growth-promoting effects and suppressive capacity the soil-borne pathogen *Sclerotinia sclerotiorum*, respectively. Microbiological analysis retrieved slight presence of some groups of problematic microorganisms (Coliform) in unventilated tea and complete absence in the aerated one. Both CTs biostimulated seed germination and seedling growth at dilution upper to 1:10 vol. and reduced wild rocket *Sclerotinia* soft rot at dilutions 1:100 and 1:10, respectively, probably due to the presence of *Bacillus*- and *Pseudomonas*-like bacterial populations. CT evaluations on wild rocket in greenhouses are ongoing.

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Studies on the pathogenicity of an *Alternaria alternata* strain isolated from infected pears (*Pyrus communis*) in Italy on fruits and its competition with *Botrytis cinerea*

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Alternaria alternata, a phytopathogenic fungus that infects species of agronomical interest worldwide (including potatoes, pears, tomatoes or strawberries), leads to food inedibility and crop losses. The study of its pathogenicity is complex, which involves correlations among the production of cell-wall-degrading enzymes (CWDEs) and host-selective (HSTs) and non-specific host (NSTs) toxins, as well as the competition with other pathogens. There is lack of information on the functions of NSTs and CWDEs while several studies have been performed on HSTs. An *A. alternata* strain isolated from infected pears (*Pyrus communis*) in Italy was evaluated in bioassays to

provide insights into its pathogenicity regarding the aforementioned issues. The fungus produced enzymatic activities against amylase, xylanase and cellulose. The chromatographic purification of culture filtrates of *A. alternata* grown *in vitro* led to the isolation of metabolites belonging to two different classes of natural compounds: perylenequinones (altertoxin I and alteichin), and dibenzopyrones (alternariol and alternariol 4-methyl ether). Only alteichin generated necrotic lesions on host-variety pears, while all showed lesions on non-host species, indicating that they are NSTs. Furthermore, *A. alternata* showed competitive exclusion (growth inhibition) to the competitor *Botrytis cinerea*. Thus, the interaction between the two species results in competition, and the most virulent fungus has an intra-host competitive advantage. Probably the mechanisms used by *A. alternata* to infect host and non-host plants and inhibit competitors are based on synergisms between enzymatic activities and the secretion of NSTs, which was corroborated by the remarked inhibition against *B. cinerea* obtained for alteichin and alternariol 4-methyl ether.

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