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ABSTRACT

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Exotic Heterobasidion Root Disease in Italy as an unexpected legacy of war

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The presentation will first review those studies that have reconstructed the early establishment and introduction in the Lazio Region of the conifer root pathogen *Heterobasidion irregulare* by U.S. troops in 1944, during the liberation of Italy in World War II. The main emphasis of the talk, though, will be on research findings that help us predict the future trajectory of this exotic disease in Italy and in the entire European continent. Although multiple studies have shown that *H. irregulare* has a greater sporulation potential, a faster growth rate in wood and a broader host range than the native Eurasian *H. annosum*, precise predictions of the future impacts of the exotic disease have been hampered both by the high fragmentation of conifer stands and by the low incidence of the congener *H. annosum* in the Lazio Region of Italy. Not unexpectedly, population genetics approaches have allowed us to predict with confidence that, as the fragmentation of conifer stands decreases, the spread rate of *H. irregulare* will increase exponentially. In addition and unexpectedly, we have experimentally shown in the laboratory that the presence of the native *H. annosum* will accelerate and not slow down the spread of *H. irregulare*. Both results paint a troublesome scenario for Central and Northern European regions (including coastal Tuscany, Liguria and the Alpine region) characterized by large contiguous conifer woodlands with high incidence of *H. annosum*. Are these predicted scenarios likely to be true? In order to answer this question, we have studied in depth the interaction between the two pathogens in the Lazio and made several discoveries. Field evidence now proves not only that *H. irregulare* is truly invasive with a spread rate of about 150 ha per year in a contiguous forest, but also that it is replacing *H. annosum*. Unexpectedly, we also have discovered that part of the genome of *H. annosum* is being replaced by genes from *H. irregulare*, thanks to gene introgression mediated by interspecific hybridization. A recent study has further shown that *H. annosum* individuals containing *H. irregulare* genes increase their transmission traits to levels comparable to those of *H. irregulare*. We conclude that Europe is facing two invasions: one by *H. irregulare* individuals and one by *H. annosum* individuals modified by the acquisition of adaptive *H. irregulare* genes. Both invasions are associated with disease that will spread at a faster rate than that of the disease caused by native *H. annosum* populations, thus increasing the estimated 800 million Euros per year of damage already caused by native Heterobasidion Root Diseases.

Tree endotherapy with *Trichoderma* spp. against the agent of chestnut nut rot *Gnomoniopsis castaneae*

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An emerging fungal disease caused by the ascomycete fungus *Gnomoniopsis castaneae* (syn. *Gnomoniopsis smithogivyi*) is responsible for nut rot of sweet chestnut. The disease is impacting chestnut production in several Italian regions, with severe economic losses in a marginal cultivation with low income such as the fruit chestnut groves. Since this fungus is endowed with an endophytic lifestyle, surviving at a latent stage in chestnut tissues, it is hard to find effective solutions for its control and management. The

considerable size of some chestnut trees and the risks for consumers and the environment arise by chemical pesticides application in chestnut groves (e.g., traditional foliar applications), have prompted the search for alternatives to traditional fungicides as well as to the conventional delivery methods of plant protection products. The exploitation of microbial natural enemies of plant pathogens as biopesticides in plant disease control is an important reality to which the European and Italian institutions strongly aim for a more sustainable and environmentally friendly agriculture. The use of biocontrol agents (BCA) for controlling forest tree diseases is still scarce. The aim of this work was to test the development and optimization of a biological control method against *G. castaneae* based on trunk injections with *Trichoderma* species. *Trichoderma* species and strains were selected in laboratory pre-trials, testing their effectiveness against *G. castaneae* in an *in-vitro* dual culture system then injected in fruit chestnut trees stem. Results showed a reduction of *G. castaneae* infection in treated stands compared to controls (untreated chestnuts), proving endotherapic treatments with BCA to be a promising control strategy against *G. castaneae* infection.

Pinus radiata – Fusarium circinatum – Phytophthora spp., a model system of a complex host plant – pathogens interaction

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This study investigated the effects of co-infections in Monterrey pine (*Pinus radiata*) seedlings determined by diverse aggressive pathogens, the fungus *Fusarium circinatum,* causing Pine Pitch Canker (PPC) disease, and the oomycetes *Phytophthora* × *cambivora* and *P. parvispora,* both causative agents of crown and root rots. *Pinus radiata* seedlings were wound-inoculated with each single pathogen and with either combinations *F. circinatum*/*P.* × *cambivora* and *F. circinatum*/*P. parvispora*. The effects of the co-infections were investigated at 4- and 11-days post inoculation (dpi), in terms of severity of symptoms and modulation of the transcriptomic profile of the pyruvate decarboxylase-(PDC)-encoding gene and three genes encoding pathogenesis- related proteins (PR3, PR5, and PAL) in pine seedlings. Results from plants inoculated singularly with pathogens, highlighted that *F. circinatum* markedly induced the up-regulation of all four genes mainly at the late stages of infection. Between the two *Phytophthora* species, only *P. cambivora* stimulated a significant up-regulations. In seedlings co-inoculated with *F. circinatum* and *P.* × *cambivora* or *P. parvispora* none of analyzed genes showed a significant up-regulation at 4 dpi. In contrast, at 11 dpi, significant up-regulation was observed for PR5 in the combination *F. circinatum*/*P.* × *cambivora* and PDC in the combination *F. circinatum*/*P. parvispora.* In conclusion, two hypotheses were formulated: i. the competition between pathogens could have delayed the infective process by *F. circinatum* and the plant defense response; ii. co-infection might have repressed the expression of defense-related genes, thus exacerbating the severity of the disease.

Biocontrol of *Botrytis cinerea* as influenced by grapevine growth stages and environmental conditions

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The effective integration of BCAs into a Botrytis bunch rot (BBR) management program should not only include understanding factors, such as berry growth stages and environmental conditions (i.e., temperature, T, and relative humidity, RH), for the pathogen growth and infection, but also for the colonization and the efficacy of the BCA. In this study, four commercial BCAs were evaluated. Turbidimetric assays were conducted to assess the BCA growth at different berry growth stages by inoculating BCA into media mimicking the chemical composition of berries. In a second study, the BCAs colony-forming units (CFUs) were assessed at the ripe berries stage by inoculating the artificial "ripe berries" medium and incubating it for 1 to 13 days at different T/RH conditions. In a third experiment, each BCA was applied to ripe berries and then incubated under different T/RH conditions. After 1 to 13 days, the berries were inoculated with *Botrytis cinerea* and incubated for 7 days, at which time BBR was assessed. The response of BCA growth to grapevine growth stages and to T/RH conditions, as well as the response of BBR control to T/RH conditions, differed among BCAs. For example, *Metschnikowia fructicola* grew better on fully ripe berries than in earlier ripening stages; *Bacillus amyloliquefaciens* showed higher temperature requirements than the other BCAs. The results obtained in this study would assist farmers in selecting the appropriate BCA for application based on the growth stages of grapes and prevailing weather conditions at the time of treatment and later.

Evaluation of the effects of *Epicoccum nigrum* on the olive fungal pathogens *Colletotrichum acutatum* and *Verticillium dahliae* by ^{1}H NMR based metabolic profiling

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Epicoccum is a genus of ubiquitous ascomycetes. *Epicoccum* species can be found in the air, soil, plants and water. Several species are plant pathogens while some are potential biological control agents (BCAs). Among the latter, *Epicoccum nigrum* is the most promising and has been shown to reduce the incidence and severity of a wide range of plant diseases. Its antagonistic activity against fungal plant pathogens has been reported in the literature and seems to be due to the production of secondary metabolites with antifungal and antibacterial activity. The aim of this study was to test the ability of *E. nigrum* to inhibit *in vitro* the mycelium growth of the fungal pathogens *Colletotrichum acutatum* and *Verticillium dahliae,* responsible for anthracnose and Verticillium wilt of olive, respectively. Dual culture assays (*E. nigrum* + *C. acutatum* and *E. nigrum* + *V. dahliae*) showed that *E. nigrum* limits the mycelia growth of both *C. acutatum* and *V. dahliae* by producing inhibition zones. The metabolomic profiles of mycelium extracts obtained from mono and dual cultures were analyzed by ^{1}H NMR Spectroscopy and Multivariate Statistical Analysis (MVA). For all microorganisms studied, aqueous and lipid extracts were analyzed and showed different metabolic patterns between mono and dual cultures. The first results show differences in relative content of sugars, aminoacids, organic acids and diunsaturated fatty acids. Although further investigations are needed, these preliminary results could represent a starting point for safeguarding the health of olive trees.

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Influence of climate change on fungal pathogens of forest plants in Italy

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Climate change, with rising temperature and recurring and persistent droughts, is causing significant changes in Italy regarding the adaptation, spread and aggressiveness of forest plant pathogens. In some cases there is a reduction in attacks of species whose spread of 'spores' and the relative infectious process are favored by rains (e.g. *Marssonina, Gloeosporium, Cylindrosporium, Phyllosticta*) or disfavoured by too mild springs (e.g. *Discella, Discosporium, Pollaccia*). Much more often, however, climate changes increase phytopathological events, favouring: (1) the adaptation of newly introduced pathogens; (2) the attacks of species characterized by xero-conidia (e.g. agents of powdery mildew); (3) the resurgence of invasive pathogens, such as *Botryosphaeria* and *Cytospora*, and especially *Phytophthora*. The latter, with an increasing number of specie adapts to ever more numerous hosts and ever more wider ranges (e.g. mountain areas); (4) the suffering of plants with the development of "weakness parasites", which often leads to the notorious complex disease of "forest decline". Among the pathogens that become more aggressive on weakened plants, there is an important expansion of: (a) agents of bark necrosis that stress induces to pass from an asymptomatic endophytic phase to an active pathogenic state (eg *Biscogniauxia, Coryneum, Fusarium, Phoma, Phomopsis, Sphaeropsis*); (b) agents of root and butt rots (e.g. *Armillaria, Ganoderma, Heterobasidion, Rhyzina, Ustulina*); (c) wood decay species (e.g. *Daedalea, Fistulina, Fomitopsis, Inonotus, Laiteporus, Phellinus*). The aforementioned suffering of plants is also often the cause of reductions or involutions of mycorrhizae, often antagonists of phytopathogens, with further depression of the plant defences and vitality.

CRISPR-Cas9 technology for dissecting *Pseudopyrenochaeta lycopersici*-tomato interaction

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The soil-borne fungus *Pseudopyrenochaeta lycopersici*, formerly *Pyrenochaeta lycopersici,* is the causal agent of corky root rot of tomato. The genome has been sequenced by Illumina and PacBio technologies and the gapless genome assembly of 62.7 Mb, obtained by PacBio technology, showed a large fraction (30% of the total bases) of repetitive sequences, including transposable elements. The most effective tool for controlling the disease is breeding for resistance, however only little is known on the molecular bases of tomato-*P. lycopersici* interaction. To investigate the underlying genetic bases, a comparative RNA Seq-based transcriptomic analysis was conducted on two tomato cultivars, Mogeor and Moneymaker, resistant and susceptible, respectively, to the pathogen. Overall, transcriptional profilings suggested that susceptibility and resistance share overlapping signalling pathways and responses. In detail, we selected a gene coding for a transcription factor which resulted expressed at a very low level in Mogeor respect to Moneymaker (in control conditions), representing a good candidate as a possible susceptibility gene. Attempts to disrupt this gene via CRISPR-Cas9 technology are in progress, with the aim to analyse its role in resistance to *P. lycopersici.*

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A two-year survey on the occurrence of the seedborne barley foliar pathogens *Pyrenophora teres* and *Ramularia collo-cygni* in Italy

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Ramularia leaf spot and net blotch, caused by the fungal pathogens *Ramularia collo-cygni* (Rcc) and *Pyrenophora teres* (Pt), respectively, are two of the main foliar fungal diseases of barley causing leaf damages and yield losses. Recently, due to their seedborne attitude, their presence has been recorded constantly in several countries around the world. Fungicide resistance has also been widely recorded in both pathogens in a number of world areas. For this purpose, a two-year survey (2019/2020–2020/2021) was carried out to understand the occurrence and distribution of these two pathogens in the main Italian barley growing areas. Two different plant tissues, kernels and leaves, were analysed with a combination of different isolation methods to obtain fungal strains which were then identified by PCR assays. DNA extracted from barley kernels was also subject to real time qPCR assays to quantify Pt and Rcc DNA. Preliminary results showed that the samples from central Italy showed a higher Pt presence, both in terms of incidence and fungal DNA in the grains. Rcc was principally isolated and quantified in the samples from northern Italy. The incidence of the two pathogens in all the analysed grain samples did not exceed 30%, and, concerning fungal DNA, Pt showed the highest amount with respect to Rcc. This study will allow to better understand Pt and Rcc occurrence in the seed and their distribution in Italy as well as to obtain isolates to be used in population studies, including fungicide resistance tests.

Presence of *Gnomoniopsis smithogilvyi* in chestnut in Campania Region and sensibility to some active ingredients

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The production of chestnuts (*Castanea sativa*) in Italy plays a pivotal role in the nut sector and especially in Campania. In 2005, the presence of *Gnomoniopsis smithogilvyi* (syn. *G. castaneae*), agent of the chestnut rot, which causes huge losses of product both at harvest and post-harvest, was reported for the first time in Italy. The extreme environmental conditions play an important role in the epidemiology of the disease. In 2021, the presence of the disease was monitored in different cultivation conditions in the Campania region. Visual samplings were carried out on the fruits which were subsequently placed in a humid chamber to favor the development of the fungi which were isolated on culture medium. All the isolates were obtained in pure culture, to proceed with the morphological and

molecular identification by PCR amplification. Eleven isolates obtained from the samples from different areas have been identified as *G. smithogilvyi* with 99.9% nucleotide sequence identity. Sensitivity tests were conducted on the eleven isolates with 4 commercial formulations, relating to 3 categories of fungicides: "biological" (tribasic copper sulphate; Eugenol + Geraniol + Thymol); succinate dehydrogenase inhibitors (Fluxapyroxad), triazoles (Tetraconazole). In addition, in vitro tests were carried out to evaluate the resistance to the various active ingredients. The results obtained in this work suggest that in addition to good agronomic practices, in the rational management of the orchard, such as the removal of residues and infected organs and the use of effective phytoiatric interventions can contribute to control the pathogen.

Dissecting foliar fitness of a *Pseudomonas mediterranea* strain nonpathogenic to citrus to understand the interaction with *Plenodomus tracheiphilus*

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Citrus mal secco disease is the most limiting factor of lemon industry. The use of biological control agents to mitigate its symptoms is based on the capability of bacteria to colonize the same ecological niche invaded by the causal agent *Plenodomus tracheiphilus.* We have investigated several strains of *Pseudomonas mediterranea* (Pme), a bacterial nonpathogenic to citrus, for the frequent and rich presence in plants' rhizosphere, the arsenal of secondary metabolites, the plant growth-promoting traits present in the genome, and the direct antagonism against a class of plant pathogens. The strain Pme 3C was tested in greenhouse and field on lemon grafted on sour orange to understand its foliar fitness and capability to reduce the penetration of *P. tracheiphilus* and to mitigate the symptoms of the disease. Results of dual tests in vitro show that the strain penetrates the leaves through the wounds and reduces the conidia germination and mycelial growth of the pathogen at different levels. Other tests, based on detection of bacterial DNA by real time PCR, evidence Pme survives epiphytically on leaf surfaces, penetrates the leaf wounds, and colonizes the mesophyll veinlets. Field tests confirm a reduction of penetration of the pathogen into the leaves, and a minor extension of the host xylem colonization, associated to a delayed expression of symptoms of the disease. Over all, the pathogen is not killed but is temporarily confined by the bacterium, and it competes for nutrient sources essential for hyphal elongation and host penetration.

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Climate change, new pathogens and multi-trophic interactions threaten forest ecosystems in the Mediterranean basin

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In recent decades, forest ecosystems of the Mediterranean basin (a renowned hotspot for climate change) have been severely impacted by climate warming. Anomalous periods of rainfall, with recurrent droughts, have strongly stressed forest trees in a variety of situations (natural/artificial forests), predisposing trees to the attack by various fungal and oomycete pathogens. While, on the one hand, there has been a resurgence of old and known diseases, on the other hand new pathogens have arrived in uncontaminated territories, heavily infecting trees and spreading at extremely rapid rates (invasive species) over forest areas. Due to a lack of host–pathogen coevolution, these alien invaders often cause huge damage, posing a serious threat to tree populations and questions about the future management of the attacked forest ecosystems. Furthermore, a combined attack by immigrant and/or resident pathogens is in some instances observed, with lethal effects on the impaired tree species. This work reports some new and emerging pathogens that are threatening some Italian forest ecosystems. There is evidence that thermophilic or thermotolerant pathogens, which normally live as opportunistic endophytes in the tissues of host plants at an asymptomatic state, are capable to extensively colonize hosts that are impaired by climate anomalies (e.g. water stress). Some examples of multiple attacks by different pathogens on the same tree host are also reported. It becomes increasingly clear that in forest ecosystems we are often dealing not with single host–pathogen interactions but with multi-trophic interactions that can seriously compromise the stability and resilience of tree populations that are already heavily compromised by climate change.

Thousand cankers disease of walnut threatens the walnut groves of the Italian peninsula

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Thousand cankers disease (TCD) of walnut is an emerging new disease of walnut caused by the ascomycete fungus *Geosmithia morbida* (Hypocreales, Bionectriaceae) and its vector insect, the walnut twig beetle *Pityophthorus juglandis* (Coleoptera, Curculionidae, Scolytinae). TCD is a disease of North American origin (USA), which in the last decade has spread to some Italian areas: first reported in the Veneto region in 2013, the disease was subsequently found in various regions of northern Italy and in 2019 it was reported in Tuscany, in the province of Florence. Main host of TCD is the black walnut (*Juglans nigra*) but in some situations the common walnut (*Juglans regia*) can also be attacked, e.g. in mixed stands with the two species growing adjacently. There is currently a strong concern that the disease may spread to other regions of our country, because the black walnut has been extensively used in tree plantations since the early nineties of the last century, with the financial support of the European Union (EU Regulation 1992/2080) aimed at boosting the cultivation of valuable hardwoods. But, being Italy the only country in which TCD is currently reported outside the USA, the concern is also high at the European level. Indeed, there is a strong fear that the disease could spread to other EU countries, where black walnut plantations have also been established with similar financial measures. For this reason, both the fungal pathogen and its insect vector have been included in the EPPO A2 List of quarantine pests. Given this alarming situation, molecular diagnostic tools have been developed to be used in phytosanitary surveillance at sensitive sites (ports, airports, etc.) and over the territory (nurseries, plantations, etc.). Such diagnostic protocols revealed the capacity to detect the DNA of the two organisms from a variety of matrices, such as necrotic areas (cankers) around insect holes, infected internal tissues (galleries), adults and insect larvae, insect frass. They therefore constitute a promising tool to try to counter the epidemic spread of the disease.

Survey of Kiwifruit Vine Decline Syndrome in Lazio region

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Kiwifruit Vine Decline Syndrome (KVDS) is a disease that affects *Actinidia chinensis* (kiwifruit) causing plant decline. Symptoms appear as root rot and branch wilting. The disease spread out in Lazio (central Italy) in 2017, while in northern Italy appeared as early as 2012. The disease probably is caused by abiotic and biotic factors. In this study, we aim to investigate the aetiological agents causing the symptoms, using both traditional, molecular and Next Generation Sequencing (NGS) approaches. Root and rhizosphere samples were collected from 20 kiwifruit orchards localized in Lazio region. Isolation from symptomatic roots revealed the presence of *Cylindrocarpon* sp. and oomycetes such as *Phytophytium* sp. In order to understand whether there is a clear difference in microbiome between kiwifruit plants affected or not by KVDS, we set up a DNA extraction method and a metabarcoding analysis using ITS2 for fungi and oomycetes. Preliminary results show that the DNA extraction method from root and soil samples works properly despite the high polysaccharides content in roots. Furthermore, NGS sequencing confirms the differences in the microbial community of asymptomatic and symptomatic samples. The next step is to analyse the other soil and root samples to confirm the difference between the symptomatic and asymptomatic plants to associate a pool of potential pathogens to the disease and a pool of microorganism to the healthy root and rhizosphere.

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Spread of Olea europaea geminivirus (OEGV) in olive trees in Sicily

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Olea europaea geminivirus (OEGV), a putative member of the *Geminiviridae* family, has been recently identified in olive trees in several cultivation areas worldwide. For this reason, we aimed to investigate the presence and spread of OEGV in Sicily. Several surveys were conducted, focusing on olive production sites in the provinces of Agrigento, Caltanissetta, Ragusa and Trapani. Specifically, 70 sample batches, each consisting of eight different trees (560 sampled trees in total) of different cultivars were collected and geo-referenced with Planthology mobile application. Positive batches were re-sampled and each plant was analysed individually. All samples were prepared with a rapid extraction method, avoiding the use of DNA extraction with commercial kits and analysed using a real-time LAMP. These analyses showed that 30 out of the 70 cultivars tested were positive for OEGV (~43%), indicating a relatively high incidence and prevalence of OEGV across the sampling locations and the cultivars. In addition, the majority of plants (235 out of 240) within positive batches resulted OEGV positive, except the 'Calatina' cv. for which only three out of eight plants tested positive. Our survey revealed a considerable presence of OEGV in olive trees in Sicily, probably due to the inadvertent movement of clonally propagated infected but asymptomatic plant material.

Detection and management of "huanglongbing" in some Caribbean countries

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"Huanglongbing" (HLB) is a bacterial citrus disease associated with huge economical damages whose most distinctive symptom is diffuse and asymmetrical mottling on the leaves. The HLB presence and management were monitored in Cuba, Guadeloupe and Jamaica during 2017–2022 where the presence of '*Candidatus* Liberibacter asiaticus' was known since several years. This bacterium was present very often in mixed infection with diverse phytoplasmas, that in some cases were the only bacteria detected in plants showing HLB. These pathogens presence was confirmed by using a next generation sequencing method specifically adapted to detect these bacteria. In Cuba the elimination of symptomatic trees at regional scale resulted in the best management strategy to reduce the disease impact. In the orchards without eradication the disease incidence ranged between 13 and 18%. After two years, the percentage of symptomatic trees was 20% lower in the area with eradication program. The use of kaolin against the vector *Diaphorina citri* was reducing the infestation at very low percentages. In Guadeloupe, under very high HLB levels and mortality, different combinations of rootstocks / varieties were selected to study the impact of polyploidy on disease tolerance. In phloem petiole observed under scanning electron microscopy, a more limited callose deposition into sieve pores was observed in triploid compared to diploid genotypes; moreover, a more limited oxidative stress was observed in triploid petiole and tetraploid root samples compared to diploids. The impact of cultivation practices as adapted nutrition and fertilization, seems to be crucial in maintaining the trees productive under HLB.

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Reverse transcription-droplet digital PCR for plum pox virus detection and quantification

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Plum pox virus (PPV) is the causal agent of sharka, one of the most detrimental diseases of stone fruits worldwide. PPV is one of the "top ten" viruses in molecular plant pathology. Currently most sensitive detection methods use reverse transcription real-time PCR (RT-qPCR), which needs a known standard to quantify the sample. Droplet digital PCR (ddPCR) is the third-generation PCR technique that overcomes this limitation, giving an absolute quantification. In this study, a validated one step RT-qPCR system was optimized and transferred to one step RT-ddPCR. Both methods were validated according to the European and Mediterranean Plant Protection Organization (EPPO) recommendations on sensitivity, specificity, selectivity, repeatability and reproducibility. PPV was detected and quantified using plant-infected RNA. Interestingly, not only RT-qPCR but also RT-ddPCR was

suitable for plant-PPV-infected crude extract, suggesting a possible faster, cheaper detection. The methods succeeded in detecting 5 different PPV strains (Marcus, Dideron, Rec, El Amar, and Sweet Cherry) coming from different countries and four *Prunus* species. The exclusivity tests confirmed there were not signals when using *Prunus*-related viruses/viroids. Comparisons between the methods demonstrated that RT-ddPCR is 10 times more sensitive than the RT-qPCR when using total RNA from infected plants, while showed the same detection limit when using crude extracts. The obtained results shed light on this useful tool for the early detection and absolute quantification of PPV strains in *Prunus* species.

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Validation of high-throughput sequencing (HTS) for routine plant virus diagnostics

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The credibility of a pathogen detection assay is measured using specific parameters including repeatability, specificity, sensitivity, and reproducibility. The use of high-throughput sequencing (HTS) as a routine detection assay for viruses and viroids in citrus was evaluated. Plant infections were established by graft inoculating healthy seedlings with a suite of viruses and viroids. Plants were sampled in triplicate and total RNA was extracted using two different methods and sent for HTS on both the Illumina and Ion Torrent platforms. The data were evaluated for biological and technical variation focussing on RNA extraction method, platform used and bioinformatic analyses. To evaluate the reproducibility of HTS, the same plants were evaluated again, one year later. The sensitivity of the HTS assay was compared to routinely used RT-PCR assays in a time course experiment. Both extraction method and sequencing platform resulted in significant differences between the data sets. The expression profiles of reference genes were also investigated to assess the suitability of these genes as internal controls to allow for the comparison between samples across different protocols. Even though the limit of detection of HTS was influenced by pathogen concentration, sample processing method and sequencing depth, HTS detection in this study was found to be either equivalent or more sensitive than RT-PCR. HTS is more comprehensive than any other assay and with the necessary validations the implementation of HTS as part of routine pathogen screening practices is possible.

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Strawberry angular leafspot: efficacy of biocontrol agents and acibenzolar-S-methyl towards *Xanthomonas fragariae*

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The angular leafspot of strawberry (ALS) is present in all areas where strawberry is cultivated, and in nurseries it represents a significant problem for the production of pathogen-free plant material. The control of *Xanthomonas fragariae* (Xf) is mainly achieved through preventive treatments based on copper compounds or antibiotics, where they are allowed. The efficacy of three bacterial antagonists (*Bacillus* sp.; D747 and QST713; *Pseudomonas fluorescens*: BB20), two essential oil (EO)-based products (Vitibiosap Plus® 458 and Vitibiosap Plus® 1R45) and the resistance inducer Bion® was tested against Xf in vitro and in vivo. All the biocontrol agents were able to directly reduce the pathogen growth in vitro, whereas Bion®, as expected, resulted ineffective. Under climatic chamber conditions, the three antagonists and the resistance inducer reduced the ALS severity and provided approx. 50% and 38% relative protection, respectively; besides, the two EO-based products provided approx. 40% relative protection. In addition, during the two years field trial, the EO-based products were able to significantly reduce ALS severity with a relative protection ranging to 60 to 80%, compared to the conventional treatments with copper compounds. Indeed, biological agents, such as bacterial antagonists or EO, and resistance inducers could represent an effective alternative in the framework of integrated control strategies.

An emerging disease challenges the use of cherry laurel (*Prunus laurocerasus* L.) as a hedge plant in urban and periurban landscapes

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In the past five years extensive dieback of cherry laurel (*Prunus laurocerasus* L.) hedges has been observed with increasing frequency in the metropolitan area of Florence, Italy. Affected plants show bark necrosis and cankers of the lignified crown down to the collar with mucilage bleeding and gummosis. In the early disease stages the hedge appears healthy from the outside, although on closer inspection the first symptoms are present on the woody crown organs. In the more advanced stages internerval leaf chlorosis is followed by marginal orange spots progressing towards the centre of the leaf blade. The leaves turn rust-coloured, dry out and remain attached to the branches. Over time, the crown dries out and the plant dies. Isolations from symptomatic branches at numerous sites over the years revealed the constant presence of *Diplodia seriata* and other associated fungal pathogens with varying frequency. Disease monitoring and assessment (over 250 hedge plants scattered in the territory) revealed the ubiquitous presence of the disease (100% of the sampled sites affected), and extensive crown damage (85% of sampled plants affected) often without apparent foliar symptoms, since they usually become evident in later disease stages. Data analysis and inoculation tests suggest multiple disease drivers including rising temperatures that may favour *D. seriata* and alter host defenses by increasing water shortage. Results suggest that greater damage will occur in the coming years. The spread of the disease and the severity of the damage call into question the customary use of cherry laurel as a hedge plant.

Peptide analogs of a *Trichoderma* peptaibol effectively control downy mildew in leaf disc assay and vineyard

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Grapevine (*Vitis vinifera* L.) is one of the main crops worldwide. *Plasmopara viticola*, the causal agent of downy mildew, causes enormous economic damage in terms of yield, quality and productivity to this crop. Although the disease control is largely based on the use of synthetic fungicides, the European Union (UE) policies promote the reduction of the reliance on synthetic plant protection products (PPPs) and the implementation of integrated approaches to crop protection. Biocontrol agents (BCA) constitute a great resource for the development of biopesticides. The fungal genus *Trichoderma* includes different species commonly used as BCAs against several crop pathogens and among their antifungal strategies, there are secondary metabolites such as peptaibols. The promise of peptaibols as agrochemicals is, however, hampered by their poor water solubility, preventing an efficient delivery for practical use in crop protection. Some water-soluble trichogin analogs with Gly to Lys substitutions were synthesized and analyzed for their antimicrobial activity against *P. viticola*. Among the peptides that proved effective in inhibiting the oomycete sporulation on leaf discs assay, the peptide 4r was selected for a two-year field trial experiment. In comparison with the untreated control, the protection level was comparable to that obtained with a cupric fungicide. The peptide did not show any phytotoxic effect and reduced downy mildew incidence and severity on leaves and bunches. Since it was demonstrated active also against the fungal pathogen *Botrytis cinerea*, the peptide 4r may be a candidate broad-spectrum fungicide whose biological properties deserve further investigations.

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The high complexity of soybean anthracnose revealed by comparative genomics and transcriptomic analyses

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Soybean (*Glycine max*) is one of the most important crops worldwide. A major limiting factor of soybean production is anthracnose, a disease caused by fungi belonging to the genus *Colletotrichum*. We performed a survey based on publicly available genomic data and showed that at least 12 *Colletotrichum* lineages are associated with soybean, with the *C. truncatum* species complex (s.c.) and *C. orchidearum* s.c. having the greatest impact and the broadest worldwide distribution. We used a combined approach of biological experiments with 'omic' technologies to gain a better understanding into the evolution of *Colletotrichum* species associated to soybean and the molecular basis of soybean resistance. The genomes of *C. truncatum*, *C. musicola*, *C. plurivorum* and *C. sojae* were sequenced and compared to eight additional *Colletotrichum* species not pathogenic to soybean. Our results show that *C. truncatum* and the three species belonging to the *C. orchidearum* s.c. did not evolve the capability to infect soybean from a common ancestor, due to the absence of effector candidates shared only among these four species. Therefore, we selected *C. truncatum* as a model system to study the molecular interaction with soybean. Pathogenicity assays revealed that the interaction between *C. truncatum* and soybean is genotype dependent, meaning that a soybean cultivar can present contrasting levels of resistance to different strains of *C. truncatum*, and a strain of *C. truncatum* can have different levels of aggressiveness in different soybean cultivars. Two soybean cultivars and two strains of *C. truncatum* were selected for cross inoculation assays. The transcriptomes of the four interactions were sequenced and analyzed in a time course experiment, revealing a coordinated pattern of gene expression over time for the resistant interactions, including the over expression of genes involved in pathogen recognition, signaling and defense responses. Our results show a strong correlation between plant/pathogen recognition and the activation of defense response opening new perspectives in the complexity of this pathosystem.

Exploiting the seed associated endophytes in *Brassica oleracea* genotypes as a potential source for plant growth promoting bacteria and biological control agents

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Plant seeds are characterised by a wide diversity of microbial communities which interact with the plant during its development. Seed associated microbial communities are influenced by genotype, environmental condition and crop management practices and may promote plant growth and act as biological control agents. Studies on seed microbiome associated with brassica seeds were mainly focused on canola, whereas there is a paucity of information on seed microbial signature in other *Brassica* genotypes. In order to explore the seed bacterial community structure, seed batches from *Brassica oleracea* groups were analysed for seed-originating endophytic bacteria with the aim to select beneficial microorganisms with potential application in suitable crop protection. Seed samples were analysed by direct isolation techniques after seed sterilisation and bacterial isolates were identified based on the PCR amplification of partial 16S rDNA gene sequences. Screening for plant growth-promoting activities revealed that most of the isolates showed PGPR traits and produced ACC deaminase, siderophores and were able to solubilize phosphate and to grow in presence of 4% and/or 8% NaCl. *In vitro* tests showed that some bacterial isolates inhibited the growth of both fungal and bacterial strains including the seed-borne brassica pathogens *Xanthomonas campestris* pv. *campestris*, *X. campestris* pv. *raphani, Leptosphaeria maculans* and *Alternaria brassicicola*. Bacterial isolates sharing PGPR traits and direct antagonistic activity against all tested pathogens were also identified. Preliminary results indicated that seeds of *Brassica oleracea* genotypes store their own beneficial inoculum which may be involved in maintaining plant health. Further studies are ongoing to analyse the *in vivo* bacterial endophyte-plant interaction after seed treatments.

In vitro exploitation of bioactivity of brassica extract on the growth of plant pathogenic bacteria and fungi

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In recent years, there has been a growing interest in exploitation and developing new environmentally sustainable solutions to counteract plant disease. Glucosinolates are

secondary metabolites found almost exclusively in Brassicaceae family and are important contributors to the health benefits of these plants. In this study, the activity of a natural brassica extract coupled with propolis was evaluated *in vitro* against plant pathogenic bacteria and fungi at three different concentrations 1.0, 1.5 and 3.0 mL/L according to the recommendations of the company who provided them. Antimicrobial activity was tested against the fungal pathogens *Alternaria brassicicola, Neocosmospora phaseoli, Fusarium oxysporum* f. sp. *lycopersici* and *Sclerotinia sclerotiorum* by the poisoned food method. The natural compound induced a reduction of mycelial growth of all tested fungal isolates with significant differences compared to the control. Inhibitory effect was dose dependent, varied according to the fungal species and decreased with the time. All the extracts at 3.0 mL/L concentration reduced the fungal growth by 70–100% up to about seven days after inoculation. No activity was observed by well diffusion assay against the plant pathogenic bacteria *Pseudomonas savastanoi* pv. *phaseolicola*, *P. syringae* pv. *tomato*, *Xanthomonas vesicatoria, X. campestris* pv. *campestris* and *Clavibacter michiganensis* subsp. *michiganensis*. The interference of the natural compound with the kinetic growth of all bacterial strains was further evaluated by an automated turbidimetry-based system and the AUC (area under curve) was calculated. All concentrations of the natural compound induced a growth reduction of all strains with a higher AUC reduction in presence of the higher concentration.

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Phytophthora: an ancient, historic, biologically and structurally cohesive and evolutionarily successful generic concept in need of preservation

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In response to evidence that the 20 genera of the obligately biotrophic, angiosperm-foliage specialised downy mildews (DMs) evolved from *Phytophthora* at least twice via convergent evolution, making the DMs as a group polyphyletic and *Phytophthora* paraphyletic in cladistic terms, a proposal has been made to split the genus into multiple new genera. We have reviewed the status of *Phytophthora* and its relationship to the DMs. Currently some 200 species are distributed across twelve major phylogenetic clades that, based on our assessment for twenty morphological and behavioural criteria, show good biological cohesion. Saprotrophy, necrotrophy and hemi-biotrophy of woody and non-woody roots, stems and foliage occurs across the clades. Phylogenetically less related clades often show strong phenotypic and behavioural similarities and no one clade or group of clades shows the synapomorphies that might justify a unique generic status. The proposal to divide *Phytophthora* appears more a device to address the issue of the convergent evolution of the DMs than the structure of *Phytophthora* per se. We consider it non-Darwinian, putting the emphasis on the emergent groups (the DMs) rather than the progenitor (*Phytophthora*) and ignoring the evolutionary processes that gave rise to the divergence. Considering the biological and structural cohesion of *Phytophthora*, its historic and social impacts and its importance in scientific communication and biosecurity protocol, we recommend that the current broad generic concept is retained by the scientific community.

Emerging *Phytophthora*-related diseases in the subalpine European vegetation

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Extensive decline and mortality of shrubs and trees typical of alpine areas has recently been observed in Southern Europe. Decline phenomena affecting different ecological niches such as riparian systems, forest margins and Alpine heathlands were characterized by a complex symptomatology including foliar necroses, shoot blight and bleeding cankers. Infections resulted in progressively widening patches of dying vegetation, suggesting the involvement of airborne *Phytophthora* species in the aetiology. Between 2019 and 2021, an in-depth study aimed to determine the impact and the diversity of *Phytophthora* species involved in these phenomena, was carried out on 47 different sites distributed in the mountainous areas of Sardinia, Tuscany Apennines and North-eastern Alpine regions among Italy, Austria, and Slovenia. A total of 286 *Phytophthora* isolates were obtained from 322 symptomatic samples collected from 26 plant species. Isolates have been identified as *Phytophthora pseudosyringae* (169 isolates), *P. plurivora* (39), *P. ilicis* (20), *P. acerina* (13), *P. alpina* (6), *P. cactorum* (6), *P. gonapodyides* (6), *P. idaei* (4), *P. cambivora* (3), *P. psychrophila* (3), *P. pseudocryptogea* (2), *P. bilorbang* (2), *P. gregata* (2), *P. hedraiandra* (2) and *P. kelmanii*

(1). The results highlighted a wide diversity of *Phytophthora* belonging to the clade 1 and 3, species able to survive under cold conditions and to cause aerial infections, due to the production of caducous sporangia. Among the different species, *Phytophthora pseudosyringae* was identified as the key pathogen in these habitats, being isolated from 22 out of 26 investigated hosts.

The fungal and bacterial bunch microbiome associated with sour rot in vineyards

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Sour rot (SR) is a grapevine disease complex that is not completely understood for both etiology and epidemiology. In recent years, SR has received special attention due to its increasing economic importance due to the heavy crop losses and reduced wine quality it can cause. In this study, the bacterial and fungal microbiota of healthy and affected (i.e., showing SR symptoms) ripe bunches were characterized across 47 epidemics (38 vineyards in 5 grape-growing areas) over a 3-year period. The 16S rRNA gene and ITS high-throughput amplicon sequencing, as well as quantitative PCR (qPCR), were used to assess the relative abundance and dynamic changes of microorganisms associated with SR. The estimators of genera richness within samples indicated that diversity was not significantly different between healthy and affected bunches. When the community composition between samples was evaluated, the bunch status (i.e., healthy and affected) was a significant source of diversity ($p < 0.05$), indicating that microbiome composition varied between healthy and affected bunches. In particular, these two categories shared 43.1% and 56.5% of fungal and bacterial genera, respectively; 31.6% (fungal) and 26.1% (bacterial) genera were associated with affected bunches only. Based on a linear discriminant analysis, the following bacteria: *Orbus* and *Gluconobacter* spp., and fungi *Zygosaccharomyces*, *Pichia*, *Issatchenkia*, and *Zygoascus* spp. were closely associated with bunches showing SR symptoms. Understanding the diversity of the microflora associated with SR affected bunches is the first step for the development of effective protection strategies.

Solid-State fermentation of organic wastes: a source of bioactive molecules

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Fungi used in biocontrol often exert their activity through the production of a wide-range of bioactive secondary metabolites, which could be exploited for the development of bio-pesticides, bio-stimulants and medicines. In this work, we evaluated the possibility of using digestate as a source for the inoculation in the open field of beneficial microorganisms, including the associated molecules with high added value, such as organic acids and enzymes. Digestate is a by-product of anaerobic digestion mainly used as a fertilizer in the agricultural field. Whole digestate, suitably mixed with agro-food waste, was used as a substrate for Solid State fermentation (SSF) by different fungal genera. The digestate-based substrates have allowed the growth of fungi belonging to the genera *Aspergillus*, *Penicillium*, *Cryphonectria* and *Trichoderma*. The aqueous crude extracts obtained from SSF of four *Trichoderma* sp. were tested for enzymes production, organic acids and bioactive molecules. Enzymatic assay revealed cellulase and esterase activities. Citric acid and other secondary metabolites produced during fermentation were detected by UPLC-QTOF-MS analysis. Moreover, experimental evaluation of germination parameters (germination index) highlighted a strong promotion of tomato seed germination and root elongation induced by *T. harzianum* and *T. atroviridae* crude extracts from SSF. This study suggests an innovative use of the whole digestate mixed with agro-food waste as a valuable substrate for growing plant growth-promoting fungi and for producing bioactive molecules. We are currently evaluating metabolites with biocontrol potential from the same extracts.

A phytocomplex obtained from a *Salvia officinalis* cell culture effectively controls the grapevine downy mildew pathogen *Plasmopara viticola*

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The impact of conventional fungicides on environmental and human health has increased the interest in safer alternatives such as plant secondary metabolites, generally having

a better toxicological profile. However, plant genetics, agronomic practices, climatic conditions and extraction methods strongly affect the quality and quantity of secondary metabolites obtained from field grown plants. These factors cause limitations to the standardization needed for industrial production. Plant cell culture technology can meet this need: totipotent cells grown in controlled conditions can provide a highly homogeneous biomass with specific chemical characteristics. A phytocomplex, with standardized rosmarinic acid content, was obtained from a selected cell line of *Salvia officinalis.* The *Salvia officinalis* phytocomplex (SOP) was tested against the grapevine downy mildew pathogen, *Plasmopara viticola*. Grapevine leaf disks were sprayed with SOP and with fresh *P. viticola* sporangia. Sporulation level on each disk was assessed after 7 days with an image processing software. SOP at 5 g/l reduced by 95% the sporulation level compared to the control treatment. SOP was significantly more effective than rosmarinic acid alone, tested at the concentration found in SOP. Persistence of the phytocomplex was also assessed: leaves were sprayed with SOP and, after a few days, they were detached and inoculated. SOP applied 5 days before inoculation reduced by 90% the sporulation level compared to the control. These results highlight the possibility for plant protection industry to take advantage of cell culture techniques to produce safer pesticides with high quality standards.

The stress related – calcium dependent – transcription factor CRZ1 is a pivotal factor in synchronising host perception and fumonisins biosynthesis in the *Fusarium verticillioides- Zea mays* interaction

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Calmodulin (CaM), the main calcium binding protein in eukaryotes, acts as a part of calcium signal transduction pathway, involved also in colony growth, stress response and pathogenicity regulation in fungi. Here we investigated the role of the fungal protein Crz1, a downstream transcription factor of the CaM pathway, in the fumonisin (FUM)-producing fungus *Fusarium verticillioides*. Previous studies have shown that the trigger for the onset of FUM synthesis in maize by *Fusarium* is related to some specific fatty acids (FAs) and oxylipins (e.g., oxidised FAs) that can play, during the infection, as intra-extra cellular signals exchanged by the pathogen and its host. In this study, we suggest the role of Crz1 as regulator and "synchronizer" of lipid metabolism and FUM biosynthesis during the *F. verticillioides-Zea mays* interaction. To investigate the possible role of the transcription factor during the infection, we found through a mass spectrometry and transcriptomic approach that the deletion of *crz1* was consistently associated with an overall reduction in oxylipin, FA and FUM expression and amount in the infected kernels, whilst this deletion did not affect the fungal lipid metabolism (under *in vitro* growth); this evidence suggests a key role for Crz1 in regulating this complex and interconnected pathways during the host–pathogen interaction. We postulate that Crz1 controls the production of secreted signals during the interaction with the host that can trigger the oxylipin pathways in maize. In turn, the oxylipins (e.g. 9-HPODE) generated by maize would provide feedback to the fungus to switch on the biosynthesis of FUM as already stated elsewhere.

Monitoring and characterisation of *Colletotrichum* species associated with bitter rot isolated from diseased apple in the Emilia-Romagna region orchards

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Italy is one of the major apple producers in Europe, ranking second after Poland. An important disease that affects apples both pre-harvest and post-harvest is bitter rot. In Europe, the species causing the most significant losses in the orchard during the vegetative period belong to the *Colletotrichum acutatum* species complex. Over the past few years, however, species of the *Colletotrichum gloeosporioides* complex are increasingly emerging as bitter rot pathogens. In Italy in 2019, a serious outbreak of fruit with bitter rot symptoms was observed in commercial apple orchards of the 'Pink Lady' variety in the Emilia- Romagna region. One of the peculiarities of *Colletotrichum* species is to infect apple fruits through lenticels in pre-harvest, remain latent and develop symptoms after months of cold storage under controlled atmosphere, leading to economic losses of the product. Therefore, the aim of this work is monitoring the incidence of bitter rot in apple orchards in the Emilia-Romagna region and bordering areas. Moreover, special attention will

be given to the phenotypic and molecular characterisation, with the application of multilocus sequencing typing, of emerging species belonging to the *C. gloeosporioides* complex isolated from leaves and fruits with symptoms related to bitter rot. Knowledge of the incidence of bitter rot in apple orchards in Emilia Romagna, as well as accurate identification of the pathogenic species will be of paramount interest in order to implement targeted disease control systems in pre-harvest. Furthermore, to date no post-harvest treatment is able to eradicate latent infection of apple fruits during cold storage, which means that monitoring of these pathogens in pre-harvest is essential to prevent post-harvest losses.

Development and validation of a mechanistic model for the prediction of hazelnut defects caused by *Diaporthe eres*

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Hazelnuts are high-value products that suffer defects sometimes associated with off flavours. They cause yield losses due to non-compliance with the required market standards. The browning of the internal tissues of kernels, which is visible when nuts are cut in half, discolouration and brown spots on the kernels surface are important defects mainly attributed to *Diaporthe eres*. Timely indications on the expected incidence of defective hazelnuts would be essential both for buyer supplies, to identify risk areas for uncompliant products and for farmers to have timely warnings of *D. eres* infection and defect outbreak. Knowledge regarding this fungus and its interaction with hazelnut is poor. Nevertheless, a mechanistic model was developed using information regarding *Diaporthe* infection cycle and *D. eres* ecological needs. The predictive model inputs hourly meteorological data (air temperature, relative humidity and rainfall). The output is the risk of hazelnut defects at the end of the growing season. Georeferenced data on the occurrence of hazelnut defects from 2014 to 2019 were collected from orchards in the Caucasus region and Turkey to validate the model. Hourly meteorological data from weather stations close to the hazelnut orchards selected were used to run the model. Then, using appropriate statistical techniques, predictions were compared with observed data (480 records). Overall, correct predictions were > 80%, with < 16% of false negative. The results confirmed the developed model's accuracy in predicting hazelnut defects caused by *D. eres*.

Scientific community actions and reactions to the predicted impact of climate change on aflatoxin

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Climate change (CC) is confirmed as a driver for emerging food/feed safety issues worldwide, and its expected impact on the presence of mycotoxins in commodities is of great concern. Aflatoxins (AF) have the highest toxicity of all mycotoxins and are regulated in food/feed worldwide. As highlighted in 2011 by a project supported by EFSA, CC is predicted to increase the risk of AF contamination in maize. In 2021 a comprehensive literature search was performed using the Scopus search engine to extract peer-reviewed studies related to this prediction. A total of 224 papers were identified after step I filtering, while step II filtering identified 25 papers for quantitative analysis. The unselected papers (199) were categorized as "actions" because they provided a sounding board for the expected impact of CC on AFB1 contamination without adding new data on the topic. The remaining papers were considered "reactions" of the scientific community because they went further in data and ideas reporting. Interesting statements taken from the "reactions" could be summarized with keywords: chain and multi-actor approach, intersectoral and multidisciplinary, resilience, human and animal health, and global vision. In addition, fields deserving increased research efforts were summarized as: i) improvement of predictive modelling, ii) extension to different crops and geographic areas, and ii) impact of CC on fungi and mycotoxin co-occurrence, both in crops and their value chains, up to the consumers. Ten-years of work after the prediction release indicated improved approaches but also emphasized that relevant actions are still requested.

Phloem-restricted phytoplasmas impair carbon fixation in tomato

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Phytoplasmas are prokaryotic obligated parasites of plants that colonize the sieve elements of their host plant, causing alterations in phloem function such as occlusion, nutrients uptake and utilization, impairment of photosynthesis and photo assimilates translocation. Symptoms have been well described and include inhibition and decline of photosynthesis efficiency and alterations in sugar metabolism. Our research group has been investigating the effects of 'Candidatus *Phytoplasma solani*' infection on tomato plants (*Solanum lycopersicum* cv. Micro-Tom) by RNA-sequencing. Analyzing tomato plants grown under different Fe regimes, we demonstrated that infection changes Fe distribution in leaves, affects photosynthetic machinery and perturbs shoot-to-root communication. Moreover, infection impacts mineral nutrient fluxes and alters ion homeostasis of K, Ca, Mg, Fe and Mn. Here, we focus our analysis on the transcriptional regulation of genes involved on carbon fixation and carbon metabolism. A gene co-expression network was investigated with a weighted correlation approach implemented in the R package WGCNA. Gene expression and protein analysis highlighted key genes of carbon fixation pathway and confirmed impairment of carbon metabolism.

Isolation and molecular characterization of endophyte bacteria in three different durum wheat cultivars susceptible to *Fusarium* Foot and Crown Rot

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Triticum durum is one of the world's most important food crops and Italy is one of the main producers and consumers of this cereal, that unfortunately is susceptible to the attack of microorganisms responsible for different diseases causing both quantitative and qualitative damages. Specifically, two main diseases that affect durum wheat in the early stages of development are *Fusarium* Foot Rot (FFR) and *Fusarium* Crown Rot (FCR). The main fungal species involved in the process belong to the genus *Fusarium*, specifically *F. culmorum*, *F. graminearum* and *F. pseudograminearum*. These species can act alone or in combination, affecting the roots and the internodal portions of the culm, and are responsible for the production of high levels of a wide variety of mycotoxins. The use of organic seed coating technique with endophytic bacteria, that colonize healthy plant tissue without causing obvious disease symptoms in host plant, may represent a reliable and safe strategy able to control soil-borne pathogens. The present investigation was carried out to analyze the microbiome of three durum wheat varieties viz. Claudio, Marco Aurelio and Odisseo to identify potential biocontrol agents. A total of 64 bacterial endophytes were isolated from crown and root tissues. Molecular characterization of all the strains was carried out by partial PCR amplification of 16S rRNA gene using the primers 27F and 1492R. Isolated endophytes are closely related phylogenetically to *Pseudomonas*, *Bacillus, Stenotrophomonas, Microbacterium, Achromobacter, Ochrobactrum, Peribacillus, Arthrobacter,* and *Rhizobium* by sequence analysis.

Pomegranate and citrus fruit rots caused by *Alternaria* species in Albania

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The genus *Alternaria* is a relevant fungal pathogen for several commodities including citrus and pomegranate fruits. On citrus, it mostly causes brown spots on fruits and leaves, whereas, on pomegranate, it primarily causes a fruit heart rot. In the present study the Alternaria rots on citrus and pomegranate fruits cultivated in Albania was assessed. Representative fruits were collected from different regions. Nineteen and thirteen *Alternaria* isolates were obtained from pomegranate and citrus samples, respectively. The isolates were identified at species and morphotype level. Micro- and macroscopic features separated isolates into four morphotypes. BLAST and phylogenetic analysis using the SCAR Marker OPA1-3 confirmed the isolate identity. All 32 isolates proved to be *Alternaria alternata* and to mostly belong to morphotype *alternata*, followed by *limoniasperae* and *tenuissima*. All *Alternaria* strains proved to possess the *pksI* gene of the mycotoxin alternariol biosynthesis. Citrus isolates were tested for the presence of genes of the biosynthesis of the phytotoxins ACT and ACR, but none of them proved to possess them. Concluding, *Alternaria* spp. might represent a treat to pomegranate and citrus production in Albania and commercialised abroad, and thus effective diagnostic and control means are needed.

Cortical necrosis of grapevine (known as "excoriose") and Diaporthe dieback: an undervalued damage to grape production

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Damages on grapevine caused by *Diaporthe* species are increasingly reported both on wine grape and table grape. Several species are detected, the most common of which is *Diaporthe ampelina*, the currently correct name of the anamorph *Phomopsis viticola*, the agent of cortical necrosis (often erroneously referred to as excoriose). The increase in the incidence of the disease in recent years is due to a series of causes such as scarce or not careful monitoring in the vineyard due to the limited importance given to fungi of this genus, considered minor pathogens compared to others associated with wood diseases of the grapevine (Grapevine Trunk Diseases, or GTDs). In Italy the main GTD is surely the well know Esca complex. In a recent survey in a vineyard where heavy damages by esca were reported a high incidence of spur dieback was noticed that was considered as one of the symptoms caused by the esca agents. Sectorial necrosis or total necrosis of the spur could be observed when cross cutting the spurs, cordon or trunk. A careful monitoring revealed a high presence of cortical necrosis symptoms in the basal internodes of the canes. Field survey and sampling proved the spur dieback to be actually strongly associated to *Diaporthe* species colonizing the wood starting from pruning wounds infections, up to causing the death of the whole cordon, that could be described as a Diaporthe dieback. The relevance of detecting the wood disease actually present in the vineyard is discussed in view of setting up efficient preventive strategies.

Complete genomes of several '*Candidatus* Phytoplasma solani' strains causing different symptoms in experimental host *Solanum lycopersicum*

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'*Candidatus* Phytoplasma (*Ca*. P.) solani' (16SrXII-A) is associated with bois noir (BN) of grapevine and stolbur disease of herbaceous plants and is primarily transmitted by *Hyalesthes obsoletus* Signoret. The aim of our study is to investigate the interaction of different '*Ca*. P. solani' strains with tomato (*Solanum lycopersicum* L. cv. Micro-Tom). Strains of '*Ca*. P. solani' were acquired by individuals of *H. obsoletus* captured on bindweed and stinging nettle in vineyards of northeastern Italy with high incidence of BN and forced to feed on tomato plants. Strains originating in stinging nettle and bindweed belonged to tuf-a and tuf-b genotypes, respectively, as expected. The genomes of four strains were shotgun sequenced by Oxford Nanopore Technology (ONT) and NovaSeq (Illumina). The complete and circular genomes of three '*Ca*. P. solani' tuf-b and one tuf-a strains with a size between 751,188 bp and 973,640 bp, were reconstructed by hybrid assembly. The number of automatically annotated CDS varied between 725 and 1064. The four genome sequences were mined for the presence of candidate effector genes by signal peptide (SP) and transmembrane signal peptide (TMSP) predictions. The protein predicted as SP from Phobius ranged between 23 and 40; in particular, all strains lacked TENGU-like proteins, whereas proteins like SAP11, SAP54/PHIL1 and SAP05 were found. Comparisons with already known phytoplasma effector proteins and putative secreted proteins evidenced strain-specific sets of effectors that may explain the quite different symptoms induced on tomato.

Insight of tomato brown rugose fruit virus (ToBRFV) dispersion applying remote RT-qPCR procedures in tomato production environment

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Tomato brown rugose fruit virus (ToBRFV) causes severe disease in tomato crops. After its outbreak in Sicily (2018), it still represents a serious threat for tomato production.

In the present work, during 2021, to assess the ToBRFV dispersion in the main tomato production Sicilian areas, five tomato commercial greenhouses with 600 tomato plants each (3 in Ragusa province, 1 in Syracuse province, 1 in Agrigento province) were monitored with the bCUBE® portable system (Hyris Ltd). The ToBRFV dispersion was studied using a network of mini-laboratories for early in-field ToBRFV detection, established and monitored by the Plant

Virology Laboratory at the University of Palermo for remote network management, data visualization, and analysis confirmation. For each greenhouse, three samples per month were collected, for eight months. Each sample, consisting of 200 plants pool (~0.5 cm^2 leaf portion from each plant), was tested by RT-qPCR developed by Panno and co-workers (2019) using the bCUBE® system. To confirm the bCUBE® analyses, 20% of the samples were also analyzed at the Plant Virology laboratory. The 25% of the samples resulted positive to ToBRFV with the bCUBE® system, confirmed by the laboratory analyses. The positive samples were sequenced in both directions, showing a 100% identity with the previously characterized ToB-SIC01/19 Sicilian isolate. The developed mini-laboratories network allowed continuous ToBRFV monitoring throughout the tomato production. Compared to previous years, a decrease of ToBRFV dispersion was observed, thanks to early in-field diagnosis using the bCUBE® diagnostic network, continuous monitoring by phytosanitary services and the prophylactic measures implementation.

Survey of main grapevine cultivars for grapevine fanleaf virus dispersion in Sicily

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Grapevine (*Vitis vinifera* L.) is one of the most important crops worldwide. Grapevine cultivation is undermined by several viral diseases, such as grapevine fanleaf degeneration disease caused by grapevine fanleaf virus (GFLV) (family *Secoviridae*). In this survey, twenty commercial vineyards in four Sicilian provinces (Italy) were investigated for GFLV presence, studying its genetic structure and molecular variability. A total of 800 grapevine samples from eleven autochthonous Sicilian cultivars (cvs) were collected and analyzed by end-point RT-PCR using specific primers for the *CP* gene. Forty-eight out of 800 tested samples resulted positive to GFLV, with 6% of the overall infection rate. The highest percentage of positive samples was recorded in cvs "Nerello Cappuccio", "Nero d'Avola" and "Catarratto", with an incidence of 26.8%, 12.3%, and 9.6%, respectively, while in cvs "Grillo" and "Moscato" no infection was found, possibly related to the different agronomic cultivation practices. Phylogenetic analyses showed that the 18 selected Sicilian GFLV-*CP* sequences were grouped in five sub-clades inside the same cluster with sequences from Italy and other countries. Recombination analyses detected only putative recombination events among Sicilian isolates, while nucleotide diversity analysis showed a very low differentiation within Italian isolates (0.1258 ± 0.008) and between isolates from Italy and from other countries. The hypothesis of negative selection was confirmed by the dN/dS ratio of 0.249. The results obtained highlighted a certain degree of variability within the sequences obtained, suggesting a different origin, probably due to the continuous interchange of plant propagation material with other Italian regions or European countries.

Genetic structure, molecular variability and spread of grapevine leafroll-associated virus 1 and 3 in Sicilian autochthonous grapevine cultivars

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Grapevine leafroll disease is one of the most important viral diseases caused by several viruses of the *Closteroviridae* family, including grapevine leafroll-associated viruses (GLRaV) 1 and 3. In this study, we investigated the genetic structure, molecular diversity and spread of GLRaV-1 and GLRaV-3 in 800 grapevine samples, collected from eleven autochthonous cultivars in four Sicilian provinces. Serological analyses were performed using GLRaV-1/GLRaV-3 polyclonal antibodies by DAS-ELISA; molecular analyses were conducted to discriminate single and/or mixed infections, using specific primers for the *CP* gene of GLRaV-1 and 3. Thirty-three and 138 samples resulted positive to GLRaV-1 and GLRaV-3, with a 4.1% and 17.3% infection rate, respectively; only 15 samples showed a mixed infection, with a 1.9% incidence. Both viruses were not detected in 'Grillo' and 'Moscato' cultivars. Phylogenetic analyses conducted on 12 GLRaV-1 selected *CP* sequences showed a close relationship with European isolates. The analyses conducted on 31 GLRaV-3 selected *CP* sequences demonstrate a close relationship between Sicilian and isolates from different countries. Regarding GLRaV-1, the discrete nucleotide differentiation and positive selection (dN/dS: 3.147) could indicate a current increase in population fitness; a certain stability of GLRaV-3 in Sicilian cultivars is suggested by the high nucleotide differentiation and

negative selection (dN/dS: 0.2519). A different incidence of both viruses was detected in the main Sicilian cultivars, and it was the highest in 'Carricante' for GLRaV-1 and 'Nero d'Avola' for GLRaV-3. The absence of GLRaV-1 in 'Grillo', 'Nerello Cappuccio', and 'Moscato' could be due to a low prevalence of infected propagation material and a possible low presence of GLRaV-1 vectors.

Insights into the grapevine virus A spread in Sicily: epidemiological and evolutionary analysis

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Sicily is one of the most important Italian regions for the grapevine industry. Grapevines are threatened by several pests and pathogens including different viral diseases. Among these, phloem-restricted grapevine virus A (GVA) of the family *Betaflexiviridae* causes losses of 5–22%. In this study, the GVA epidemiology, genetic structure and molecular variability were ascertained in twenty commercial vineyards of four Sicilian provinces. Eleven autochthonous cultivars were investigated and a total of 800 samples were analyzed by end-point RT-PCR assay, using specific primers for the *CP* gene. Only forty-nine positive samples were detected, with an infection rate of 6.1%. Ten out of 11 cultivars analyzed resulted positive to GVA infection; a higher incidence was observed in 'Nerello Mascalese', 'Carricante', 'Perricone' and 'Nero d'Avola', with an infection rate of 30%, 12.8%, 7.7% and 4.8%, respectively, while in 'Moscato' cultivar no infection was found. Evolutionary relationships showed that the sixteen GVA-*CP* selected sequences were separated into two clusters. All Sicilian isolates were grouped into the same sub-clade with the other three Italian GVA isolates, showing a low variability. Recombination analyses identified putative recombination events among the Sicilian isolates analyzed, while the analysis of the nucleotide diversity showed a low differentiation within Italian isolates (0.0681 ± 0.015). The dN/dS ratio of 0.099 confirmed the hypothesis of negative selection. This study revealed a low variability degree among the Sicilian isolates, and the absence of candidate recombination events, suggesting a common origin, probably due to the exchange of infected propagation material within the Italian territory.

Inoculation of *Citrus* relatives ornamental rutaceous and rootstocks highlights a different replication rate of local CTV isolates

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The study of the effects of Citrus tristeza virus (CTV) on different host plants has been attempted in many citrus countries worldwide. Within the project SIRPA several ornamental rutaceous grafted onto seedling rootstocks were bark patch inoculated in greenhouse with a severe SY-VT isolate (P7/2) and its homologous and asymptomatic variant, cross protective on sour orange (M39D). Young plantlets included seven ornamentals rutaceous (*Afraegle paniculata, Atalantia ceylanica, Clausena excavata, Eremocitrus glauca, Microcitrus australis, M. papuana, Severinia buxifolia, S. disticha*), three species of *Fortunella* (*F. margarita, F. obovata,* and *F. polyandra*) and four species of *Citrus* (*C. micrantha* var. *microcarpa, C. hystrix, C. madurensis* and *C. jambhiri*). All the plants were monitored for 6 – 12 months and tested by ELISA and/or RT-real time PCR. ELISA tests of young new flushes taken at different times showed a fast and high replication in *A. ceylanica, M. australis* and *M. papuana, Fortunella* and *Citrus* species, whereas no replication was detected in *A. paniculata, E. glauca, S. buxifolia.* and *S. disticha*. Additional inoculations carried out on Troyer citrange and *Poncirus trifoliata* seedlings discovered a different replication rate of a subset of representative CTV isolates belonging to VT and T30 genotypes. RT-real time PCR tests, specific for detection of resistance breaking isolates, were negative. The study highlights the risk of aphid transmission in a system which includes many potential host reservoirs of the virus.

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Microbiome-informed selection of biocontrol bacteria from the tomato endophytome

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Metagenomics by next generation sequencing (NGS) provided a window into the microbial community of cultivated plants and changed our belief of the complex interactions in the microbiome. Keystone operational taxonomic units (OTUs) in this context represent highly connected taxa, co-expressed in the same conditions, which exert a considerable influence on microbiome structure and could be identified by microbial co-occurrence network analysis. Here we present the data analysis of tomato root bacterial communities obtained by amplicon-based metagenomics and the identification of core taxa in two different growing conditions, i.e. either in soil or soilless cultivation. The properties of the network structure were evaluated. Microbial networks were compartmentalized into several "network modules" and the keystone OTUs identified. The next step was to align the sequences of the OTUs to those from a cultivable bacterial collection obtained from the tomato endosphere to find their role in the network. Purpose of the third step was to select a subset of bacterial endophytes based on bacterial community data such as abundance (either highly abundant or rare taxa), number or type of connections in the co-occurrence network as well as in vitro plant growth promotion (ACC deaminase, siderophores, phosphate solubilization, etc.) and biocontrol activities against tomato pathogens (bacteria and fungi). Microbial consortia to be used as bioinoculants were formed from these data after the evaluation of the in vitro growth compatibility between the selected bacteria. Individual and sum effects of microbial consortia are being evaluated in vivo for biotic and abiotic stresses.

Temperature plays a decisive role in the ability of *Pseudomonas syringae* strains carrying a recognized avirulence gene to induce a hypersensitive response in *Arabidopsis thaliana*

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The phytopathogenic species complex *P. syringae* has a very important economic impact, causing diseases on many important crops and woody plants. The *P. syringae* complex is currently divided into phylogenetic groups, and according to a certain recognized host specificity, they have been divided into more than 50 pathovars. A considerable research effort is invested in understanding how effector repertoires could determine the range of plants that a given strain can infect. Recently, we showed that the incapacity of *P. syringae* pv. *actinidiae* to induce the hypersensitive response (HR) in *A. thaliana* is due to its incapability to inject effectors rather than to the absence of a recognized effector. In this context, we report here results from the comparison of different *P. syringae* strains, belonging to different phylogroups and carrying the same plasmid-borne avirulence gene, for their capacity to induce an HR in *A. thaliana* Col-0, at different temperatures. *Pto* DC3000 and *Pma* M6 consistently trigger a strong hypersensitive cell death, while the other strains induce an HR, at different intensities, significantly dependent on temperature. Surprisingly, differences were also observed among quasi-clonal strains. These results i) highlight the necessity to study bacterial virulence in a broader set of strains and reveal that *Pto* DC3000 is a reliable model strain but not representative of the *Pseudomonas* complex, ii) support the notion that the presence/absence of effectors is not sufficient to predict the outcome of plant-bacteria interactions, and iii) indicate that temperature may play a crucial role in regulating effector injection.

Effect of different light spectra on *Zymoseptoria tritici* wheat colonization

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Light plays a crucial role in the growth and development of fungi. *Zymoseptoria tritici*, the causal agent of Septoria tritici blotch, is capable to sense and respond to specific wavelengths by modulating gene expression. To study the effect of light on *Z. tritici* infection, bread wheat plants of the susceptible variety A416 were inoculated with a conidial suspension of 10^7 ml^{-1} and grown under 100% red (627 nm) (R100), 100% blue (470 nm) (B100), blue:red (B50:R50), and white lights (W), with a photoperiod of 12 h, as well as in the dark (D). The overall photon flux density in all light treatments was 200 micromoles m^{-2} s^{-1}. Fungal DNA was detected by qPCR at seven days after inoculation in leaf samples from all treatments. To investigate if light directly affected *Z. tritici*, fungal cultures were grown on PDA under the same light conditions and measured after 14 dpi. The highest disease severity was observed in B100 plants, followed by B50:R50 and W ones, while no symptoms were

observed on R100 plants. In agreement with symptom expression, a steady increase of *Z. tritici* DNA amount was recorded in B100 and B50:R50 plants up to 21 dpi, while the lowest fungal DNA concentration was detected in R100 plants. In contrast to the *in planta* results, the B100 treatment reduced the *in vitro* growth of the pathogen, while no significant differences were observed among R100, W and B50:R50 treatments. These results suggest that the reduced infection levels obtained under the red wavelength is probably related to the host response, as already observed for other foliar pathogens on rice plants.

LIFE MICROFIGHTERS: an EU funded project for the implementation and use of innovative Zeo-biopesticides, based on beneficial microorganisms, as an alternative to the use of copper-based products

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Copper, as a pesticide, has been used in agriculture for more than 150 years. During the last three decades, limitations in its use have been imposed by the European Commission, in order to avoid its accumulation in agricultural ecosystems. Meanwhile, alternatives to copper–based chemicals have been suggested, *e.g*. by using microbial biocontrol agents or by applying natural zeolites to the crops. The LIFE MICROFIGHTER project was conceived to demonstrate in open field, and on different crops, the effectiveness of innovative pesticide formulations based on the intimate association of bacterial antagonists with zeolites micro-particles, which will support bacterial viability and efficacy after their spray on the crop canopy. The pathogen-crop association considered by the project are: *Plasmopara viticola* and grapevine; *Pseudomonas syringae* pv. *tomato* and *Xanthomonas vesicatoria* on tomato; *Pseudomonas savastanoi* pv. *savastanoi* and olive. The Zeo-biopesticides will be used to control pathogens in vineyards, tomato fields and olive groves located in Italy, Croatia and Spain, and results will be compared to those obtained by using copper compounds according the local IPM. Copper dynamics, as an environmental pollutant, will be studied in all fields where the experiments are conducted, in order to confirm its reduction in the agricultural environments. The project has a duration of 42 months and 9 partners are participating: Symbiagro (coordinator, Roncadelle, Italy), UNIMORE (Reggio Emilia, Italy), UNIFE (Ferrara, Italy), UNIZD (Zadar, Croatia); Astra Innovazione e Sviluppo (Faenza, Italy), Consorzio Agrario di Ravenna (Cotignola, Italy), Ibe-CNR (Bologna, Italy), Consorcio Cooperatives Agroalimentaries (Valencia, Spain); CO&SO (Firenze, Italy).

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In vitro efficacy of natural compounds on plant pathogenic bacteria affecting kiwi and olive crops

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Plant bacterial diseases have always led to major economic losses for producers worldwide. In Italy, two relevant outbreaks are still affecting *Actinidia* crops: kiwifruit canker and blight disease, caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) and *Pseudomonas viridiflava* (Pv), respectively. Another severe epidemic is represented by the ongoing Olive Quick Decline Disease (OQDS) and the control of his causal agent, *Xylella fastidiosa* (Xf), continues to be a priority in the management of Apulian olive crops. Nowadays, the use of copper-based formulations and an early disease diagnosis are the only two strategies for a preventive control. It is therefore important to choose a more eco-compatible approach. For this purpose, we investigated the *in vitro* antibacterial effect of natural molecules and resistance inductors (BABA®, Atholio Bio®, *Trametes versicolor* extract, chitosan, algae and essential oil based formulations, clove oil, free-fatty acids, diacylglycerides and oxylipins) and synthetic compounds with low environmental impact (Biozon®, Dentamet® and propionic acid). A preliminary screening of different methodologies was carried out to evaluate the best one for Xf, a slow-growing bacterium difficult to cultivate. Among the several assessed methods (broth macrodilution, broth microdilution, agar dilution assay, agar disk diffusion and a novel bacterial culture method based on microfluidic channels), broth macrodilution is the most accurate for Xf. On the other hand, microfluidic channels permit a real-time observation of the bacterial behaviour under continuous-flow conditions. The overall results highlight that most of the tested products show a good antimicrobial efficacy, suggesting a possible future open field application.

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Viroid classification: Reassessment of the species demarcation criteria

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Viroids are small (240–430 nt), circular, single-stranded, non-coding RNAs that can infect plants and induce symptoms. Since their discovery over 50 years ago, more than forty viroids have been identified. Similarly to viruses, viroid populations in a single host exist as a group of variants closely related but slightly differing from each other, thus behaving as a quasi-species. To take into account the intraspecies variability, the early demarcation threshold for viroid species was set at 90% of sequence identity on the overall genome in addition to divergent biological evidence (i.e., host range, symptoms) compared to the closest related viroid. High-throughput sequencing technology allowed the identification of many new viroids not eliciting symptoms in their hosts with narrow experimental host range, thus making difficult the fulfilment of the biological criteria needed for the establishment of a new viroid species. To overcome this problem, pairwise identity scores (PWISs) thresholds at genus level were tested as potential species demarcation criteria. We demonstrated that applying these thresholds as a major species demarcation criterion did not modify the current classification and allowed the assignment of the unclassified viroids to a known or a new species. Starting from 2021, the proposed thresholds have been adopted by the International Committee on Taxonomy of Viruses (ICTV). The adoption of these demarcation criteria allowed the establishment of ten new viroid species. Such approach based on pairwise identity matrices will facilitate the classification of new viroids, limiting the need of providing biological evidence only to the most complicate cases.

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Molecular characterization and effectiveness evaluation of *Aureobasidium* spp. strains against brown rot of stone fruit

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Given the need to reduce the use of chemicals during the postharvest phase, the study aimed to exploit the natural diversity of *Aureobasidium* spp. strains, identifying new Biocontrol Agents (BCAs) as more suitable or simply alternative or complementary to those already available. For these reasons, an alternative defence strategy was investigated. A population of 40 epiphytic and endophytic strains of *Aureobasidium* spp., isolated from common plants (e.g., olive, laurel, plum tree) during the winter season at low temperatures, was molecularly characterized by multi-*locus* sequence analysis (ITS, ELO and EF1α). The most representative strains, belonging to every single cluster and different subspecies, were tested as active BCAs by *in vitro* and *in vivo* assays against *Monilinia* spp. of stone fruit. Molecular results displayed, in a population consisting mainly of *A. pullulans*, the presence of an unusual species: *A. namibiae* (UC14 strain). According to the results of *in vitro* and *in vivo* assays, the selected strains showed a certain ability to control brown rot diseases at different levels of effectiveness. In fact, our results demonstrated that *Aureobasidium* spp., isolated during the winter season, could be more capable of withstanding low temperatures, a key feature for the storage. In addition, from the unusual source of isolation, we detected a greater and more interesting potential of species diversification.

PvDCL1/2 dsRNA as an eco-friendly approach for downy mildew control in grapevine

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Downy mildew caused by *Plasmopara viticola* is the most serious fungal disease of grapevine and its control requires the use of a large amount of pesticides throughout the vegetative season with a consequent high environmental impact. During the last decades, the RNA interference (RNAi) mechanism was exploited for the development of eco-friendly approaches for pest management in crops paving the way toward innovative and sustainable alternatives to traditional toxic Plant Protection Products (PPP). In this work, we show the potential of dsRNA targeting *P. viticola* Dicer-like (DCL) homolog genes for grapevine protection

against downy mildew. The topical application of the chimeric dsRNA construct PvDCL1/2 at different concentrations (75, 100, and 125 ng/µl) was correlated with a significant reduction of the pathogen virulence. In particular, grapevine leaves treated with PvDCL1/2 and subsequently inoculated with a *P. viticola* suspension ($1*10^5$ sporangia/ml) exhibited lower downy mildew incidence and severity compared to those subjected to treatments with water (negative control) or unspecific dsRNA (BcDCL1/2, positive control). The RNAi mechanism exerted by PvDCL1/2 dsRNA was confirmed by the significant reduction of PvDCL1 and PvDCL2 transcript levels in infected tissues previously treated with these molecules compared to control samples. Besides the preventive effect, PvDCL1/2 dsRNA also exhibited a curative action by reducing downy mildew progression of already established infections. Therefore, our results suggest that PvDCL1/2 dsRNA is efficient in controlling *P. viticola* infections and can be considered as a promising candidate to substitute toxic pesticides.

Use of RNAi-based protection strategies against fungal pathogens

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Plant pathogenic fungi represent a serious and constant threat to the agri-food sector worldwide and traditionally their control has been approached with chemical fungicides, harmful to both the environment and human health. During the last decades, the urgency of their replacement with eco-friendly tools led to the investigation of control strategies based on the RNA interference (RNAi), a post-transcriptional silencing mechanism that, acting in a very specific manner, allows to downregulate the expression of key genes and consequently arrests the infective process of plant pathogens. In particular, two main approaches have been explored: Host-Induced Gene Silencing (HIGS) and Spray-Induced Gene Silencing (SIGS). HIGS relies on the development of Genetically Modified (GM) crop plants expressing siRNAs that target pathogen key genes, while SIGS is based on the topical application of interfering dsRNA molecules in replacement of toxic fungicides. Of course, the high specificity of the RNAi mechanism requires the case-by-case identification of genes and gene regions efficient to properly arrest the infective process. Our studies focus on the detection of the most efficacy sequences to be used to develop RNAi-based strategies to control main pathogens (*Plasmopara viticola*, *Botrytis cinerea*, *Phytophthora infestans*, *Stemphylium vesicarium*, *Colletotrichum* spp., and *Podosphaera aphanis*) of economically strategic crops for our country, such as grapevine, tomato, pear, and strawberry. Results suggest that this approach represents a promising candidate for the implementation of sustainable pathogen management strategies, although several issues remain to be addressed for their field application.

Microbial BCA-PGP inoculants on food crops in Kenya and evaluation of rhizosphere microbiota

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In Kenya, agronomic techniques used in small farms are manual and fertilizers are manures applied to soils, the availability of water is strongly related to the two rainfall seasons. Microbial inoculants may improve the assimilation of nutrients/water in conditions of abiotic stress, enhance plant fitness and disease control. In order to test small-scale agriculture systems operating in limited-water conditions, the seed of two food crops (green gram and sorghum) were treated with fungal spore suspensions of *Trichoderma afroharzianum* strain T22 and *T. asperellum*, known biocontrol agents (BCA) and plant growth promoters (PGP). The aim of this study was to characterize the rhizosphere microbiota of plants treated with manure and *Trichoderma* after short or long rain seasons in Kaliku-Kenya. DNA was extracted from soil rhizosphere samples, bacterial and fungal diversity were assessed by Illumina amplicon sequencing, then analyzed by Qiime software. Actinobacteria were the most abundant bacterial phylum in both crops, followed by Firmicutes and Proteobacteria; in particular, all these phyla and Chloroflexi were reduced during the long-rain season, while Acidobacteria, Planctomyces and Gemmatimonadetes increased. Ascomycota was the most abundant fungal phylum, with *Dothideomycetes* and *Sordariomycetes* demonstrating a seasonal pattern in the rhizosphere of green grams, with a negative correlation to the rainfall lengths. *Trichoderma* abundance increased in long-rains and there was a reduction in the relative abundance of the soilborne phytopathogen *Rhizoctonia*,

which was absent in sorghum and T22-treated green gram. Thus, there was a variation of rhizospheric microbiota due to rain seasonality and microbial inoculants.

Molecular markers for differentiation of a '*Candidatus* Phytoplasma solani' strain associated with "bois noir" disease in Sicily

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"Bois noir" (BN) associated with the presence of '*Candidatus* Phytoplasma solani' is one of the most important and widespread grapevine yellows diseases in the Euro-Mediterranean region. Its epidemiology is complex since it is related to interactions of the pathogen with host plants and insect vectors and its spread through propagation material. During monitoring in a vineyard located in Palermo province (Sicily) symptomatic grapevine plants sampled tested positive for '*Ca*. P. solani '. The detected phytoplasmas were then subjected to a multigenic characterization on the *tuf*, *stamp*, *vmp1* and *secY* genes to study their molecular variability in relationship with possible environmental and epidemiological aspects. The *vmp1* gene provided 3 restriction profiles (V4-V11-V12) confirming results reported in Sicily, Sardinia and central western Italy. No variability was present on the 16S rRNA and *tuf* genes (tuf type b1). On *stamp* and *secY* sequences it was possible to verify the presence of a molecular variant having two SNPs to the reported St4 and 99.75% identical to strain P-TV on *secY* gene. The *stamp* gene encodes a membrane protein involved in phytoplasma adaptation to different vector species, and the Sicilian variant is different from those identified in various Italian regions. The BN vector *Hyalesthes obsoletus* is not common in Sicily and this variant might suggest phytoplasma adaptation to insect vectors other than *H. obsoletus*. This *stamp* and *secY* variant is therefore an excellent molecular marker for epidemiological studies that are the basis to improve BN prevention and control in Sicily.

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Characterization of micotoxins produced by *Fusariun proliferatum* and *F. sacchari*

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The genus *Fusarium* is responsible for many serious pre- and post-harvest diseases of agricultural crops. In this respect, product losses along the post-harvest supply chains (storage, transport, and final distribution) also result in severe consequences for product quality. Harvesting and handling of the fruit is, therefore, an extremely important process, due to the risk that lesions may promote the development of fungal agents that rapidly deteriorate the fruit by also producing mycotoxins. In an open-air market in Catania (southern Italy), lady finger banana fruit showed dark circular spots from which *Fusarium sacchari* and *F. proliferatum* were isolated. The aim of this study was to test the pathogenicity of *F. sacchari* and *F. proliferatum*, and to characterize the mycotoxins produced by both species. The characterization of the mycotoxins was carried out by individually inoculating with either species, *F. sacchari* and *F. proliferatum,* corn and barley. Seven mycotoxins were detected from the samples inoculated with *F. sacchari* (ENB2, ENB3, ENB4, FA1, FB2, HFB1 and FC), of which 2 (ENB2 and FC) in both matrices, while FA1 only in maize and ENB3, ENB4, FB2 and HFB1 only in barley. On the other hand, seven mycotoxins (FUS, FA1, FA2, FB1, HFB1, FC and MON) were detected from the samples inoculated with *F. proliferatum*, of which FHB1 and MON only from barley.

Genome-barcoding (NGS) of quarantine bacterial pathogens relevant to the European Union: development and application with Nanopore methodology

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Globalization and climate change are accelerating the spread of plant pathogens in new areas. Keeping the expansion of these pathogens under control is fundamental because they impact both the conservation of ecosystems and nations'

economy. Their spread often comes from asymptomatic plant materials so, to prevent it, it is necessary to improve detection and identification methodologies. In this study, quarantine relevant pathogens *Xylella fastidiosa*, *Xanthomonas citri* and *Pantoea stewartii* have been considered. It is key to identify these pathogens at sequence-type, pathovar and subspecies levels, as these provide insight on their host specificity as well as potential damage. Therefore, this work aims at developing a sensitive and specific diagnostic system for a precise identification even in asymptomatic conditions. We decided to rely on MinION from Oxford Nanopore Technology, a portable, fast, and easy to use device, paired with an ad hoc bioinformatics pipeline. An amplicon-Nanopore sequencing, based on selected housekeeping genes (e.g. MLST), has been developed. These selected genes allow the distinction of the pathovar, subspecies or ST of the different tested bacteria. Several plant species have been spiked with different bacterial suspensions at known concentrations. Then, a multiplex- (for *Xylella fastidiosa*) and duplex- (*Xanthomonas citri*) PCR amplification of housekeeping genes has been developed. The prepared samples were finally sequenced with Nanopore to test the workflow. Preliminary results indicate that this approach is promising for the detection and identification of these priority pathogens. Further study will be done to determine the limit of detection and specificity.

LAMP assay for in-field detection of Flavescence dorée phytoplasma in *Scaphoideus titanus* vector

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Flavescence Dorée is one the most notorious diseases of grapevine, caused by EPPO quarantine Flavescence Dorée phytoplasma (FDp) belonging to the 16Sr-V group. Although many molecular techniques such as Loop-Mediated Isothermal Amplification (LAMP) have frequent use in the rapid detection of FDp in infected grapevine plants, there is still a lack of developed isothermal amplification protocol for FDp detection in the insect vectors. In this study, a simple in-field real-time LAMP assay was developed for the detection of FDp in the *Scaphoideus titanus* vector. The LAMP assay was optimized to work with crude insect extracts obtained with 5 min-manual shaking of a single insect in the buffer. An easy, sensitive, specific, low-cost and point-of-care LAMP assay was able to detect FDp in *S. titanus* in less than half an hour directly in the infected Piedmont vineyards. These results enable new possibilities for FDp detection in both, plants and vectors, directly in the field without the requirement of highly specialized personnel, at relatively low operating costs.

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Psa3 virulence reduction by natural molecules: insights on subtle regulation mechanisms

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Pseudomonas syringae pv. *actinidiae* (Psa) is the causal agent of kiwifruit bacterial canker disease, and currently, the disease management relies on copper-based compounds, which could force the insurgence of copper-resistance Psa strains, as already observed with other plant pathogens. Thus, new control strategies should target bacterial virulence mechanisms to weaken pathogens rather than prevent their growth *in planta*. One of the most important factors of virulence is the ability of the bacterium to inject effectors into the plant cells through the type III secretion system (T3SS). Thus, exploiting a reporter system based on the GFP expression driven by the promoter region of the *hrpA1* gene, which encodes one of the major components of the T3SS, a chemical library composed of 502 natural molecules was screened looking for those able to reduce the T3SS induction in the most aggressive Psa biovar 3, without affecting its growth. Among selected candidates, dicoumarol showed the ability to reduce the hypersensitive response (HR) in *Arabidopsis thaliana*, further supporting the hypothesis of T3SS inhibition. Moreover, a proteomic analysis revealed that dicoumarol reduces the secretion of proteins by Psa3 cultured in minimal medium, in particular the injectisome proteins HrpZ and proteins of the flagellum-related T3SS, thus suggesting the inhibition of a common mechanism involved in the regulation of both secretion systems. The possible target of dicoumarol will be discussed.

eCPMV nanoparticles: the potential of a bio-inspired strategy for plant protection

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Nano-agrochemicals are an unprecedent opportunity for crop disease management since they can provide a valuable alternative to common pesticides, due to a targeted and enhanced efficacy, resulting in a lower dosage of active substances and a reduction of possible off-target effects. Plant viruses can play an important role in this context since they are naturally occurring proteinaceous nanoparticles able to carry, protect, and deliver a cargo. Moreover, the biosafety concerns about the use of plant viruses for crop protection have been exceeded by the development of nucleic acid-free virus nanoparticles, named empty virus-like particles (eVLPs). (CPMV) can be produced as eVLPs (eCPMV) at high titres through transient expression in *Nicotiana benthamiana* plants. Additionally, eCPMV is prone to a vast array of functionalization by chemical and genetic engineering. In this work, eCPMV nanoparticles have been exploited in an attempt to develop new products for crop protection, combining the physical stability of the eCPMV capsid with natural bioactive peptides and molecules. Particularly eCPMV has been exploited for three purposes: (i) exposure of antimicrobial peptides (AMP); (ii), as a plant immunity-triggering nanoparticle, and (iii) eCPMV as a functional nanocarrier. Overall, the data enforce to reconsider the paradigms regarding eCPMV functionalization, in particular in terms of peptide features required for genetical modification. Moreover, this work supports the idea of eCPMV as a promising tool to develop new nano biopesticides applicable in agriculture.

Evaluation of copper, zinc and organic fertilizers for their side-effect against *Xylella fastidiosa* subsp. *pauca in vitro* and on naturally infected olive plants

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Xylella fastidiosa (Xf), a dangerous xylem-limited bacterium, can infect more than 600 plant species. The bacterium was first discovered in Europe in 2013 in the Apulia region (southern Italy) where the outbreak of *X. fastidiosa* subsp. *pauca* (*Xfp*) raised major concerns due to the deadly disease caused on infected olive trees (*Olea europaea*). The lack of effective therapeutic tools to cure infected plants is also a key factor boosting research for practical solutions to contain the infections. In this framework, the screening for new antibacterial products such as zinc and copper phosphites, and an organic fertilizer based on a balanced mixture of seaweeds and vegetal polyphenols, evidenced a broad-spectrum antibacterial activity *in vitro* against various species of phytopathogenic bacteria. Through the optimization of specific protocols, the selected products were then tested *in vitro* also for their antimicrobial activity against *Xf* strains belonging to different subspecies. Although with some differences related to *Xf* strain or subspecies, results confirmed the broad antibacterial activity of the tested compounds. Subsequently, in the framework of an integrated control approach, these products were tested in the field in naturally *Xfp*-infected olive orchards in the Salento Peninsula. Upon two years of field assessments, positive effects of the application were recorded in terms of appreciable improvement of vegetative, productive and phytosanitary conditions of the treated plants. However, further observations are needed to assess the long-term effect and sustainability of selected products application, while additional researches are needed to optimize their formulation/application and understanding the mechanism of action.

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A rapid lab screening to select biological and integrated tools to be used against *Phytophthora cinnamomi* root rot of holm oak and other woody plants

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Phytophthora species, as well as other telluric pathogens, are harmful microorganisms very difficult to control with conventional means. The low effectiveness of chemical formulates is principally due to the development of strains resistant to fungicides. Furthermore, the use of resistant plants has several limitations due to the extreme ability of *Phytophthora* species to mutate and overcome host resistance. These factors together with the risks and negative impact on human health and the environment underline the need to develop new screening methods and sustainable control strategies. In this work, antagonistic bacterial strains and fertilizers with collateral antimicrobial activity, already selected for their antagonistic/inhibitory activity against fungal and/or bacterial pathogens, were evaluated against *P. cinnamomi*. Assays were carried out *in vitro*, on agarized media as well as in vivo on pot grown young holm oak plants. Moreover, as a new rapid lab screen *in vivo* we proposed the use of apple fruit. More specifically, surface sterilized apples were artificially wounded, treated with biocontrol or chemical products and inoculated before (curative tests) or after (preventive test) with the pathogen. Several experiments have shown a good agreement between the rapid fruit screening and the classic plant test method based on treated and artificially inoculated olm oak plants. Therefore, the fruit method could be used as a new alternative model *in vivo* to pre-select/evaluate new biological or integrated tools/strategy.

First report of *Fusarium pseudograminearum* causing root and crown rot in the halophyte *Salicornia europaea*

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Salicornia europaea L. (glasswort), a euhalophyte in the Amaranthaceae family, is a valuable green vegetable. In July 2021, an outbreak of root and crown rot disease occurred on *S. europaea* grown in peat-filled pots under greenhouse. Symptoms appeared on 20–25% of 6-month-old plants. The fungus was identified as *F. pseudograminearum* by means of morphological observations and molecular sequence analysis based on *tef-1α* gene (EF-1/EF-2) and using species-specific PCR primers (Fp1-1/Fp1-2). This pathogen is known as the causal agent of crown rot in cereals and has sporadically been reported on wheat plants and seeds of soybean and vetch in Europe. A pathogenicity test was then conducted in a growth chamber to fulfill Koch's postulates. Forty-eight seedlings (57 days after sowing) were grown in aerated non-saline nutrient solution in which a suspension of *F. pseudograminearum* macroconidia had been poured (final concentration 10^5 ml^{-1}). Other 48 plants (controls) were grown hydroponically in a separate growth chamber and inoculated with sterile distilled water. Twenty-four days after inoculation (dpi), half of control and inoculated plants was transferred into a new sterile nutrient solution while the other half was transplanted into pots filled with sterilized peat. After 80 dpi, 100% of pot-grown plants showed root and crown rot symptoms whereas only 70% of infected hydroponically-grown plants developed symptoms. No evidence of disease was observed in the controls. *F. pseudograminearum* was consistently re-isolated from diseased plants in both cultivation systems (64.5–83.0%). Further investigations are in progress on this new pathosystem in saltwater hydroponics.

Advances in plant disease detection: pulse thermography as new tool to predict *Botrytis cinerea* infection in pepper and tomato

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Botrytis cinerea is a necrotrophic pathogen characterized by short life cycle, high reproduction and genetic variation. Infrared thermal imaging is a non-destructive and fast technique, which holds great promise for the detection of pathogen attacks in plants. Herein, we evaluated and predicted the development of gray mold in pepper (*Capsicum annum*) and tomato (*Solanum lycopersicum*) plants by pulse thermography of the leaves in the pre-symptomatic phase. For this purpose, pepper and tomato plants were inoculated with different concentrations of *B. cinerea* or *Trichoderma harzianum* spores, a beneficial fungus employed as non-pathogenic control. Thermographic measurements, carried out during seven days after infection, revealed specific thermal patterns in the infected leaves after a few hours (6 – 48 h) and earlier than the appearance of the characteristic lesions caused by *B. cinerea*. Diagnostic parameters, such as specificity, sensitivity, positive and negative predictive thermographic values, confirmed a good reliability of the pulse thermography technique in the early detection of *B. cinerea* infections. In order to better understand the mechanisms underlying the thermographic patterns caused by the gray mold, stomatal opening and conductance as well as

expression of genes typically involved in plant-pathogen interactions were evaluated. Altogether, our data, supported by physiological, cellular and molecular evidence, demonstrate that pulse thermography imaging is a valid and reliable diagnostic tool for the rapid detection of *B. cinerea* infection and, possibly, to other necrotrophic fungi, to be applied for a rapid plant phenotyping of resistance/tolerance as well as for a more automatized, and sustainable plant disease control.

Yet another valuable trait of the biological control agent, *Aurebasidium subglaciale*

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Aureobasidium subglaciale is a psychrotolerant yeast isolated from subglacial ice that is able to grow at low temperatures. In fact, due to such feature it is an excellent biocontrol candidate that can be applied to long postharvest storage. The potential of *A. subglaciale* (strain AS13) against different fungal pathogens was previously verified by the assessment of the production of various metabolites, including both VOCs (volatile organic compounds) and non-VOCs. Furthermore, a more in-depth look at the bioactive compounds produced by strain AS13 was investigated by using preparative high-performance liquid chromatography (HPLC) in combination with nuclear magnetic resonance (NMR) spectroscopy. The biological activity of the strain AS13 and the fractionated extracts were tested in *in vitro* and *in vivo* assays against *Penicillium* spp., confirming the strain's potential as a biocontrol agent against green and blue molds. Furthermore, the most potent purified fraction consisted of an unsaturated triglyceride similar to glyceryl tripalmitoleate ($C_{51}H_{92}O_6$), which is known to be physiologically active and able to bind to the lipid bilayer and other components of the microbial cell membrane. With new findings on the type of compounds produced by AS13 further applications of the strains or derived compounds can be envisioned, providing a framework to develop environmentally-friendly and sustainable alternatives to current commercial pesticides.

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Identification and characterization of *Xanthomonas arboricola* pv. *pruni* strains isolated from almond in Sicily

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Xanthomonas arboricola pv. *pruni* (*Xap*) is the causal agent of bacterial spot disease of stone fruits, severely affecting health and productivity of many fruit and ornamental *Prunus* species. In the EU, the pathogen is regarded as a Regulated Non-Quarantine Pest (RNQP). In Italy, severe outbreaks have been reported on plum and peach in the north-east of the country. On almond it has been recently described in Apulia. Samples consisting of young sprouts and fruits of almonds from a commercial almond orchard near Agrigento (Sicily) were delivered to the laboratory of phytobacteriology of the University of Catania, Italy. Symptoms consisted in leaf and fruit spots. The symptoms on almond's fruits were very characteristic, with sunken, corky lesions and oozing gum. Colonies with typical *Xap* morphology were isolated both from leaf and fruit samples. *Xap* identification was achieved by duplex PCR and supported by *gyrB* and *rpoD* gene sequence analysis. The pathogenicity of representative isolates was confirmed by prick-inoculation of young almond stems and by a detached-leaf assay. This latter assay also suggested differences in resistance amongst six Sicilian almond cultivars, with no immunity. The draft genome of *X. arboricola* pv. *pruni* strain PVCT262.1 was obtained. Genome-wide average nucleotide identity with other *Xap* from different hosts and countries was above 99%. However, it clustered more tightly with *Xap* almond strain CITA99 than CITA33 both isolated in Spain, in 2006 and 2009, respectively. No other genomes from Italian strains are available to date. Pathogenicity and virulence genes genome mining results will be discussed.

Virulence and host-specificity in *Fusarium oxysporum* ff. spp. interactions

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Pathogenic fungi use effectors to colonize plants. These molecules regulate the interaction between pathogen and host, manipulating the ability of the pathogen to induce disease on plants. For example, *Verticillium dahliae* (*Vd*), a well-known pathogenic fungus, can infect a wide variety of plants, while *Fusarium oxysporum* (*Fo*), is able to infect only one or a few related plant species and therefore each strain is subcategorized in *formae speciales* (ff. spp.). Recently, an essential effector protein involved in the interaction *Vd*-cotton was identified in *Vd* isolates and named *d-Vd*. Additionally, we found that the *d-Vd* gene has many homologs in different ff.spp. of *Fo*. We were able to express the *d*-homolog protein in a bacterial heterologous system for the version from *Fo* f. sp. *vasinfectum* that infect cotton, for *Fo* f. sp. *radices-cucumerinum* that infect cucurbits and for *Vd* non-cotton infecting strains. Subsequently, we set an assay using plants growing in a protein solution and check the effect of the different homologs. The results showed that the *d-Vd* and the *d-Fov* alone can induce wilting on cotton plants while plants grown in a solution containing the *nd-Vd* or *d-Forc* did not show any symptoms. Additionally, we generate a deletion mutant for the *d-Forc* homolog and test the virulence of the mutant on different plants. Interestingly, deletion mutants of *d-Forc* showed a reduction of virulence on cucumber and watermelon after infection while no difference was observed on melon plants. All the obtained results show that the *d*-homologs may have different effect in different interactions rather than a common target.

Characterization of fungi associated with olive fruit rot in central Italy

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Olive (*Olea europaea* L.) is one of the most cultivated tree species in Mediterranean countries with a major social, economic and environmental importance. Olive is commonly affected by diverse fungal pathogens causing fruit rot and thus, diminishing productivity and oil quantity and quality in oil-producing varieties. In Autumn 2021, a survey was carried out in an oil mill in Tuscia area (Viterbo Province). Fruit rot symptoms on olive fruits were observed with 13% prevalence. Depressed, round, and ochre or brown lesions leading to fruit rot were observed on the Italian olive cultivars Leccino, Canino and Frantoio collected from four different municipalities (Viterbo, Carbognano, Caprarola and Sutri). Visual, stereoscopic, and microscopic observations were carried out. Fungal strains isolated from symptomatic fruits were characterized by morphological and molecular analyses. Fungal species were identified belonging to seven different genera: *Didymella, Botryosphaeria, Colletotrichum, Diaporthe, Epicoccum, Alternaria* and *Fusarium* by the analysis of their internal transcribed spacer regions of ribosomal DNA region. Pathogenicity tests on punctured and non-punctured fruits were carried out for selected isolates. Further molecular analyses are ongoing on additional housekeeping genes for an accurate species-level identification of the isolated fungi. This research allowed to define a list of the most frequent fungal pathogens affecting olive drupes in Lazio region in central Italy.

Evaluation of alternative seed treatments for the management of seed-borne diseases

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The European project H2020 BRESOV "Breeding for Resilient, Efficient and Sustainable Organic Vegetable production" aims to improve the competitiveness of three vegetable crops (tomato, broccoli and snap beans) when grown in an organic production system. In particular, workpackage 4 aims at developing protocols and tools to maximize yield and ensuring high sanitary quality and high genetic purity of organic seeds. In this framework, alternative seed treatments on different pathosystems are being evaluated. Here we report on the efficacy of seed treatments with three microbial consortia (*Azotobacter*, *Bacillus*, *Pseudomonas*, *Streptomyces*, *Glomus*, *Trichoderma*) and two natural compounds (chitosan and glucosinolates based). Organic seeds of two cultivars were inoculated in each trial. Snap beans and broccoli seeds were artificially inoculated with *Neocosmospora phaseoli* (10^6 conidia ml^{-1}) and *Alternaria brassicicola* (10^5 conidia ml^{-1}), respectively, before the treatment. Only one of the microbial consortia and the chitosan based product reduced significantly symptoms caused by *A. brassicicola* on broccoli cotyledons and brown discoloration of hypocotyl of snap beans induced by *N. phaseoli*. Trials on the tomato-*Xanthomonas euvesicatoria* pv. *perforans* pathosystem

aimed at evaluating a possible biopriming effect of the seed treatment. To this aim 1-month-old tomato seedlings were spray inoculated with a bacterial suspension of the pathogen (10^8 cfu ml^{-1}) and symptoms evaluated two weeks after inoculation. Results showed a reduction of bacterial disease severity with some of the treatments with MCs and NCs although the chitosan was the most effective.

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Pigmented wheat genotypes as innovative tools against Fusarium Head Blight disease

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Fusarium head blight (FHB) is one of the main severe cereal diseases, including bread wheat (*Triticum aestivum*). To date, few fungicides to counteract this disease are available, thus there is a high priority need to find new protective compounds. Polyphenols are secondary metabolites that act as stress protecting agents, playing a significant role in plant resistance against plant pathogens. Pigmented wheat genotypes have flavonoid-rich kernels due to the presence of anthocyanins in the external layers (pericarp and aleurone). The aim of the present work was to evaluate whether the pigmented wheat genotypes show an increased resistance to FHB and if the development of new crop protection products based on nanomaterials obtained from pigmented wheat wastes (such as bran) can be successfully used to counteract FHB. The work was articulated in several steps: 1) evaluation of the resistance level of five pigmented wheat genotypes (two blue-aleurone genotypes, Purendo and Skorpion and three purple-pericarp genotypes, Rosso, Vanilnoir, and Indigo) compared to a resistant genotype (Sumai3) and to a control non pigmented cultivar (Rebelde); 2) synthetize cellulose nanocrystals (CNCs) from cellulose of wheat bran to be used as crop protection products. Results highlighted that blue aleurone genotypes showed high susceptibility to FHB, purple pericarp genotypes are less susceptible and Vanilnoir showed a resistance level comparable to Sumai3. CNCs was successfully obtained by pigmented wheat bran and further analysis to verify its antimicrobial activity (both *in vitro* and *in vivo*) has already been planned.

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Endophytes isolated from maize Lombard landraces: new perspectives for the control of *Fusarium verticillioides*

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Maize is one of the most important crops in our country. However, in the past years yields have gradually decreased due to reduced cultivated areas and climate change. GEMMA project aims to study the endophytic bacterial community of landraces and their relationship with *Fusarium verticillioides*, one of the most widespread toxicogenic fungal pathogens in Lombardy region. Recent studies show how endophytes have abilities to inhibit the development of pathogens. Over the past three years four traditional landraces (Nero Spinoso Valcamonica, Spinato Gandino, Rostrato Rosso Rovetta, Fiorine Clusone, preserved at CREA Bergamo Genebank) and a pure line (B73) were grown in different locations (Landriano, Bergamo, Verderio, Carvico) with low-input farming techniques. Embryo of seeds harvested in 2019 allowed the isolation of 96 bacterial strains, mostly Firmicutes. *In vitro* antifungal assays against *F. verticillioides* allowed the selection of 12 strains with inhibition percentage higher than 50% for *in vivo* test. Only 2 isolates (*Bacillus* and *Psychrobacillus*) significantly reduced fungal infection *in vivo*. In 2021, experimental inoculations with *F. verticillioides* conidia were carried out in field during flowering. Values of infected seeds/ear and % infected area/ear show that Spinato di Gandino is the most susceptible variety while Nero Spinoso is confirmed as being the least susceptible. The study of microbiota and resistance traits from landraces shows potential for future organic maize production where biocontrol agents and resistant genotypes can contribute to a better, and more sustainable, response to stresses that will allow a higher maize yield even in the scenario of climate change.

Basalt-based novel agrochemicals demonstrated *in vitro* antimicrobial activities against fungal and bacterial plant pathogens

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Basalt-based flours are natural products of volcanic origin, admitted in organic agriculture, obtained by a grinding process and rich in silicon, potassium, iron, calcium, and magnesium, which restore nutrients for soil fertility and can also potentially protect plants directly and indirectly as a physical barrier against pathogens and thanks to their biostimulant and nutritional properties. The aim of this study was to test *in vitro* the antimicrobial potentiality of three different particle size basalt-based flours, denominated as Farina di Basalto® XF, Farina di Basalto® F and Farina di Basalto® XM, 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 olive knot. The three basalt-based products were assayed at five concentrations (10–15-20–25-30 g/L). The direct antimicrobial properties have been assayed by broth and agar assays and the highest concentration demonstrated high antimicrobial activity compared to the mock controls. The indirect antimicrobial properties were assayed by evaluating the ability to form a barrier against the pathogens and the physical interaction between the basalt particles and the pathogen cells. In particular, the basalt-based flours formed an effective barrier against Fg and Psav, inhibited the biofilm biosynthesis in Fg and Psav, inhibited the adhesion of cells and favoured their aggregation, thus confirming that such basalt-based particles can mechanically interact with the pathogen cells to reduce their contact surface with the host. Further investigations will be focused on validating such evidences by *in vivo* experiments.

Potato late blight: detached leaf and *in planta* assays to evaluate *Phytophthora infestans* colonization in several potato varieties

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Late blight caused by the oomycete *Phytophthora infestans* is one of the most devastating diseases affecting potato (*Solanum tuberosum* L.). Under favorable conditions, *P. infestans* can infect leaves and tubers and severely spread in field. The use of resistant cultivars, when available, is considered a sustainable option to control the disease. However, the main strategy is still the oomycide application. Different potato varieties, all cultivated in central Italy, were tested in these experiments. The first aim of this work was to develop a reliable method to phenotypically evaluate the susceptibility of potato varieties to late blight with a detached leaf assay, leaves from adult plants were inoculated with a sporangial suspension of *P. infestans* and after 3 days foliar lesions were measured. The second aim was to evaluate the development of the infection caused by *P. infestans in planta*. Molecular approach (qPCR for pathogen quantification) coupled with an analytical approach (HPLC–MS/MS for plant hormones detection) was used. For the *in planta* assay, 2-week-old seedlings were inoculated with sporangia and kept under controlled conditions in a phytotron. The infection was monitored for 10 days and leaves samples were collected at early, middle and late stages for DNA and plant hormones extraction. The molecular analysis via qPCR was performed in order to estimate *P. infestans* colonization based on the infection stage and presence/absence of symptoms, while the quantification of phytohormones (salicylic acid and jasmonate) was conducted for a better comprehension of plant response to the infection of hemibiotrophic oomycete *P. infestans.*

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The evolution of the Plant Health Service diagnostic activity: the role of an Official Laboratory

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In December 2019, the Regulation (EU) 2016/2031 on protective measures against plant pathogens and pests entered into force and became applicable. The Regulation introduced the new plant health regime to reduce the risk of introduction into and spread within the territory of the European Union of invasive organisms, that can be responsible of substantial

economic losses in both agricultural and forestry sectors. The Legislative Decree No. 19/2021 redefined the new national phytosanitary system and established a Network of Laboratories including the National Reference Laboratory, the Official Laboratories and others operating on the national territory in the plant protection sector. The Official Laboratories perform analyses, tests and diagnoses in the context of official controls and other official activities. To ensure highest standard results, these Laboratories must be accredited according to standard EN ISO/IEC 17,025. In this context, the Official Laboratory of Plant Health Service of Lombardy Region performs analyses for detection, identification and characterization of bacteria, fungi and oomycetes, insects and mites, nematodes, viruses, viroids and phytoplasmas. The diagnostic activity encompasses from traditional to advanced molecular techniques. In the era of climate change and globalization, the early detection and the accurate identification of pathogens and pests represents one of the most important goals of Plant Health Service. Therefore, we present our experience as Official Laboratory including the accreditation process, the contribution to development of new standard diagnostic protocols, and the collaboration with national and international research centers.

Development of a new LAMP assay for the fast diagnosis of *Gnomoniopsis castaneae*

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Gnomoniopsis castaneae (syn. *Gnomoniopsis smitholgivyi*) is an emerging fungal pathogen causing nut rot in sweet chestnut (*Castanea sativa*) but also reported as agent of cankers on twigs as well as of necroses on galls and leaves. Nut rot disease was observed in northern Italy since the second half of the nineteenth century, but only recently its incidence and severity was associated with outbreaks of *G. castaneae.* The little knowledge about the disease epidemiology, its almost ubiquitous distribution in chestnut groves and its endophytic occurrence in chestnut organs make it difficult to find valid solutions for its management and control. The possibility of a fast and accurate diagnosis could represent a valuable alternative to monitor pathogen occurrence both in pre- and in post-harvest conditions. Among the strategies today available to counter the disease, molecular detection tools are the most effective for disease diagnosis with high sensitivity and specificity. However, molecular detection often requires a well-equipped laboratory to be applied, limiting the speed and user-friendliness of the diagnosis. The aim of this work was to develop a new LAMP-based tool to be applied directly on site for the early detection of *G. castaneae*. The assay, optimized on the portable instrument Genie III (Optigene, UK) and based on the Ef1-α target region, can recognize the pathogen with a high level of specificity and sensitivity in about 20 min. Application of this method in chestnut orchards and in the subsequent processing steps of the fruit and derived products (e.g. chestnut flour) might provide a new, intriguing perspective for disease prevention and control directly in the field and in the chestnut processing chain.

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Trichoderma application for reducing copper-based fungicides use and controlling phytopathogens

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Copper (Cu)-based agricultural products are widely used as antimicrobial compounds in different pathosystems. However, their frequent applications can cause indiscriminate Cu accumulation in soil and water sources, negatively impacting micro- and macro-organisms. On 22th June 2022, European Commission proposed a 50% reduction in Cu pesticide applications by 2030, as part of its mandate for sustainability and biodiversity preservation. The development of effective alternative products which can contribute in minimizing pollutant release in the agroecosystem is needed. The present research was aimed at testing the efficacy of *Trichoderma*

sp. in combination with reduced doses of copper-based fungicide ($CuSO_4$, Cupravit Bioadvance®, Bayer) for controlling *Botrytis cinerea* and *Alternaria alternata* on tomato. In order to select *Trichoderma* Cu tolerant strains, the growth of 4 isolates was monitored on PDA enriched with increasing concentrations of fungicide, ranging from 0.18 to 1.8 g/L. *T. afroharzianum* T22 and *T. asperellum* T25 were able to growth on media contained up to 90 mg/L of Cu (metal), resulting as the best candidates for *in vivo* biocontrol assays. A consistent disease severity reduction was observed on plants treated with one-half and one-quarter doses of Cupravit® (0.45 g/L and 0.9 g/L) when combined with either *Trichoderma* strains. T25 applied by soil drenching with 0.45 g/L $CuSO_4$ was more effective than the full fungicide dose in reducing *Alternaria* diseases symptoms. The data suggested that Cu tolerant *Trichoderma* strains can be used in combination with Cu-based fungicides for the effective management of phytopathogens, reducing drastically the Cu released in the environment.

Pathogenicity assay and genome assembly of *Fusarium oxysporum* f. sp. *melongenae* that causes *Fusarium* wilt in eggplant

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Fusarium oxysporum f. sp. *melongenae* (FOMG) is the causal agent of *Fusarium* wilt in eggplant (*Solanum melongena*). FOMG is a tracheomycotic fungus that penetrates roots and invades plants systematically, causing leaf chlorosis and dropping of leaves, followed by wilt and finally plant death. FOMG is a soil-borne plant pathogen that can spread widely, it can persist for many years in the soil causing severe diseases on eggplant and seriously affecting the production. To better understand FOMG pathogenicity in FOMG-eggplant interaction, we set up a fast and easy pathogenicity *in vitro* assay. The test was used in order to rapidly identify pathogenic strains among *Fusarium* isolates from symptomatic eggplants; moreover, the test was used for molecular analyses on *in vitro* inoculated plants. We performed whole genome assembly of an Italian FOMG strain (FOMG-2267) and RNA-seq analysis in order to identify fungal genes involved in FOMG—eggplant interaction. Future reverse genetics analyses will be performed in order to assess the role of these genes in the interaction.

This work was founded by the Italian Ministry of Agriculture (MiPAAF) within the research project QUALIMEC 'Miglioramento delle proprietà qualitative in melanzana e carciofo mediante approcci di genome editing e cisgenesi'.

The introduction of new cultivar and the risk of new virus: the case study of cherry necrotic rusty mottle virus (CNRMV) in northern Italy

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Sweet cherry trees (*Prunus avium* L.) can be infected with various viruses, the presence of which can lead to production losses and orchard decline. In Trentino region in northern Italy the most important cultivars are Kordia and Regina but from 2016 for commercial purposes it was introduced over a large area a cv. "Giant Red (Mariant)" on Gisela 5 rootstocks. During spring in 2019 a high number of trees displaying virus-like disease symptoms such as brown angular necrotic spots on leaves of which can drop out giving a shot-hole appearance, yellow alone along the veins and necrosis on the margin, on the bark sometimes necrosis with gum spill on. Symptomatic and asymptomatic plants of sweet cherry cv. Giant Red planted in the previous 1–2 years were collected in several orchard in 2019. Total RNAs were extracted and analyzed by RT-PCR for detection of prunus necrotic ringspot virus (PNRV), prune dwarf virus (PDV), apple chlorotic leaf spot virus (ACLSV), cherry virus A (CVA), cherry leaf roll virus (CRLV) and cherry necrotic rusty mottle virus (CNRMV) with diagnostic method already published. Only CVA and CNRMV were identified, and relative amplicons obtained were purified and sequenced in both directions. The sequences were analyzed using blast. The analysis confirmed the only presence of CVA and CNRMV. While CVA is generally considered a latent virus, this result suggests that CNRMV is the cause of observed symptoms. This finding underscores the importance of virus regulation that should be highlighted and points again the risk for virus distribution utilizing infected propagation material. To our knowledge, this is the first report of CNRMV causing necrotic rusty mottle of cherry in Italy.

Paraconiothyrium fuckelii, Diaporthe eres and *Neocosmospora parceramosa* causing cane blight of red raspberry in Northern Italy

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Red raspberry (*Rubus idaeus*) is considered a crop of global significance and its production has largely increased during the last decade. In Piedmont, the increasing interest in raspberry cultivation has led to a progressive intensification of the production systems and to the adoption of new cultivars to supply the consumer demand. However, the consequent consistent movement of plant materials and fruit, along with the presence of favourable climate conditions, resulted in the emergence of diseases previously not reported in this area. Thus, a monitoring activity was conducted in red raspberry orchards over a three years period (2019–2021) to investigate the etiology and pathogen diversity in association with cane blight, the most common symptom observed and one of the most diffused and serious fungal diseases of raspberry. Isolates were collected from symptomatic plants of the cultivars 'Diamond Jubilee' and 'Grandeur'. Three fungal species were identified: *Paraconiothyrium fuckelii*, *Diaporthe eres* and *Neocosmospora parceramosa*. The identification was achieved through morphological features assessment and multi-locus phylogenetic analyses on four different genomic loci (ITS, *tef1*, *tub2* and *rpb2*). All the species found were confirmed as pathogenic and *P. fuckelii* was the most aggressive. This study provides the first insight on raspberry cane blight in Italy. This preliminary knowledge can be applied to further epidemiological and diagnostic studies in order to adopt effective integrated strategies to control and prevent the disease spread.

Kiwifruit Vine Decline Syndrome: the roles of microbiome and abiotic stress in plant health

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Kiwifruit vine decline syndrome (KVDS) has been threatening kiwifruit cultivation in Italy for the last decade. Symptoms lead to severe root decay leading to irreversible wilting that develops with the high temperatures of mid-late summer when kiwi vines have the highest transpiration rates. Root symptoms include disappearance of root hairs, breakdown of root cortex, blocked vessels, hypertrophy, and phloem detachment from the cortex. Among the microorganisms involved in the disease development, there are soilborne oomycetes belonging to the genus *Phytopythium*. High summer temperatures and flooding play a key role in the development of the disease. Trials performed in controlled conditions revealed a high virulence for the species *P. helicoides*, which showed a higher temperature tolerance even if the most isolated species is *P. vexans*. The use of a naturally infected soil to reproduce the symptoms revealed higher disease severity compared to the soil inoculation of a single species. For this reason, the soil, rhizosphere, and root microbiota of eight kiwifruit orchards, both healthy and KVDS-affected. Total DNA was extracted, and the population dynamics of fungi, bacteria and oomycetes was analyzed through metabarcoding. Defining the interplay between the factors involved will contribute to the understanding of multifactorial diseases where biotic and abiotic components simultaneously or sequentially affect plant health.

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A gnotobiotic approach to investigate microbiome functions in grapevine and lettuce

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Plant microbiome plays a key role on impacting plant health, however knowledge on the exploitation of certain beneficial microorganisms to improve crops is still partial. The aim of the project is to investigate timing and patterns of the plant microbial colonization and the effect of microorganisms on stress tolerance, fungal pathogenesis and plant growth by using gnotobiotic plants. Gnotobiotic plants, defined as

plants with a characterized microbiome, could represent a new system to study microbial interactions and their benefits against abiotic and biotic stresses. For this study, grapevine (*Vitis vinifera* cv. Chardonnay) and lettuce (*Lactuca sativa*) were selected as representative crops for woody and herbaceous systems. Firstly, colonization from selected Biome Agents (BAs) and their efficacy in biocontrol against fungal pathogens were evaluated in controlled conditions. We tested bacterial strains isolated from lettuce leaf endosphere against biotic stress using lettuce plants inoculated with two races of the wilting agent: *Fusarium oxysporum* f. sp. *lactucae*. The efficacy assay was performed *in vivo* by assigning the disease index at 12-, 21- and 28-days post inoculation. The average biomass weight was also evaluated for *in vivo* trials. The identified best performing agents will be evaluated using gnotobiotic conditions. Gnotobiotic plants of both species were obtained by testing different sterilization methods. The gnotobiotic conditions are maintained by using smart boxes able to completely isolate the plants from the external environment while monitoring humidity and plant ecophysiological parameters in real time.

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The GPI-anchored protein HAM-7 regulates root adhesion in *Fusarium oxysporum*

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The fungal cell wall is a dynamic structure protecting cells from environmental stresses, providing cell type-specific morphology and functioning as a physio-chemical rheostat for the transmission of extracellular signals through a large set of cell-wall-anchored proteins. HAM-7 is a highly conserved protein present in all filamentous ascomycetes, and it is known to form a sensor complex at the cell wall/plasma membrane interface for the activation of the MAK-1 cell wall integrity mitogen-activated protein kinase (MAPK) pathway in *Neurospora crassa*. Additionally, absence of the GPI-anchored cell wall protein HAM-7 in *N. crassa* leads to severe defects in cell-to-cell fusion and sexual development. BlastP searches using the *N. crassa OR74A* HAM-7 protein as a bait identified a single 233-amino-acid long orthologue with 64.19% identity in the *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) genome. Here, we genetically dissected the contribution of *Fol ham-7* gene in the regulation of stress response, vegetative hyphal fusion, hyphal agglutination, plant root adhesion and virulence. Similarly to *N. crassa*, *Fol ham-7Δ* mutants are severely impaired in vegetative hyphal fusion, but not in vegetative growth under stress conditions (*i.e.* cell wall, hyperosmotic and heat stress). Importantly, despite being unable to undergo hyphal agglutination and plant root adhesion *ham-7Δ* mutants only showed minor however not significant defects in plant virulence.

BNYVV genomic formula changes during host infection and vector transmission

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Multipartite viruses possess genomes split in more than two genomic segments each one packaged into independent particles. One of the benefits of being a multipartite virus is the gene expression modulation via changes of the segment copy number. The soil-borne beet necrotic yellow vein virus (BNYVV) may be considered a model of multipartitism since, with its 4 to 5 genomic RNAs, it has the highest number of genomic segments among RNA viruses infecting plants. In this work, we investigate the ratio of the four genomic segments of BNYVV type B in different host types analyzing tissues from infected roots and leaves by a validated protocol of dual step reverse transcriptase droplet digital (RT-dd)-PCR. BNYVV genome formula was also calculated within the vector *Polymyxa betae* after zoospore purification from infected *Beta vulgaris* roots evaluating the plant rate of contamination. Results showed that some viral gene segments accumulate at low frequency, whereas others dominate. BNYVV segment copy numbers change according to the type of host and organ infected, moreover the virus seems to reach a dedicated set-point genome formula also within its vector. These data together with the biology of this virus raise questions about the genome integrity preservation of BNYVV during the host infection and transmission by the vector.

Current status of *Botryosphaeriaceae*: fourteen years surveys among nursery, urban and agro-ecosystems in Sicily

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Botryosphaeriaceae represents a wide fungal family of which most members are known to be serious plant pathogens worldwide. During 2007–2022 numerous investigations have been conducted in Sicily to ascertain the etiology of many diseases of different crops. Specifically, ornamental crops, nut trees, fruit trees, and urban trees showing symptoms of dieback, cankers, shoot blight, fruit rot, and leaf spot, were observed, collected and furtherly investigated. Fungal species isolated from symptomatic samples were fully characterized morphologically and molecularly. Sequencing of the internal transcriber spacer region (ITS) of the nuclear ribosomal RNA cluster, part of the translation elongation factor 1α gene (*tef1*-α), and partial β-tubulin gene (*tub2*) was performed to infer phylogenetic analyses of concatenated gene datasets (multi-locus approach) for a reliable and robust characterization. Phylogenetic analyses, based on Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI), led us to identify different genera and species belonging to *Botryosphaeriaceae* including *Botryosphaeria dothidea*, *Lasiodiplodia* spp., *Neofusicoccum* spp., *Neoscytalidium dimidiatum*. Pathogenicity tests, under greenhouses and field conditions, were always performed for all the species characterized among the years to ascertain their role as plant pathogens on the target hosts. Presence of *Botryosphaeriaceae* in Sicilian ecosystems represents an important epidemiological information since these group of fungi are known to be latent pathogens, cosmopolitan and having neutral host behavior. Monitoring of *Botryosphaeriaceae* among different hosts is a crucial step in order to assess effective control strategies.

Preliminary metabarcoding analysis of potential plant pathogens transmitted by hazelnut pollen

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Pollination is a vital process in the reproduction of most spermatophytes and requires the transfer of pollen to the plant carpel, which contains the ovule, in order that fertilisation can occur. It is well known that many insects are vectors for plant viruses by contamination of pollen with these pathogens when feeding, while there are a few reports of fungi associated with pollen. However, pollen grains are nutritionally rich and represent an important source of food for fungi. Pathogenic fungus can be spread with pollen as it has been reported for *Citrus sinensis* pollen that may, in fact, play a role in the spread of C*olletotrichum acutatum* in citrus orchards. To clarify if fungi causal agents of rotten hazelnut can be transmitted with pollen, we analyzed the fungal populations present on pollen by metabarcoding. To this aim, we extracted DNA from microorganisms present on pollen of two commercial hazelnut "Tonda Gentile Trilobata" and "Tonda di Giffoni", performing a mild extraction to avoid pollen breakage and plant DNA extraction. Metabarcoding was performed by amplification of the Internal Transcribed Spacer (ITS) region of rDNA followed by short-reads Illumina sequencing. Preliminary analysis showed a high number of fungal microorganisms, and the most represented fungus is *Colletotrichum lagenaria* both in GF (26.73%) than in TGT (19.41%), but also different species of *Fusarium* sp. including *F. lateritium*, *Alternaria* sp., *Cladosporium* sp., *Ramularia* sp., (especially TGT) are present, in addition to different epiphyte/saprophyte fungi.

Rapid detection of *Eremothecium coryli* from kernel hazelnut and *Halyomorpha halys*

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Eremothecium coryli (*Nematospora coryli*), a yeast pathogen of some fruit trees, cotton, and vegetables (especially tomatoes and Lima beans) was first isolated from *Corylus avellana* by Peglion in 1897. Since 2017, an intense survey on Italian hazelnut orchards has been carried out to investigate defects of hazelnut kernel. In these studies, *E. coryli* was isolated in Piedmont and Campania regions, from kernels that presented the typical damage caused by Pentatomids bug (e.g., *Halyomorpha halys*) and showing an altered area with symptoms of dry rot. To study the presence, transmission and diffusion of *E. coryli* on hazelnut, we developed a simple, fast, reliable and sensitive method for detection and quantification of the pathogen. We set up a real-time quantitative PCR (qPCR) on total DNA extracted directly from kernel hazelnut or from insect, with primers ERM-F1/

ERM-R1 developed on the ITS region. Specificity analysis of this primer pair demonstrated that fungi that usually are isolated from hazelnuts, such as *Alternaria* spp., *Colletotrichum* spp., *Botryosphaeria* spp., *Phomopsis* spp., *and Didymella corilicola,* did not give cross reaction. Conversely, the phylogenetically close yeast *Saccaromyces cerevisiae* gave cross reaction. For this reason, qPCR for *E. coryli* was run in parallel with *S. cerevisiae* primers S14F/S17R, to exclude the presence of the latter. The efficiency and reliability of the qPCR protocol was evaluated on kernel hazelnut and on different body parts of the stink bug *Halyomorpha halys*. Results show the presence of *E. coryli* in the mouthparts of *H. halys* fed with infected hazelnuts, suggesting that the transmission of this fungus probably occurs through this way.

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)".

Biochar potentialities for suppression of diseases caused by viruses, bacteria, oomycetes, fungi and parasitic plants

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Soilborne pathogens and pests in agroecosystems represent a serious problem limiting crop yield. With a view to developing more ecologically sustainable agriculture, the possibility of using biochar to control the damaging agents has been increasingly studied in recent years. This work provided a general overview of the suppression of diseases and infestations by biochar. We presented an update of literature from 2015 to 2022 based on 61 articles, including 117 experimental cases. We assessed how several biochar production feedstocks, pyrolysis temperatures, application rates, and the examined pathosystems affect diseases and infestations incidence. Fungal pathogens represented 55% of the case studies, followed by bacteria (15%), insects and nematodes (8%), oomycetes and viruses (6%), and only 2% parasitic plants. The most studied fungal species is *Fusarium oxysporum* f. sp. *radicis lycopersici*, *Ralstonia solanacearum* for bacteria, *Meloidogyne incognita* for nematodes, *Epitrix fuscula* for insects, *Phytophthora capisci* for oomycetes and *Phelipanche aegyptiaca* as the only parasitic plant. Biochar showed 85% suppression efficiency for fungi, 50% for oomycetes, 60% for viruses, 70% for bacteria and 50% for nematodes. Most studies used an application rate between 1 and 3%, a pyrolysis temperature between 500 °C and 600 °C, and a feedstock based on sawdust and wood debris. Different mechanisms were proposed to explain disease suppression by biochar, including induction of systemic resistance, improving rhizosphere competence of microbial community, and sorption of phytotoxic compounds of plant and/or microbial origin. Overall, it is important to standardize biochar feedstock and rate application and to improve beneficial effect on plant in terms of disease control.

Survey on the presence of fungal epiphytic, saprophytes, and leaf pathogens present on a collection of grapevine rootstocks in Italy

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Vitis rootstocks are cultivated in Italy on about 2,500 Ha, with a predominance of 5 varieties constituting 95% of the total (SO4, 110 Richter, 1103 Paulsen, 140 Ruggeri and K5BB). Besides conferring resistance to *Daktulosphaira vitifoliae*, recent breeding efforts have been devoted to new rootstocks tolerant to salinity, nematode, and water saving. No data are available on the characterization of fungal species potentially affecting the health of the propagation material. The aim of the present work is to provide a preliminary characterization of fungal species associated with different leaf symptomatologies, ranging from discolorations to necroses of variable extent. A first survey was conducted in 2020 at the CREA-VE experimental vineyard. Conventional diagnosis through isolation of Potato Dextrose Agar amended with antibiotics, and pathogenicity test on detached *Vitis vinifera* leaves, allowed to identify fungal species (*Botrytis cinerea, Coniella diplodiopsis, Pilidium concavum, Diaporthe helianthii, Neopestalotiopsis* spp.)

already known as pathogenic on other crops. For a second surveys, done in 2021 in the same farm, a metabarcoding strategy using Illumina MiSeq amplifying the ITS2 region, allowed to classify a total of 110 OTUs; 27.7% of the total reads were identified as *Pseudopezicula tracheiphila*, agent of red fire disease of grapevine, followed by *Cladosporium* sp., *Alternaria alternata*, and *Saccotheciaceae* sp., a family including potentially phytopathogenic genera. The use of some rootstocks as donors of genetic resistance towards the most common grapevine diseases (powdery mildew, black rot blight), could imply the risk of transfer, through crossing, susceptibility to these neglected but potentially dangerous fungal species.

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SUStaINable use of bioactive compounds from Brassicaceae and Solanaceae wastes for CEReal crop protection (SUSinCER)

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Defatted seed meals of oleaginous Brassicaceae, such as *Eruca sativa*, and potato peel are excellent plant matrices to recover potentially useful biomolecules from industrial processes in a circular economy perspective aiming at crop protection. These biomolecules, mainly glucosinolates for Brassicaceae and glycoalkaloids for potato, have been proven to be effective against pests like bacteria and fungi. Their role in plant protection is investigated, together with the molecular basis of their synthesis in plant, and their mechanisms of action. Possible genetic and biotechnological strategies are presented to increase their content in plants. The application of these bioprotectors will deal with new global challenges, such as reducing food waste and increasing sustainability and food safety for the consumer. SUSinCER project aims to investigate the biological activity of secondary metabolites from defatted seed meals of oleaginous Brassicaceae and potato peel, against development of fungal pathogens and mycotoxin production by fungi that contaminate cereals, mainly maize and wheat. In particular, during the first year of project activity a radial growth inhibition assay has been performed in vitro to evaluate the effect of different bioactive compounds on *Fusarium verticillioides* and *Fusarium graminearum*, important mycotoxigenic fungal pathogens responsible for maize and wheat rot. In field activities are also in course for wheat and maize to study the effect of bioactive compounds on resistance to fungal diseases and agronomic performance.

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Multispecies *Trichoderma* consortium in combination with hydrolyzed lignin extract improve growth, yield, and nutritional quality of tomato

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The introduction of beneficial synthetic communities with functional and metabolic complementarity can modify the rhizosphere microbial composition and substantially improve plant yield and pathogenic microorganism containment. Many studies aimed to evaluate the potential association of microbial consortia and bioactive molecules belonging to "botanicals", plant extracts able of inhibiting pathogen development, inducing systemic resistance and promoting plant growth. The aim of this research was to develop new biological formulations combining *Trichoderma* spp. multispecies consortia with a lignin-derived polyphenolic mixture, for improving plants health status and productivity, both in quantitative and qualitative terms. To this

scope, the compatibility between strains belonging to *T. asperellum*, *T. virens*, *T. atroviride* and commercial hydrolyzed lignin extract (Solargo UPM Solargo™) was tested *in vitro* and *in vivo* assays. The effect of lignin-*Trichoderma* formulations was assessed in greenhouse and field experiments, by evaluating the main plant biometric parameters, yield, and quality of tomato fruits. *T. virens* GV41 + *T. asperellum* + *T. atroviride* + Solargo formulation was the most effective in terms of growth promotion, increasing root and stem dry weight compared to control (45.4 and 43.9%, respectively). The same formulation determined a 63% increase in yield, compared to the control, resulting the better performing treatment also compared to single or coupled constituents. In addition, significant differences in terms of lycopene, GABA, ornithine, total, essential and branched-chain amino acid contents were revealed in fruits from treated tomato plants, confirming that beneficial microorganisms and polyphenolic mixture can positively interact in improving crop nutritional quality.

Is the combination of DNA metabarcoding and conventional leaf-baiting isolation techniques the best strategy to unravel the *Phytophthora* variability in natural, semi natural and horticultural ecosystems?

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Over the past two decades, molecular diagnostic tools for identification of cultured *Phytophthora* specimens supported by traditional isolation techniques provided efficient tools for the monitoring of *Phytophthora* communities within different kinds of ecosystems. More recently, metabarcoding approaches emerged as new paradigms for the detection and surveillance of target organisms within an environmental sample. In this study, the Illumina metabarcoding and a conventional baiting isolation technique complemented each other to describe the variability in *Phytophthora* communities from three small-scale ecosystems: (i) Nature Reserve, (ii) Botanical Garden, (iii) Citrus Orchard. Overall, 28 out of 39 collected rhizosphere soil samples, processed by both leaf baiting and Illumina metabarcoding, were classified as *Phytophthora*-positive. In total, the 1,406,613 ITS1 sequences obtained by metabarcoding together with the 155 baited isolates made it possible to record 21 *Phytophthora* taxa, five exclusively by baiting (*P. bilorbang*, *P. cryptogea*, *P. gonapodyides*, *P. parvispora* and *P. pseudocryptogea*), 12 exclusively by metabarcoding (*P. asparagi*, *P. occultans*, *P. psycrophila*, *P. syringae*, *P. aleatoria*/*P. cactorum*, *P. castanetorum*/*P. quercina*, *P. iranica*-like, *P.* unknown taxon 1, *P.* unknown taxon 2, *P.* unknown taxon 3, *P.* unknown taxon 4, *P.* unknown taxon 5) and four with both techniques (*P. citrophthora*, *P. multivora*, *P. nicotianae* and *P. plurivora*). Results suggested that the combined use of an efficient leaf baiting technique and a reliable metabarcoding detection method is the best approach to unravel the variability of *Phytophthora* communities from both natural and managed ecosystems.

High Throughput Sequencing testing of small RNAs empowers the simultaneous detection of citrus viruses and viroids

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Citrus are affected by a relevant number of viruses and viroids routinely tested by bioindexing and molecular assays. Anyway, the selection of virus free mother trees candidate for the foundation block requires a process of shoot tip grafting and biological indexing in greenhouse and molecular assays. A challenge which needs adequate professional and technical resources. Recent papers have shown the adoption of High Throughput Sequencing (HTS) technology is effective for the simultaneous detection and identification of viruses and viroids in *Citrus* and other crops, as pre-screening to conventional detection methods to solve ambiguous results. To explore the reliability of a potential HTS-based virus detection protocol in association of bioinformatic strategies, within the project Novarancia we developed a pilot testing of different field trees in parallel with molecular and biological methods. Three sweet orange trees and two alemow seedlings previously indexed by conventional methods were re-analyzed by HTS of small RNAs. High-quality reads, depleted of the *Citrus sinensis* genome, were aligned with reference genomes of 13 viruses and 6 viroids and analyzed by a bioinformatic pipeline. A genome coverage above 90% was assumed indicative of the presence of a specific genotype, and coverage > 50% of potential presence of variants not represented in the read mapping reference list. The results show the implementation of conventional certification scheme with HTS sequencing of

small RNAs speeds up the time over all required and reduces the greenhouse footprint, labor, time, and cost needed for bioindexing.

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Differences in the endophytic microbial communities of two olive cultivars infected by *Pseudomonas savastanoi* pv. *savastanoi*

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Olive knot disease (OKD) seriously affects olive production in the Mediterranean basin. The characteristic symptom is the formation of tumorous overgrowths deeply colonized by *Pseudomonas savastanoi* pv. *savastanoi* (*Psv*). Microorganisms in nature live as members of complex multispecies communities and an articulated pathobiome has been described for OKD. During a 3-years survey at the International Olive Germplasm Collection "Villa Zagaria" (Sicily, Italy), difference in resistance to *Psv* has been detected in trees harbouring a similar epiphytic bacterial population titre. In order to investigate on the possible interactions with endophytic microbial communities the cultivars Zaituna and Giarraffa, showing high and low resistance to OKD, respectively, were selected for an amplicon-based metagenomics analysis. The DNAs were extracted from surface sterilised twigs of field-grown olive trees. The endophytic bacterial and fungal communities were analyzed using Illumina-based sequencing of the V3–V4 hyper variable regions of bacterial 16S rRNA genes and the ITS2 regions of fungal rRNA genes, respectively. In the whole, *Proteobacteria* and *Ascomycota* were the most abundant bacterial and fungal phyla respectively. A significantly higher bacterial richness was detected in the shoots of the susceptible 'Giarraffa' although with a lower diversity. The opposite trend was observed for fungal communities. Differential abundance analysis of bacterial communities showed that *Amnibacterium* (*Actinobacteria*), unidentified *Mollicutes* (*Tenericutes*), *Methylobacterium* and *Sphingomonas* (*Proteobacteria*) were enriched in 'Zaituna' whereas *Pseudomonas* (*Proteobacteria)*, unidentified *Plankthothricoides* and unidentified *Microcoleaceae* (*Cyanobacteria*) were depleted. Moreover, in 'Zaituna' the fungal community resulted enriched in *Neofabraea* (*Ascomycota*) and unidentified *Tremellomycetes* (*Basidiomycota*) and depleted in *Alternaria*, *Neofusicoccum* and *Pseudocercospora* (*Ascomycota*).

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Biological control of *Pseudomonas savastanoi* pv. *savastanoi* by using an extract from pomegranate by-products

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Olive knot disease (OKD) seriously affects olive production inducing the formation of tumorous overgrowths. *Pseudomonas savastanoi* pv. *savastanoi* (*Psv*) is the causal agent and lives epiphytically on the surface of leaves and twigs. The pruning of infected material and copper treatments are the unique currently available strategies to control further spread. Because sometimes copper treatments can have a reduced efficacy and can cause undesirable effects, alternative biological methods are under evaluation. A standardized dry powdered pomegranate extract (PPE) derived from the by-products (peels, exhausted arils and seeds) of pomegranate juice industrial processing, developed by the researchers of CREA (Acireale), has been tested to contain *Psv* epiphytic population in olive plants. Preliminary *in vitro* experiments revealed relevant inhibitory activities of PPE at different concentrations (10%, 5%, 2.5%, 1%, 0.5%) against *Psv* (10^8–10^6 log cfu ml^{-1}). Furthermore, *in vivo* treatments were applied on the canopy of 'Biancolilla', 'Moresca' and 'Calatina' olive plants with different susceptibility to OKD. Three treatments of PPE at 5% concentration were applied during spring (after 30 days each), few days after pruning. The overall epiphytic bacterial population obtained by plate count on King's B medium showed no statistical differences between PPE, copper treatment and control (water). A significant reduction of epipthyic *Psv* population, estimated by quantitative real-time PCR, has been detected in 'Biancolilla' and 'Calatina' treated with PPE and copper (2.2 log cfu mL^{-1}) in comparison to the control (3.7 log cfu mL^{-1}). No efficacy was detected in 'Moresca'. The *Psv* population counts were statistically different among the different groups and were influenced by the fixed factors taken into account (i.e. cultivar, treatment, cultivar x treatment).

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Gene expression of sour oranges seedlings inoculated with an aggressive seedling yellow CTV and a homologous asymptomatic isolate

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Citrus tristeza virus (CTV) is a phloem-restricted aphid-transmitted virus, responsible for tremendous economic losses of sweet oranges and grapefruits grafted on sour orange (SO), leading many countries to the use of tolerant rootstocks. In earlier studies SO seedlings inoculated with a severe VT-SY isolate (SG29) showed severe seedling yellows, stunting and reduced root apparatus, while those inoculated with a homologous asymptomatic variant (M39) remained very similar to control non-inoculated plants. Moreover, the asymptomatic isolate inhibited the superinfection with the severe one. To understand the basis of this cross protection-like reaction we have investigated the transcriptomic response of SO seedlings inoculated with SG29 or the homologous asymptomatic and protective variant M39. Analysis of transcriptome obtained through Illumina RNA-seq, in comparison to uninoculated controls, showed 2,869 differentially expressed transcripts (DETs) after infection with SG29 and only 7 with M39. A total of 517 DETs were exclusively present in SG29 plants relative to M39, whereas 1,739 were shared also to the uninoculated control. Many DETs consist of immune receptors (i.e., nucleotide-binding-leucine-rich repeat proteins) able to initiate a resistance response through a cascade consisting of MAP kinases and chaperone protein complex (heat shock proteins). Transcripts associated to senescence, hormone metabolism (auxin, ethylene), cell wall and ribosomal composition are also included. These data reveal that SG29 is responsible of the activation of an effector-trigged immunity (ETI) response, giving rise to the marked symptomatic hypersensitive reaction observed in plants. Only few DETs linked with ETI are upregulated in M39 inoculated plants.

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Evaluation of copper-alternative products to control citrus anthracnose and Alternaria brown spot in Sicilian citrus orchards

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Colletotrichum and *Alternaria* species, causal agents of pre- and postharvest anthracnose and Alternaria brown spot have been recently reported as emerging fungal pathogens on citrus in the Mediterranean area. The limitation on the use of copper-based fungicides in most European countries, in both integrated and organic farming, due to its demonstrated noxious effect on the environment, makes the control of these pathogens difficult. Thus, alternative products able to reduce/phase out copper in citrus farming are needed. In this work, copper-alternative products, previously identified and evaluated *in vitro* and in pre- and post-harvest trials, were tested for 2 consecutive years (2020–2021) in two integrated and one organic citrus orchards in different pedo-climatic conditions to control natural infections caused by *Colletotrichum* spp. and *Alternaria* spp. The alternatives, including basic products, active substances, biocontrol agents and their combinations, were applied on oranges cultivar "Tarocco Scirè" and "Tarocco Tapi" and on lemon cultivar Femminello 2KR. Even under different disease pressure levels, the basic products (chitosan and equisetum) and the active substance (sweet orange essential oil), alone and in mixture, significantly reduced disease incidence and severity compared with the untreated controls, often showing similar or better efficacy than copper compounds. The good efficacy of alternative products encourages their further evaluations in other *in vivo* trials and indicates the potential of their sustainable and large-scale use, useful for replacing or reducing the use of copper in integrated and organic citriculture in view of future limitation of its use.

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Genome-wideanalysis of G-type lectin family in *Fragaria vesca* and functional characterization of *FaMBL1* gene in defense to fungal pathogens

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Strawberry (*Fragaria*×*ananassa*) is one of the most consumed and produced fleshy fruits in the world but due to its characteristic softening is highly susceptible to many fungal pathogens. Anthracnose and gray mold are two of the most destructive diseases of strawberry which lead to serious fruit rot. White unripe strawberry fruits are more resistant to *Colletotrichum acutatum* than red ripe fruits. Previous transcriptional analysis conducted during early stage infection has shown that a mannose-binding lectin gene FaMBL1 was the most upregulated in the white stage compared to the ripe susceptible one. FaMBL1 belongs to the G-type lectin family, having important roles in plant development and defense process. In this study genome-wide identification of G-type lectin gene family was carried out in *F. vesca*. Overall, 133 G-lectin genes were characterized for domain arrangement and expression patterns. Active transcription of G-lectin genes during development and under stresses suggested a potential role of G-lectin genes in strawberry defense. Hence, stable transgenic strawberry plants overexpressing the FaMBL1 gene were generated. Transformed strawberry plants were selected and molecularly characterized through droplet digital PCR and RT-PCR analysis. In total four overexpressing lines (OE) with different copy numbers were obtained and used for the subsequent studies, including the evaluation of disease-related phytohormones content and their reaction to biotic stresses. Accordingly, jasmonic acid (JA) was found decreased in OE-lines compared to wild type (WT). OE-plants petioles inoculated with *C. fioriniae* had lower disease incidence than WT, and leaves challenged by *B. cinerea* showed remarkably smaller lesion. Furthermore, expression of defence related genes as chitinase 2–1 (FaChi2-1) gene showed higher expression in OE-lines than in WT during *B. cinerea* infection development. Our results showed that G-type lectin genes, a big gene family in *F. vesca*, are involved in strawberry response to biotic and abiotic stresses, and in particular, FaMBL1 gene plays a role in inducing disease resistance, presumably in a JA-dependent pattern.

Fitness of Venturia inaequalis strains associated with resistance to different fungicides classes

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Venturia inaequalis, the causal agent of apple scab, represents one of the major threats to apple production. The repeated use of fungicides increases the selection pressure in favour of individuals resistant to different fungicide classes. To limit the spread of resistant individuals, it is mandatory to characterize their competitiveness inside the population. In this study, 35 V. *inaequalis* strains sampled in Lombardy and previously phenotyped for resistance towards five fungicides (dodine, cyprodinil, trifloxystrobin, boscalid, myclobutanil) of different classes, were characterized for their fitness components, to evaluate the spread and persistence potentials of resistant. For each isolate, the mycelial growth and conidial production, in terms of concentration and size, were evaluated and related to the phenotype and environment. Generally, the results highlighted the absence of significant differences between resistant and sensitive strains in terms of fitness. Only strains resistant to cyprodinil showed a significantly higher mycelial growth than sensitive ones, while strains resistant to dodine showed a significant reduced conidia size compared to sensitive ones. Moreover, the environmental conditions seemed to influence the fitness components, especially conidia differentiation. Indeed, strains sampled at higher elevation produced more conidia with reduced size, suggesting that genetic background and environmental conditions due to altitude could induce the pathogen to differentiate a higher number of conidia, providing a greater diffusion in the environment and increasing the probability to infect the host. Therefore, the resistant strains could persist and spread throughout the population, thus making essential to adopt adequate control strategies, taking into account anti-resistance practices.

The rules behind plant microbiome assembly: a big data approach

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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. In this study, we leverage on the massive amount of publicly available data generated by over 14,000 studies (timespan 2017–2021), which we collected and re-analyzed under a common framework. Using a variety of metrics (microbiome diversity, structure, composition, co-occurrence networks) and approaches (Bayesian modelling, network comparison, machine learning), we tested several questions, including: (i) which are the major factors contributing to the assembly of plant microbiomes? (ii) do specific stressors (e.g., pathogens, drought) generate unique changes in the plant microbiomes? (iii) does plant phylogeny reconciliate with the diversity/structure of plant microbiomes? We also carefully searched each article, highlighting important methodological limitations that need to be considered in the future research endeavors. Our results contribute to a more general understanding of plant-microbiome interactions, which are thought to be key towards eco-sustainable plant protection, ecosystems conservation, and successful ecological restoration.

Selection of informing spectral bands and hyperspectral indices for the downy mildew (*Plasmopara viticola*) detection on *Vitis vinifera*

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Downy mildew, caused by the obligate biotrophic oomycete *Plasmopara viticola*, is one of the most serious grapevine diseases worldwide, predominantly controlled by the application of copper-based fungicides. The large application of these products is responsible for copper (Cu) accumulation in the upper layers of soils and water sources and may cause phytotoxicity. With limitations in the use of Cu-based products imposed for organic agriculture by the European Union, research for alternative sustainable products is strongly encouraged. This project aims at reducing Cu usage by combining the application of microbial agents and natural compounds with the monitoring of disease appearing and progression by hyperspectral sensors. In this work informative spectral bands and vegetation indices describing differences between healthy and infected leaves were selected. Hyperspectral images of symptomless and diseased leaves were acquired by using the SPECIM IQ camera (Specim, Spectral Imaging Ltd., Oulu, Finland) working in the range of 400–1.000 nm. The presence of disease determined a flattening and a strongly reduction of reflectance between 570–670 and 710–1.000 nm, respectively. Vegetation indices discriminating healthy leaves were related to water content (WI, FWBI1, FWBI2) while diseased leaves were exhaustively described by chlorophyll and vegetation-associated indices (RGRcn, G, VOG3, PRI, PSRI). Spectral signature produced in this preliminary stage of research will be applied to estimate the effectiveness of bioformulation applications in ongoing field trials.

Hyperspectral imaging elucidates the ability of *Trichoderma harzianum* T22, *Pseudomonas simiae* WCS417r, and their combination to control wild rocket foliar bacterial blight through induction of systemic resistance

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Selected members of plant microbiota can protect plants against pathogenic microorganisms through different mechanisms, including the induction of systemic resistance (ISR). *Pseudomonas simiae* WCS417r and *Trichoderma harzianum* T22 are two model beneficial microbes and their ability to induce ISR has been demonstrated on multiple plant species and against a wide range of phytopathogens. In this work we evaluated their ISR activity, in individual and combined applications on wild rocket against *Xanthomonas campestris* pv. *campestris* (*Xcc*) by assessing hyperspectral signature,

ISR-associated gene expression and disease severity. Fifteen-dayold plantlets were transferred to peat mixed with T22, WCS417r and T22 + WCS417r, while untreated peat was used as control. After 2 weeks, 75 plants per treatment were infected with *Xcc* by shoot dipping. Starting from 24 h post inoculation (hpi) and for the following 4 days, each 24 h plants were subjected to hyperspectral image acquisition, disease monitoring and snap frozen for further gene expression analyses. Antagonists significantly reduced disease severity of 40 and 50% compared to the infected control, at 96 and 168 hpi, respectively. ISR activation was confirmed by analyzing marker genes of different hormone pathways which resulted differentially expressed compared to infected untreated control. Hyperspectral vegetation indices (22 out of 54 analyzed) proved to be able in discriminating different disease levels (none, low, intermediate, and high). The dynamic of vegetation indices resulted time-dependent and allowed the significant differentiation of treatments up to 72 h, before symptom appearing, thus favoring the early disease detection.

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Isolation and partial characterization of fungi associated with cankers and necrosis on young holm oak (*Quercus ilex*) stands in the Salento Peninsula of Southern Italy

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Emerging plant diseases are an increasingly common problem for forest plants globally, and climate change is most likely a major predisposing factor. Such diseases may be caused by latent microorganisms cryptically associated with plants such as many fungal endophytes, originally described as generalist or widespread species and recently recognized as pathogens. The transition to a pathogenetic stage in fungi may depend on physiological alterations of the host, environmental changes and/or stress factors. The *Botryosphaeriaceae* family is one of the most representative examples of a very diverse group of fungi, including well-studied endophytes and latent pathogens of woody plants that typically cause stressor associated diseases. A sudden decline of young holm oak plants (*Quercus ilex*) has recently been observed during our inspections in the Salento peninsula (Apulia region, Italy). Samplings and isolations were carried out from symptomatic holm oak branches and trunks with cankers and necrosis. Representative fungal isolates were morphologically characterized and used in pathogenicity tests on artificially inoculated young holm oak plants. The result confirmed the aggressiveness of collected fungal isolates and their belonging to the *Botryosphaeriaceae* family, which include genera and fungal species generally known as weak pathogens. However, under favourable conditions, e.g., in plants affected by other biotic and/or abiotic factors, these organisms can cause serious infections, debilitating the host even to death. Further investigations are underway to identify the most virulent species of fungi involved and to better understand their role in the decline of holm oak stands.

H.J. Maree

Huanglongbing has been observed in South Africa for nearly 100 years. Unusual symptoms of yellow branches and green fruit were reported in the late 1920's, and were linked to significant crop losses. The disease was named Greening with the aetiological agent unknown. They had no idea that these symptoms would later become part of an almost global pandemic of diseases collectively called HLB. The disease was shown to be graft transmissible and vectored by *Trioza erytreae*. It took more than 50 years of research to identify the causative agent as '*Candidatus* Liberibacter africanus' (CLaf). Greening has been detected in more African countries and is spreading through infected planting material and the insect vector. Five CLaf subspecies have been identified in various Rutaceous species with one CLaf subsp. clausenae being the only subspecies for which a biovar was detected in citrus.

heat sensitive that prevents the establishment of Greening in unfavorable climatic regions. Greening has been successfully managed in regions deemed unsuitable for citriculture, due to established populations of CLaf and *Trioza erytreae*, through coordinated area-wide management strategies.

efforts are focused on the development of sensitive detection methods and monitoring strategies for HLB causing liberibacter species and their vectors.

Effects of mulch biofilms and biostimulants based on *Trichoderma* and *Ascophyllum nodosum* on industrial tomato plants

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The European Green Deal aims to reduce the use of synthetic chemical pesticides by 50% by 2030. Thus, novel strategies using low inputs of chemical products are highly desirable. Bioformulates consisting of living beneficial microbes or natural products used to improve crop production or control phytopathogens represent fundamental issues of an Integrated Pest Management (IPM) program. In this work two commercial products based on *Trichoderma afroharzianum* strain T22 (Trianum P®, Koppert), and a seaweed extract from *Ascophyllum nodosum* (Phylgreen®, Trade Corporation International) were tested on industrial tomato plants (*Solanum lycopersicum* var. Heinz 5108) in a field experiment. The effects of single and combined applications of microbial and plant biostimulants on the productive and qualitative traits were evaluated on plants grown on two biodegradable plastic mulch films. Results showed that mulch films increased up to 30% marketable yield compared to control, while the combined application of biostimulants increased yield up to 25% compared to untreated plants. Treatments affected also qualitative parameters, such as fruit texture and colours, and concentration of bioactive compounds (total polyphenols, carotenoid, lycopene). Finally, a LC–MS Q-TOF metabolomic analysis revealed that plant metabolic profiles varied according to the presence of biofilm as well as to the application of biostimulants. An increase in the concentration of some metabolites (alkaloids, flavonoids) in tomato berries and leaves was observed following the application of *Trichoderma*-based product.

Characterization of fungal pathogens associated with canker and dieback of apple trees in Northern Italy

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Italy is one of the largest apple (*Malus* × *domestica*) producers in Europe, with 57.025 ha cultivated and more than 2 million tons of apple produced in 2021. Wood infections represent a serious threat to apple cultivation worldwide. Several pathogens can infect trunks, branches and shoots causing cankers, twig blight, wood rot and death of trees. Currently, the main fungal species found in association with canker and dieback of apple trees belong to Botryosphaeriaceae, Diaporthaceae and Diatrypaceae families. Considering the impact of wood pathogens and the scarcity of studies on wood diseases of apple in Italy, different surveys were conducted in Piedmont during 2020 – 2022. Six apple orchards and eight cultivars were surveyed. The species characterization was achieved through morphological features, optimum growth temperature assessment, and multilocus phylogenetic analyses. Seven fungal species were found: *Botryosphaeria dothidea*, *Cadophora luteo-olivacea*, *Diaporthe rudis*, *Diplodia seriata*, *Eutypa lata*, *Kalmusia longispora* and *Paraconiothyrium brasiliense*. A broad optimum temperature range was observed among the species (21 to 29 °C). Pathogenicity tests were conducted on plants of the representative cultivar 'Gala' and the virulence of all the fungal species was confirmed. During this study, the species *Cadophora luteo-olivacea*, *Diplodia seriata*, *Eutypa lata*, *Kalmusia longispora* and *Paraconiothyrium brasiliense* were reported for the first time as causal agents of apple canker and dieback in Italy. Moreover, the demonstrated insights on apple wood diseases lay the basis for further epidemiological and diagnostic investigations to assess the phytosanitary status of plant material and to implement effective preventive management strategies.

Development and validation of a SYBR Green qPCR assay for early and specific detection of *Colletotrichum ocimi*, causal agent of black spot on basil

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The genus *Colletotrichum* includes several important species pathogenic on several plant hosts. *Colletotrichum ocimi* is the causal agent of black spot of basil (*Ocimum basilicum* L.) and represents a serious threat to basil producers and seed-production companies as it can affect both leaves and seeds. Considering the importance of basil

cultivation in the Mediterranean area and the absence of diagnostic methods to identify *C. ocimi*, a SYBR Green real-time PCR assays was developed to detect the pathogen on basil leaves and seeds. Two primer sets were designed on the partial *β-tubulin* (*tub2*) region. The selected primers pair, TubOc68fw – TubOc_197rev, produced amplicons of 130 bp. The developed SYBR Green real-time PCR assay was validated for specificity, sensitivity, selectivity, repeatability and reproducibility. The assay was specific for *C. ocimi* with respect to 10 *Colletotrichum* spp. and to another 12 species known as pathogens of basil plants. The sensitivity of the method was 1 pg μl^{-1} of genomic fungal DNA. The amplification analyses were not influenced by basil genomic DNA. The primer set was able to detect and quantify *C. ocimi* on artificially inoculated basil leaves and on artificially inoculated seeds. To our knowledge, this is the first specific primer set for the identification of *C. ocimi* and the developed assay can be useful for diagnostics on plants and seeds, with an implemented extraction method, thus contributing to improve the early detection of the pathogen on seeds or seedlings and the adoption of effective preventive management strategies.

Insights on grapevine varietal susceptibility to Flavescence dorée in relation with the vector *Scaphoideus titanus*

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Flavescence dorée (FD) of grapevine is a disease associated with the homonymous phytoplasma (FDp), causing severe losses to European viticulture. The main insect vector is *Scaphoideus titanus* (Ball), a Nearctic leafhopper introduced into Europe. To investigate the role of the grapevine cultivar in contrasting the disease, 14 varieties were exposed to FDp, using infectious *S. titanus* for a one-week Inoculation Access Period (IAP). Five and eight weeks after the IAP, the presence of FDp was assessed by qPCR, drawing a complete susceptibility range for these 14 cultivars. To exclude the possibility that low susceptibility to FD may be due to poor vector fitness on the specific grapevine genotype, *S. titanus* fitness parameters and feeding behaviour were measure on three *Vitis* varieties selected to represent the extremes of the FD-susceptibility range. In particular, *S. titanus* fitness was studied on Barbera as FD-susceptible, Brachetto, and Moscato as FD-tolerant. Female prolificacy was chosen as fitness parameter, by means of number of beared mature eggs and vitellogenin expression at three different times after emergence. In parallel, also nymph and adult survival were measured on the three *Vitis* genotypes. Overall, *S. titanus* performed and fed better on the FD most susceptible cultivar. On the other hand, impaired vector feeding behaviour and performance may explain the poor susceptibility to the disease of the least susceptible cultivar, possibly through antibiosis and antixenosis defense mechanisms against the vector.

Molecular characterization of an Apulian isolate of tomato leaf curl New Delhi virus

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Tomato leaf curl New Delhi virus (ToLCNDV) is an emerging begomovirus (*Geminiviridae* family) whose economic importance in the Mediterranean basin correlates with recurrent outbreaks in tomato and in cucurbits with estimated losses between 30 and 100%. In Apulia (southern Italy), the virus was isolated in 2018 in commercial fields of zucchini squash and melon and in 2020 in protected crops of zucchini squash with heavy infestations of aleyrodids. ToLCNDV genome consists of two circular single-stranded DNA molecules of about 2.7–2.6 kb, referred to as DNA-A and DNA-B, encapsidated in geminate particles. Betasatellites could be associated with DNA affecting replication, movement within host, horizontal transmission between plants and inducing severe disease symptoms and suppression of transcriptional gene silencing. We sampled zucchini squash during 2020 outbreaks and obtained the whole sequence of the DNA-A of one of the isolates for which the name of ToLCNDV-Le is proposed. Phylogenetic analysis of the CP sequences of ToLCNDV-Le with those of 42 ToLCNDV isolates from different countries and plant species, retrieved from the public database placed ToLCNDV-Le among the Spain strains (ToLCNDV-ES) of the virus as it shares more than 94% nucleotide identity, according to ICTV strain demarcation criteria. Characteristics of the fully sequenced DNA-A of ToLCNDV-Le using the Illumina 2 × 150 bp reads platform and WGS application with a 100 × depth of coverage will be reported. Since recombination has been previously reported for ToLCNDV, we examined the ToLCNDV-Le DNA-A for any evidence of recombination as well as for its association with betasatellites.

Grafting to manage tomato leaf curl New Delhi virus in cucurbits

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Tomato leaf curl New Delhi virus (ToLCNDV) is an emerging begomovirus (*Geminiviridae* family) listed in the EPPO Alert-list 2, present in the Mediterranean area and in Italy, where it was reported in 2015 in Sicilian courgette and since then its recurrent outbreaks generated justified concern among growers. The virus is particularly harmful in cucurbits, where it causes 100% production losses, thus a sustainable and environmentally friendly approach must be adopted. Genetic resistances have been identified in *Cucurbita moschata* and *Luffa cylindrica*, but the graft could provide a faster and more flexible solution inducing tolerance rather than resistance, as shown in tomato crops by grafting susceptible commercial tomato varieties onto the tomato wild ecotype Manduria (Ma). Here we report results of a screening among twenty-one local cucurbit ecotypes to evaluate tolerance levels against mechanical transmission of ToLCNDV. Results will lead to the identification of potential rootstocks to attain suitable levels of tolerance against the virus in commercial cucurbit varieties. Plants were challenged with ToLCNDV isolated in Apulia and observed for disease symptoms development and viral DNA accumulation by quantitative dot-blot assays at 14 and 28 days after inoculation. *C. melo* var. Retato standard (F1 commercial hybrid) and *C. pepo* var. Scuro di Milano proved the most susceptible, whereas *C. melo* var. Barattiere and *C. pepo* accession 5 the most tolerant. Tolerant plants did not show disease symptoms and very low level of virus accumulation, suggesting their use as rootstocks of grafted cucurbits against ToLCNDV outbreaks.

Raman spectroscopy detection of virus infection in asymptomatic tomato and grapevine plants

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Plants are exposed to a huge variety of biotic stresses caused by different pathogens and react to them by activating several metabolic pathways. Among pathogens, viruses are the most difficult to control and their reliable detection in the early stages of the disease may help to reduce their spread and alleviate the economic impact. Besides laborious, costly and destructive diagnostic serological and molecular techniques, Raman spectroscopy (RS) is an innovative alternative method for a quick, cheap and non-destructive pathogen detection by the creation of a sample chemical fingerprint. In this study, the efficiency of RS (in combination with chemometric analysis) in virus detection in asymptomatic samples and in the monitoring of the virus infection progress in two selected agricultural crops was investigated. Tomato plants infected by tomato yellow leaf curl Sardinia virus (TYLCSV) and tomato spotted wilt virus (TSWV), and grapevine plants infected by grapevine fanleaf virus (GFLV) and grapevine rupestris stem pitting-associated virus (GRSPaV) were analysed. RS successfully differentiated the RS profiles of healthy and virus-infected asymptomatic plants with 70 and 85% precision for TYLCSV and TSWV, respectively, and with 80 and 100% accuracy for GRSPaV and GFLV in grapevine, respectively. Metabolic changes in chlorophylls, carotenoids, and polyphenolic compounds occurring in asymptomatic infected leaves were identified as the principal biomarkers. The potential uses of this emerging and cutting-edge technique for real-time in-field virus detection in crops will be outlined.

Plant pest surveillance program and survey results in Italy

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Since the second half of the nineteenth century, the introduction of plant pests has been a critical issue for national agricultural production. First in Italy, Umberto I of Savoy created a network of *consortia* with the purpose of monitoring phylloxera infestations. Then, processes such as trade globalization and climate change have greatly facilitated the introduction of non-native pathogens. Nowadays, EU legislation obligates Member States for annual plant health

surveys carried out by the Regional Phytosanitary Services. The aim of the surveys is to confirm the pest-free status in areas where their presence is not known, and that any infections in the territory can be promptly eradicated. In 2021, Italy surveyed 82 pests, 34 of which included fungi, bacteria, viruses, and phytoplasma listed either as priority pests (i.e., pests subject to Union emergency measures) or as pests listed in parts A and B of Annex II of Regulation (EU) 2019/2072, as well as other pests of EU and/or national interest. The methodology for pathogen surveys consisted primarily of visual examinations supported by sampling in case of suspicious symptoms. Trapping have been performed for pathogen vectors, such as *Philaenus spumarius, Pityophthorus juglandis,* and *Scaphoideus titanus*. Samples were analyzed in official laboratories, with specific tests such as morphological identification, serological and molecular tests. The major survey activity concerned *Xylella fastidiosa, Phyllosticta citricarpa*, *Pantoea stewartii* subsp. *stewartii*, potato bacteria, *Flavescence dorée* and tomato brown rugose virus. In case of new findings, official emergency measures have promptly applied.

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Evaluation of *Gnomoniopsis castaneae* incidence in Campania region under different agricultural systems

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Gnomoniopsis castaneae (Gnomoniaceae, Diaporthales) is an ascomycete fungus capable of colonizing different plant species, belonging to the families Fagaceae, Onagraceae and Rosaceae. It is the major rot agent on chestnut fruits, also associated with cankers on both chestnut and hazelnut, as well as necrosis on leaves and chestnut galls caused by *Dryocosmus kuriphilus*. In the last 10 years, the presence of *G. castaneae* caused a severe plant health emergency in Campania region, so that some chestnut productions were totally lost. Aim of this work was to evaluate the best field treatment (organic or IPM) against *G. castaneae* diffusion in chestnut cultivation. Different field sites were treated following: organic protocol, Integrated Pest Management (IPM) and no treatments, sampling in two different seasons: autumn 2020 and 2021. The disease incidence was assessed both by visual inspections of chestnut fruits and by evaluating the occult presence of the pathogen (identified as potential rot) by axenic isolation of *G. castaneae* from asymptomatic chestnuts. Molecular analyses by PCR amplification of ITS region of ribosomal DNA, confirmed the identity of morphologically identified *G. castaneae* isolates. Results demonstrated that both treatments are effective in controlling *G. castaneae* development in chestnut fruits. In chestnut fields treated following organic system, visible infection reached 15% and the potential rot was around 50%. In IPM treated fields a very low percentage of chestnut showed visible rot (3–4%) while 30–35% were asymptomatic but infected samples. In control field, were no treatment were carried out, visible chestnut rot was higher than 50% and the potential rot reached 100% of samples.

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Trichoderma afroharzianum causing seed rot on maize in Italy

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Maize (*Zea mays* L.) is a cereal crop of great economic importance in Italy. *Trichoderma* species are widespread filamentous fungi in soil, well known and studied as biological control. In September 2020 during a routine seed testing procedure to check the phytosanitary condition of seeds of a yellow grain hybrid (class FAO 700, 132 days) collected from an experimental field located in Carmagnola (TO, Italy: GPS: 44°53′11.0"N 7°40′60.0"E) the presence of green mycelium typical of the genus *Trichoderma* over the 400 seeds tested was greater than 50%. Due to the high and unexpected percentage of decaying kernels, representative strains were identified through morphological features assessment and by sequence comparison of ITS, *rpb2*, and *tef-1α* gene fragments, revealing 100% identity to *Trichoderma afroharzianum*. Pathogenicity tests were carried out by injecting into the silk channel 1 ml of the conidial suspension (10^6 conidia/ml) obtained from the strain of *T. afroharzianum,* seven days after the silk channel emergence (BBCH 65). Ears were removed four weeks after inoculation and disease severity, reaching up to 75% of the kernels of the cobs, was visually assessed. *T. afroharzianum* has been already reported on maize in Germany and France as causal

agent of ear rot of maize. The potential production of mycotoxins and the losses that can be caused by the pathogen during post-harvest need to be explored. To our knowledge this is the first report of *T. afroharzianum* as a pathogen of maize in Italy.

Identification of apple rubbery wood virus 1 and apple rubbery wood virus 2 infecting pear and apple in Campania and development of a detection assay for rubodviruses

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Apple rubbery wood virus 1 (ARWV-1) and apple rubbery wood virus 2 (ARWV-2) are two negative-stranded RNA viruses recently discovered in apple and pear. Both viruses have been classified as *Apple rubodvirus 1* and *Apple rubodvirus 2* in the genus *Rubodvirus,* which includes other two species *Grapevine rubodvirus 1* and *Grapevine rubodvirus 2,* identified in grapevine. In 2021, during a survey to assess the sanitary status of pear and apple trees in Campania region (southern Italy), leaves were collected and assessed by RT-PCR using primer pairs designed for the specific detection of ARWV-1 and ARWV-2. Amplicons of 312 and 272-bp expected for ARWV-1 and ARWV-2, respectively, were obtained in pear and apple samples. Of the 50 tested pear trees, one resulted mixed infected by ARWV-1 and ARWV-2 and 19 by ARWV-2 only. Of the 70 analyzed apple trees, four were infected by ARWV-1, 11 by ARWV-2 and three by both ARWV-1 and ARWV-2. Virus identity was confirmed by sequencing the amplicons, which shared 95.1–97.7 and 95.6–99.6% nucleotide identity with ARWV-1 and ARWV-2 sequences from GenBank, respectively. To our knowledge, this is the first report of ARWV1 and ARWV-2 in Italy and the first report of ARWV1 in pear. A degenerate primer pair, targeting a conserved domain in the RNA-dependent RNA polymerase of known rubodviruses was designed to develop a RT-PCR assay able to diagnose these viruses at genus level. Preliminary results showed that the assay allows the detection of ARWV-1 and ARWV-2 in single and mixed infection. Further studies are necessary to evaluate the ability of the assay to detect the rubodviruses from grapevine and eventually new rubodviruses from other host plants.

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Results of three-year survey conducted on EU quarantine pests of *Citrus* in Campania territory

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In order to cope with the increasing introduction and spread of new harmful organisms, the European Union has issued a series of regulations that are the basis of the "new plant health regime". For the Member States, territorial controls are foreseen through the preparation of surveys both for EU quarantine pests and for the 20 priority pests (Regulation (EU) 2019/1702). For a better phytosanitary surveillance of the territory, the Campania region has activated a partnership with the Department of Agriculture of the University of Naples "Federico II", with the National Research Council – IPSP, Unit of Portici and the Council for Research in Agriculture and the Analysis of agricultural economics—CREA, going to establish the Regional Unit of Phytosanitary Coordination—URCoFi. The regional survey program includes specific activities (i.e., visual surveys, sampling, laboratory analysis, use of traps) to be carried out on about eighty quarantine pests. Given the agricultural, environmental and territorial importance of citrus and the high risks of introduction of new harmful organisms through the large quantities of citrus fruit imported from third countries, particular attention is paid on the protection of this culture. Here, we present the results of a large-scale survey carried out during 2019–2021 in Campania, southern Italy, on the occurrence of *Phyllosticta citricarpa,* the agent of citrus black spot disease, *Candidatutus* Liberibacter asiaticus, *Ca.* L. africanus and *Ca.* L. americanus, the agents of Huanglongbing, and their two vectors *Diaphorina citri* and *Trioza erytreae,* and non-EU genotypes of citrus tristeza virus (CTV) and its vector *Toxoptera citricidus*.

Nanoplate-based digital PCR for early detection of different quarantine pathogens of ornamental plants

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The early detection of quarantine pathogens is pivotal to reduce the risk of its introduction into new ecosystems, where they may find susceptible hosts and/or environments favoring their pathogenic behavior, especially in the context of growing global mobility and trade. In this scenario, we tested the applicability of nanoplate-based digital PCR (dPCR) for early, accurate, and quantitative detection of different quarantine pathogens, including bacteria, oomycetes, and viruses, that can infect ornamental plants. The selected pathogens were *Erwinia amylovora*, *Ralstonia solanacearum*, *Phytophthora ramorum*, and tomato spotted wilt virus (TSWV). The assays were carried out using nucleic acids samples of healthy plants spiked with known quantities of bacterial DNA or, for obligate pathogens, nucleic acids extracted from experimentally infected plants. Results were compared with those obtained with validated quantitative PCR (qPCR) techniques. The nanoplate-based dPCR provided reliable detection and quantification for three of the four selected pathogens (*E. amylovora*, *P. ramorum*, TSWV), with a limit of detection of 10 target copies per microliter and 100% accuracy, sensitivity, and specificity. For the last pathogen, *R. solanacearum*, on the other hand, the results did not reach the expected performance criteria, therefore, other tests are needed in order to optimize the diagnostic assay. In conclusion, our results suggest that nanoplate-based dPCR could become an efficient upgrade of qPCR for the early detection of quarantine pathogens on ornamental plants.

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Agrobacterium mediated production of CanCV particles in *N. benthamiana*

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Cannabis cryptic virus (CanCV) belongs to the *Partitiviridae* family and to the *Betapartitivirus* genus that includes virus infecting both plants and fungi. CanCV is bipartite and has a dsRNA genome encapsidated in small, isometric, non-enveloped particles. Each single sense strand RNA (ssRNA(+)) is monocistronic: one codes for the RNA dependent RNA polymerase while the other for the coat protein. The lack of movement proteins makes these viruses incapable to move systemically in mechanical inoculated plants. CanCV life cycle is restricted to the cytoplasm and the virus propagates exclusively during cell divisions. Other betapartitiviruses infecting fungi are able to propagate in new cells during processes of anastomosis and sporogenesis. In this work, we demonstrate the possibility of obtaining CanCV particles using a *Nicotiana benthamiana* plants as bioreactors. For this purpose we cloned the cDNA copy of each CanCV ssRNA(+) into an binary expression vector to be used by *Agrobacterium tumefaciens*. Agroclone infectivity was tested by their co-infiltration in mature leaves with or without the transient expression of tomato bushy stunt virus p19 used to suppress the sense transgene-induced post-transcriptional gene silencing. The system turns out to be very economical and the particles yield, using purification techniques, is far higher than what could be obtained from the same amount of biological material infected by a natural infection. We believe this system could be applied to other partitivirids allowing to obtain a high number of viral particles to be used in future experiments of characterization/transfection in fungal or plant protoplasts.

Fungi associated with a trunk disease in young grapevine plants in Sicily (Italy)

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In spring 2022, in the territory of Sommatino (CL), 10-year-old plants of grapevine cv. Sangiovese and Insolia grafted on 140 Ruggeri, showed late sprout, stunted and chlorotic vegetation and evident xylem browning, starting from the rootstock. In the field, this syndrome affected about 20% of the plants of both cultivars. At the Plant Pathology laboratories of the SAAF Department symptomatic samples were submitted to isolation tests aimed at identifying associated fungal microorganisms. In particular, 3 Sangiovese and 3 Insolia grapevines were dissected and subcortical tissue fragments of approximately 2–3 mm were placed in Petri dishes, on PDA. The fungal colonies were identified with traditional (macro- and microscopic observations) and molecular methods (ITS and partial β-tubulin gene sequences). The results of these observations ascertained the presence of

Neofusicoccum vitifusiforme and *Phoma* sp. in the plants of cv. Sangiovese and *Arthrinium arundinis*, *Libertasomyces platani* and *Seimatosporium vitis* in those of cv. Insolia. Among these fungi, only a few *Phoma* species, *N. vitifusiforme* and *S. vitis* have been reported as causal agents of grapevine trunk diseases. Further investigations in the field, to study the evolution of the disease, and in the laboratory, both to evaluate the spread of isolated fungal microorganisms and to satisfy Koch's postulates, will clarify the role of these fungi in the manifestation of the described disease.

Aspergillus contaminating food and feed: biocontrol assays and new diagnostic method (LAMP)

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Contamination by *Aspergillus* is considered the main cause of spoilage in food and feed and of toxicosis in consumers. A previous study aimed to assess fungal contamination in 48 feed samples allowed us to isolate 47 strains belonging to 5 sections and characterized by cellulolytic activity. In order to develop biocontrol strategies, dual culture assays were carried out by using four fungi (*Penicillium italicum* Pi*, Trichoderma atroviride* P1*, T. harzianum* T22*, T. pleuroticola* Tp) and a bacterium (*Bacillus amyloliquefaciens* AG1) against 7 *Aspergillus* isolates. AG1 showed the highest antagonistic activity against all the aspergilli, with percentage growth inhibition (PGI %) ranging from 46.53% to 60.92%. The ability of AG1 to reduce aspergilli growth and sporulation was also confirmed by turbidimetric assay. In the poison agar assay, T22 culture filtrate induced a percentage of growth inhibition from 0 to 27.41%. In *in vivo* test conducted on artificially contaminated maize kernels, *Aspergillus* growth was reduced when P1 was inoculated three days before fungal contamination. Moreover, in order to rapidly detect *A. flavus*, the most common aflatoxin-producing species worldwide, LAMP assay based on the amplification of its *rmt-A* gene, involved in the production of conidia and sclerotia as well as in the expression of regulators in the aflatoxin gene cluster, was developed. Assay specificity was tested using the genomic DNA of 10 *Aspergillus* species. Among them only genomic DNA of *A. flavus* was amplified and the detection limit of the assay was 1.03 pg of DNA/reaction and 1 conidium/reaction in artificially contaminated samples.

Pleurotus ostreatus associated with white rot decay in a monumental specimen of *Ficus macrophylla* subsp. *columnaris* in Palermo

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Among the most famous monumental trees in Italy, the giant specimen of *Ficus macrophylla* subsp. *columnaris* (syn.: *Ficus magnolioides* var. *magnolioides*) which grows in Palermo, in the Giardino Garibaldi (Piazza Marina), is considered the largest European tree (30 m high, more than 21 m of circumference at the base of the trunk and 50 m of foliage diameter). Originating from Australia and planted in the 1863, the tree grew quickly and luxuriantly. Recently, however, some crashes of entire branches have raised serious concerns about the plant health. In the summer of 2021, pleurotus-like basidiomata were also observed, associated with white rot in the wood near the large wounds caused by the breaking of the branches. Samples of these basidiomata, subjected to microscopic observations, showed basidia, spores and cystidia typical for shape and dimension to *Pleurotus ostreatus*. Isolation tests permitted to obtain white fungal colonies with dense, fluffy mycelium and abundant dark orange exudates. Preliminary molecular analyzes, based on the amplification of the ITS region, confirmed the morphological identification. *P. ostreatus*, reported for the first time on this host, poses a serious risk to the stability and health of the monumental tree. Therefore, constant and accurate monitoring of the entire plant is considered necessary in order to define the most appropriate intervention strategies aimed to maintaining the best vegetative state of the tree.

Fungi associated with wood decay in olive trees in Trapani province (Sicily, Italy)

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Recently, in several localities of the province of Trapani (Sicily, Italy), many olive growers have reported cases of decay of olive trees cv. Nocellara del Belice, characterized by defoliation, drying of the branches, browning of the internal xylem tissues and reduced production. Internal symptoms consisted also in white and brown wood rot, starting from the base of the trunk. These alterations were observed in plants irrigated with a pipe system at the trunk with spray sprinklers. A collaboration was therefore started with the Ente di Sviluppo Agricolo of the Sicilian Region to investigate on the presence of fungi associated with this syndrome. Isolation tests were started on sections of symptomatic plants. In particular seven decaying olive trees were selected in the territories of Castelvetrano and Campobello di Mazara. Three trunk disks (thickness 10 cm) for tree were taken from the collar, at about 100 cm and 200 cm from soil. For each trunk disk, five plates containing PDA were used, placing five wood fragments (3 × 3 mm) for plate. Among the grown colonies, the most recurrent were identified by morphological (macro- and microscopical observations) and molecular (ITS and β-tubulin amplification) analyses. The first results showed the presence of *Coriolopsis gallica* and *Fomitiporia mediterranea*, two basidiomycetes well-known as agent of white wood rot, and *Pleurostomophora richardsiae*, already reported as an aggressive fungal pathogen of decaying olive trees in Southern Brazil and in Puglia (Southern Italy). Regarding *C. gallica* and *P. richardsiae*, this is the first report in olive trees in Sicily.

Survey of oomycetes associated with the Kiwifruit Vine Decline Syndrome using a baiting approach

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Kiwifruit Vine Decline Syndrome (KVDS), initially detected in northern Italy in 2012, is a severe destructive disease currently spread within the major kiwifruit growing areas in Italy. The syndrome causes the collapse of plants due to root rot. The etiology of KVDS is still far from being clearly defined, although symptoms and pattern of spread suggest that biological agents might be involved. Different microorganisms have been associated with the syndrome, although oomycetes are those more frequently isolated, in particular species of *Phytopythium*. Based on these observations, we used a leaf baiting approach to characterize the oomycete community of the rhizosphere soils from the Gioia Tauro plain (Reggio Calabria, Italy), one of the major national kiwifruits growing areas. The use of bating with fresh leaves of carob (*Ceratonia siliqua*) was strategic to aid the isolation of pathogens with motile spores, which might be hindered by other fast-growing microorganisms on culturing media. The survey was carried over spring 2021 and 2022, and confirmed the abundant presence of potential plant-pathogenic oomycetes, preferentially but not exclusively associated with plants showing severe KVDS symptoms. Interestingly, the identification of representative isolates through barcoding the ITS1-5.8S-ITS2 region of the rDNA highlighted the abundant presence of *Phytophthora* species, including *P. citricola, P. pseudocryptogea, P. plurivora, P. crassamura,* and *P. megasperma.* A high incidence of *Phytopythium vexans* was also detected, while *Pythium* species were less frequent. The prevalence of *Phytophthora* species, widely known as aggressive plant pathogens, might contribute to clarify the etiology of KVDS.

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Conservation Detection Dogs for the detection of *Tilletia indica* and *Xylella fastidiosa*: a training project

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In recent years, dogs are increasingly used in the search for biological targets of economic interest, otherwise difficult to detect. Actually, phytosanitary is one of the fields of application of dogs mainly developed, in which dogs are a useful tool both for the interception of infected plants across borders and to detect and characterize possible outbreaks of new diseases. Only a few cases of dogs trained to recognize plant pathogens are known, including *Xanthomonas citri*, *Candidatus* Liberibacter asiaticus, some species of the *Phytophthora* genus and *Xylella fastidiosa* (unpublished data). The low number of studies concerning quarantine plant pathogens is probably due to the difficulties in their handling, mainly related to the need to manipulate these organisms only in accredited quarantine laboratories with

specific security protocols that could be incompatible with training. A workflow aimed at training two dogs that will target two quarantine pathogens, *Tilletia indica* and *Xylella fastidiosa,* is proposed. As a first step, the imprinting phase will be carried out transferring the target odor of both *T. indica* and *X. fastidiosa* to an adsorbent support or using naturally infected plants as target (only for *X. fastidiosa*). Subsequently, the discrimination phase will be carried out by teaching the dogs to locate *X. fastidiosa* in association with various plant species and to distinguish *T. indica* from other species of the genus *Tilletia* which can be present in grain loads. The correspondence between the VOCs of the target species and the targets used for training will be validated using an electronic nose.

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Bois noir disease incidence is reduced by grafting of shoots from recovered grapevines

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Bois noir (BN), the most widespread disease of the grapevine yellows complex, is associated with '*Candidatus* Phytoplasma solani' (CaPsol). Due to its multifaceted ecology, BN control is extremely difficult. Several studies showed that BN recovery can be elicited by abiotic stresses and treatment with resistance inducers. In this study, field trials along with molecular analyses have been conducted to evaluate if grafting of shoots from recovered grapevine plants can increase the BN recovery rate in symptomatic grapevines and decrease the new CaPsol infection rate on asymptomatic grapevines. Field trials were performed in two BN-affected vineyards (cv. Chardonnay/Kober 5BB) in Franciacorta (Lombardy Region, northern Italy). Grafting effects were evaluated for three consecutive years by symptom observation and CaPsol detection by nested-PCR amplification of *stamp* gene and compared with non-grafted control vines. Obtained data showed that BN incidence was lower in grafted plants, mainly due to a statistically significant increase of recovery rate, four times higher than in non-grafted plants. These data indicated that grafting of recovered shoots can efficiently induce BN recovery, opening an interesting scenario for its utilization in sustainable strategies of vineyard management.

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Establishment of a new concept agronomic protocol to push eco-sustainability in the hazelnut agroecosystem

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Hazelnut (*Corylus avellana*) cultivation has become predominant over other crops, so much so that in some areas of Campania region it is considered ubiquitous. As the most adopted approach for hazelnut cultivation is intensive agriculture, the aim of the present study is to create a synergy between anthropogenic action with the natural balance of the agroecosystem, optimizing the use of productive inputs and maximizing environmental and economic sustainability. To this end a new eco-sustainable agronomic protocol, carrying out environmental and agronomic monitoring aimed at assessing the establishment of new ecological balances was applied. The experimental protocol is taking place in one hectare hazelnut grove located in Presenzano (CE), compared to the organic and adopted one of the partner company. The experimental protocol involves different aspects, as well as soil management by using grassing and adding to the soil manure previously inoculated with biostimulants (*Trichoderma* spp., rhizosphere bacteria, mycorrhizal fungi). To control pests and pathogens biofungicides, bioinsecticides and natural bactericides conjugated to corroborants are used. Evaluation of the eco-sustainability of the experimental protocol, is carried out with meta-genomics analysis after DNA extraction from manure and soil, in order to characterize the microbiome, pre- and post-treatment and denote any increase in microbial biodiversity and its new, possible interactions. During the trial, agro-climatic conditions at the site are monitored with a micro-climatic station equipped with a decision support system by correlating soil climate data with all the microbiome recorded data in order to better define the new concept agronomic crop management.

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Viroid-like RNAs with hammerhead ribozymes in both polarity strands identified in a metagenomic study on citrus

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Applying a specific pipeline aimed to search for hammerhead ribozymes (HHRzs) in RNAseq libraries, a novel small (550 nt) non-protein coding viroid-like RNA (Vd-LRNA) containing a HHRz in each polarity strand and adopting a rod-like conformation was identified in a library generated from young leaves and stem tissues of a *Citrus reticulata* tree. The new Vd-LRNA was named hammerhead viroid-like RNA 1 (HVd-LR1) and its existence confirmed by Northern-blot hybridization assays. HVd-LRNA1 circular forms of both polarity strands were detected. Cloning and sequencing of full-length amplicons generated by RT-PCR using three pairs of specific primers showed a high sequence variability preserving the rod-like secondary structure of minimal free energy and the conserved motifs of HHRzs. Self-cleaving activity of HVd-LR1 mediated by the HHRz in each polarity strands was confirmed during transcription and in the absence of any protein, providing evidence of their major role during replication through a symmetric pathway of the rolling circle mechanism. The existence of a DNA counterpart of HVd-LR1 was excluded based on PCR amplification assays. HVd-LR1 was not graft-transmissible to other citrus indicator seedlings (sour orange and grapefruit) and attempts of identifying it in the original citrus source tree by RT-PCR and/or HTS failed so far. Altogether these data show that HVd-LR1 was only transiently associated with the field tree, thus excluding that it is a new plant viroid. The possibility that Hvd-LR1 may be an infectious agent of another organism (i.e., a fungus) will be discussed.

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Bio-based treatments with laminarin, chitosan and *Trichoderma harzianum* to prevent alternariosis and grey mold on tomato in Mediterranean greenhouse system

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Tomato is one of the most widespread crops in the Mediterranean basin, where new protocols for managing plant diseases with the use of bioprotection means is required to increase sustainability. In this work, three spray treatments with the resistance inducers, laminarin (Vacciplant, 2‰), chitosan (Biorend, 4‰), and *Trichoderma harzianum* strain TL23 (10^6 spores mL^{-1}), were compared to chemical (cyprodinil + fludioxonil (0.6‰) and untreated controls in preventing *Botrytis cinerea* and *Alternaria alternata* leaf infections of tomato cv. Crovarese. One-day after the treatment, pregerminated fungal spores (5 mL, 10^5 spores mL^{-1}) were inoculated on 5 leaves *per* plant. The design included, for each pathogen, 5 treatments replicated 7 times (pots) each containing 3 plants accounting for a total of 35 pots, 105 tomato plantlets and 525 inoculated leaves; the trial was repeated for 5 cycles. Three days after inoculum the developed necrotic area was measured. The disease control effect of the fungicides was, on average, 31 and 10%, for *B. cinerea* and *A. alternata* spots, respectively. Although, with a variability among the cycles, the control efficacy of the bio-based ingredients, *Trichoderma*, chitosan and laminarin, proved to be 17, 18 and 10%, respectively, for *B. cinerea* and 4, 5 and 13% for *A. alternata* infections. Levels of air relative humidity and temperature recorded continuously during the assays, were used to develop a forecasting model able to alert when favorable conditions for pathogenesis of the two fungi occur. The model was validated against natural infection events in a dataset of tomato greenhouse.

This research is carried out in the framework of the PRIMA project iGUESS-MED.

Mobile DNA elements within the genome define *Pseudomonas syringae* pv. tomato race-specific genotypes

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Bacterial speck of tomato is an important disease that can result in severe crop losses in all the tomato growing areas. For *Pseudomonas syringae* pv. tomato, the causative agent of this disease, two different races have been described: race 0 and race 1. While race 0 strains have avirulence genes for the expression of type III system-associated effectors AvrPto and AvrPtoB that are recognized and targeted by the effector-triggered immunity by tomato cultivars which have the *Pto* race-specific resistance gene, race 1 strains instead lack the *avrPto* and *avrPtoB* genes, and are therefore capable of infect also the resistant cultivars with *Pto*. In this work we have performed the sequencing and the analysis of the whole genome of the strain DAPP-PG 215, which was isolated and described as race 0 strain in 1996. Its whole genome comprises a 6.2 Mb circular chromosome and two plasmids (107 kb and 81 kb). The results indicate also that the strain is phylogenetically closely related to the T1 strain. The chromosome encodes race 1-associated genes like *avrA* and *hopW1* and lacks race 0-associated genes like *hopN1*, giving it a race 1 genetic background. However, the genome harbors a complete ortholog of *avrPto1*, that allows the strain to display a race 0 phenotype. DAPP-Pg 215 is not the first strain reported to show intermediate virulence characteristics between race 0 and race 1, but comparative genomics with several PG1A genomes revealed that mobile DNA elements are potentially involved in the evolution of the two different races.

Identification of the main hemp diseases in Italy within the UNIHEMP Project

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The recent and rapid expansion of hemp cultivation has seen the increase of various phytosanitary problems. Many diseases and/or pests are already known, while other unreported are emerging and constitute a challenge for their identification and management. Within the UNIHEMP project, one of the aims is the identification of the main diseases affecting the cultivation of industrial hemp in open fields in different areas of Italy. Inspections were carried out in the experimental fields of the various partners involved in this project (Caserta, CREA-CI; Battipaglia (SA), CREA-DC; Lecce and Rutigliano (BA), CREA-AA; Foggia, CREA-CI; Rovigo, CRE-CI) and laboratory analysis completed the diagnosis. A complex phytopathological picture was highlighted, in which the main adversities encountered were at the root system (southern blight, *Athelia rolfsii*; fusarium wilt, *Fusarium* spp.) and at the stem (corn borer, *Ostrinia nubilalis*), with extensive damages to cultivation. Less harmful symptoms were found at the foliar and inflorescence level (caused by *Alternaria* spp.), with the related problematic of possible seed-borne diseases outbreak. It was also found that the phytosanitary state of the crops is linked to the cultivation area, with the southern fields (Caserta, Battipaglia, Lecce and Bari) mainly affected by *A. rolfsii* that can even cause the total loss of plants and harvest. In areas with different climatic conditions, such as Rovigo, the root system is instead more subject to root rot caused mostly by fusarium wilt causing plants wilting and death. On the other hand, the presence of the corn borer *O. nubilaris* is widespread throughout the territories.

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Persistence of *Aspergillus flavus* MUCL54911, active ingredient of the aflatoxin biocontrol product AF-X1, in maize fields in Italy

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AF-X1 is an aflatoxin biocontrol product containing the atoxigenic strain of *Aspergillus flavus* MUCL54911, endemic to Italy, as an active ingredient. The strain belongs to the vegetative compatibility group (VCG) IT006. AF-X1 is commercially available in Italy since 2015 with a temporary authorisation for use to prevent aflatoxin contamination in maize. The aim of this study was to evaluate the persistence

of VCG IT006 in the soil after treatment. Soil samples were collected in 2020 and 2021 from seven locations placed in North Italy. Fields were selected as follows: i) not treated, intended as field where AF-X1 was never applied; ii) treated with AF-X1 in the year n-2, iii) treated with AF-X1 in the years n-2/n-1, and iv) treated in the year n-1. A total of 399 isolates of *A. flavus* were recovered from soil samples following serial dilution technique. Densities of *A. flavus* ranged between 54 to 191 Colony Forming Unit (CFU)/g, significantly ($p < 0.005$) influenced by location and treatment. The VCG analysis showed IT006 occurrence in the studied fields treated n-2/n-1 and treated n-1 ranging between 20–83% and 40–93%, respectively, whilst in not-treated fields IT006 incidence ranged from 0 to 50%. The data suggests that weather conditions and cropping context impact on the strain recovery. The active ingredient persists over the years, its dispersal in untreated fields is possible, but in some treatments/locations there was a decrease of IT006 occurence. Therefore, it is still suggested a yearly application of AF-X1 in order to provide adequate protection to aflatoxin contamination.

PLANTHEAD - A Remote PLANT HEAlth Diagnostic network to foster sustainable agricultural intensification in Eastern and Western Africa

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African countries suffer from high levels of food and nutrition insecurity exacerbated by an increasing impact of climate change on agricultural production. The H2020 EWA-BELT project (https://www.ewabelt.eu/) aims to design and implement research activities involving relevant stakeholders willing to adopt innovations based on sustainable intensification (SI) approaches in order to increase agricultural yields, while preserving natural resources. In collaboration with the University of Nairobi and OCCAM, a remote PLANT HEAlth Diagnostic (PLANTHEAD) network is being developed, based on a platform hosting photographic database and georeferentiation. An image repository is being built-up with vouchered pictures of symptoms of the most relevant diseases and pests affecting major crops as indicated by the African partners. The local farmer sends an alert by mobile phone directly to the central HUB, providing relevant information such as localization, crop management, pesticide treatments, along with pictures (macro- or microscopic) and a short description of the problem. If the HUB can solve the problem, the platform sends the diagnosis and suggested actions directly to the farmer. If not, the platform sends the request to the first node, which takes charge of it. The request goes to a higher node if the lower one is unable to provide a solution. The node can be local, national or international. Once the solution is found, the competent node formulates a diagnosis and the farmer receives a notification of successful response. This response is stored in the database for future use and the images feed an AI-based image recognition system.

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Intracellular, intercellular and vascular trafficking of plant viral RNAs

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To overcome the rigidity of the cell wall, plant viruses normally take advantage of the way of life of different biological vectors to enter and replicate within plant cells. Once inside the cell, plant viruses hijack endogenous host transport machinery to aid their intracellular spread. In addition, taking advantage of the characteristic symplastic continuity of plant cells, viruses need to remodel and/or modify the restricted pore size of the plasmodesmata (channels that connect plant cells). In a successful interaction for the virus, it can reach the vascular tissue to systematically invade the plant. We use one of the simplest multicomponent transport systems identified to date, such as the *Carmovirus* genus, which is extremely reduced in the case of melon necrotic spot virus (MNSV), as a model to study intra- and inter-cellular movement of plant viruses. The MNSV genome codes for five functionally characterized proteins and two small proteins are directly involved in the virus movement, p7A and p7B. p7A shows RNA-binding capabilities whereas p7B has a single-TMD domain, which allows protein insertion into the ER-derived microsomal membranes obtained *in vitro*. By the other hand, MNSV CP localizes to both mitochondria and chloroplasts in ectopic expression and during MNSV infection. By using different experimental approaches, we will show results that collectively demonstrate that a functional Golgi-mediated secretory pathway is essential for the intra- and intercellular movement of a plant virus and that a specific region/domain of MNSV CP can act as an ambiguous transit peptide driving dual targeting of MNSV CP to mitochondria and chloroplasts.

Molecular investigations on the role of extracellular polysaccharides released by the antagonist yeast *Papiliotrema terrestris* PT22AV in the biological control of *Penicillium expansum*

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Exopolysaccharides (EPS), secreted by microbes during their growth play a wide range of biological roles, including protection against environmental stresses such as salinity and drought, adherence to surfaces, cell-to-cell interactions, nutrient storage, etc. Some plant-associated microbes are capable to synthesize EPS during vegetable surface colonization extending the benefits also for their plant hosts. While enough is known about the role of microbial EPS in overcoming abiotic stress in plants, little is known about the involvement of these compounds in plant protection against pathogens. The yeast *Papiliotrema terrestris* strain PT22AV is a strong producer of mannose-based EPS characterized for its functional properties and as biomaterial. The present study aimed to elucidate key factors in the modulation of EPS biosynthesis in PT22AV, and the role of these complex compounds in the protection of apple wounds from *Penicillium expansum* infection. For this purpose, two PT22AV-derived random mutant strains unable to synthesize EPS were selected and characterized for their biocontrol activity. Moreover, the EPS production in the wild-type strain was characterized under different nutritional conditions identifying the nutrients that mainly affect this biosynthetic process. Our results show as the yeast mutants defective in EPS production have a reduced ability to protect apple wounds against *Penicillium expansum* infection compared to the parental strain (PT22AV), especially when these strains are applied at low cells concentration. Furthermore, we found that the EPS production was strongly stimulated in nitrogen limiting conditions, especially when there is a marked imbalance between sugars and nitrogenous substances.

Hyperspectral imaging coupled with microclimatic-based alert help targeted management of downy mildew (*Hyaloperonospora parasitica* (Pers.:Fr) Fr.) of wild rocket (*Diplotaxis tenuifolia* L. [D.C.])

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Wild rocket, *Diplotaxis tenuifolia* L. (D.C.) (Brassicaceae), is a widely cultivated baby-leaf salad crop for the fresh high-convenience food chain. Downy mildew, caused by *Hyaloperonospora parasitica* (Pers.:Fr) Fr. here identified by Internal Transcribed Spacer region sequencing, is the key disease of this crop, favored by environmental and agronomic conditions occurring during cultivation. Digital monitoring by optoelectronic sensors coupled with the assessment of microclimatic parameters can help more targeted disease management. A plastic tunnel trial, carried out comparing plot treatments with *Trichoderma atroviride* TA35, laminarin and cell-wall extract of *Saccharomyces cerevisiae* LAS117, to fungicides and no treatment, was subjected to hyperspectral imaging and recording of microclimatic parameters, with the aim of defining symptom spectral features on the canopy and setting phyto-pathological alert, respectively. Correlation analysis between spectral signatures and disease incidence allowed for the selection of four hyperspectral vegetation indices, ZTM, VOG1, Red-Edge NDVI and HVI (working in the range 692–750 nm) able to distinguish early symptoms from intermediate and advanced ones. A Random Forest machine learning model including a few of predictive variables (400, 536, 696, 756, 948, 963 and 962 nm), allowed to classify healthy or diseased samples with 92% accuracy. The analysis of the micrometeorological data allowed the spatial–temporal characterization of the experimental area, with the definition of the points necessary for the correct monitoring of the parameters for the evaluation of the spatial trend in disease risk. In addition, a leaf wetness estimation model and a disease alert model were implemented in MATLAB.

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Steps to improve molecular detection of barley loose smut (*Ustilago nuda*) in seedlings

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Agricultural policies encourage a reduction of synthetic chemical plant protection products (PPPs). Cereal seeds are often prophylactically treated to prevent seed-borne diseases, including the internally located barley loose smut (*Ustilago nuda*). Cultivar resistance or biological seed treatments can contribute to chemical PPP reduction, but evaluating the effectiveness of new varieties or treatments can be cumbersome. *U. nuda* infections are symptomless in seeds and plants until its teliospores develop at emergence. Previously described detection methods are laborious and/or yield a non-target product. We investigated steps to improve *U. nuda* detection by optimizing seedling growth and qPCR conditions, including using filter paper as a substrate, growing the seedling without light, shortening the growth time, and testing new primers. The protocol alterations were tested on seedlings with either a low or high *U. nuda* infection level. Untreated, infected seed was directly used for the high infection rate. To reduce the infection, the same seed was treated with warm water, an effective *U. nuda* treatment. The less laborious seedling growth conditions, including on filter paper and without light, seems to yield more *U. nuda* DNA than growth in soil. Preliminary results suggest that the seedling growth period can be reduced. The newly designed COX3 primers appeared to be more sensitive than the ITS primers. Using the optimized seedling growth procedures and COX3 as the target gene can potentially streamline the molecular detection of *U. nuda* in seedlings, which in turn, can aid in the evaluation of new seed treatments or varieties.

Chemical characterization and biological properties of essential oils from different bioformulations treatment sweet basil plants

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The development of innovative formulations as a replacement to synthetic chemicals is an increasing interest to improve agricultural production and resource use efficiency. Alternatives can consist of natural products containing beneficial microorganisms and bioactive metabolites able to prevent or reduce plant pathogens infection. The efficacy of the bioformulations can be improved by polymers as adjuvants or carriers. The variation in the chemical composition of essential oils (EOs) from sweet basil (*Ocimum basilicum* L.) after the treatments with *Trichoderma afroharzianum* T22 (T22), *Azotobacter chroococcum* 76A (76A), and 6-pentyl-α-pyrone (6PP), singularly or in a consortium, with or without a carboxymethyl cellulose-based biopolymer (BP) were performed. Moreover, the results of the application under *in vitro* conditions of the EOs against plant pathogen bacteria, showed that the oil treated with T22 + 76A, which contains a higher concentration of linalool compared to the untreated essential oil, has the highest antimicrobial activity against *Xanthomonas campestris* pv. *campestris* and *Citrobacter freundii* (80% of inhibition growth). Furthermore, the EOs under investigation displayed the same activity as the linalool standard, one of the major constituents in basil essential oil and a wellknown antimicrobial compound. This antimicrobial activity was also confirmed by cellular material release assay. The results are in line with the antimicrobial test, samples treated with T22 + 76A displayed a release of nucleic acids similar to the control treated with ethanol. In addition, the anti-biofilm activity of the essential oils on mature biofilm was performed and demonstrated that T22 + 76A reduced the biofilm biomass by 90%, greater than pure linalool.

Effect of field plant coverage on virus infection of seed potato (*Solanum tuberosum* L.) tubers in two Italian up-land environments

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Potato is an economically important crop, which is severely affected by virus infection. The transmission of viruses, through seed potato tubers used as propagation material and by aphid vectors, represents a serious threat to potato yield production worldwide. In the frame of "RESILIENT" project, two multiplication techniques: 1) open field; 2) plant coverage (BBCH 31 301, beginning of crop cover growth stage: 10% of plants meet between rows) with woven fabric or insect-proof net tunnels were compared to establish the potential of seed potato tuber production in two up-land environments. Certified virus-free potato seeds of cv. Desirée and cv. Kennebec were evaluated in two areas in northern Italy

(Romagnese, PV, 800 m asl and Madesimo SO, 1550 m asl) under two growing conditions. Sprouted seed tubers were analyzed by Double Antibody Sandwich—Enzyme-Linked ImmunoSorbent Assay (DAS-ELISA) for the presence of potato virus Y (PVY), potato leafroll virus (PLRV) and potato virus S (PVS). Statistical analysis highlighted different infection rates depending on the three variables: PVY was found prevalent, with higher incidence in 'Kennebec' and in the area at lower altitude (where aphid vectors are more widespread). Generally, a low impact of the cultivation technique on the infection rate was observed. PLRV was not detected in any sample, while a low PVS infection rate was found only in 'Kennebec' cultivated in Romagnese in open field. Thus, this study concluded that a higher altitude and prompt insect-proof plant coverage (until crop emergence from the soil) play a key role in the production of seed tubers with low rates of virus infections.

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Neofusicoccum parvum causal agent of bot gummosis on lemon trees in Italy

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Bot gummosis, traditionally considered a minor citrus disease, has been found to be common and widespread in lemon groves in southern Italy. It is a disease whose causative agent causes premature aging of the plant with a consequent reduction in productivity. A fungal pathogen belonging to the *Botryosphaeriaceae* family, was isolated from the sampling carried out on symptomatic lemon plants in Sicily and Calabria. The main symptoms associated with the disease were gummy cancers on the trunk with abundant exudate and on the branches of the scaffolding of the trees. The identification of *Neofusicoccum parvum* was performed on the basis of morphological characters and the phylogenetic analysis of three loci, i.e., the internal transcribed spacer of nuclear ribosomal DNA (ITS) as well as the translation elongation factor 1-alpha (TEF1) and β-tubulin (TUB2) genes. To confirm Koch's postulates pathogenicity tests were performed on 'Femminello 2kr', 'Monachello' and Citrange 'Carrizo' cultivars. Symptoms (14 d.a.i.) were more severe on lemon cultivars 'Femminello 2kr'and 'Monachello' while less on Citrange 'Carrizo'. This is the first report of *N. parvum* as a pathogen of citrus in Italy. The incidence and frequency of these outbreaks are expected to increase in the future as a result of both climate change and the introduction of more sensitive lemon cultivars. It is suspected that nursery plants are the main vehicle for the wide spread of this fungus whose lifestyle as a latent pathogen may have favoured its spread through asymptomatic plants.

Role of enniatins as emerging mycotoxins and their association with deoxynivalenol in plant, insect, animal and human systems

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Deoxynivalenol (DON), produced by *F. graminearum* (FG), a causal agent of *Fusarium* Head Blight (FHB) of wheat, is notoriously toxic to animals and humans. Enniatins (ENNs), produced by *F. avenaceum* (FA), have also received attention for chronic exposure to contaminated feeds and foods but little is known about their effects. Therefore, this project aims to investigate the role of ENNs [enniatin B (ENB) in particular] and their association with DON: 1) in fungal virulence towards wheat; 2) in fungal competition; 3) towards the wheat microbiome and biocontrol agents (BCAs); 4) on wheat pests and their natural enemies; 5) on dairy cows health; 5) on human intestinal barrier permeability. So far, our research has shown that ENB plays a marginal activity in FA virulence but its role in defense priming is under evaluation. ENB also shows involvement in FA competition against FG. *Streptomyces* strains modulate DON production by FG in wheat being effective as BCAs. The effect of DON + ENB on BCAs fitness and on the whole microbiome are under investigation. While DON shows a toxic contact effect towards wheat aphids, ENB does not play the same activity. DON + ENB show a toxic effect on the topical application on lacewing larvae. ENNs in livestock diets are primarily from consuming cereal grains. Little information

is available on their effects on dairy cows concerning chronic or acute exposure and well-balanced or acidotic diets. ENB and DON exert time and concentration-dependent toxic effects on human intestinal Caco2 cells. However, when combined, no additive effects are observed.

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Use of tomatine extracts from tomato industry wastes against *Botrytis cinerea* highlights potential for production of biopesticides from circular economy workflow

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Tomato is one of the most developed horticultural commodities in Italy. The tomato industry produces several wastes: pomaces have been widely investigated as substrates for reutilization, while residues of the washing and sorting selection (WSR) have received little interest. Despite this, the WSR include unripe tomato fruits, stems and leaves, boasting a high concentration of tomatines. Tomatines are defense glycoalkaloids produced by tomato plants that can reduce the incidence of biotic stresses thanks to the toxicity that these compounds possess against a broad range of pathogens and pests. Most of the studies carried out in the past focused on using standard molecules, while information regarding tomatines extracted from plant residues is currently very limited. In this study we tested two different extracts from tomato WSR for their efficacy in reducing the growth of *Botrytis cinerea*, a major pathogen that affects tomato both in field and after harvest, *in vitro*. The results obtained using different concentrations of the two extracts (ranging from 0.06 mg/mL to 2 mg/mL) showed antifungal effect, obtaining inhibition of fungal growth that ranged from 50 to 100% on two different *B. cinerea* strains. These results were comparable to those obtained using a mix of tomatine and tomatidine analytical standards with similar concentrations and ratios as those found in the extracts. These results suggest that the use of these extracts, obtained in the context of a circular economy approach, could see application towards the reduction of the environmental impact of the defense of horticultural crops.

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Short-term effects of biological and chemical treatments against *Heterobasidion irregulare* on bacterial and fungal communities of *Pinus pinea* stumps

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Stumps are the targets of chemical or biological treatments against airborne infections of *Heterobasidion* spp. Here, we report on the short-term impact of biological (Proradix®, the cell-free filtrate of *Pseudomonas protegens* [strain DSMZ 13134], the conidial suspension of *Phlebiopsis gigantea* [strain MUT 6212], Rotstop®) and chemical (urea) treatments targeting the alien invasive *H. irregulare* on both bacterial and fungal communities inhabiting the surface of *Pinus pinea* stumps. Microbial communities of 15 stumps per each treatment and for controls were characterised four months after treatments using amplicon metagenomic sequencing of 16S and ITS gene. Our results demonstrated that treatments with Rotstop® and urea had a significant impact on fungi, decreasing significantly both diversity and richness of fungal communities compared to other treatments and control stumps. Fungal communities inhabiting stumps were less sensitive to the other tested treatments. The levels of diversity and richness in bacteria were never significantly different from those of control stumps, revealing a short-term stability of bacterial communities. However, correspondence analysis indicated that the composition of both bacterial and fungal communities was affected by treatments. Our findings provide additional insights on the risk associated with stump treatments against *Heterobasidion* spp. on natural ecosystems and improve our understanding on microbial communities inhabiting stumps of *P. pinea*.

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Polyphasic characterization of *Xanthomonas* strains isolated from hazelnut symptomatic plants

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The *Xanthomonas arboricola* (Xa) species has a high genetic variability and, among the 9 pathovars, pv. *corylina* causes the bacterial blight of hazelnut which is one of the main bacterial diseases present in all areas where hazelnut is cultivated. In this study, 19 Xa and 21 *X. arboricola* pv. *corylina* (Xac) strains isolated in Chile from European hazelnut symptomatic plants, were characterised through Rep-PCR analysis, where 13 *X. arboricola* reference strains belonging to the pathovars *celebensis, pruni, juglandis* and *populi* were used as controls, while DISTAL 9081 strain of *X. axonopodis* pv. *vitians* as outgroup; then, Biolog analysis, bioassays (hypersensitive response) and pathogenicity tests were carried out. The UPGMA cluster analysis of Rep-PCR fingerprints discriminated the 51 *X. arboricola* strains into 5 statistically significant groups corresponding to the relative pathovars: *pruni*, *corylina*, *juglandis*, *celebensis* and *populi*. The *X. arboricola* pv. *corylina* strains showed a high genetic variability (approx. 45%); similarly, the Rep-PCR fingerprints of the *X. arboricola* strains isolated from symptomatic hazelnut shoots and leaves gave similar results with a similarity value of approx. 50% among strains which were included in the *corylina* pathovar. The hypersensitive response on French bean resulted positive for all Xa/Xac strains, and the selected Xa/Xac strains resulted pathogenic on hazelnut plants confirming the Koch's postulates. At last, most of metabolic activity data obtained with Biolog GenIII™ analysis confirmed the molecular characterization of Xac strains, pooled in the same group. Furthermore, the Xa isolates were here combined in a sub-group of the Xac cluster.

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Monitoring Calabrian (Southern Italy) citrus orchards for *Phyllosticta citricarpa*

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Citrus Black Spot (CBS) is a foliar and fruit disease caused by a quarantine fungus *Phyllosticta citricarpa* (McAlpine). It is one of the most important disease affecting many commercial *Citrus* species. CBS was first reported in Australia in 1895 and subsequentially observed in many citrus producing areas of Asia, Africa, Australia, North and South America. *P. citricarpa* produces lesion on fruits and leaves; it has an economic impact because the external blemishes it generates, make fruits unsuitable for the market. The pathogen has not been reported in Europe until 2017 when it was described in Italy, Malta and Portugal. A network funded by European Food Safety Authority (EFSA) was created for the surveillance of this pathogen in the reported areas, including the Southern Italian area of Trebisacce (Calabria). Since leaves are reported to be a source of primary inoculum in CBS disease, we focused our analysis on both green leaves and leaf litter, developing an efficient DNA extraction method. From 2019 to 2021 leaf litter and green leaves of citrus trees were collected in Torre Albidona (Trebisacce), the site corresponding to geographical coordinates indicated in the first report of *P. citricarpa* in Italy. The vegetal material was analyzed by conventional methods (humid chamber and fungal isolation) and by molecular methods (qPCR). Samples analysed were negative for the presence of *P. citricarpa* fruiting body after morphological observation, as well for the presence of *Phyllosticta citricarpa/paracitricarpa* DNA after molecular test.

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Application of *Trichoderma* strains and Benzothiazole derivatives to tomato plants (*Solanum lycopersicum*) as biostimulants

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Synthetic chemical compounds are commonly used for the resistance induction in plants to provide crop protection, even if many disadvantages are known such as toxic effects to human health, environment and soil biodiversity. In recent years, alternative choices to chemical use in agriculture includes the use of beneficial microorganisms from the rhizosphere. The aim of this work was to evaluate different application strains of *Trichoderma* spp. in combination with benzothiazole compounds to obtain healthy and

disease-resistant plants. Experiments were conducted using three *Trichoderma* strains: *T. afroharzianum* strain T22, *T. harzianum* strain M10, *T. atroviride* strain P1 according to their biocontrol abilities against plant pathogens and plant growth-promoting effects. Three derivatives of benzothiazole, CH, BTH (BION, Syngenta), and ILAGRO, are known as inducers of plant resistance. The first part evaluated compound compatibility with *Trichoderma* strains by *in vitro* tests. Results showed that the growth of M10 and T22 were not inhibited by the presence of benzothiazole compounds, and even promoted in some cases. Subsequently, plants were treated singly and in combination with treatments of *Trichoderma* strains (soil irrigation) and the compounds (foliar spray); with water and/or only fungal strains were used as controls. Plant growth promotion was confirmed by in vivo tests on tomato plants cv. San Marzano nano in the greenhouse by measuring biometric parameters (fresh and dry weight of stem and root; stem and root lengths; number, fresh and dry weight of leaves). These results may contribute to develop improved beneficial microbes to be used as plant protection products or pant biostimulants.

Extracellular acidification is not necessary for *Beauveria bassiana* biocontrol activity

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Entomopathogenic fungi (EPF) belonging to *Beauveria bassiana* (*Bb*) apart from being widely used in agriculture for the control of insect pests they also play different roles in natural agroecosystems, including endophytism, plant growth promotion and disease control. Rhizosphere pH alkalinization represents a renowned pathogenicity mechanism of several fungal pathogens including the soil-borne ascomycete *F. oxysporum* f. sp. *lycopersici* (*Fol*), the causal agent of vascular wilt disease on tomato plants. Here, to understand if *Bb* biocontrol activity against fungal pathogens might be related to its ability to modify rhizosphere pH, we characterized the pH modulating and biocontrol activity *in vitro* and *in vivo* of ten *Bb* isolates. *Bb* acidification in vitro was evaluated by using the pH indicator bromophenol blue and an acidification index was calculated for each of the tested isolates. Nine isolates out of ten were able to acidify the culture medium and six of them produced a more intense acidification halo around the colony. However, when biocontrol activity was tested in vitro only three highly-acidifying and one non-acidifying isolate greatly inhibited *Fol* growth. Unexpectedly, when *Bb* biocontrol activity was evaluated *in vivo* against *Fol*, all isolates similarly protected tomato plants from wilting, thus suggesting that rhizosphere acidification might be only one of the biocontrol mechanisms used by *Bb* in vivo. Further experiments are required to determine which additional mechanisms apart from acidification are involved in *Bb* biocontrol activity.

Root and collar rot caused by *Phytophthora nicotianae* and *Phytophthora palmivora*, a new disease of *Paulownia* in Europe

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Paulownia species, characterized by fast growth, ability to re-sprout rapidly after cutting as well as a good tolerance to drought and high soil acidity, are trees native to China. They are grown in managed plantings in several European countries for the production of wood and biomasses. In 2018, 40% of trees of a 2-year-old commercial planting of *P. elongata* × *P. fortunei* in Calabria (Southern Italy) were affected by a severe disease characterized by wilting, stunting, leaf yellowing, and final collapse of the entire tree as a consequence of root and crown rot. *Phytophthora* species were consistently recovered from basal stem bark, roots and rhizosphere soil of symptomatic trees and identified as *Phytophthora nicotianae* and *P. palmivora*. The identification was performed on the basis of both morphological characteristics and phylogenetic analysis of rDNA ITS sequences. To confirm Koch's postulates, potted paulownia saplings were transplanted into infested soil and stem-inoculated by wounding. Both *Phytophthora* species were pathogenic and caused root rot and stem cankers. Even though *P. palmivora* was the only species recovered from roots of naturally infected plants, in pathogenicity tests through infested soil *P. nicotianae* was more virulent. This is the first report of Phytophthora root and crown rot of *Paulownia* in Europe. The use of healthy nursery plants is crucial for preventing this disease in commercial plantations. Strategies to prevent this emerging disease include the use of healthy nursery plants, well-drained soils for new plantations and proper irrigation management.

Identification of the genes involved in early response to Flavescence dorée infection in the model plant *Arabidopsis thaliana*

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Flavescence dorée (FD) is a grapevine disease associated with a quarantine phytoplasma, representing a severe threat to the European wine-growing areas. FD management, consisting mainly of compulsory insecticide treatments and roguing of infected plants, has relevant environmental and economic impacts. Even if grapevine cultivars show different degrees of susceptibility to the disease, no genetic resistance to FD has been described yet. *Arabidopsis thaliana* (plant)/FD phytoplasma (FDp, pathogen)/*Euscelidius variegatus* (FDp insect vector) was the model system adopted to study the plant early response to FDp exposure. Literature search and a bioinformatic pipeline to analyze RNAseq data of healthy vs FD-infected *A. thaliana* and *Vitis vinifera* plants allowed selection of a set of 15 genes putatively involved in plant response to FDp infection. The expression of the selected genes was analyzed by Real-Time PCR comparing phytoplasma-exposed and not-exposed plants. The involvement in FDp infection of a subset of 3 differentially expressed genes (*jaz*7, *pad*4 and *gsl*5) was further investigated using knockout *A. thaliana* mutants in a reverse genetics approach. Phytoplasma multiplication and infection rates were measured in the mutant lines and compared with the wild type. Moreover, *E. variegatus* feeding behavior on wild type and a double-mutant line *Pad4(-)/Pmr4(-)* was investigated and described. Developing sustainable strategies for FD control requires expanding the knowledge of plant-pathogen interactions. Identifying genes involved in susceptibility to FDp infection in a model system is a preliminary step for the characterization of grapevine genes potentially involved in disease resistance.

Lessons learned from plant viruses: SARS-CoV-2 nsp3, nsp4 and nsp6 expression in *Saccharomyces cerevisiae*

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Positive-strand RNA [(+)RNA] viruses are agents of important diseases in humans, animals and plants, including COVID-19. Regardless of the host, the replication of all (+)RNA viruses occurs in association with the host endomembrane system. Based on this common replication mechanism, yeast has been successfully employed as a surrogate host to study plant (+)virus replication. In this work the yeast model was used to express the replication-associated proteins of human SARS-CoV-2, having a role in the formation of the double-membrane vesicles and in virus replication, to decipher virus-membrane interactions. (+)RNA SARS-CoV-2 non-structural proteins nsp3, nsp4 and nsp6 were cloned under the control of the inducible *GAL1* promoter and the effects of their expression were studied in *Saccharomyces cerevisiae* strains W303-1B and YPH499. Yeast cells expressing nsp3 showed a low but significant decrease of growth rate, whereas nsp4 expression dramatically reduced cell growth. Moreover, nsp4-expressing cells showed a loss of cell viability compared to control cells harboring empty plasmid. Nsp6 expression did not have significant effect on cell growth and viability. All proteins sedimented in a membrane-enriched protein fraction. Immunofluorescence analysis showed that nsp3, nsp4 and nsp6 localize on the endoplasmic reticulum. Elucidating (+)RNA virus-host cell interaction complexity will allow the identification of novel druggable targets for the development of broad-spectrum antivirals.

Tomato-*Beauveria bassiana* interaction for bioinspired strategies of pathogen control

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Plants are central to the complex networks of multitrophic interactions. Increasing evidence suggests that beneficial microorganisms (BMs) may be used as an alternative to chemically synthesized pesticides to control pathogens and pests while improving plant fitness and stress tolerance. Among BMs the entomopathogenic fungus *Beauveria bassiana* can endophytically colonize plant tissues, but its effect on plant physiology and resistance to pathogens has been poorly investigated. In particular, it is not known whether the colonization of *B. bassiana* changes plant

photosynthesis, stomatal opening and the emission of Volatile Organic Compounds (VOCs) released by plants constitutively or in response to abiotic or biotic stresses. In the present work we used tomato (*Solanum lycopersicum* L.) to investigate whether endophytic colonization by *B. bassiana* strain ATCC 74,040 (Naturalis®) affects plant physiology and induces resistance against the pathogen *Botrytis cinerea*. The efficacy and persistence of endophytic colonization of *B. bassiana* was confirmed by PCR and colony formation on Potato dextrose agar (PDA) medium. Tomato plants responded to *B. bassiana* treatment with a significant but transient (1–2 day-long) reduction of stomatal conductance and photosynthesis, indicating rapid activation of defensive (rejection) responses, followed by host recognition and onset of a symbiotic relationship. We are now examining whether *B. bassiana* can control *in vitro* the infection of *B. cinerea*. Finally, we aim to investigate whether *B. bassiana* changes VOCs blend emitted by tomato and whether such a change is also a factor improving plant resistance to *B. cinerea*.

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Tomato yellow leaf curl Sardinia virus confers tolerance to drought in tomato

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Viruses can alter the response to abiotic stresses of plants and their ability to tolerate them. Geminiviruses, particularly begomoviruses, have been shown to increase tolerance to heat and drought in *Arabidopsis thaliana*. In this study, we investigated the drought tolerance in tomato (*Solanum lycopersicum* L.), which is considered one of the most important crops in agriculture. Tomato plants infected by the begomovirus tomato yellow leaf curl Sardinia virus (TYLCSV) and mock-inoculated plants were grown under well-watered, water-stressed, and recovered conditions. Morphological and physiological parameters, and hormone levels were investigated in both groups of plants. In addition, the transcriptional response of candidate genes known to be involved in the synthesis and degradation of water-stress related metabolites (abscisic acid, auxin, proline, and salicylic acid) was studied by qRT-PCR. Based on the results obtained, the TYLCSV infection positively influenced the ability of tomato plants to tolerate drought by postponing the onset of stress-related traits, altering transcriptional responses of the water-stress pathway, enhancing the plant water use efficiency, and permitting the plant quick recovery after the rehydration phase. These results open up new possibilities to design new strategies allowing to cope with climate change in agriculture, particularly in locations with limited access to water and high temperatures.

Phytophthora spp. associated with kiwifruit vine decline syndrome in north-eastern Italy

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Since 2012, kiwifruit vine decline syndrome has been threatening several orchards in the most important growing areas of north-eastern Italy. A field survey, aimed to detect the presence of *Phytophthora* spp. in roots of symptomatic plants, was conducted during the 2021 growing season in 5 orchards located in the Veneto and Friuli Venezia Giulia (FVG) regions. Pathogen isolation was performed on selective PDA media (supplemented with: 100 ml/L of carrot juice, 0.05 g/L of hymexazol, and 0.013 g/L of pimaricin) from decaying kiwifruit roots obtained from 39 and 25 plants collected in Veneto and FVG orchards, respectively. *Phytophthora* spp. isolates were identified by morphological and DNA-based techniques. In both regions, the species most frequently isolated were *P. cinnamomi* and *P. citrophthora*, while the least was *P. nicotianae*. Besides, *P. acerina, P. plurivora,* and *P. palmivora* were rarely isolated only from samples collected in Veneto. To fulfill Koch's postulates, the pathogenicity and aggressiveness of *Phytophthora* spp. were tested on one-year old plants of *Actinidia chinensis* var. *deliciosa*, following a previously set-up protocol consisting of inoculation and 3 waterlogging events. The species *Phytopythium vexans* and *Phy. chamaehyphon,* whose pathogenicity was already demonstrated, were used as positive control. After three months from the inoculation, the most aggressive species caused the same symptoms observed in field, showing a root rot disease severity ranging from 70 to 100%. The results obtained in this study could help to better understand the complex etiology of this disease and to develop a reliable integrated strategy for its management.

A mechanistic weather-driven model to predict *Puccinia graminis* f. sp. *tritici* infection and disease development in wheat

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Stem rust (or black rust) of wheat, caused by *Puccinia graminis* f. sp. *tritici*, is a re-emerging, major threat to wheat production worldwide. A mechanistic, weather-driven model for the stem rust pathosystem was developed in order to quantitatively synthesize the literature knowledge and to provide a tool to guide tactic decisions for disease management. The model considers infections caused by uredospores that are responsible for disease epidemics in temperate climates and assumes that sites of the crop move from being healthy, latent, infectious, and finally removed during the epidemic. The model was implemented and validated in STELLA®, a visual programming language for modeling system dynamics. The ability of the model to predict the first seasonal infections was evaluated using field data collected in three wheat-growing areas of Italy (Emilia-Romagna, Apulia, and Sardinia) from 2016 to 2021. The model showed good accuracy and specificity, with correct predictions occurring in the 90% of the cases. The model's ability to predict disease progress during the growing season was evaluated by using data of six epidemics occurred in the USA between 1968 and 1986. Comparison of observed versus predicted data gave a concordance correlation coefficient of 0.96 and an average distance between real data and the fitted line of 0.09. The model could therefore be considered accurate and reliable for predicting epidemics of wheat stem rust and it has the potential for being used for scheduling fungicide applications to control the disease.

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Study of the oxylipinic profile of the model plant *Arabidopsis thaliana* artificially infected with *Xylella fastidiosa*

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Recently we demonstrated that oxylipins are hallmarks of *Xylella fastidiosa* (Xf) subsp. *pauca* infection in olive; in particular the 13-LOX derived oxylipins accumulated in Xf-infected *Olea europea* cv. Ogliarola salentina, one of the most susceptible variety. This study aims at deciphering how and if plant 13-LOXs can modulate Xf virulence in the model plant *Arabidopsis thaliana* that has been proposed as potential host for Xf. The first issue was to evaluate the ability of Xf subsp. *pauca* De Donno and subsp. *fastidiosa* Temecula 1 to colonize *A. thaliana.* The infection of *A. thaliana* ecotypes Col-0 and the mutant *lox2* deleted for a 13-*lox* with Xf was confirmed by real-time PCR evaluating the ability of Xf to move from the inoculation point and colonize systemically the tissue plant. Oxylipins profile and the modulation of oxylipin genes of infected plants were monitored at different time intervals after the pathogen infection under controlled conditions. Preliminary results report that the bacterium is able to colonize the plant tissue systemically. In the infected tissue the oxylipins generated by 9-lipoxygenase and 13-lipoxygenase is down modulated in the infected tissue in Col-0 and in mutant. In the mutant infected by Xf it is possible to observe a down modulation of different lipidic entities.

Protein hydrolysates of different origins to improve resistance to stresses

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In the last two decades, several products generically referred as biostimulants appeared on the market to promote plant growth and induce defence responses against biotic and abiotic stresses. Particularly, protein hydrolysates (PHs), processed from the protein fractions of both plant and animal matrices, are commonly used in the food and cosmetic industry as the hydrolysis process allows to obtain amino acids while destroying allergens. Furthermore, in recent times they gained popularity as plant biostimulants for their efficacy and ease of use. In the present overview, the activity of soybean and casein protein hydrolysates, both applied before and after harvest, against biotic and abiotic stresses is reported and discussed. These activities seem mainly related to the bioactive peptides released after enzymatic degradation process, displaying multifunctional properties rooted mainly to influence plant hormonal activity; in response, plants may initiate or increase physiological processes that end

up as resistance to stresses. Specific hints on their mode of action are provided.

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Streptomyces spp. on wheat reduce disease impact and deoxynivalenol accumulation caused by *F. graminearum*

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Fusarium Head Blight causes important losses to wheat production due to reduced productivity and contamination with trichothecenes, in particular deoxynivalenol (DON). The disease is caused by different *Fusarium* species, among which the most significant and widespread worldwide is *F. graminearum.* Effective biocontrol against FHB shall limit both the disease incidence as well as DON accumulation in grains. *Streptomyces* spp. can act as endophytes of the wheat plant. Moreover, they produce a wide range of secondary metabolites that can hinder specifically toxin production by the fungus and/or limit the growth of the fungus. Our previous work identified some strains effective against the disease and toxin accumulation in controlled conditions. The aim of the work was to assess the efficacy of three strains of *Streptomyces* spp. to limit disease and DON accumulation in the field (using two types of treatment: seed coating and spike inoculation). The field trial, carried out in 2021, exploited artificial inoculation of the pathogen to obtain a high level of infection and toxin accumulation. The disease and the DON content were compared to the untreated control on a durum wheat and a spring wheat cultivar. In both cultivars, the three streptomycetes showed the ability to reduce disease and DON accumulation. Higher efficacy of the strains was observed in spring wheat (DON reduction > 60%) compared to durum wheat (DON reduction > 40%). The three *Streptomyces* strains under scrutiny showed promising results under field conditions. Future research will focus on the mechanism of action of the strains.

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Pantoea stewartii subsp. *stewartii* and seed trade: "trick or treat" for Italian sweet corn?

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Pantoea stewartii subsp. *stewartii* (Pss) caused Stewart's vascular wilt of sweet corn and maize and it is responsible for serious crop losses. Pss, indigenous of North America spreads with maize seeds and its presence was notified in Italy since 2015. The risk assessment of the entry of Pss in the EU from the USA through seed trade is in the order of magnitude of hundred introductions *per* year. Several methods, based on molecular or serological methodology, were developed for the detection of Pss for commercial seed certification and official analysis but some of these are unable to correctly distinguish between *P. stewartii* subsp. *indologenes* (Psi), with Pss. Psi is avirulent on corn and it is not a regulated corn pest even if occasionally present on corn seed. The *galE* gene can differentiate Pss from Psi based on SNPs. Pal and colleagues develop a real-time PCR assay for the detection of Pss without the cross reaction of Psi, from corn seeds without the need for bacterial isolation. In this study, we characterized the Italian isolates recovered in 2015 and 2018 by molecular, biochemical, pathogenicity tests and their genome was assembled through hybrid MinION and Illumina – sequencing procedures. Through this, we were able to individuate by *in silico* analysis a sequence that can detect Pss until 10^3 CFU/mL in spiked seeds (bypassing bacteria isolation). The assay was proven specific for the sole Pss. This method with good sensitivity and specificity can improve the detection in maize seed to go through a critical problem associate to the import of maize seed potentially affected by Pss coming from region where Stewart's disease is endemic.

Cellulose Nanocrystals as innovative and sustainable tool to control bacterial plant pathogens

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Among the huge variety of nanomaterials usable in crop protection, recent promising results were obtained studying the antibacterial properties of Cellulose Nanocrystals (CNC). CNC can be obtained through enzymatic and chemical protocols, even from agro-food lignocellulosic biomasses. CNC have an acicular shape with dimension ranging from 80–400 nm and 5–20 nm for length and width respectively. CNC have showed some interesting antimicrobial properties when tested against several plant pathogenic bacteria: 0.5%

w/v CNC suspension is able to inhibit the *in vitro* growth of *Pseudomonas syringae* pv. *tomato* (Pst), *Pseudomonas savastanoi* pv. *savastanoi* (Psav), *Xanthomonas arboricola* pv. *corylina* (Xac), *Xylella fastidiosa* subsp. *pauca* (Xf). The biological mechanisms behind these properties are still largely unknown; however, it has been recorded the ability of CNC to inhibit Pst and Psav biofilm formation at 24 h when applied at concentration lower than 1% w/v; while no generation of intracellular ROS was observed in treated cells. When *in vivo* applied, CNC showed also the capability of suppressing the epiphytic survival of Psav and Pst on olive and tomato leaves respectively, while a severity reduction was observed in Xac-artificially inoculated hazelnut seedlings. These results open a new scenario in bacterial disease management since CNC can be also used as nanocarriers for other active molecules. Promising results were obtained in controlling tomato bacterial speck disease with nanostructured microparticles containing CNC, starch and chitosan, by reducing the symptoms in a comparable way to copper. Similar results were obtained against Psav and Xf using CNC and thyme extract nanocapsules.

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Hormonal and antioxidant orchestrated responses of canker-resistant and susceptible *Cupressus sempervirens* clones inoculated with *Seiridium cardinale*

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Cypress Canker Disease is a pandemic affecting several species in the family Cupressaceae, caused by the invasive necrotrophic fungus *Seiridium cardinale*. As a result of a genetic improvement program a series of canker-resistant clones of *Cupressus sempervirens* have been patented. The present study intends to increase the understanding of early biochemical changes in the response to *S. cardinale* infection of canker-resistant and susceptible cypress clones. In infected ramets of the resistant material, a production of ethylene was observed at 3 and 13 days post inoculation (dpi) in the bark (more than twofold higher than in uninoculated controls). Conversely, an accumulation of salicylic acid was reported at 1 dpi in the foliage, twinned with an increase of abscisic acid which reached a maximum at 9 dpi (more than tenfold). These phytohormones and signaling molecules were able to counteract oxidative burst and prevent lipid peroxidation by offering protection to bark tissues and foliage. In infected ramets of the susceptible material, a concomitant synthesis of ethylene and jasmonic acid was recorded at 3 and 4 dpi in the bark (more than fourfold higher), whereas a partial and transient redox signaling activation was observed in the foliage. However, the synergistic interaction between phytohormones and antioxidants was not able to modulate the defense responses and limit the progression of the oxidative pressure (as confirmed by the increase of hydrogen peroxide levels and malondialdehyde-by products at 13 dpi) so suggesting an explanation of the fungal detrimental effects in the susceptible clone.

Laboratory investigation of the bacterial microflora of the seed of *Linum usitatissimum* L.

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In Italy, textile and oilseed flax seeds can be marketed only if they comply with the certification standards. The national legislation refers to the provisions of EU regulation 2016/2031 and EU 2017/625. For the phytosanitary aspect, the reference protocol adopted is the ISTA 7–007 protocol. Bacteriological analyses are not foreseen. In 2021, bacterial colonies were detected during the mycological screening of a sample. The presence of *Pseudomonas lini* was initially hypothesized. From the sequencing of the 16S rRNA gene, the strain is not associated with the genus *Pseudomonas*. It was decided to extract the bacterial colonies from the seed. Since there is no specific ISTA protocol, 7–019 (b) was used as a reference. The changes were the use of substrates such as Nutrient Agar and King's medium B. Standard methods were used to characterize colonies at the genus level. Based on some morphological and biochemical characters, the isolate is identified as belonging to the genus *Pantoea*. Pathogenicity was tested *in vivo* on flax plants. Control plants were asymptomatic. The bacterium was re-isolated to demonstrate Koch's postulates. To date, there are no studies or reports that demonstrate the spread of this pathogen in Italy. The environmental conditions of our areas seem not to be predisposing for the development of the disease. The high level of homoplasia in the sequences of the 16S rRNA gene

complicates the phylogenetic support of the polyphyletic genus *Pantoea*. The future prospect is therefore an in-depth study of DNA gyrase sequences.

Transcriptome profiling of sanitized artichoke ecotypes and characterization of genes involved in the biosynthesis of secondary metabolites

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Globe artichoke (*Cynara cardunculus* L. var. *scolymus*) is gaining commercial interest for the high content of health-promoting bioactive compounds (BACs) such as inulin, polyphenols and antioxidant molecules. Availability of ecotypes sanitized by viral and fungal infections, characterized by vegetative vigor, productivity and quality has relaunched the crop in the market. We report the effect of sanitation protocol, based on *in vitro* culture and thermotherapy, on BACs accumulation by transcriptomic analysis of two late-flowering artichoke ecotypes Locale di Mola tardivo and Troianella. The analysis highlighted remarkable differences in genetic, environmental information processing and primary cell metabolisms between sanitized and wild-type plants with about 2% of differentially expressed genes (DEGs) mainly involved in biosynthesis of phenylpropanoid, carotenoid and other secondary metabolites. Comparison between the two sanitized ecotypes showed only 75 DEGs, with respect to a higher percentage of DEGs between wild-type ecotypes. This difference could be related to plant response and oxidative burst against pathogen infection in wild-type plants. BACs analysis by HPLC–DAD showed a significant decrease of polyphenols accumulation in sanitized-plants compared to wild-type, speculating a different modulation of the biosynthetic pathway by sanitation protocol. A different response of the two ecotypes was also observed by the analysis of peroxidase activity. A similar approach is being conducted on the early-flowering ecotype Brindisino. This study represents the unique investigation of transcriptome profile and BACs accumulation in artichoke ecotypes exposed to sanitation protocol and aims to promote the soilless cultivation of vigorous and pathogen-free artichokes, even for BACs extraction.

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Metabolomics for the selection of beneficial microorganisms and/or their metabolites for a new generation of plant protection products

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Due to an increasing awareness about negative impacts to the environment, there has been a shift from conventional to sustainable agriculture, that has led to a change in regulations and the promotion in the use of microbial-based products as alternatives to synthetic chemical products. Metabolomics is a useful tool to guide the selection of beneficial microbes (single-strain inoculants, synthetic consortia, co-application of two or more microorganisms) and their secondary metabolites as active substances of new bio-formulates, and to evaluate treatment performance. With the aim to discover new formulations for agricultural uses, a metabolomic approach was used to investigate the compatibility of different beneficial microbial strains (i.e., *Trichoderma, Streptomyces*, *Azotobacter*) and test selected combinations in applications to different crops (parsley, basil, olive drupes). Moreover, the chelating properties of harzianic acid (a *Trichoderma* siderophore) were studied in order to enhance soil nutrient quality. Results showed that the tested combinations had significant beneficial effects on crops, in terms of pathogen control and improved nutritional value. Statistical analysis of plant extracts revealed a modulation of metabolic profiles depending on the treatment based on beneficial microbe application. It was possible to identify several metabolites (e.g., petroselinic acid, quinic acid, caffeic acid, rosmarinic acid, coumaric acid, oleuropein, luteolin and other phenolic compounds), whose relative abundance was increased in treated samples compared to the water control. Moreover, the analyses of the formulations consisting of harzianic acid and bivalent metal cations (biologically relevant or toxic), highlighted the formation of neutral of charged complexes depending on pH of the solution.

A novel methoxychromone derivative from *Trichoderma harzianum* M10

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Fungi of the genus *Trichoderma* are characterized by a high versatility and some selected species are plant symbionts, capable of producing multiple positive effects on plants, such as disease control, growth promotion, increased resistance etc. Several strains of *Trichoderma* are well-known producers of bioactive secondary metabolites (SMs). Recently, it has been demonstrated that *Trichoderma* produces natural compounds that can be involved in the beneficial interactions directly established with the plants. In the course of an ongoing search for bioactive secondary metabolites from *T. harzianum* M10, a methoxychromone derivative has been isolated. After growing M10 in an inductive liquid media for 21 days, the cultural filtrate was exhaustively extracted with ethyl acetate and the crude extract was fractionated by direct phase column chromatography (silica gel, atmospheric pressure). A compound, obtained as pure crystal from one of the fractions, was identified as 5-hydroxy-2,3-dimethyl-7-methoxychromone by X-ray analysis (from data collected at the XRD1, Elettra Synchrotron, Trieste) and spectroscopic studies. The compound, isolated for first time from a *T. harzianum* culture, was tested at different concentrations for antifungal activity against the phytopathogenic agent *Rhizoctonia solani*. The methoxychromone showed a 45% of growth inhibition after 24 h of incubation at a concentration of 100 ng plug $^{-1}$ whereas the maximum inhibition percentage was found to be 61% at 200 µg plug^{-1}. These results suggest a possible role of 5-hydroxy-2,3-dimethyl-7-methoxychromone in antibiosis mechanism of *T. harzianum* M10 against fungal pathogens.

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Visualization and analysis of grapevine downy mildew disease progress data on partially resistant varieties

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Grapevine downy mildew (DM), caused by *Plasmopara viticola,* is a threatening disease in viticulture, especially when weather conditions are conducive and the host is highly susceptible. DM-resistant cultivars express varying degrees of partial resistance and are important for integrated disease management. Still, they require plant protection interventions throughout the season to prevent yield losses and protect the durability of host resistance. Most studies on grapevine varieties' resistance durability and variability were conducted in controlled conditions with monocyclic experiments, artificial inoculations and primarily on leaves. Therefore, an understanding of the disease progress on the resistant varieties on both leaves and clusters under field conditions, where many infection cycles are concatenated, would add precision to the scheduling of fungicide interventions. At the experimental vineyard (University campus), we monitored weekly DM infections on leaves and clusters for four years, from the bud break to the harvest of 16 varieties registered in the Vitis International Variety Catalogue (VIVC). Using a Linear Mixed Model (LMM), we analyzed the Area Under the Disease Progress Curve (AUDPC) to assess the influence of different varieties on disease development. Resistant varieties such as Bronner, Cabernet Volos, Calardis Blanc, Johanniter, Merlot Kanthus, Merlot Khorus, Regent, or Solaris had a lower AUDPC compared to Calandro, Felicia, Fleurtai, Reberger, Rkatsitelli, and Villaris, with Palava and Merlot showing the highest AUDPCs. The resistant varieties carrying quantitative treat loci (QTLs) such as *Rpv* 3, *Rpv* 10 and/or *Rpv* 12 reduced the AUDPC on leaves and clusters by > 90% on average, compared to the susceptible Merlot.

The complete genome of *Fusarium musae* strain F31, pathogen of banana fruits

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F. musae is a recently described species belonging to *Fusarium fujikuroi* Species Complex and sister to *F. verticillioides*. It causes crown rot on banana fruits and it can also be opportunistic human pathogen. By exploring its genome we aim to explore diversities that might facilitate the identification of *F. musae* species within the genus *Fusarium*. Identification of pathogenic genes and comparative genomic studies could help us to understand peculiarities of this pathogen. We obtained complete genome at chromosome level of the representative strain of *F. musae* F31, isolated in 2013 in Dominican Republic from a diseased banana. The genome

is 44.07 Mb, divided into 12 chromosomes (11 have both telomers), the circular mitochondrial DNA (mtDNA) and one unplaced contig. Functional annotation led to a total of 13,963 annotated genes, of which 13,661 were proteins and 302 transfer RNA (tRNA) for nuclear DNA. EffectorP 3.0 estimated the presence of 214 effectors (probability 0.9 or higher) and antiSMASH (version 6.1.1) analysis predicted 44 secondary metabolite clusters. F31 potentially produces mycotoxins typical of Fusarium species such as fusarin and fusaric acid but also pigments such as fusarubin and bikaverin. The fumonisin biosynthesis gene cluster, distinctive of *F. verticillioides* strains, is absent in *F. musae*, whereas a gibberellin biosynthesis gene cluster is present (usually absent in its "sister species"). Genome data allowed the generation of fluorescent *F. musae* reporter strains expressing E2-Crimson under control of the constitutively active *F. musae* enolase promoter *Peno1*. Reporter strains will be used to explore the infection mechanism in bananas.

Response of seed germination to heat treatment required to reduce the risk of '*Candidatus* Liberibacter solanacearum' vertical transmission in Apiaceae crops

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In Europe and the Mediterranean basin, '*Candidatus* Liberibacter solanacearum' (Lso) is associated with vegetative diseases of Apiaceae crops, mainly carrot (*Daucus carota* L.), inducing yellow or red leaf discoloration, reduction in size of the main root with lateral root proliferation. In some countries (e.g. Japan and Australia), mandatory heat treatment (50 °C) or PCR negative testing for Lso is required as a phytosanitary measure for carrot seed importation. Carrot seed movement is economically important for the Mediterranean region, and Lso is extensively widespread in Apiaceae seeds. Hence, heat treatment plays a key role as a disinfection method for ensuring international seed trade; nonetheless, the treatment could reduce seeds germination. In this work, the impact of heat treatment on germination of 41 Apiaceae seed lots (24 carrot, 10 parsley, 5 fennel, and 2 celery) belonging to different varieties and hybrids was investigated: healthy and Lso-infected seed lots were heat-treated and then germinated according to International Seed Testing Association. Germinability data of treated and untreated seeds were compared in both healthy and infected lots; overall, the heat treatment significantly decreased the germination percentage in 17% of the analysed seed lots of carrot, 30% of parsley, 40% of fennel, and 50% of celery. Our data show that heat treatment negatively affects the germination rate of Apiaceae seeds, although to a different extent depending on the species and variety. These results may envisage an economic burden not only for the heat treatment procedure per se, but also for decreasing the seed germinability.

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Detection and absolute quantification of the fungus *Fusicladium oleaginum* in olive tree by droplet digital-PCR (ddPCR)

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Olive leaf spot, caused by the fungus *Fusicladium oleaginum* (= *Venturia oleagina*), is a leaf disease, widespread in all olive-growing regions of the world. This pathogen is often present also in asymptomatic leaves depending on environmental factors (e.g. temperature, humidity). In appropriate conditions the attack is severe and it can lead to massive defoliation, reduced productivity and poor oil quality. The aim of this study was to develop real time-qPCR (qPCR) and droplet digital PCR (ddPCR) for an accurate, early and robust detection of *F. oleaginum*. ddPCR indeed overcomes some limitations of qPCR (e.g. the need of a standard, the influence of inhibitors). Symptomatic leaves of 28 olive trees from 10 different Italian regions were collected. The ITS FusiR *Fusicladium*-specific and the ITS5 primers enabled to amplify the region ITS 1—5.8S rDNA—ITS2, without fungal isolation. The obtained sequences showed that this region is highly conserved among different fungal isolates. Primer and probes were designed to be suitable for both EvaGreen®, TaqMan®-qPCR and ddPCR techniques. All genomic extracts, obtained from symptomatic leaves, tested positive in all developed methods. The exclusivity was tested against twenty-eight epiphytic and endophytic olive leaf fungi, isolated from symptomatic and asymptomatic leaves. Tests are underway to fully develop and validate these methods. Monitoring climate parameters together with a sensitive molecular detection of *F. oleaginum* will be crucial for leaf spot control by establishing timely chemical interventions, which combines effectiveness and dose reduction.

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Population genetics and fitness of *Plasmopara viticola* in the winegrowing region of South Tyrol

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Grapevine downy mildew, caused by *Plasmopara viticola*, is considered one of the most devastating oomycete diseases globally, causing significant yield losses in vineyards. It is generally accepted that effective control of the pathogen relies on solid knowledge of *P. viticola* population genetics for each specific wine-growing region. Up to date, no data are available about the genetic composition of *P. viticola* populations in South Tyrol (Northern Italy). For this reason, the aim of the present study was to investigate the population genetics and fitness of *P. viticola* in dependence on distinct farming management systems differing in the chemical classes and dosages of applied fungicides. Nine vineyards located in an area of 30 km of linear distance were selected. Fifteen samples per vineyard were collected in 2020 at the end of the growing season and genotyped with 15 microsatellite markers. The fitness parameters were evaluated in vitro by the leaf disk assay. A total of 35 different alleles were found in the dataset, with an average of 2.33 alleles per locus. Among the 135 isolates, 125 distinct multilocus genotypes were identified. The elevated genetic variability of *P. viticola* within vineyards points to a high degree of sexual reproduction in the area. Instead, population differentiation between the vineyards was low and no differentiation was found among the different management systems, also concerning the fitness parameters, suggesting the presence of a single metapopulation. An in-depth analysis of molecular data will allow us to elucidate processes taking place in the *P. viticola* populations of South Tyrol.

Hybrid assembly and comparative genomics of three different isolates of *Gnomoniopsis castaneae*

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Gnomoniopsis castaneae (syn. *G. smithogilvyi*) is the causal agent of the brown rot of chestnut fruits and is responsible for leaf, shoot blight, and bark cankers as well. The pathogen spends part of its life cycle as an endophyte in the host tissues. Symptoms on fruits are mainly expressed post-harvest when peculiar conditions of temperature and humidity occur. Thus, healthy fruits may hide a latent infection that can rapidly switch to symptoms expression. Recently, a rapid and reliable detection method based on a qPCR assay was developed to reveal the presence of the pathogen in all the symptomatic and asymptomatic specimens. Nonetheless, to better understand the genetics behind the endophytic behavior and the pathogenic mechanisms of this fungus, here we provide the first complete genome sequence of three different isolates of *Gnomoniopsis castaneae*: the Italian ex-type MUT-401, a second Italian MW494885 isolate and the ICMP 14,040 isolate from New Zealand. The three genome sequences were obtained through a hybrid assembly using both short Illumina reads and long Nanopore reads, the coding sequences annotated and compared with other genomes of the Diaporthales. The identification of specific traits related to the three isolates with different geographic origins could give insights into the interaction between the pathogen, its host, and the environment. These premises would also define the foundation for future analysis, with particular focus on the relationship between the endophytes and the outer world and on developing customized control strategies.

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Rapid molecular assay for the evaluation of seed treatments with clove essential oil against wheat common bunt

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Common bunt, caused by the fungus *Tilletia laevis,* is an important disease of wheat, causing considerable yield loss

and reduction in seed quality. The spread of organic and low-input farming, led to the increase of the disease incidence and to a growing search for bio-compounds replacing chemical treatments for pest management. In this study, the antifungal activity of clove oil was evaluated as seed treatment against *T. laevis*. The trial was set up with 6 different treatments, performed either by seed immersion or spray application, consisting in clove essential oil and two clove oil-based experimental formulations, used in different concentrations, plus two controls (infected/untreated and infected/copper-treated). For each treatment 90 seeds of durum wheat (cultivar Grifoni) naturally infected were sown in 3 repetitions, for a total of 30 seeds/ pot. The germination rate was evaluated: the clove oil treatment concentrated at 0.5% v/v showed phytotoxic effects. Before the tillering stage, 25 seedlings were sampled for each pot and separated in 5 bulks of 5 plants each; the DNA was extracted from each bulk. A qPCR test has been developed for the specific quantification of *T. laevis* in the seedling bulks, obtaining optimal results both for amplification efficiency (95–110%) and for the detection sensitivity (10 fg). The results were statistically analysed (ANOVA) and showed that immersion and spray treatments with both the experimental formulations significantly reduced the percentage of plant infection. Given the promising results, further tests are ongoing to confirm the data obtained.

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Metabolomic characterization of a *Clonostachys rosea* strain active against nematodes

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The rhizosphere is occupied by a variety of organisms that coexist by sharing space and nutrients. Beneficial microbial communities are well-known for their capacity to improve plant growth and resistance to biotic and abiotic stresses and studied to achieve a better understanding of microbe-microbe, microbe-plant and microbe-pathogen interactions. Since secondary metabolites, which are released in the rhizosphere, play an important role in these interactions, a detailed characterization of microbial metabolome is essential. For this study, soil from selected vineyards located in Avellino province (Campania region, southern Italy) were collected to characterize the microfauna associated to grapevines rhizosphere by extensive application of morphological and molecular techniques. Among the beneficial microbes, a *Clonostachys rosea* strain isolated in suppressive soil, showed a significant *in vitro* nematicidal activity. Therefore, to chemically characterize the exo metabolome of this isolate, the crude extracts of cultural filtrates were fractionated by direct and indirect chromatography to identify the bioactive secondary metabolites. Mass spectrometry, NMR and crystallography techniques were used to characterize the isolated natural compounds. The fraction containing a mixture of diketopiperazines exhibited a strong nematicidal activity demonstrating that these exometabolites have a significant role on the improvement of soil quality and to control pests and/or pathogens on vineyard production. More investigations are ongoing in order to obtain pure molecules for *in vivo* test against plant pathogens and nematodes.

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First evidence of *Pantoea ananatis* infections on strawberry in Italy

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Recently, symptoms resembling fire blight such as brownish lesions, necrosis, and production of exudates were observed on a new cold-resistant strawberry variety (Ania®). Thus, the aim of this study was to isolate, identify and characterize the causative agents. Bacterial isolation was carried out from symptomatic plant tissues (leaves and stems) collected from four strawberry plants. Tissues were surface-sterilized, homogenized in 0.85% (w/v) NaCl and serially diluted onto Nutrient Agar. One hundred twenty bacterial isolates were tested for the induction of the hypersensitive response (HR) in tobacco leaves. Bacterial isolates with yellow colonies able to induce HR were identified as *Pantoea ananatis* by 16S rDNA gene sequencing. PCRs using the primer pair PANA_1080 specific for *P. ananatis* species validated these results. Subsequently, pathogenicity tests were carried out by wounding detached ripe pseudo-fruits from strawberry plants (Ania®) and inoculating five µL of bacterial cell suspension (~1×10^8 CFU/mL) of a selected *P. ananatis* strain. Distilled water and *Escherichia coli* DH5α were used as

control treatments. Strawberries were incubated in the dark at 27 °C and 90% RH and appearance of symptoms was monitored daily. No symptoms were observed on the control treatments. On *P. ananatis* inoculated strawberries, symptoms appeared after five days and the bacterial strain was re-isolated from the sympotmatic pseudo-frutits. Furthermore, *P. ananatis* strains were evaluated for the production of plant cell wall degrading enzymes (i.e. cellulases). To the best of our knowledge, this is the first report of *P. ananatis* on strawberry in Italy.

Insights on KP4 killer toxin-like proteins of *Fusarium* species in interspecific interactions

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KP4 killer toxins are secreted proteins inhibiting cell growth and inducing cell death in target organisms. First identified in *Ustilago maydis*, homologues have been also found in yeasts, in one species of moss, and in ascomycetes. In *Fusarium graminearum*, KP4-like (KP4L) proteins contribute to fungal virulence in wheat seedling rot and are expressed under stress conditions and during Fusarium head blight development. However, fungal KP4L proteins are also hypothesized to support fungal antagonism by permeabilizing cell walls of competing fungi, thus enabling penetration of toxic compounds. Here, we report differential expression patterns of four KP4L genes of *F. graminearum* (*Fgkp4l-1*, *-2*, *-3* and *-4*) in a competitive interaction, using *Trichoderma gamsii* as antagonist. Results from time-course experiments performed in dual cultures revealed that *Fgkp4l-3* and *Fgkp4l-4* could participate in the recognition at distance of the antagonist, while all the four *Fgkp4l* genes were highly activated in the pathogen during the physical interaction of both fungi. When on wheat spikes, only *Fgkp4l-4* was differentially expressed during the interaction with *T. gamsii*. This suggests a possible role of KP4L proteins in supporting *F. graminearum* interspecific interactions, even in living plant tissues. Database searches enabled the identification of KP4L orthologous proteins in 21 *Fusarium* genomes. Distribution of KP4L proteins across five phylogenetic groups within the genus *Fusarium* revealed they are more represented in species with broad host-plant range than in host-specific species. Phylogeny inferred provides evidence that KP4L genes evolved through gene duplications, gene loss and sequence diversification in the genus *Fusarium*.

The diversity of *Xanthomonas* spp. from Brassicaceae, diagnostics and disease control

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Plants from the Brassicaceae family can be attacked by different *Xanthomonas* pathogens. *Xanthomonas campestris* pv. *campestris* (Xcc), the cause of black rot of brassicas, is one of the most important diseases of crops like cabbage and cauliflower worldwide. Ornamental Brassicaceae plants like wallflowers and garden stocks and some common weeds can also be infected by *X. campestris* pathogens including *X. campestris* pv. *incanae*. In contrast to pvs *campestris* and *incanae*, that cause vascular diseases, pv. *raphani* causes leaf spots in a larger range of Brassicaceae and some other hosts including tomato. At least two of these pathovars, *campestris* and *raphani*, have race structures defined by the ability of causing disease in different brassica differential accessions. A disease that causes symptoms in watercress that resemble black rot, is caused by a different species, *X. nasturtii*. We are characterising the pathogenicity and sequencing a collection of *Xanthomonas* spp. isolates from different origins and spanning over 60 years, including the different pathovars of *X. campestris* and also the watercress pathogen, *X. nasturtii*. The results of our work will allow us to clarify the taxonomy of *X. campestris* and related species and to design diagnostic testing for some important species, pathovars and possibly some groups of races of *Xanthomonas*. We are screening brassica collections to identify useful sources of resistance and we are developing chlorophyll imaging and whole plant imaging techniques to allow us to study Xcc infections. We aim to characterise and map resistances and identify resistance-linked markers that can assist future breeding.

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Evaluation of tolerance of a walnut progeny (*Juglans regia* x *J. major* x *J nigra* and *J. microcarpa*) to crown and root rot disease caused by *Phytophthora cinnamomi*

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The cultivation of common walnut (*Juglans regia*) is very sensitive to *Phytophthora cinnamomi*. Several commercial walnut orchards were decimated by *P. cinnamomi* in north-eastern Italy. In response to *Phytophthora* attacks, several *Juglans* species in addition to *J. regia* were considered as rootstocks. The Italian Ministry of Agriculture financially supported a project named PORTNOC addressed to respond to this issue. After an intense selection, 4 *Juglans microcarpa* genotypes and 5 hybrids showed good level of tolerance towards *P. cinnamomi*. A total of 392 progenies derived from these mother plants were subjected to artificial inoculation with *P. cinnamomi* (isolate CREADC-Om274). Based on field observations and necrosis length caused by the pathogen, 23 progenies of hybrid 10/43 (*J. regia* x *J. major* x *J. nigra*), 11 progenies of hybrid 6/22 (*J. regia* x *J. major* x *J. nigra*) and 3 progenies of *J. microcarpa* M7 showed significantly higher tolerance levels towards *P. cinnamomi*. In addition, evaluation of seedling progenies of tolerant genotypes grafted with commercial walnut varieties is currently underway in naturally infected fields and will provide further information for successful usage.

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Influence of carbon and nitrogen sources on *Trichoderma* pH modulation and biocontrol activity

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Trichoderma spp. are the most widely used biocontrol agents in agriculture protecting plants against pathogens through different mechanisms: mycoparasitism, secretion of lytic enzymes and bioactive metabolites, nutrient competition or antibiosis. The *Trichoderma harzianum* isolate M10 is known to produce harzianic acid (HA), a metabolite with both antimicrobial and plant growth promoting activities. To test the environmental conditions influencing the production of HA and thus its activity against plant pathogens, M10 was grown in different liquid media (rich or minimal media). Here, we found that M10 initially induces a slight extracellular acidification followed by a strong alkalinization at later time points in rich media. On the contrary, in poor media the pH is maintained constant over time. Since rhizosphere pH alkalinization is an important mechanism used by plant pathogens to infect the host plant, we decided to test if different concentrations of carbon sources (glucose or sucrose) or different nitrogen sources (nitrate or ammonia) influence M10 extracellular alkalinization and bicontrol activity *in vitro*. Our results suggest that M10 mediated alkalinization is independent of the carbon source but not of the nitrogen source supplemented to the medium. Indeed, ammonia supplements reverted extracellular alkalinization to acidification. Importantly, ammonia-mediated acidification also results in higher biocontrol activity against the soil born fungal pathogen *Fusarium oxysporum*. Further experiments are required to provide a putative correlation between extracellular pH modulation and HA production in M10.

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Volatile-mediated inhibitory activity of the biocontrol agent *Lysobacter capsici* AZ78: a result of multiple factors interaction

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Plant beneficial rhizobacteria are able to inhibit the growth of soilborne phytopathogenic fungi and oomycetes through the release of a relevant number of volatile compounds. Based on this, we investigated the ability of the biocontrol agent *Lysobacter capsici* AZ78 (AZ78) to produce volatile organic compounds (VOCs) that may contribute to its efficacy in controlling soilborne phytopathogenic fungi and oomycetes. AZ78 significantly reduced the growth of *Pythium ultimum*, *Rhizoctonia solani* and *Sclerotinia minor* through the release of VOCs in split Petri dish assays. The GC–MS analysis revealed that AZ78 produced 22 VOCs and most of them were putatively identified as mono- and dialkylated methoxypyrazines. Exposure to 2,5-dimethylpyrazine, 2-ethyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine determined a drastic reduction of *P. ultimum*, *R. solani* and *S. minor* mycelium growth in split Petri dish assays. However, a remarkable difference between the toxicity of the pyrazines and the AZ78 total volatile blend was observed. This difference led us to further investigate the volatile-mediated inhibitory activity of the biocontrol bacterium. Further experiments revealed the ability of AZ78 cells to produce ammonia that caused the alkalinization of the physically separated growth medium in split Petri dishes assays. As a consequence, the mycelium growth of the tested phytopathogenic fungi and oomycetes was negatively affected on the alkalinized growth medium. Results achieved in this work clearly showed that volatile-mediated inhibitory activity of AZ78 mainly relies on the interaction between the toxicity of VOCs, ammonia and the alkalinization of growth medium.

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Genetic characterisation of *Fusarium culmorum* isolates from Çanakkale, Turkey

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Fusarium head blight (FHB) is one of the most important fungal diseases of small grain cereals worldwide. *Fusarium culmorum* is the predominant causal agent of FHB in many Mediterranean regions, leading to reduction in crop quality and quantity. Mycotoxin contamination of grain, primarily with deoxynivalenol and its derivatives, may occur in fields hit by FHB epidemics. In this study, a detailed genetic characterisation of *F. culmorum* isolates obtained from scabby wheat samples collected from the Çanakkale province (Turkey) in the year 2021, was carried out. A total of 45 monosporic cultures were obtained and were identified at species level by morphological characteristics. *Tef1-α* sequencing was also carried out to confirm the identification at species level. For this purpose, genomic DNA was extracted from 7-day-old potato dextrose agar (PDA) cultures. A portion of the *Tef1-α* gene was amplified from all isolates and then sequenced. The sequences were subjected to BlastN analysis. All 45 monosporic isolates, obtained from Sutlucekoy and Bayirkoy regions (Çanakkale Province), were identified as *F. culmorum*. Class B chemotype assays were also carried out in Turkish *F. culmorum* isolates by PCR assay. The *TRI12* gene was amplified to distinguish isolates as 3-acetyldeoxinivalenol (3-ADON) or nivalenol (NIV) producers. All isolates belonged to 3-ADON chemotype and to MAT1-2 idiomorph. Further studies will be extended to a larger number of isolates from different regions in order to provide a comprehensive set of data on the *Fusarium* populations causing FHB in this area.