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ABSTRACT

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Fungicides activity against mycotoxigenic fungi associated to wheat diseases and their involvement in trichothecene production

C. Abi Saad[1], M. Masiello[2], S. Somma[2], A. De Girolamo[2], S. Cervellieri[2], S.M. Sanzani[1], A. Moretti[2]

[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy; [2]Institute of Science of Food Production-ISPA, Research National Council—CNR, Via Amendola 122/O, 70126 Bari, Italy. E-mail: Charlie.abisaad@uniba.it

Fungicides are the most effective tools to protect wheat against pathogenic fungi, including the mycotoxigenic *Fusarium* and *Alternaria* species associated with Fusarium Head Blight, Fusarium Crown Rot, and Black Point diseases. These species, responsible for mycotoxin accumulation in grains, represent both phytopathological and toxicological issues. Multiple *Fusarium* species can co-occur on wheat, producing various mycotoxins. The most important are trichothecenes: type-A, including T-2 and HT-2 toxin, and type-B, including deoxynivalenol (DON), nivalenol (NIV) and their acetylated forms. The sensitivity of *F. graminearum, F. culmorum, F. poae, F. langhsethiae, F. sporotrichioides, F. avenaceum*, and *A. alternata* to eight fungicides, containing Tebuconazole, Metconazole, Difenoconazole, Prothioconazole, Azoxystrobin, Fluazinam and Folpet has been evaluated by mycelial growth assay. All tested *Fusarium* strains showed sensitivity to azole fungicides, being Prothioconazole, used alone or in mixture with Difenoconazole or Azoxystrobin, the most effective. *Alternaria* strains showed sensitivity to all tested fungicides lower than *Fusarium* species. The sub-lethal dose effect of Prothioconazole in mixture with Difenoconazole or Azoxystrobin on trichothecene production has been studied. Although a strict correlation between fungicide doses and mycotoxin production was not observed, an increased NIV amount was detected when *F. graminearum* and *F. culmorum* strains were grown in a liquid medium amended with Difenoconazole (0.75 mg/L) and in *F. graminearum* strains grown in presence of 0.5+0.375 mg/L of Azoxystrobin+Prothioconazole. Furthermore, a different distribution of mycotoxins in supernatant and fungal biomass was observed, with NIV detected only in mycelial biomass and DON mainly in supernatant.

Unravelling the impact of *Armillaria mellea* biocontrol agents on root and rhizosphere soil bacterial microbiome of olive plants

M.M. Aci, G. Pelle, A. Malacrinò, L. Schena, G.E. Agosteo

Department of Agriculture, Università degli Studi Mediterranea di Reggio Calabria, Località Feo di Vito, 89124 Reggio Calabria, Italy. E-mail: miyassa.aci@unirc.it

Armillaria mellea poses a significant threat to the stability of several agricultural ecosystems. Bacterial biocontrol agents (BCAs) offer a promising alternative to chemicals in soil-borne pathogens management, but their impact on soil and plant microbiomes remains poorly studied. In this study, we selected four distinct BCAs (three *Pseudomonas* sp. and one *Burkholderia* sp.) for their strong *in vitro* antagonistic effect on *A. mellea*, and we tested their influence on the structure of the bacterial communities of rhizosphere soil and roots samples in olive plants, and their impact on root and shoot biomass. Amplicon metagenomics analyses have shown that our BCAs significantly altered the microbial diversity in roots and rhizosphere soil, and many differentially abundant taxa were identified between control and treated plants. In turn, the structure of root and rhizosphere microbial communities remained unaltered in response to the BCAs. In addition, all BCAs significantly affected the biomass of olive roots and shoots. The Structural Equation Model approach revealed that the increase of root and shoot biomass of olive plants was mediated directly by the presence of BCAs. In contrast, BCAs did not influence the bacterial community composition in both compartments. Thus, these BCAs represent excellent candidates for future trials, as they show *in vitro* antagonistic activity against *A. mellea*, do not significantly impact the plant microbiome, and can promote plant growth. Overall, this approach linking classic biological control with the plant microbiome and plant fitness might be a promising tool for the next generation of sustainable plant protection strategies.

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AcOTApks* gene-based molecular tools to improve quantitative detection of the mycotoxigenic fungus *Aspergillus carbonarius

A. Agnusdei, D. Gerin, F. Dalena, R.M. De Miccolis Angelini, V. Montilon, F. Faretra, S. Pollastro

Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy. E-mail: donato.gerin@uniba.it

Aspergillus carbonarius is the main producer of Ochratoxin A (OTA) in grape, a mycotoxin frequently detected in grapes and derivatives and classified as a possible human carcinogen by the IARC. Several molecular methods are available for *A. carbonarius* detection and quantification, although the correlation between the consistency of fungal population and OTA contamination needs to be improved. This study aimed to develop new quantitative PCR (qPCR) and digital droplet PCR (ddPCR) to quantify *A. carbonarius* OTA-producing strains based on the key gene *AcOTApks* (*otaA*). Different primers/probe sets targeting the *AcOTApks* gene were obtained and validated for their specificity *in silico* and then using the gDNA of different OTA-producing *Aspergillus* spp. and a panel of bacteria and fungi commonly associated with grapes. The method allowed to quantify up to 100 fg μL^{-1} (Cq=37) and 10 fg μL^{-1} (0.38 copies$\cdot\mu L^{-1}$) of gDNA extracted from *A. carbonarius* mycelium in qPCR and ddPCR, respectively. The sensitivity with artificially contaminated must samples was up to 100 conidia (Cq=38) and 1 conidium (0.13 copies$\cdot\mu L^{-1}$), with qPCR and ddPCR, respectively. The methods were validated on naturally infected grape must samples, and the quantification of the fungus, in both cases, highly correlated (r= 0.8) with OTA amount in the samples. In conclusion, we report new molecular diagnostic methods in ddPCR and qPCR, targeting the *AcOTApks* gene, which can be helpful in the management of OTA contamination in grapes and derivatives to ensure wine safety with a sustainable approach.

This work was partially carried out in the framework of the projects Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)– MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4– D.D. 1032 17/06/2022, CN00000022) and Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union– NextGenerationEU; Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title "ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security– Working ON Foods". Angelo Agnusdei was granted by UE under the Programme PON– Project Code DOT1302377 (CUP: H99J21010190001).

Fludioxonil loaded liposomes *vs Botrytis cinerea*: a new perspective for sustainable management of grey mold

A. Agnusdei[1], D. Gerin[1], S. Pollastro[1], F. Faretra[1], A.M. Maurelli[2], L. Catucci[2], V. De Leo[2]

[1]Department of Soil, Plant and Food Sciences, (DiSSPA), University of Bari "Aldo Moro", Bari, Italy; [2]Department of Chemistry, University of Bari "Aldo Moro", Via Orabona 4, 70126 Bari, Italy. E-mail: donato.gerin@uniba.it

Botrytis cinerea is a ubiquitous phytopathogenic fungus responsible for grey mold, affecting worldwide important crops in the field and in postharvest. Different strategies are used to manage *B. cinerea*, but chemicals remain the most consolidated. To address the challenge of sustainable agriculture, nanotechnology has been applied to improve fungicide delivery. Here, liposomes loaded with the fungicide Fludioxonil (FLUD) were developed through the micelle-to-vesicle transition method or extrusion technique. Three types of vesicle composition (plain, PEG-coated, and cationic vesicles) were compared for Encapsulation Yields, Drug Loading Content, and Zeta potentials. The extruded and PEGylated liposomes were the most stable over time and, together with the cationic ones, showed a significantly prolonged FLUD release capacity. Liposomes' biological activity was evaluated on *B. cinerea* conidial germination, germ tube elongation, and colony radial growth. In *in vitro* assay with different concentrations of FLUD technical grade or FLUD-loaded liposomes, the Extruded PEGylated liposomes were the optimal compromise for active substance release and pathogen control. Following a slight control of conidial germination, although less effective than FLUD (22.7% at 0.3 $\mu g \cdot ml^{-1}$), the formulation showed higher effectiveness than FLUD in the subsequent stages of germ tube elongation and mycelial growth (respectively 7.7% and 68% at 0.01 $\mu g \cdot ml^{-1}$). By improving the fungicide stability, the liposomal formulation can increase its persistence, extend the application interval, and target distinct stages of pathogen development. Furthermore, the liposome's ability to adhere to membranes mediates a targeted release, improving effectiveness, and should represent a new tool for sustainable control strategies.

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Distinct roles of histone methyltransferases *Acdot1* and *AcrmtA* in growth regulation, secondary metabolism and stress response in *Aspergillus carbonarius*

A. Agnusdei[1], A. González-García[2], D. Gerin[1], S. Pollastro[1], F. Faretra[1], L. González-Candelas[2], A.R. Ballester[2]

[1]Department of Soil, Plant and Food Sciences, (DiSSPA), University of Bari Aldo Moro, Bari, Italy; [2]Instituto de Agroquímica y Tecnología de Alimentos, IATA-CSIC, Paterna, Valencia, Spain. E-mail: donato.gerin@uniba.it

Histone methyltransferases are involved in gene expression regulation by modulating the methylation of histone residues. We investigated the role of the histone methyltransferases *Acdot1* and *AcrmtA* in the mycotoxigenic fungus *Aspergillus carbonarius*, obtaining knockout or overexpression mutants through *Agrobacterium tumefaciens* mediated transformation (ATMT). *Aspergillus carbonarius* is responsible for grape-bunch rots, representing the major source of Ochratoxin A (OTA) contamination on grapes. In morphogenesis assay, deletion of *Acdot1* or *AcrmtA* upregulated the growth only on a minimal medium. In mycotoxin biosynthesis, the role of the genes was different and strongly affected by culture conditions. In liquid culture, Δ*Acdot1* increased OTA production on rich media (+41%) as compared to wild type (WT), while Δ*AcrmtA* reduced OTA biosynthesis in both rich (−43%) and minimal media (−93%). OTA production in OE*rmtA* was like WT on both media, while a lower OTA amount was reported for OE*dot1* only on a minimal medium (−74%). The expression of *AcOTAp450* (*otaC*), *AcOTAnrps* (*otaB*), *AcOTAhal* (*otaD*), key genes of the OTA biosynthetic pathway, was reduced in the Δ*AcrmtA*. In the presence of oxidative stress, Δ*Acdot1* or Δ*AcrmtA* were slightly inhibited in growth and increased OTA production (+34% and +59%, respectively), while, on the contrary, WT resulted slightly upregulated in mycelium growth. In conclusion, *Acdot1* and *AcrmtA* are differently involved in the growth, mycotoxin biosynthesis, and stress response in *A. carbonarius*. Due to its involvement in OTA biosynthesis regulation, especially *AcrmtA* represents a potential target for preventing OTA contamination.

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Tolerance/resistance assessment to ToBRFV infection in different local Sicilian tomato ecotypes

G. Agrò, E. Yahyaoui, A.G. Caruso, C.M. La Marra, S. Davino, S. Bertacca, A. Ragona, S. Panno

Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Viale delle Scienze, Ed. 5, 90128 Palermo, Italy. E-mail: stefano.panno@unipa.it

Tomato brown rugose fruit virus (ToBRFV) represents a threat to tomato crop worldwide, causing severe production losses, aided by its highly contagious nature, that makes its spread extremely rapid and difficult to contain. Selective pressure towards specific phenotypes has caused a dramatic reduction in genetic diversity of tomato plants, resulting in greater vulnerability to pathogens. In contrast, ancient ecotypes and autochthonous varieties can have an increased resistance to biotic and abiotic stresses. This study aimed to evaluate the response of some selected Sicilian tomato ecotypes towards ToBRFV infection. First, 37 tomato local ecotypes seeds were preliminary tested by molecular analyses to confirm the absence of tomato mosaic virus (ToMV), pepino mosaic virus (PepMV) and ToBRFV. Subsequently, ten seeds per ecotype were sown and grown under greenhouse conditions. Afterwards, eight seedlings per ecotype were mechanically inoculated with ToB SIC01/19 ToBRFV isolate, while two non-inoculated seedlings per ecotype were maintained as negative controls. Symptoms were screened by visual inspection at 7, 14, 21 and 28 days post-inoculation (dpi), and virus replication was evaluated by RT-qPCR. The outcome of the current investigation revealed that all inoculated seedlings resulted positive to ToBRFV and exhibited severe symptoms except for one ecotype, that showed mild symptoms until 28 dpi, suggesting a high level of tolerance to the virus. The identified Sicilian tomato ecotype could be a candidate for genetic breeding programs to develop new ToBRFV-tolerant cultivars, representing a new perspective to reduce crop damage and yield loss and control the pathogen dispersion.

Identification and characterisation of *Monilinia* spp. on stone fruits in Sicily

E. Ahmed[1], L. Oualguirah[2], F. Valentini[2], F. Santoro[2], S. Gualano[2], A. Ippolito[3], A.M. D'Onghia[2], M. Gallo[2]

[1]Department of Civil, Environmental, Land, Building Engineering and Chemistry (DICATECh), Polytechnic University of Bari, Via Orabona, 4, I-70125 Bari, Italy; [2]Centre International de Hautes Etudes Agronomiques Méditerranéennes Bari (CIHEAM Bari), Via Ceglie 9, 70010 Valenzano, Bari, Italy; [3]Department of Soil, Plant, and Food Sciences (Di.S.S.P.A.), University of Bari - Aldo Moro, Via Amendola 165/A, I-4, 70126 Bari, Italy. E-mail: gallo@iamb.it

Brown rot is a significant disease of stone fruit both in the field and after harvest, caused by *Monilinia fructicola* (MF), *Monilinia laxa* (ML), *Monilinia fructigena* (MFG), and *Monilinia polytroma* (MP). MF is recommended for regulation as a quarantine pest by the European and Mediterranean Plant Protection Organization, being included in the A2 list of quarantine pests. In Italy, *M. fructicola* was first detected in Piemonte and then in Emilia-Romagna, Lazio, Basilicata, Campania and Puglia. This study was conducted in four orchards in the municipalities of Maniace and Bronte, Sicily (Italy). The orchards studied were peach, plum, and apricot. Based on the morphological characteristics of colony colour, growth rate, concentric rings of spore production, lobed margin of colonies, rosettes, and black arches associated with rosettes, three typical morphological profiles of *Monilinia* were obtained. Respectively, for 26 isolates, two belonged to MF, seven to ML, and 17 to MFG. As for the plant organs subjected to isolations: one MF and five ML from flowers, one MF from twigs, two ML and two MFG from fruits, and 16 MFG from fruit mummies. In addition, the sequences of the ITS-5 and ITS-4 of six selected representative isolates, representing the three morphological profiles of *Monilinia*, belonged to MF, ML, and MFG with more than 99% similarity. The sequences were deposited in GenBank with accession numbers MN049476 to MN049483. This study is the first to describe the occurrence and distribution of *Monilinia* spp. on stone fruit, flowers, and twigs in Sicily.

Post-winter leaf and debris removal reduces overwintering inoculum of strawberry powdery mildew and delays disease onset

A. Aldrighetti, I. Pertot

Center of Agriculture, Food, Environment, University of Trento, San Michele all'Adige, Italy; E-mail: anna.aldrighetti@unitn.it

Powdery mildew is an important strawberry disease, commonly controlled with repeated fungicide applications, which may lead to the selection of resistant pathogen populations and a negative impact on the environment. The causal agent, *Podosphaera aphanis*, overwinters as mycelium on infected plant tissue, with chasmothecia serving as a critical inoculum for primary infections in colder climates. Consequently, post-winter plant sanitation could significantly reduce disease incidence by diminishing primary inoculum levels. In a two-year study, we assessed the effectiveness of removing infected leaves and debris in tabletop strawberry production systems. High temperatures in late summer negatively impact the formation of chasmothecia, leading to reduced overwintering inoculum and severity of primary infections. The removal of infected leaves resulted in a 95% reduction in disease incidence compared to the control and delayed disease onset. Although some chasmothecia may detach from leaves and remain in the substrate, they were insufficient to initiate infection once the leaves were removed, suggesting a marginal role in the epidemiology of the disease. This study demonstrates that integrating agronomic practices, such as post-winter plant sanitation, can significantly reduce the reliance on chemical fungicides in strawberry farming.

Control of Fusarium wilt on lettuce and tomato in the greenhouse by insect frass

L. Alfarano[1,2], M. Pugliese[1,2]

[1]University of Torino– DiSAFA, Grugliasco, Italy; [2]University of Torino– Agroinnova, Grugliasco, Italy. E-mail: luca.alfarano@unito.it

Fusarium oxysporum is a soil-borne fungus that causes severe diseases in tomatoes and lettuce. The main control strategies include the use of resistant cultivars, fungicides, physical methods, and agronomical practices. This study aimed to investigate the capacity of frass produced by *Hermetia illucens* to suppress Fusarium wilt on tomato and lettuce. Frass was obtained from *H. illucens* larvae, fed with the Gainesville diet, at the experimental insect facility of DiSAFA– University of Torino. The frass, both heat-treated (70 °C for 60 minutes) and untreated, was mixed with peat at dosages of 0, 1, 2, 5, 10, and 20% (v/v) in nursery trays and tomato and lettuce seeds were sown immediately after. The plants were transplanted about three weeks later into 2 L pots filled with a peat substrate previously inoculated with *F. oxysporum* f. sp. *lycopersici* race 1 or *F. oxysporum* f. sp. *lactucae* race 1. Disease severity and above-ground plant biomass were evaluated, and data were analysed using ANOVA to identify which dosage was effective. Untreated frass reduced Fusarium wilt in both crops, while heat-treated frass partially lost its suppression capacity, suggesting a potential role of the native microflora in the frass in disease control.

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU, PNRR CN00000022.

Effectiveness of a novel integrated solarization system to control soilborne pathogens of strawberry and effects on non-target microorganisms in northern Italy

F. Aloi[1], V. Battaglia[2], G. Dardani[1], M. Garello[1], M. Cermola[2], P. Mormile[3], D. Spadaro[1,4], E. Lahoz[2], V. Guarnaccia[1,4]

[1]Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Università degli Studi di Torino, Grugliasco, Italy; [2]Consiglio per la Ricerca in Agricoltura e l'Economia Agraria (CREA), Centro di ricerca cerealicoltura e colture industriali, Caserta, Italy; [3]Green App, Benevento, Italy; [4]Centro Interdipartimentale Agroinnova, Università degli Studi di Torino, Grugliasco, Italy. E-mail: francesco.aloi@unito.it

A novel integrated solarization system, referred to as the Solin® method, offers a promising alternative to fumigants or pesticides for managing soilborne plant pathogens. The technology, inspired by solar panels, creates a black matrix that absorbs more solar radiation, raising the temperature of the superficial soil. The technology has been applied to control strawberry soilborne plant pathogens in northern Italy. To assess its effectiveness, the study employed small polyethylene bags (0.02 μm membrane sun bags) containing wheat seeds colonized by fungal strains of *Neopestalotiopsis* spp., along with temperature probes, which were buried in plots covered with different types of solarization films. Furthermore, soil samples were collected to conduct a metagenomic analysis of microbial communities, focusing on fungi, bacteria, and oomycetes. The experimental design included four treatment plots: (i) untreated soil (control), (ii) standard solarization with PET film, (iii) solarization with multilayer thermal film (Polysolar), and (iv) the Solin method. The study showed evidence for the enhanced efficacy of this innovative approach, as indicated by a significant increase in soil temperature and a faster achievement of the thermal sums suitable for reducing the inoculum potential. The evaluation of temperature, survival of fungal pathogens, and analysis of resident microbial communities across the treatment plots demonstrated the potential of solarization as an effective tool to control soilborne plant pathogens without disrupting the soil microbial community. Notably, these findings challenge the conventional assumptions regarding the problematic application of solarization in regions characterized by less favourable climatic conditions, such as northern Italy.

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Optimization of seed coating formulations based on biochar, agro-industrial by-products, and beneficial microbes for plant growth promotion and disease protection

G. Amoroso[1], G. Iacomino[1], L. Di Costanzo[1], R. Spaccini[1], M. Idbella[2], G. Bonanomi[1,3]

[1]Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Naples, Italy; [2]Laboratory of Biosciences, Faculty of Sciences and Techniques, Hassan II University, Casablanca 28806, Morocco; [3]Task Force on Microbiome Studies, Università degli Studi di Napoli Federico II, 80138 Napoli, Italy. E-mail: giandomenico.amoroso@unina.it; mohamed.idbella@unina.it; giuliano.bonanomi@unina.it

In intensive crop cultivation, plant pathogens thrive and can quickly become highly virulent, requiring timely and costly interventions. One effective method to fortify plants is using seed coating, which enhances germination and shields against pests and diseases, promotes uniform plant growth, delivers nutrients effectively, and supports environmental sustainability. Biochar in agriculture improves soil quality by adsorbing fertilizers, enhancing nutrient retention, and fostering habitat for beneficial microbes, thereby bolstering sustainability efforts. To this end, we set up a biochar-based seed coating integrating agro-industrial byproducts, including olive, pomace, and buffalo digestate as nitrogen sources, beneficial microbiomes from natural ecosystems (*i.e.*, oak, beech, pine forest), and microbial consortia containing

Trichoderma species. First, chemical and spectroscopic analyses were performed on the seed coatings using 13C NMR spectroscopy and SEM-EDS to characterize their composition. Subsequently, these coatings were tested on *Solanum lycopersicum, Zea mays*, and *Lactuca sativa* in open field conditions, employing volcanic soil infested by *Rhizoctonia solani, Sclerotinia sclerotiorum*, and *Fusarium oxysporum*. Results indicate distinct advantages for seeds coated with natural microbiomes or *Trichoderma* compared to those coated solely with pomace or buffalo digestate. In particular, beneficial microbes from beech and occasionally oak forests are among the most valuable natural microbiomes for the tested species, except *Z. mays*, as they provided higher germination and accelerated growth rates. Conversely, microbes from pine forests did not exhibit positive effects. Further analysis of seed coating microbiomes will be conducted to clarify the microbiological mechanisms underlying the observed results.

Ornamental trees: cortical macro and micromycete pathogens found on trunk lesions from sunburn related to climate change

N. Anselmi[1], A. Anselmi[2]

[1]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Via S. Camillo de Lellis, snc I-01100, Viterbo, Italy; [2]Istituto Tecnico Agrario Statale Fabio Bocchialini, Viale Piacenza, 14, Parma, Italy. E-mail: anselmicasa@live.it

Increasingly high summer temperatures linked to climate change show to induce an increased incidence of sunburn on the trunks of ornamental plants, often resulting with infections by various pathogens. In order to detect the incidence of micro and macro fungal pathogens on trunk bark damaged by sunburn, starting from the very hot and dry summer of 2017, systematic observations were carried out on urban trees in 10 different cities of northern central Italy. The identification of fungal species was carried out through macroscopic and microscopic observations and, in some cases, molecular analyses. Sunburn on the trunk, which has really increased compared to the past, has been found on numerous different species of ornamental trees, mainly in the area of the trunk exposed to South-West, especially in paved or asphalted areas. Among the micromycetes, *Biscognauxia* spp. was detected in several sites on *Quercus, Acer, Fraxinus* and *Carpinus* species, while *Cytospora chrysosperma* and *Diaporthe* spp. were detected on *Populus* and *Salix* spp. In almost all sites *Anthostoma decipiens* on *Carpinus betulus* was found. The following basidiomycetes causing wood decay were found, with some differentiation based on the site: *Schizophyllum commune* on all tree species; *Bjerkandera adusta* on *Populus nigra, Acer, Alnus* and *Platanus; Cerrena unicolor* on *Acer* and *P. nigra; Fomitiporia mediterranea* on *Alnus* and *Populus alba; Coriolopsis trogii* and *Trametes hirsuta* on *P. nigra; Condrostereum purpureum* and *Phellinus pomaceus* on *Prunus; Trametes versicolor* on *Acer, Alnus, Carpinus* and *Quercus*; and finally *Gloeophyllum trabeum* and *Dacrymyces deliquescens* on *Carpinus* and *Alnus.*

Aeromonas sp.: a plant growth-promoting bacterium (PGPB) strain with biocontrol potential against *Phytophthora infestans* on tomato plants

C. Antonelli[1], D. Nicolai[1], N. Benfradj[1], A. Testa[2], A.M. Vettraino[1]

[1]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Via S. Camillo de Lellis snc, I-01100 Viterbo, Italy; [2]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania 'Luigi Vanvitelli', Caserta, Italy. E-mail: vettrain@unitus.it

Tomato (*Solanum lycopersicum* L.) is one of the most commercially important vegetable crops in the world, with a harvested area of 4.9 million ha, an annual production of 175.9 million tonnes and an average yield of 35.9 t/ha (2011–2021). However, it is known that the ability of agriculture to provide food is often unsustainable if soils are deficient in one or more essential plant nutrients, resulting in insufficient plant development. To overcome this problem and increase yields, farmers have become increasingly dependent on chemical fertilizers, with negative consequences for the environment and human and animal health. Fertilizers containing PGPB can minimize costs and environmental impact, and influence soil fertility through mechanisms of decomposition, mineralization, and nutrient storage/release. Beneficial bacteria can stimulate plant growth and vigour through direct mechanisms by promoting the nutrient acquisition and modulating plant growth hormone levels, and indirectly by increasing plant resistance to abiotic and biotic stresses. This work aimed to use an *Aeromonas* sp. strain, characterized by PGP traits (*e.g.*, IAA production, phosphate, and potassium solubilization) and tolerance to abiotic stresses (salt, pH and osmotic stress), as inoculum for tomato plants exposed to abiotic and biotic stresses. Plants were first inoculated with the bacteria and then subjected to drought stress or inoculated with the plant pathogen *Phytophthora infestans*. Data on seedling height, dry weight, and disease incidence and severity showed the bacterial strain efficacy both as a PGPB and as a biological control agent.

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AGRIVITA - Protecting Italian citrus groves from the tracheomycotic fungus *Plenodomus tracheiphilus*

M. Aragona[1], S. Di Silvestro[2], P.F. Roversi[1]

[1]Council for Agricultural Research and Economics, Research Centre for Plant Protection and Certification (CREA-DC), Rome, Italy; [2]Council for Agricultural Research and Economics, Research Centre for Olive, Fruit and Citrus Crops (CREA-OFA), Acireale (CT), Italy. E-mail: maria.aragona@crea.gov.it

Mal secco is a disease of citrus crops, particularly of lemons, caused by the tracheomycotic fungus *Plenodomus tracheiphilus*, reported in Sicily in the early 1900s and widespread exclusively in some regions of the Mediterranean basin. In Sicily, which produces over 90% of Italian lemons, a progressive spread of the disease has been detected over the last decade, with a growing number of plants affected and the resurgence of attacks even in the most valuable areas characterized by the main quality brands. Despite the disease having been detected some time ago, data and technical-scientific information are still quite incomplete, also due to the lack of specific large-scale research projects to date. In this context, it is essential to acquire data on control strategies, biology, genetics of the fungus, and plant-pathogen interaction to deal adequately with the disease. The main objective of AGRIVITA project is to make the use of new genotypes and innovative crop protection products together with environmentally and economically sustainable cultivation techniques available to farms. The two-year project started on December 27, 2023 and it includes five Work Packages, the first for coordination and four for research and experimental activities: WP2, evaluation of existing germplasm (IGP) and selection of rootstocks; WP3, genetic improvement; WP4, innovative methodologies for the rapid recognition of the disease; WP5, biological/integrated defence and management of citrus groves. The coordinator of the project is CREA-DC, other partners involved are CREA-OFA, CREA-CI, CREA-VE, CREA-GB, CREA-IT, CREA-PB, University of Catania and the University of Reggio Calabria.

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Exploring the role of rubellin D in *Ramularia collo-cygni* virulence during interaction with barley

E. Balducci[1], L. Roehrig[2], F. Tini[1], G. Beccari[1], M. Sulyok[3], N.D. Havis[2], P. Benincasa[1], L. Covarelli[1]

[1]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy; [2]Scotland's Rural College (SRUC), King's Buildings Campus, West Mains Road Edinburgh EH9 3JG, UK; [3]University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology (IFA-Tulln), Institute of Bionalytics and Agro-Metabolomics, Tullin, Austria. E-mail: emilio.balduccii@unipg.it

Ramularia collo-cygni is the causal agent of Ramularia leaf spot (RLS) of barley, a disease posing an increasing threat to farmers globally. The development of RLS symptoms has been potentially linked to the biosynthesis of the non-host-specific phytotoxin rubellin D by the pathogen. However, further investigation is needed to confirm and better understand this involvement. In this study, three *R. collo-cygni* strains (SC19, RccPG4, and NZ62) with different rubellin D *in vitro* production were artificially inoculated into two barley cultivars (Fairing and Planet) with diverse susceptibilities to RLS. Infection development was monitored at three time points (7, 15, and 21 days post inoculation) by evaluating symptoms (visual observation), chlorophyll content (SPAD), fungal biomass accumulation (qPCR) and secondary metabolite biosynthesis (LC-MS/MS). Generally, strains SC19 and RccPG4 exhibited higher virulence in both barley varieties, accompanied by increased rubellin D production. Positive correlations between RLS symptom expression and rubellin D biosynthesis were observed in the most susceptible cultivar Fairing. Correlations between fungal biomass accumulation and the other considered variables were also detected. The obtained results provide further evidence for the role of rubellin D in fungal virulence, especially in the more susceptible variety. This study also confirms that, besides rubellin D, *R. collo-cygni* can produce a broad spectrum of secondary metabolites both *in vitro* and *in planta*.

Advancing sustainable agriculture: the use of stationary application systems in apple cultivation

M. Baraldo, U. Spitaler

Department of Plant Protection, Research Centre Laimburg, Vadena, Italy. E-mail: Mattia.Baraldo@laimburg.it

The integration of stationary application systems in apple cultivation presents an innovative solution for environmentally friendly and efficient plant protection strategies. Unlike traditional mobile sprayers, these systems utilize fixed nozzles installed along cultivation areas to deliver localized treatments. One of the primary challenges in modern agriculture is balancing the need for effective plant protection with the goals of sustainability and reduced environmental impact. Stationary application systems address this challenge by avoiding soil compaction while ensuring precisely timed application of the product. Furthermore, these systems enhance operational safety by eliminating the need for machinery to navigate hazardous terrains, thus mitigating the risk of accidents. The automation potential also reduces labour intensity and exposure, leading to substantial time savings and increased efficiency in farm operations. Stationary application systems are being currently researched and optimized at the Laimburg Research Centre in South Tyrol. In field trials conducted at the Laimburg Integrated Digital Orchard (https://lido.laimburg.it/), nozzle resistance to clogging with sulphur lime and the efficacy of chronic administration of entomopathogenic fungi in controlling aphid populations are tested. The trials aim to provide further insights into the operational effectiveness and use of stationary application systems. In conclusion, stationary application systems have the potential to improve plant protection by enhancing safety, reducing time dependency, and maintaining the high efficacy of plant protection strategies against pests and diseases.

A recombinant tobacco mosaic virus-based vector for functional genomic studies in plant pathogenic fungi

N.F. Barnaba, T. Mascia, R.M. De Miccolis Angelini, R. Spanò, S. Pollastro, F. Faretra, F. Nigro

Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy. E-mail: tiziana.mascia@uniba.it

Plant viruses have proven to be very versatile tools for biotechnology. They have been used as vectors for protein expression in plants and to trigger gene-silencing mechanisms using recombinant virus vectors carrying fragments of the target gene. The latter process, known as virus-induced gene silencing (VIGS), can be used for functional genomic studies. In this work, we used a plant virus-based vector, tobacco mosaic virus (TMV), carrying the green fluorescent protein (GFP) gene as a reporter for the infection of taxonomically different phytopathogenic fungi, namely *Botrytis cinerea, Fusarium oxysporum* f. sp. *lycopersici, Monilinia fructicola*, and *Verticillum dahliae*. Confocal microscope observations and molecular analysis at 6, 9, and 13 days post-inoculation (dpi) showed that TMV can replicate and persist in *V. dahliae* and *B. cinerea,* whereas it is unable to enter and replicate in *F. oxysporum* f. sp. *lycopersici* and *M. fructicola*. Total RNA preparations extracted at 6, 9 and 13 dpi from infected *V. dahliae* and *B. cinerea* mycelia were used to estimate the accumulation of viral RNA and the expression of two key genes involved in the RNA silencing pathway, namely Dicer Like 1 and Argonaute 1, by RT-qPCR. Results indicate that the TMV vector could be a functional VIGS approach to silence constitutive pathogenesis-related genes in *V. dahliae* and *B. cinerea*.

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Phytosanitary aspects and control strategies of the main chestnut fungal and oomycete diseases

V. Battaglia[1], L. Luongo[2], F. Maione[1], I. Mercuri[2], S. Vitale[2], E. Lahoz[1], A. Haegi[2]

[1]CREA - Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria Centro di Cerealicoltura e Colture Industriali, Via Torrino 32, 81100 Caserta, Italia; [2]CREA - Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria Centro di Difesa e Certificazione, Via C.G. Bertero 22, 00156 Roma, Italia. E-mail: valerio.battaglia@crea.gov.it

European sweet chestnut (*Castanea sativa* L.) is an economically important cultivated species widely planted for both fruit and timber production. Chestnut production in Italy plays a pivotal role in the nut sector, especially in the Campania region. Some fungal diseases of chestnut trees can cause extensive damage and losses. *Phytophthora* spp. is the causal agent of chestnut decline, while on the stems and other parts of the wood, significant damages can be induced by *Cryphonectria parasitica*, the etiological agent of chestnut blight. In 2005, the presence of *Gnomoniopsis smithogilvyi* (syn. *G. castaneae*), the causal agent of the chestnut rot, which causes relevant product losses both at harvest and post-harvest, was reported for the first time in Italy. In 2024, the presence of the diseases was monitored in chestnut orchards and forests in the Campania and Lazio regions. Visual assessments were carried out on the woody plant parts and symptomatic fruits, and then some samples were analysed to collect fungal isolates. The monoconidial or mono hyphal cultures were obtained in pure culture, and identification was achieved through the analysis of morphology, ITS sequencing, and/or specific real-time qPCR. Characterization of hypovirulent strains of *C. parasitica* isolates is in progress. For *G. smithogilvyi, in vitro* tests were carried out to evaluate the resistance to five different active ingredients with four distinct modes of action. Preliminary results in this research suggested that rational management of chestnut orchards could reduce the onset of fungal plant pathogens.

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Presence of *Plenodomus tracheiphilus* in lemon orchards in Campania region and sensitivity to some active ingredients

V. Battaglia[1], G.E. Pio[1], F. Maione[1], G. Capriolo[2], A. Lizzio[1,2], M. Petriccione[2], E. Lahoz[1]

[1]CREA - Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria Centro di Cerealicoltura e Colture Industriali, Via Torrino 2, 81100 Caserta, Italia; [2]CREA - Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria Centro di Olivicoltura, Frutticoltura e Agrumicoltura, Via Torrino 2, 81100 Caserta, Italia. E-mail: valerio.battaglia@crea.gov.it

Lemon cultivation is mainly in the South of Italy (99%), where Campania is the second most important region, with approximately 1,233 hectares representing about 5% of the national production. In Campania there are two lemon crops with Protected Geographical Indication: "*Limone costa d'Amalfi*" and "*Limone di Sorrento*". Mal secco, caused by the fungus *Plenodomus tracheiphilus*, is a vascular disease representing a limiting factor for lemon production. In 2024, a survey was done in the two main cultivation areas. Visual assessments were carried out on branches sampled from symptomatic plants from four farms, and isolates were obtained from 50 plants. All the isolates were obtained in pure culture, to proceed with the morphological and molecular identification by PCR amplification. Thirty isolates obtained from the samples from different farms have been identified as *P. tracheiphilus* with 99.9% nucleotide sequence identity. Sensitivity tests were conducted on the thirty isolates with four formulates relating to three fungicide categories. In addition, *in vitro* tests were carried out to evaluate the resistance to the various active ingredients. Field trials were also conducted to verify the effectiveness of the pruning cuts and on the plants of active substances applied with an adjuvant of natural origin based on galactomannans extracted from locust bean gum (*Ceratonia siliqua*). The results suggested that, besides good agronomic practices, the rational management of orchards, such as removing residues and infected organs and using effective phytoiatric interventions, can contribute to controlling the pathogen.

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Biocontrol of *Trichoderma* spp. against *Meloidogyne enterolobii* and *Rotylenchulus reniformis* in cucumber (*Cucumis sativus* L.)

H. Bayardo-Rosales, M.B. Contreras-Soto, J.A. Carrillo-Fasio, J. B. Heredia, J.P. Manjarrez-Quintero, J.M. Tovar-Pedraza

Centro de Investigación en Alimentación y Desarrollo, Coordinación Regional Culiacán, Laboratorio de Fitopatología, Culiacán, 80110, México. E-mail: hbayardo123@estudiantes.ciad.mx

In this study, the biocontrol activity of *Trichoderma afroharzianum* and *T. longibrachiatum* against the nematodes *Meloidogyne enterolobii* and *Rotylenchulus reniformis* in cucumber plants under greenhouse conditions was evaluated. The treatments were established in pots, where three concentrations of each *Trichoderma* species (1×10^4, 1×10^6, and 1×10^8 conidia/mL) were evaluated. Three weekly applications of 50 mL per plant were made for each *Trichoderma* species, and the number of nematodes per 100 g of soil, the damage caused by each nematode on the roots, as well as plant height, root length, and weight, were evaluated 30 days after nematode inoculation. For *R. reniformis*, the number of females per gram of root and the percentage of necrosis were evaluated, while the number of galls per root was determined for *M. enterolobii*. The results showed that the three applications of *T. afroharzianum* and *T. longibrachiatum* at concentrations of 1×10^6 and 1×10^8 conidia/mL significantly reduced the number of galls induced by *M. enterolobii*. Additionally, a reduction in the percentage of root necrosis, the number of *R. reniformis* females per gram of root, and a decrease in nematodes per 100 grams of soil were observed. Regarding the growth-promoting effect, an increase in plant height, root weight, and length was observed in treatments with all three concentrations of *Trichoderma* spp. These results showed that *T. afroharzianum* and *T. longibrachiatum* are viable alternatives for the biocontrol of *M. enterolobii* and *R. reniformis* in cucumber.

Climate change impacts on crop pests and pathogens

D. Bebber

Department of Biosciences, University of Exeter, UK. E-mail: D.Bebber@exeter.ac.uk

Global food security is threatened by climate change, both directly through responses of crop physiology and productivity, and indirectly through responses of plant-associated microbiota including plant pathogens. While the interactions between host plants, pathogens, and environmental drivers can be complex, recent research is beginning to indicate certain overall patterns in how plant diseases will affect crop production in future. There are three general methodological approaches to investigating climate change impacts on plant disease: large-scale observational studies, process-based disease models, and experimental comparisons of pathosystems under current and future conditions. Observational studies have tended to identify rising temperatures as the primary driver of disease impact. Process-based models suggest that rising temperatures will lead to latitudinal shifts in disease pressure, but drying conditions could mitigate disease risk. Experimental studies suggest that rising atmospheric CO_2 will exacerbate disease impacts. Plant diseases may therefore counteract any crop yield increases at higher latitudes due to climate change.

Foliar application of a *Streptomyces* sp. on tomato plants: an insight into the induction of defence-related genes through RNA-Seq analysis

F. Bellameche[1], S. Cassanelli[1], F. Caradonia[1], M. Cortiello[1], S. Perez[2], E. Stefani[1], D. Giovanardi[1]

[1]Department of Life Sciences, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy; [2]Institute of Agri-Food, Animal and Environmental Sciences (ICA3), Universidad de O'Higgins, Ruta 90 km. 3, 3070000 San Fernando, Chile. E-mail: fares.bellameche@unimore.it

Microbial biocontrol agents (mBCAs) protect plants against a broad spectrum of pathogens through various mechanisms of action, including the activation of innate defence systems. The investigation of molecular mechanisms of plant resistance triggered by mBCAs is crucial for implementing sustainable disease management. Streptomycetes have drawn considerable attention for their potential as mBCAs; however, their use in foliar treatments to elicit plant resistance is still poorly investigated. RNA-Seq analysis was conducted to assess differential gene expression in tomato plants within 24 hours after applying *Streptomyces* sp. DLS2013. Our results showed a time-course up-regulation of genes related to defence pathways that are either SA or JA-dependent, and promoting the synthesis of antimicrobial metabolites (*e.g.,* polyamines and phenolics). These findings suggest that DLS2013 preconditions tomato plants, therefore arming them against future challenging pests. To confirm this, foliar application of DLS2013, followed by the inoculation with the hemibiotrophic pathogen *Pseudomonas syringae* pv. *tomato* (Pst), significantly reduced bacterial speck severity by 67%, while no antagonistic effect of DLS2013 against Pst was observed *in vitro*. Lastly, the transcription profiling of six genes tied to different defence mechanisms showed an enhanced expression of genes involved in SA-dependent defence signalling (*e.g.,* PR-1), alongside a decrease in JA-dependent defence responses in pre-treated plants challenged or not with Pst, possibly mediated by JAZ-25 repressor protein. Further studies are ongoing to investigate disease resistance induced by *Streptomyces* sp. DLS2013 against other pathogens and the potential regulatory mode of JAZ genes.

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Preliminary characterization and mode action of *Pseudomonas synxantha* DLS65 as the active ingredient of Microfighter, an innovative bio-pesticide

F. Bellameche[1], F. Modica[1], L. Fagioli[2], D. Giovanardi[1], E. Stefani[1]

[1]Department of Life Sciences, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy; [2]Consorzio Agrario di Ravenna, via Madonna di Genova 39, 48033 Cotignola, Italy. E-mail: fares.bellameche@unimore.it

The everyday use of copper-based pesticides in agriculture resulted in its accumulation in the agro-environment, thus posing a threat to its safety, human health, and biodiversity. Effective alternatives are being investigated, and using beneficial microbes is a promising approach to reduce the use of copper. The EU-financed project "LIFE Microfighter" is currently checking the efficacy of *Pseudomonas synxantha*, strain DLS65 (DLS65), coupled with natural zeolites, to lower the impact of fungal (grapevine downy mildew) and bacterial (olive knot and tomato speck) diseases, together with the reduction of copper inputs in vineyards, olive groves, and tomato fields. DLS65 showed tolerance to copper (500 ppm) in liquid and solid medium, thus confirming its viability and activity in IPM strategies with reduced copper use: such tolerance was molecularly confirmed through the detection of the *cusABL* genes cluster. Traits linked to a plant growth promotion activity by DLS65 were observed, demonstrating its ability to produce siderophores, ammonia, and solubilize phosphates. The antimicrobial activity of DLS65 appears to be linked to the production of volatile organic compounds (VOCs), since the growth and virulence traits of *Pseudomonas syringae* pv. *tomato* and *Pseudomonas savastanoi* pv. *savastanoi* were severely affected. Finally, DLS65 coupled with natural zeolites, reduced the incidence of grapevine downy mildew in semi-field experiments, although its control mechanism(s) remain largely unknown. Our findings confirmed strain DLS65 as a promising biocontrol agent against both bacteria and the oomycete.

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Botryosphaeriaceae and *Phytophthora* species contribute to *Fraxinus* decline in the Mediterranean area

A. Benigno[1], C. Bregant[2], C. Aglietti[1], E. Falciani[1], M. Marcolongo[2], L. Montecchio[2], B. Tolio[2,3,4], B.T. Linaldeddu[2], S. Moricca[1]

[1]Department of Agricultural, Food, Environmental and Forest Sciences and Technologies, University of Florence, Piazzale delle Cascine 28, 50144 Firenze, Italy; [2]Department of Land, Environment, Agriculture and Forestry, University of Padua, Viale dell'Università 16, 35020 Legnaro, Italy; [3]Skogforsk, The Forestry Research Institute of Sweden, Svalöv, Sweden; [4]Southern Swedish Forest Research Centre, Swedish University of Agricultural Sciences, Alnarp, Sweden. E-mail: alessandra.benigno@unifi.it

The Mediterranean basin, renowned for its rich biodiversity, faces increasing vulnerability to significant threats from climate change. Elevated temperatures and irregular precipitation patterns heighten the susceptibility of Mediterranean forests to diseases, pests, fires, and habitat degradation. In the Mediterranean area, *Fraxinus* species like *Fraxinus excelsior, Fraxinus ornus*, and *Fraxinus angustifolia* are suffering widespread decline and dieback. The investigations conducted in this study on these tree species unveiled a complex phytopathological scenario, elucidated through the isolation and pathogenicity testing of symptomatic and asymptomatic plant material. Besides *Hymenoscyphus fraxineus*, several other pathogenic species played significant roles in the onset of ash dieback. Key microbial taxa included the fungal and oomycete plant pathogens *Botryosphaeria dothidea, Diplodia fraxini, Diplodia subglobosa, Phytophthora acerina*, and *Phytophthora plurivora*. The impact of these plant pathogens was more severe in areas where ash trees endured environmental stress, such as mild, dry winters and hot summers accompanied by prolonged droughts. Additionally, anthropogenic factors like silvicultural management and wildfires further compromised tree vigor. In contrast to *H. fraxineus*, which thrives in cooler, wetter valleys of the Alps and central-northern Apennines, these newly identified pathogens thrive under warmer conditions, exacerbating their impact in the studied areas. Our findings underscore the urgent necessity for integrated forest management strategies aimed at mitigating the dual threats of climate change and pathogen invasions. Such strategies are crucial for safeguarding the resilience and sustainability of *Fraxinus* in Italy's dynamic environmental landscape.

Climate stressors and Botryosphaeriaceae infections are key factors in the dieback of *Fraxinus ornus* in northern-central Italy

A. Benigno[1], C. Bregant[2], C. Aglietti[1], E. Falciani[1], M. Marcolongo[2], L. Montecchio[2], B. Tolio[2,3,4], B.T. Linaldeddu[2], S. Moricca[1]

[1]Department of Agricultural, Food, Environmental and Forest Sciences and Technologies, University of Florence, Piazzale delle Cascine 28, 50144 Firenze, Italy; [2]Department of Land, Environment, Agriculture and Forestry, University of Padua, Viale dell'Università 16, 35020 Legnaro, Italy; [3]Skogforsk, The Forestry Research Institute of Sweden, Svalöv, Sweden; [4]Southern Swedish Forest Research Centre, Swedish University of Agricultural Sciences, Alnarp, Sweden. E-mail: alessandra.benigno@unifi.it

A severe dieback of flowering ash (*Fraxinus ornus* L.) has been observed recently in several areas of northern-central Italy. Symptoms were more evident at the beginning of the growing season and more severe on young regeneration, with sunken, light-brown cankers on the stems and branches, vascular discoloration, tip and shoot die-off, and foliar necroses. Six Botryosphaeriaceae species were consistently isolated from symptomatic tissues: *Botryosphaeria dothidea, Diplodia fraxini, Diplodia subglobosa, Dothiorella iberica, Dothiorella omnivora*, and *Neofusicoccum parvum*. Among these, *B. dothidea* and *D. fraxini* were the most aggressive and prevalent species, most frequently associated with cankers. The other Botryosphaeriaceae were less virulent and occurred primarily on succulent branch tips and foliage. Isolate identification was through morphological and molecular methods, including colony and conidial phenotyping, and rDNA-ITS genotyping. Phylogenetic analyses revealed congruent relationships between the six taxa and their closest relatives. Pathogenicity tests, carried out on two-year-old seedlings, confirmed the higher virulence of *B. dothidea* and *D. fraxini*. Multi-year field studies and observations at various sites suggest that climatic factors such as heat, water stress, and drought significantly impaired tree health and vigor, thereby facilitating infection and colonization by these Botryosphaeriaceae. Environmental stressors are, therefore, crucial in triggering lethal infections by these fungal pathogens on flowering ash.

Combined infections of Botryosphaeriaceae and *Phytophthora* species in a declining apple orchard in Terre d'Adige (Trento, northern Italy)

A. Benigno[1], C. Bregant[2], E. Falciani[1], M. Marcolongo[2], L. Montecchio[2], B.T. Linaldeddu[2], S. Moricca[1]

[1]Department of Agricultural, Food, Environmental and Forest Sciences and Technologies, University of Florence, Piazzale delle Cascine 28, 50144 Firenze, Italy; [2]Department of Land, Environment, Agriculture and Forestry, University of Padua, Viale dell'Università 16, 35020 Legnaro, Italy. E-mail: alessandra.benigno@unifi.it

In 2023, as part of a legal dispute, we were asked to express an opinion on the possible causes of a severe dieback occurring in an apple orchard in Nave San Rocco, a hamlet of Terre d'Adige, Valdadige, Trento. To clarify the aetiology of the phenomenon, symptomatic and asymptomatic plant tissues were sampled from apple trees (Red Spur variety). Samples included: a) portions of stem and branches with cortical cankers and necroses; b) portions of collar and roots with rot symptoms at various stages of severity. Water (from an irrigation canal) and soil portions were also sampled. The identity of microorganisms associated with the aforementioned samples was ascertained in the laboratory using conventional and molecular diagnosis. Several phytopathogenic fungi and oomycetes of the genera *Diplodia, Neofusicoccum, Phytophthora* (*P.*), and *Phytopythium* (*Ph.*) were isolated. In particular, *Diplodia seriata, Neofusicoccum parvum, Phytophthora cactorum, P. multivora* and *P. pini* were isolated from stem and branch samples. Three species of *Phytophthora*, namely *P. cactorum, P. multivora,* and *P. pini*, and one species of *Phytopythium, i.e., Ph. vexans*, were obtained from collar, roots, and rhizosphere samples. *Neofusicoccum parvum* was the species most frequently isolated from branches with sunken cankers and necroses. *Phytophthora cactorum* and *P. pini* were the species most commonly associated with root rot symptoms and collar necroses. From this research it emerged that the severe dieback of the apple trees on the plantation under investigation was the result of multiple attacks by Botryosphaeriaceae and *Phytophthora* species, prolonged over time.

Innovative biocontrol strategies for sustainable disease control in chestnut groves

A. Benigno[1], E. Falciani[1], C. Aglietti[1], S.O. Cacciola[2], S. Moricca[1]

[1]Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Plant Pathology and Entomology Section, University of Florence, Piazzale delle Cascine 28, 50144 Florence, Italy; [2]Department of Agriculture, Food and Environment, University of Catania, 95123 Catania, Italy. E-mail: alessandra.benigno@unifi.it

Chestnut groves provide high-quality fruits and play a significant role in soil protection and biodiversity conservation. Sustainable management of these valuable tree crops encompasses agroforestry practices useful for the long-term maintenance of tree health and productivity as well as for protecting the environment. This research focused on the use of *Trichoderma* biocontrol agents to: 1) protect pruning lesions from wound parasites like, for example, the fearsome chestnut blight pathogen *Cryphonectria parasitica*; and 2) inhibit the agent of brown or chalky rot of nuts *Gnomoniopsis castaneae*. Selected strains of *Trichoderma* spp., known to have broad-spectrum biocontrol capabilities, were tested for their ability to protect pruning wounds through spray treatments of freshly cut surfaces. The same *Trichoderma* strains were administered by endotherapy, which involves the direct injection of the biocontrol agent into the tree's vascular system. In the following two years, no trace of fungal infections was observed on the wounds protected by spray treatment with the biopesticide. Endotherapic treatments, conducted for two consecutive growing seasons, significantly reduced the incidence of brown or chalky rot on nuts harvested in autumn. This study demonstrated that the combination of spray and endotherapic treatments with *Trichoderma* spp. effectively reduces chestnut diseases and also improves tree health, allowing us to give up chemical treatments that are dangerous for the environment and human health. Continued research and collaborative interactions among farmers, researchers, and policymakers are necessary to promote a wider diffusion of this sustainable and environmentally friendly plant protection strategy.

Method and evaluation of dog training in the search for *Tilletia indica*

V. Bergamaschi[1,2], A. Infantino[1], C. Costa[3], A. Matere[1], L. Orzali[1], P.F. Roversi[4], F. Tocci[3], M.T. Valente[1], F. Mosconi[1]

[1]Council for Agricultural Research and Economics - Research Centre for Plant Protection and Certification (CREA - DC), Via C.G. Bertero 22, 00156 Rome, Italy; [2]Department of Environmental Biology, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy; [3]Council for Agricultural Research and Economics Research Centre for Engineering and Agro-food Processing (CREA - IT), Via della Pascolare 16, 00015 Monterotondo Scalo, Italy; [4]Council for Agricultural Research and Economics - Research Centre for Plant Protection and Certification (CREA - DC), Via Lanciola 12A, 50125 Impruneta, Italy. E-mail: fabio.mosconi@crea.gov.it

The use of detection dogs in plant protection is a methodology that has been constantly growing in recent years and is the subject of ever-increasing attention. In fact, dogs are an excellent "early detection" tool to be used for plant pathogens or pests (*i.e.*, fungi, bacteria, insects, etc.) before the onset of specific symptoms. This allows the use of dogs both at the "entry points" (*e.g.*, ports or airports) to prevent the entry of plants containing pathogens or pests and in the surveillance activities to assist in identifying new outbreaks or containment and eradication of pathogens or pests. However, for quarantine organisms, which must be handled only within special laboratories with specific containment and control structures, the different stages of dog training present different, obvious difficulties, which could make them impossible to achieve. The results of the work carried out in training a dog to search for the wheat quarantine fungus *Tilletia indica* are presented here, with particular reference to: descriptions of specific tools and methodology for transferring the pathogen odour onto cotton support to be used for the training; preliminary analysis of the volatile organic compounds (VOCs) of the plant pathogen and the odour transferred to the support; training results measured through tests carried out in different conditions, *i.e.*, in a controlled experimental setting *vs* a setting that simulates the actual conditions in which the dog will work (*i.e.*, with target odour mixed in the wheat).

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Find a match: isolated versus molecular-detected oomycetes involved in the kiwifruit vine decline syndrome

C. Bernardini[1], S. Mosca[2], A. Malacrinò[2], P. Bianco[1], M. Zillet[1], M. Martini[1], L. Schena[2], P. Ermacora[1]

[1]Department of Agriculture, Food, Environmental and Animal Sciences (Di4A), Università degli Studi di Udine, Udine, Italy; [2]Department of Agriculture, Università degli Studi Mediterranea di Reggio Calabria, Reggio Calabria, Italy. E-mail: chiara.bernardini@uniud.it

In the past decade, kiwifruit vines have been affected by a sudden decline named kiwifruit vine decline syndrome (KVDS), which, in the worst cases, leads to plant collapse with significant losses in fruit production. The symptoms of this syndrome appear on the canopy once the roots have been compromised due to the decay of fine roots and root hairs. Recent amplicon metagenomic analyses have identified *Phytopythium* and *Phytophthora* species as possible causal agents of KVDS. Nevertheless, in some cases, we still need a match between the results of metagenomic analyses and microorganisms isolated from corresponding samples. An oomycete closely related to *Phytophthora sojae* provides the perfect example: amplicon metagenomics and PCR detection with specific primers have shown its presence in soil and roots from kiwifruit orchards with KVDS. However, until now, it has never been isolated *in vitro*. In this study, we analysed soil and roots from KVDS-affected fields, and we compared results from molecular assays with those obtained through *in vitro* isolation using baiting and cultivation on different selective media. We hypothesize that the putative plant pathogen does not grow on commonly used selective media or grows slowly and is concealed by other fast-growing microorganisms, making it detectable only by molecular tools. Obtaining an alive culture is a mandatory step to satisfy Koch's postulates and confirm the role of *P. sojae*-like species as a possible causal agent of KVSD.

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Tomato yellow leaf curl disease: epidemiological survey of the extant population of viral agents in Sicily

S. Bertacca[1,2], S. Davino[1], D. Marian[2], C. Sacco Botto[2], S. Matic[2], L. Miozzi[2], G.P. Accotto[2], A.M. Vaira[2], E. Noris[2]

[1]Department of Agricultural, Food and Forest Science, University of Palermo, 90128 Palermo, Italy; [2]Institute for Sustainable Plant Protection, National Research Council of Italy (CNR), 10135 Torino, Italy. E-mail: emanuela.noris@ipsp.cnr.it

Tomato yellow leaf curl disease (TYLCD), caused by viruses belonging to the genus *Begomovirus*, family *Geminiviridae*, provokes high economic losses in tomato crops worldwide. Its rapid spread is driven by the efficient persistent transmission of the whitefly *Bemisia tabaci* and the dynamic nature of the geminivirus genomes, which are prone to a high level of mutations and recombinations. In Italy, two TYLCD-related viral species are present: tomato yellow leaf curl Sardinia virus (TYLCSV), first detected in Sardinia in 1989, and tomato yellow leaf curl virus (TYLCV), detected in Sicily in 2002. Their co-existence originated different recombinants. Moreover, the use of tomato cultivars carrying resistance genes, generally *Ty-1*, contributed to shaping the population dynamics of these viruses with the generation of resistance-breaking recombinants. In this work, we investigated the population of TYLCD-related viruses in 70 symptomatic plants collected from seven fields in the Ragusa province (Sicily) in 2020, using multiplex PCR analysis. TYLCD-related viruses were detected in 57% of the samples. An almost complete displacement of TYLCV was manifested in favour of TYLCSV and TYLCV/TYLCSV recombinants. Among the different types of recombinants detected by the multiplex PCR, the prevailing ones were those of the resistance-breaking IS76-type, which occurred even in a single infection, *i.e.*, in the absence of TYLCV or TYLCSV parentals. This study provides valuable epidemiological results regarding the impact of growing *Ty-1* resistant cultivars in an intensive tomato growing area.

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Seed transmission of phytoplasmas: a hidden threat in agriculture

A. Bertaccini[1], E. Satta[1], S. Mateeti[1], N.A. Messina[2], F. Pacini[1]

[1]Department of Agricultural and Food Sciences, Alma Mater Studiorum - University of Bologna, 40127 Bologna, Italy; [2]Agronomist, Crotone, Italy. E-mail: assunta.bertaccini@unibo.it

Extensive research has demonstrated the presence in seeds of endophytic bacteria that can directly or indirectly interact with germination and seedling growth. Phytoplasmas are cell wall lacking bacteria for which the transmission by seed was reported under laboratory conditions since almost ten years for several species including tomato. Recently, phytoplasma transmission was also verified directly in tomato fields, which are severely affected nearly every year in some important southern Italy tomato-producing areas. In these cultivations, identification of '*Candidatus* Phytoplasma solani' and '*Ca*. P. asteris' presence was demonstrated in both tomato mother plants and their seedlings for different varieties. Other '*Ca.* Phytoplasma' species were verified as seed-transmitted by testing seedlings between 30 to about 200 days after germination in corn, wheat, tomato, carrot, eggplant, and alfalfa. In the latter case, the analysis of infected seeds conserved for more than 20 years showed a low germination percentage (below 20%) but a consistent detection of phytoplasmas in the seedlings. However, since the detection of these bacteria is not always accompanied by the presence of symptoms, seed transmission represents a hidden and dangerous risk for many horticultural crops, considering also that the international seed trade does not consider the possible presence of these pathogens. Phytoplasmas have transmission percentages between 1 to 3%; however, the widespread presence of insect vectors (leafhoppers and psyllids) further enhances their field spreading facilitating epidemic outbreaks that could lead to strong economic losses.

The FEM grapevine crossbreeding program for resistance to the main ampelopathies

P. Bettinelli, L. Zulini, D. Nicolini, A. Zatelli, M. Dallaserra, C. Dorigatti, G. Betta, R. Filippi, A. Campestrin, M. Calovi, S. Clementi, L. Costantini, T. Tomasi, M. Stefanini, S. Vezzulli

Research and Innovation Centre, Grapevine Genetics and Breeding Unit, Fondazione Edmund Mach, San Michele all'Adige, Italy. E-mail: paola.bettinelli@fmach.it, silvia.vezzulli@fmach.it

The technique of crossing, free or controlled, has always been a source of variability, allowing the selection of new varieties with improved fitness. Therefore, one promising strategy towards sustainable viticulture is crossbreeding for resistance traits to biotic stresses under climate change conditions to reduce pesticide usage and improve resilience. With this awareness and vision, the Edmund Mach Foundation (FEM) began its grapevine genetic improvement program in the 1990s. Later, in 2010 with the exploration of the genetic pool of resistance loci to downy and powdery mildew, a group of accessions was selected as donors. Next, genotypes with stacked ("pyramided") loci were generated through marker-assisted parental selection (MAPS) with up to seven loci combining resistance to both mildews. Then, upon protocol optimization highly efficient marker-assisted seedling selection (MASS) was established, allowing since 2019 to overcome phenotypic screening and revealing inter- and intra-population effects. Upon multi-year agronomic surveys, grape quality evaluations, and wine tastings, in 2018, four new varieties were registered for their novel organoleptic characteristics and tolerance to gray mold. In 2020, four (mid)- resistant varieties to mildews were patented, and in 2024, another four are in the process. In the meantime, resistance to other "emergent" ampelopathies, such as black rot, is being introgressed. Various collaborations are in place across the national territory to exploit the superior parental lines. Lately, the genetic and phenotypic characterization of the FEM germplasm (ca. 3,000 accessions) has been completed so that the scouting process within such biodiversity is continuously ongoing.

Structural characterization of ViuB a siderophore interacting protein of *Erwinia amylovora*

S. Bharti[1], I. Polsinelli[2], I. Bento[3], T. Schneider[3], S. Baric[4,5], S. Benini[1]

[1]Laboratory of Bioorganic Chemistry and Bio-Crystallography, Free University of Bozen-Bolzano, Piazza Università 1, 39100 Bolzano, Italy; [2]Institute of Molecular and Translational Cardiology, IRCCS Policlinico San Donato, Milan, Italy; [3]EMBL Hamburg, Notkestrasse 85, 22607 Hamburg, Germany; **[4]***Laboratory for Phytopathology, Free University*

of Bozen-Bolzano, Italy; [5]Competence Centre for Plant Health, Free University of Bozen-Bolzano, Italy. E-mail: sbenini@unibz.it

Erwinia amylovora is the causative agent of fire blight in apples and pears. The main pathogenicity factors of *E. amylovora* are amylovoran, the type III secretion system, and siderophore-mediated iron uptake. Under iron-deficient conditions, *E. amylovora* secretes hydroxamate-type siderophores, which overcome iron scarcity by acquiring ferric iron from the host. The siderophore-mediated iron acquisition involves outer membrane receptors, periplasmic binding proteins, and inner membrane proteins that transport the iron-loaded siderophore into the cytoplasm. Once the siderophore complex enters the cytoplasm, iron must be released from the siderophore to be available for the bacterium. This process requires an oxidoreductase enzyme (ViuB), which facilitates the reduction of Fe(III) in the complex, releasing Fe(II) in the cytoplasm of *E. amylovora*. As ViuB is vital for iron utilization by the bacterium, it represents a potential druggable target. In this work, we present the three-dimensional structure of ViuB from *E. amylovora*, determined by X-ray crystallography at 2.3 Å. The protein has two domains connected by a 21-residue linker. The structure also revealed the presence of cofactor FAD, which facilitates the electron transfer steps in the reduction process. Conclusively, ViuB from *E. amylovora* has the typical structure of siderophore-interacting proteins, but the long linker connecting the two domains suggests that ViuB has the potential to accommodate different types of siderophore analogs. The overall structural information of protein could contribute to designing specific inhibitors against the enzyme, potentially leading to the development of novel, sustainable chemical controls for fire blight disease.

Influence of microelements on the growth and enzymatic activity of *Fomitiporia mediterranea* and its role in Esca Complex of Diseases

F. Bigazzi, G. Carella, L. Mugnai

Department of Agricultural, Food, Environmental, and Forestry Science and Technology (DAGRI), Plant Pathology and Entomology section, University of Florence, P.le delle Cascine 28, 50144 Firenze, Italy. E-mail: francesco.bigazzi1@unifi.it

Esca Complex Disease remains a very damaging grapevine disease, with unclear causal factors. Recently, the role of fungi causing white rot, such as *Fomitiporia mediterranea* (Fmed), has been more deeply investigated. For instance, removing the rotten wood through curettage showed significant symptom reduction. One hypothesis is that the wood-degrading activity of Fmed produces secondary metabolites and toxins, which, once reaching a certain threshold, can trigger the characteristic leaf-stripe symptoms. Recent studies have shown that foliar symptoms are absent under soil drought conditions, while the application of fertilizers containing specific microelements may increase symptom expression. These observations suggest that the physical and chemical properties of the soil, which have not been thoroughly investigated in relation to this grapevine disease, may play a role in foliar symptom expression. This study aims to determine whether Fmed exhibits increased growth, enhanced wood-degrading activity, and greater enzymatic production when grown on substrates enriched with different microelements. In our initial experiment, we focused on growing Fmed on PDA with grapevine sawdust enriched with manganese at various concentrations. Manganese is of particular interest because manganese peroxidase, a key enzyme in wood degradation, might be produced in greater quantities in the presence of increased manganese levels. This could result in greater fungal degradation activity, leading to increased production of byproducts or toxic compounds. Preliminary results indicate that Fmed grows faster on manganese-enriched substrates. Visual observations also suggest differences in the secretome compared to the control. Ongoing trials investigate other microelements and conduct detailed analyses of the secretome.

Investigating the potential of microbial consortia in the control of *Fusarium oxysporum* in tomato plants

M. Birhanu, D. Palmieri, P. Travaglini, F. De Curtis, G. Lima

Department of Agricultural, Environmental and Food Sciences, University of Molise, via F. De Sanctis snc, 86100 Campobasso, Italy. E-mail: lima@unimol.it

Vascular wilt caused by subspecies of the common soilborne plant pathogen *Fusarium oxysporum* severely damages many crops both in the field and greenhouse. Species of this plant pathogen also produce a variety of mycotoxins, pigments, and phytohormones. Soilborne fungal pathogens are generally very difficult to counteract and the selection of antagonistic microbes could provide a practical solution for their control. Suppressive soils are the primary source of effective antagonistic microorganisms for use as biocontrol agents. The present study aimed to isolate and characterize active bacterial strains from olive oil mill-waste biocompost, to define an effective microbial consortium in combination with *Trichoderma harzianum* T22. From the biocompost, we collected approximately 200 bacterial isolates, subsequently characterized for their *in vitro* antagonistic activity against *F. oxysporum* as well as for phosphorus solubilization, auxin, and siderophore production. The most promising bacterial strains were further characterized *in vivo*, alone and in combination, on tomato plants by evaluating both their plant growth-promotion and the antagonistic activity against *F. oxysporum* f. sp. *lycopersici*. The compatibility and the possible synergistic activity with *T. harzianum* T22 are under study.

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Bacillus amyloliquefaciens **treatment can mitigate the effect of abiotic stresses on pathogen infection in pomegranate**

A. Bolzonello[1], F. Meggio[2], A. Pitacco[2], L. Sella[1], B. Linaldeddu[1], R. Musetti[1], F. Favaron[1], A. Solla[3], S. Tundo[1,2]

[1]Department of Land, Environment, Agriculture and Forestry (TESAF), University of Padova, 35020 Legnaro, Italy; [2]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, 35020 Legnaro, Italy; [3]Faculty of Forestry, Institute for Dehesa Research (INDEHESA), Universidad de Extremadura, 10600 Plasencia, Spain. E-mail: silvio.tundo@unipd.it

Pomegranate (*Punica granatum* L.) in Italy is threatened by several biotic and abiotic stresses. Among biotic stresses, *Coniella granati* and *Phytophthora palmivora* are two emerging fungal pathogens. It is ignored whether the virulence of these pathogens is influenced by previous plant stress conditions such as heavy rainfall or intense drought. In a first experiment, we evaluated the effect of drought (D) or waterlogging (W) concerning regular watering (C) conditions on the aggressiveness of *C. granati* and *P. palmivora*. Two-year-old pomegranate plants were stem-inoculated with the two pathogens either individually or simultaneously. We observed a significant increase of *C. granati* lesion length in presence of W, while D significantly decreased *P. palmivora* lesions. Moreover, *P. palmivora* infection increased *C. granati* lesions but not vice versa. Subsequently, we demonstrated that the treatment with Amylo-X LC® (active ingredient *B. amyloliquefaciens* subsp. *plantarum* strain D747) effectively decreased *P. palmivora* lesions and prevented the boosting effect of the oomycete on *C. granati* under C conditions. This prompted us to assess the impact of Amylo-X LC® under water stress conditions used in the initial experiment. The treatment prevented the exacerbation of *C. granati* symptoms when co-inoculated with *P. palmivora*, while the aggressiveness of *P. palmivora* confirmed to be negatively affected only by D. This study demonstrates the influence of abiotic stress on *C. granati* and *P. palmivora* aggressiveness in pomegranate and identifies *B. amyloliquefaciens* as a promising strategy for mitigating pomegranate decline.

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Role of the chitin-binding domain (CBD) of a grapevine class IV chitinase against *Botrytis cinerea*

A. Bolzonello[1], S. Tundo[1,2], N. Poussereau[3], S. Vincenzi[2], M. Marangon[2], I. Battisti[2], L. Sella[1], F. Favaron[1]

[1]Department of Land, Environment, Agriculture, and Forestry (TESAF), University of Padova, Viale dell'Università 16, 35020 Legnaro, Italy; [2]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale Dell' Università 16, 35020 Legnaro, Italy; [3]Univ Lyon, Université Claude Bernard Lyon 1, CNRS, Bayer SAS, INSA Lyon, UMR5240, Microbiologie, Adaptation et Pathogénie, 14 Impasse Pierre Baizet, F-69263 Lyon, France. E-mail: angela.bolzonello@.unipd.it

During plant infection, fungal chitin deposition at the hyphal tip is counteracted by host chitinases. We studied the interaction between *Botrytis cinerea* and a class IV chitinase, constitutively expressed in mature grapevine berries. The fungus proteolytically removes the chitin-binding domain (CBD) early during fungal growth. This removal results in a 50% reduction in the enzymatic activity of the chitinase and a complete loss of its botryticidal activity. Despite this, the pattern of chitin degradation by the native and the cleaved chitinase remains similar. We also detected the expression of this class IV chitinase in grapevine leaves during *B. cinerea* infection. Analysis of chitinase activity in leaf samples at various infection stages revealed that while this chitinase is not the most active among all chitinases, it is the most abundant at the protein level, with its expression correlating with infection progression. The analysis of specific bands after SDS-PAGE confirmed the presence of both the native and CBD-deprived forms of the chitinase in the infected leaves, indicating that CBD removal observed *in vitro* also occurs during infection. Given the relatively low activity of this chitinase, as shown by zymogram, we further investigated the role of CBD against *B. cinerea*. We transiently expressed the CBD in *Nicotiana tabacum* and heterologously expressed it in *Pichia pastoris* to assess its function. These findings highlight the significant role of CBD in the chitinase's antifungal activity and its potential application in enhancing plant resistance to fungal infections.

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Microsatellite analysis of Italian isolates of *Pyricularia oryzae* over the years

S. Bosco[1,2], L. Bergonzi[1], P. Ruffa[1], D.T. Marinoni[1], D. Tenni[3], E. Cadei[3], M. Romani[3], V. Guarnaccia[1,2], M. Mezzalama[1,2], D. Spadaro[1,2]

[1]Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, TO, Italy; [2]AGROINNOVA– Centre of Competence, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, TO, Italy; [3]Ente Nazionale Risi, 27030 Castello d'Agogna, PV, Italy. E-mail: davide.spadaro@unito.it

Rice blast, caused by *Pyricularia oryzae* Cavara, is the main threat to Italian rice cultivation. The choice of resistant varieties and the use of chemical fungicides are the main management strategies adopted against the disease. Pathogen evolutionary potential, a measure of the speed needed to overcome fungicide sensitivity and host resistance genes, can be evaluated through DNA fingerprinting analyses. The first study characterising European *P. oryzae* populations was performed in 1997 by Roumen et al., giving crucial information for rice breeding based on a combination of pathotyping and DNA fingerprinting through the RFLP method. The use of microsatellites (SSRs) as fingerprinting markers was explored to define the *P. oryzae* composition in Italy in 2012 and 2013, but the results were not published. This work aimed to collect and molecularly characterize the genetic variability of 96 Italian *P. oryzae* isolates, obtained from diseased rice panicles collected from Piedmont, Lombardy, Emilia Romagna, and Sardinia over the years 2020, 2021, and 2022. Isolation was carried out using the monoconidial method, and DNA fingerprinting was performed using the same SSR regions chosen in 2013 to compare the populations of 2012–13 with those of 2020–22. Reference strains were included in the analysis. This study set the basis for elucidating the genetic variability of *P. oryzae* in Italy over the years.

In vitro efficacy of fungicides against Glomerella Leaf Spot

F. Bottegoni[1], E. Deltedesco[1], E. Rizzolli[1], S.M. Primisser[1,2], S. Öttl[1]

[1]Laimburg Research Centre, Auer/Ora, Italy; [2]University of Turin, Department of Life Sciences and Systems Biology, Torino, Italy. E-mail: sabine.oettl@laimburg.it

Glomerella Leaf Spot (GLS) is an emerging apple tree disease caused by several *Colletotrichum* species. In Italy, occurrence of GLS was first observed in 2019, and recently, *C. chrysophilum* was identified as the causative agent for GLS in the province of South Tyrol. Effective management of GLS is crucial to prevent significant yield losses and ensure high fruit quality. This study evaluates the susceptibility of *C. chrysophilum* to several commercially available fungicide active ingredients through *in vitro* screening, aiming to develop more effective GLS control strategies. Sensitivity was assessed by measuring radial mycelial growth inhibition on Potato Dextrose Agar of *C. chrysophilum* with the following active substances at different concentrations (0.01, 0.1, 1, 10, and 100 ppm): boscalid, captan, dithianon, fludioxonil, fluxapyroxad, potassium phosphonate, metiram, penthiopyrad, pyraclostrobin, pyrimethanil, and trifloxystrobin. Results showed that *in vitro C. chrysophilum* is highly sensitive to penthiopyrad and fludioxonil, which caused more than 57% inhibition of mycelial growth even at lower concentrations. Penthiopyrad and metiram both caused 100% inhibition at 100 ppm. Captan and fludioxonil showed 79.84% and 58.48% inhibition at 100 ppm, respectively, while all other fungicides showed less than 60% inhibition of mycelial growth at 100 ppm. EC50 was calculated, and the dose-response curves were constructed. In addition, field doses per hectare were calculated and compared with the results obtained from the *in vitro* experiment. These preliminary results provide the basis for further trials with the respective active substances on seedlings in the greenhouse and in the field.

A promising strain of *Trichoderma gamsii* for the biological control of *Colletotrichum* species

F. Brugneti, S. Turco, A. Mazzaglia

Department of Agricultural and Forest Science, Università degli Studi della Tuscia, 01100 Viterbo, Italy. E-mail: angmazza@unitus.it

Due to the increasingly stringent national and international regulations on pesticides, developing alternative, sustainable strategies is of paramount importance. *Trichoderma* is one of the most important fungal genera used in agriculture as a biocontrol agent against plant pathogens and as biostimulant. In this study, a strain of *T. gamsii* was isolated from the surface of a hazelnut twig. Being a species known for its antagonistic activity, it was tested *in vitro* against six *Colletotrichum* species responsible for anthracnose diseases on several economically important crops and ornamental plants worldwide. The biocontrol capacity was tested by double growth and by volatile (VOCs) and non-volatile (N-VOCs) metabolites production. Overall, the preliminary results show a growth inhibition of up to 81.5% in double growth, 73.1 % for the VOCs and 71.2 % for the N-VOCs, suggesting a highly remarkable *in vitro* efficacy as a biocontrol agent against the tested *Colletotrichum* species. The strain, named TG-AK47, was morphologically and genetically characterized. Further tests on its biological control effectiveness against other pathogenic fungal species of interest are ongoing, along with the biochemical characterization of VOCs and N-VOCs. The outcome of these experiments could better refine these preliminary results, leading to a promising and effective product to be used as a biofungicide.

Systemic endophytic establishment and long-term persistence of *Trichoderma atroviride* SC1 and *Pseudomonas chlororaphis* subsp. *aureofaciens* M71 in grapevine plants

G. Brussi[1], G. Puopolo[1,2], S. Di Marco[3], L. Mugnai[4], I. Pertot[1,2]

[1]Centre Agriculture Food Environment (C3A), University of Trento, San Michele all'Adige, Italy; [2]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy; [3]Institute of BioEconomy, National Research Council, Bologna, Italy; [4]Plant Pathology and Entomology Section, Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), University of Florence, Florence, Italy. E-mail: greta.brussi@unitn.it

Grapevine protection against trunk diseases is complex and some of them are still not fully understood. Due to the wood colonization of the plant pathogens, management is mainly based on preventive strategies to limit the new infections of the plant. Endophytes can significantly contribute to plant protection and the use of beneficial microorganisms for endotherapy represents a promising method to combat plant diseases. This approach benefits from the active and persistent colonization of these microorganisms within the plant, allowing them to reach areas that are otherwise inaccessible to the passive movement of injected chemicals. This work aimed to verify if injected beneficial microorganisms (*Trichoderma atroviride* SC1 and *Pseudomonas chlororaphis* subsp. *aureofaciens* M71) can establish themselves as systemic endophytes, both in cuttings in the greenhouse and in plants in the vineyard. Experiments were conducted to evaluate the colonization efficiency, translocation within the plant, and long-term persistence. Preliminary data indicated successful colonization by both microorganisms, with significant translocation observed throughout the plant tissues. Long-term colonization results showed that these beneficial microorganisms could persist within the grapevine cuttings over an extended period of at least 60 days. Further research will be needed to optimize injection protocols and to assess the practical applications of this method under field conditions.

Botanic Garden of Catania, Dr Jekyll or Mr Hyde? Global warming matters

C. Bua[1], M. Riolo[1], S. Conti Taguali[1,2], R. Parlascino[1], G. Polizzi[1], G. Giusso del Galdo[3], A. Pane[1], F. Drizou[4], F. La Spada[1], S.O. Cacciola[1]

[1]Department of Agriculture, Food and Environment (Di3A), University of Catania, Catania, Italy; [2]Department of Agricultural Science, Mediterranean University of Reggio Calabria, 89122 Reggio Calabria, Italy; [3]Department of Biological, Geological and Environmental Sciences, University of Catania, Catania, Italy; [4]Institute of Mediterranean Forest Ecosystems, Athens, Greece. E-mail: olga.cacciola@unict.it

The successful establishment of exotic pathogens originating from tropical or subtropical areas may be favoured by global warming. As a major task of Botanic Gardens is to preserve plant diversity, they host rare plant species, most introduced over decades from diverse continents. In recent years, researchers have focused on Botanic Gardens as both potential sources and reservoirs of exotic plant pathogens as well as sites where both endemic and exotic pathogens encounter new host plants, the last acting as 'sentinels'. The Botanic Garden of Catania, founded in 1853, houses a rich collection of plant species, including exotic and native flora. Notable collections include the '*Tepidarium greenhouse*' with 160 tropical species, a succulent collection of over 2,000 species of succulents, and the 'Sicilian *hortus*' comprised of indigenous plants. In the past decade, several plant species in the garden have shown severe decline symptoms indicative of root decay. The Molecular Plant Pathology Laboratory at Di3A, University of Catania, made a survey to identify the Oomycetes inhabiting the rhizosphere soil of declining plants. Numerous aggressive *Phytophthora* and *Phytopythium* species were identified, including *P. nicotianae, P. plurivora, P. multivora, P. syringae, P. parvispora,* and *Ph. vexans* as well as new associations pathogen-host.

Global warming, the mesophilic nature, and polyphagy of most of these pathogens pose a threat to plants in the Garden. Actions are being taken to prevent the introduction of new exotic pathogens or their spread through the distribution of propagation material to nurseries and amateurs.

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Molecular and morphological characterization of *Colletotrichum* species, causing apple bitter rot and Glomerella leaf spot in northern Italy orchards

M. Calì[1], E. Cappelletti[1], G. Amaral Carneiro[1], F. Rabbi[1], A. Menicucci[1], R. Baroncelli[1], R. Bugiani[2], A. Prodi[1]

[1]Dipartimento di Scienze e Tecnologie Agro-alimentari,Università di Bologna, Viale Fanin 40, 40127 Bologna, Italy; [2]Servizio Fitosanitario Regione Emilia-Romagna, via Andrea da Formigine 3, 40128 Bologna, Italy. E-mail: antonio.prodi@unibo.it

Italy is one of Europe's major apple producers, ranking third after Poland and Russia. Bitter rot and Glomerella leaf spot are two significant pre- and post-harvest diseases caused by *Colletotrichum* species. In Europe, the dominant bitter rot pathogens during the growing season belong to the *Colletotrichum acutatum* species complex (CASC). However, lately, there has been a growing emergence of species from the *Colletotrichum gloeosporioides* species complex (CGSC) as significant contributors to fruit decay and defoliation. Therefore, the present work aims to investigate the *Colletotrichum* species diversity in northern Italy apple orchards through morphological and multi-locus analysis and, secondly to confirm the virulence of the main isolated species via pathogenicity test on apple fruits. During 2021 - 2023, about 700 colonies of *Colletotrichum* spp. were collected from apple fruits and leaves of 8 different areas. Multi-locus analysis revealed the presence of 3 complexes (CGSC, CASC, and *Colletotrichum orchidearum* species complex COSC), notably the following species: 61% of *C. chrysophilum*, 23% of *C. fiorinie*, 14% of *C. siamense* and 1% of both *C. sojae* and *C. grossum*, never reported in *Malus domestica*. A pathogenicity test was performed on wounded and unwounded Gala apple fruits, with 10^4 spores/mL inoculum suspension of 7 representative *Colletotrichum* species, in which *C. siamense* has been the most aggressive, while *C. sojae* did not cause any lesions. This knowledge is fundamental for a deeper understanding of the *Colletotrichum* community composition, its biology, and tissue preferences, to develop more efficient control strategies.

Genetic diversity and population differentiation of *Botrytis cinerea* in raspberry plantations in Jalisco, Mexico

A.J. Camacho-Alonso[1], A.R. Solano-Báez[1], G. Rodríguez-Bautista[2], G. Márquez-Licona[1]

[1]Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional (CEPROBI-IPN), Morelos, México; [2]Centro Universitario de la Costa Sur, Universidad de Guadalajara, Autlán de Navarro, Jalisco, México. E-mail: gmarquezl@ipn.mx

Mexico is the second-largest producer of raspberries globally, with a cultivated area of 7,767 hectares and an annual revenue of $ 2.3 billion. *Botrytis cinerea*, the causal agent of gray mold, can cause losses of up to 70% in production. In 2022, an increase in the severity of the disease and greater tolerance to fungicides were observed in commercial plantations in the state of Jalisco. This research aimed to determine the genetic diversity of the plant pathogen. To achieve this, 33 isolates were obtained and grouped into seven morphotypes, from which 13 were selected based on their geographic origin. Phylogenetic reconstruction was performed using the genes *g3pdh, hsp60*, and *rpb2*. Morphological and phylogenetic analyses confirmed that all isolates belong to *B. cinerea*. However, some isolates formed clades separate from the reference sequences, indicating the possible presence of haplotypes. Reference sequences of the *rpb2* gene of isolates from Mexico, Italy, the Netherlands, California, and China were used to identify them. Sequence alignment was performed with Mega (version 7.1) and population analysis with DnaSP (version 5) using the Tajima Test. The haplotype network was visualized with PopArt using the TCS Network analysis. The 13 isolates were grouped into four of the six identified haplotypes, with haplotype 3 being the most abundant, including the type species. The detection of haplotypes indicates a wide genetic diversity and population differentiation among the isolates, which may explain the failures in pathogen control.

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Predicting the effects of climate change on the fitness and impact of native and alien *Heterobasidion* spp. in Europe

S. Canna[1], G. Lione[1,2], M. Garbelotto[3], P. Gonthier[1,2]

[1]Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Grugliasco, Italy; [2]Interdepartmental Centre for Innovation in the Agro-environmental Sector (AGROINNOVA), University of Torino, Grugliasco, Italy; [3]Department of Environmental Science, Policy and Management (ESPM), University of California-Berkeley, USA. E-mail: paolo.gonthier@unito.it

Climate change is one of the main threats to forest ecosystems and a major driver of the impact of many plant diseases. *Heterobasidion* spp. cause root rot and wood decay in conifer stands and are among the most destructive fungal forest pathogens worldwide. Predicting whether and to which extent the fitness and impact of *Heterobasidion* spp. may vary in the framework of climate change scenarios might be pivotal to implementing strategies to mitigate the effects of such pathogens and increase the resilience of forest ecosystems. In this study, we designed and tested risk models to predict the fitness and impact of the European native *H. abietinum, H. annosum* and *H. parviporum*, and the North American invasive *H. irregulare* on their preferential hosts. *In vitro* mycelial growth experiments using five genotypes per each of the four *Heterobasidion* species were conducted in climatic chambers set at different temperatures. Generalized additive models (GAMs) were fitted and tested to assess the relation between temperature and mycelial growth rate. The best-performing GAMs were used to derive a temperature-dependent risk score appraising the fitness and potential impact of *Heterobasidion* spp. across the whole geographic distribution of host tree species in Europe. Models predictions accounted for different climate change scenarios based on forecasts from the Global Climate Data platform WorldClim until 2100. Results suggest that the impact of *Heterobasidion* spp. may dramatically increase with rising temperatures, especially in some geographic areas that will likely become more suitable to the pathogens.

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From seed to spike: exploring biological control strategies to mitigate mycotoxin contamination in durum wheat affected by *Fusarium* diseases

E. Cappelletti, D. Alberoni, M. Calì, G. Amaral Carneiro, F. Rabbi, D. Di Gioia, A. Prodi

Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna, Viale Fanin 40, Bologna. E-mail: antonio.prodi@unibo.it

Durum wheat faces significant threats from *Fusarium* diseases, resulting in yield and quality losses, prompting the search for greener alternatives to current farming practices. To tackle this issue, the study focuses on bolstering durum wheat's resilience against *Fusarium* diseases using biological strategies involving beneficial bacteria or essential oils (EOs). *In vitro* screening of 91 bacterial strains, 75 isolated from durum wheat, against *Fusarium* strains unveiled promising candidates mainly from *Bacillus, Pseudomonas*, and Lactic Acid Bacteria (LAB) genera. EOs from clove (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) showed fungistatic effects, with clove exhibiting fungicidal properties even at low concentrations. Seed priming with clove EO, *Bacillus*, and LAB strains proved effective against Fusarium Crown Rot and Fusarium Foot Rot *in vitro*. In controlled environment trials, a LAB strain could control *F. culmorum* at crown level, stimulating plant growth. Greenhouse tests at the head level indicated limited antagonistic abilities of bacterial strains, likely due to high *Fusarium* inoculum doses. Two years of open-field trials with LAB strains showed effects in Fusarium Head Blight control, with a strain of *Lactobacillus plantarum* significantly reducing deoxynivalenol levels in grain. Metabolomic profiling highlighted the presence of antifungal compounds, such as surfactin and fengycin from *Bacillus* species, and benzoic acid and hydrocinnamic acid from LAB. *In vitro* observations demonstrated varied mycotoxin production in the presence of bacterial metabolites, indicating complex interactions. Overall, this integrated approach offers valuable insights into using beneficial bacteria to manage *Fusarium*-related diseases in durum wheat.

Deciphering Kiwifruit Vine Decline Syndrome: insights into microbial communities, gene expression, and hormonal dynamics

A. Cardacino[1], L. Pereira-Dias[2,3], P.R. Oliveira-Pinto[3], C. Santos[3], G.M. Balestra[1]

[1]Department of Agriculture and Forest Sciences, University of Tuscia, Viterbo, Italy; [2]Instituto de Conservación y Mejora de la Agrodiversidad

Valenciana, Universitat Politècnica de València, Valencia, Spain; [3]Department of Biology, Faculty of Sciences, University of Porto, Portugal. E-mail: antonella.cardacino@unitus.it

Since 2012, Kiwifruit Vine Decline Syndrome (KVDS) has been spreading across Italian orchards, significantly impacting production and causing substantial economic losses. This new disease is evidently complex, involving various abiotic and biotic stressors as causal agents. This study aimed to characterize the microbiome colonizing the roots' endosphere, analyse gene expression modulation, and quantify the vine's hormonal response to understand the plant's fitness and adaptability to the disease. Root samples were collected from symptomatic plants at two critical points in the growing season, at the end of flowering (June) and during the fruit set (July), with roots from asymptomatic plants serving as controls. Metagenomic analysis revealed significant differences in microbial communities (bacteria, fungi, and oomycetes) between diseased and asymptomatic samples, confirming the involvement of specific genera previously identified as putative causal agents of KVDS. The relative expression of 17 genes involved in plant resistance responses (ROS detoxification, PR proteins and R proteins synthesis; PRRs, and hormone biosynthesis and signalling) showed significant differences between asymptomatic and KVDS samples in all pathways examined at both time points. Finally, to further understand the hormonal regulatory networks activated in response to the disease and to establish a link with plant fitness, the concentrations of GA3, 6-BA, IAA, SA, ABA, JA, and MeJA were measured using HPLC. These results contribute to a better understanding of the epidemiology of KVDS and could provide clues for effective disease management.

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Diaporthe species do not only cause Phomopsis cane and leaf spot but also contribute to spur dieback in an Italian vineyard

G. Carella, F. Bigazzi, G. Resti, L. Mugnai

Department of Agricultural, Food, Environmental, and Forestry Science and Technology (DAGRI), Plant Pathology and Entomology section, University of Florence, P.le delle Cascine 28, 50144 Firenze, Italy. E-mail: giuseppe.carella@unifi.it

Cortical necrosis caused by *Diaporthe* species, also known as Phomopsis cane and leaf spot (PCLS), is increasingly present in Italian vineyards. Several species have been reported to cause damage to the first internodes and leaves, leading to dieback and shoot blight. The development of cortical necrosis symptoms was monitored over three years in a vineyard cv. Cabernet Sauvignon located near Florence, Tuscany. During this period, increasing damage to the wood, characterized by sectorial necrosis, decline, and death of the spurs, was recorded. Isolates obtained from sampled spurs exhibiting symptoms of wood necrosis or cankers were characterized morphologically and identified using internal transcribed spacer (ITS) region of rDNA. Further identification was performed using the EF1-α, TUB2, and CAL gene when necessary. *Diaporthe* species isolates were found in 43.7% of the spurs, whereas isolates belonging to the Botryosphaeriaceae family were identified in 81.2% of the samples. Other wood pathogens were isolated at lower frequencies: *Phaeomoniella* (6.2%), *Phaeoacremonium* (6.2%), *Cadophora* (9.4%), and *Fomitiporia* (12.5%). Therefore, Botryosphaeriaceae and *Diaporthe* species were prevalent in samples exhibiting symptoms of cankers and necrosis. Notably, *Diaporthe* species were consistently isolated from dead spurs showing PCLS symptoms on the new canes and leaves, suggesting a strong correlation between *Diaporthe* infection, wood cankers, and following spur mortality. This finding underscores the critical role of *Diaporthe* in the observed dieback of grapevine spurs, together with Botryosphaeriaceae species. To the best of our knowledge, this is the first report of cankers and spur dieback caused by *Diaporthe* species in Italy.

Metabolic changes in grapevine leaves affected by *Flavescence dorée* in a Tuscany vineyard

M. Carli[1,2], G. Bianchi[1], L. Cotrozzi[1], C. Nali[1], A. Panattoni[1], E. Pellegrini[1], A. Pedrelli[1,3], D. Rizzo[4]

[1]Department of Agriculture, Food, and Environment (DAFE), University of Pisa, Italy; [2]Department of Forestry, Environment, and Agriculture Technology, University of Florence, Italy; [3]Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy; [4]Regional Phytosanitary Service, Laboratory of Phytopathological Diagnostics and Molecular Biology, Pistoia, Italy. E-mail: marco.carli1@phd.unipi.it

Flavescence dorée (FD) is Europe's most threatening grapevine yellows disease, with severe FD outbreaks reported in major viticulture areas, including Tuscany (central Italy). This study aimed to elucidate the biochemical responses of grapevine (*Vitis vinifera* cv. Sangiovese) to the infection of flavescence dorée phytoplasma (FDp, also reported as '*Candidatus* Phytoplasma vitis'; confirmed by molecular diagnosis, *i.e*., qPCR), analysing several parameters (*e.g*., hydrogen peroxide, H_2O_2; malondialdehyde, MDA; photosynthetic pigments; amino acids and sugars; resveratrol) in leaf samples monthly collected from July to September in a Tuscany vineyard located in Lucolena, Greve in Chianti (Florence). Although FD symptoms first occurred in September (leaf discoloration and reddening) and developed, becoming harsher and more evident in October (also with downward curling of leaves), FD-induced oxidative stress was already reported in July (MDA and H_2O_2 levels increased by 4- and 2-fold, respectively, compared to FDp negative leaves). However, most metabolic changes occurred later in September, as specific variations of both pigment (*e.g*., chlorophyll *a* increased by more than 2-fold, while zeaxanthin and neoxanthin decreased by more than 50%) and amino acid and sugar profiles were reported. Unexpectedly, no changes were noted in resveratrol content. Overall, this research provides valuable insights into the metabolic alterations in FDp-infected grapevine leaves, which could suggest strategies for managing FD and guide the selection of cultivars with enhanced tolerance.

New potential challenges to manage fungal diseases by *Streptomyces* strains

A. Carlucci, T. Conte, M.G. Morea, G. Ricciardi, F. Lops

Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Foggia, Italy. E-mail: antonia.carlucci@unifg.it

The *Streptomyces* genus represents a relevant source of bioactive compounds, antibiotics, and extracellular enzymes. It is undoubtedly the most abundant and important actinomycete used in agriculture, showing promise as both a biological control agent (BCA) and a plant growth-promoting agent (PGPA). Recent studies by our research group have yielded very positive results, as the strain CARA17 used effectively inhibited the growth of several fungal diseases on horticultural crops and during grape postharvest storage. Notably, CARA17 suppressed the mycelial growth of severe fungal soilborne plant pathogens, namely *Athelia rolfsii, Fusarium oxysporum, Plectosphaerella ramiseptata, Sclerotinia sclerotiorum*, and *Verticillium dahliae*, in both dual cultures and when bioactive crude extracts (BCEs) were added to culture media. Greenhouse experiments further demonstrated that CARA17 significantly reduced disease severity and promoted better plant development in fennel and lettuce seedlings artificially inoculated with these plant pathogens. Furthermore, the *Streptomyces* sp. strain CARA17 effectively protected grape berries during postharvest storage from *Botrytis cinerea* and *Monilinia laxa*. This protection was comparable to a fungicide (fludioxonil) and two commercial resistance-inducing products. As the biocontrol activity of CARA17 likely stems primarily from its bioactive crude extracts, further studies are going on to identify the specific metabolites (*e.g*., polyphenols, terpenes, fatty acids) responsible for this protective effect against plant pathogens.

Bioremediation and plant protection: Bacilli consortia reduce metal toxicity and fungal pathogens in wheat

S. Castaldi[1], M. Correggia[1], I. Staiano[1], T. Reitz[2], R. Isticato[1,3]

[1]Department of Biology, University of Naples Federico II, Complesso Universitario Monte S. Angelo, Naples, Italy; [2]Department of Soil Ecology, Helmholtz Centre for Environmental Research - UFZ, Halle, Germany; [3]National Biodiversity Future Center (NBFC), 90133 Palermo, Italy. E-mail: stefany.castaldi@unina.it

Due to natural and anthropogenic processes, heavy metal contamination of soil represents a considerable risk for humans and the biosphere, negatively affecting ecosystem health. Traditional soil remediation technologies are complex processes that reduce soil fertility and are ineffective in treating low-concentration, large-scale heavy metal contamination. As a result, an environmentally friendly and safe strategy to ensure agricultural productivity is required. Among alternative approaches, Plant Growth-Promoting Bacteria (PGPB) are receiving increased interest. PGPB enhances plant growth through various mechanisms and protects plants from several biotic and abiotic stresses.

Moreover, these beneficial bacteria create symbiotic relationships with plants and can alleviate the toxicity of heavy metals through metal biosorption, bioaccumulation, redox reactions, mobilization, precipitation, and transformation. In this study, we tested two different consortia of Bacilli PGPB to alleviate copper and nickel contamination in wheat plants. Both consortia can tolerate up to 1000 ppm of $CuSO_4 \cdot 5H_2O$ and $NiCl_2 \cdot 6H_2O$, and ICP-MS analysis demonstrated the ability of the PGPB to reduce the concentration of metals in the media. When inoculated in metal-contaminated soil, both consortia significantly improved the survival of wheat plants, increasing root and shoot growth and total fresh weight. The consortia were also able to inhibit the growth of pathogenic fungi in wheat plants in the presence of heavy metals. One of the two consortia results was more efficient and could be proposed as a bioinoculant for the metal bioremediation process and as an alternative to chemical pesticides and fertilizers.

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Aspergillus flavus occurrence and aflatoxin contamination in hazelnut orchards throughout the growing season

A. Casu[1], G. Chiusa[1], E. Zagottis[2], G. Genova[2], P. Battilani[1]

[1]Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Piacenza, Italy; [2]Soremartec Italia S.r.l, Piazzale Pietro Ferrero 1, Alba (CN), Italy. E-mail: paola.battilani@unicatt.it

Hazelnuts (*Corylus avellana L.*) are widely used for direct consumption and as ingredients in the confectionery and chocolate industry. They are ranked fifth in terms of production among tree nuts, with a worldwide production of almost 1,200k metric tonnes. Hazelnuts however can be infected by *Aspergillus flavus,* which negatively impacts hazelnuts' market value and poses severe health risks connected to aflatoxin contamination. Azerbaijan in particular is currently facing a high level of aflatoxin contamination. The time of infection of hazelnut by aflatoxigenic fungi is not defined, and it should be clarified if it could occur in the orchard before harvest, during harvesting and/or more specifically during storage after cracking of the shell. This 3-year study (2022 to 2024) investigated four different hazelnut growth stages, from female flowering (BBCH 70) to ripened fruit (BBCH 89), in three different production areas of Azerbaijan, with the aim of describing *A. flavus* occurrence in hazelnut orchards. The research revealed that *A. flavus* infection started in the early stages of crop growth, in a phenological stage corresponding to the ovary enlargement of hazelnut female inflorescence. *Aspergillus flavus* remained present in all the examined growth stages, with a mean value of 10^2 UFC/g. Aflatoxin contamination was detected only in the ripening stage of hazelnut fruit (BBCH 89). *Aspergillus flavus* is present in hazelnut orchards before nut development and its occurrence can lead to aflatoxin contamination before post-harvest handling. These findings support the importance of applying measures in the field to prevent aflatoxin contamination.

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Effects of sustainable agronomic measures on potato tuber dry rot under climate change

A. Catalani[1], G. Chilosi[1], M. Jasarevic[1], C. Morales-Rodríguez[1], E. Radicetti[2], R. Mancinelli[3]

[1]Department for Innovation in Biological, Agrofood and Forest Systems (DIBAF), University of Tuscia, 01100 Viterbo, Italy; [2]Department of Chemical, Pharmaceutical and Agricultural Sciences (DOCPAS), University of Ferrara, 44121 Ferrara, Italy; [3]Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, 01100 Viterbo, Italy. E-mail: chilosi@unitus.it

Potato dry rot caused by different fungal pathogens affects tubers in the field and during storage and represents the primary postharvest loss. In a three-year trial (2021–2023), this study aimed to determine whether a sustainable and circular agricultural model, based on conservative tillage techniques and compost as organic fertilization, could reduce the severity of dry rot compared to traditional ploughing and mineral fertilization. In the 2022 cropping season, a high rate of dry-rotted tubers was collected due to high temperatures. *Fusarium brachygibbosum* was the preponderant virulent fungus found on symptomatic tubers. In 2023, the thermophilic fungus *Macrophomina phaseolina* was also recorded. In 2022, we observed that spading combined with compost fertilization caused a significant decrease in dry rot; furthermore, fertilization with compost, mainly when combined with subsoiling, decreased the frequency of *F. brachygibbosum. Trichoderma atroviride* and *T. gamsii* were also isolated from symptomatic tubers, particularly from organically fertilized plots. The saprophytic and antagonistic fungus *Linnemannia elongata* was isolated on tubers from subsoiling. These antagonist fungi are crucial to reducing crop losses even if *Fusarium* and *Macrophomina* isolates developed consistently at 30 and 35 °C. In contrast, the development of *Trichoderma* isolates strongly decreased at these temperature values. A high percentage of the arbuscular mycorrhizal fungus *Paraglomus occultum* was observed in the roots of plants from organically fertilized plots. Sustainable and circular agriculture management systems can maintain or restore soil fertility through conservative tillering and organic fertilization and minimize losses due to diseases such as the dry rot of potato tubers.

Trichoderma gamsii ICC080 and *Trichoderma asperellum* ICC012 (active ingredients of the commercial Remedier®) to control Fusarium head blight (FHB) on wheat

M. Cesarini[1], A. Petrucci[1, 2], E. Hotaj[1], G. Venturini[3], R. Liguori[3], S. Sarrocco[1]

[1]Department of Agriculture, Food and Environment, University of Pisa, Italy; [2]Department of Plant and Environmental Sciences and Copenhagen Plant Science Centre, University of Copenhagen, Denmark; [3]Gowan Novara Isagro S.p.A.– Research Center Renato Ugo, Novara, Italy. E-mail: marco.cesarini@agr.unipi.it

FHB represents a significant threat to wheat production due to food security and safety risks. The present work aimed to assess the capability of *Trichoderma gamsii* ICC080 and *Trichoderma asperellum* ICC012 - active ingredients of Remedier® - to control FHB on wheat. The ability of ICC012 and ICC080 to colonize wheat roots was preliminary investigated, resulting in 100% endophytism followed by a significant modulation of defence-related genes *pr1, sod, pgip2,* and *pal1.* When the expression of the same genes was investigated in spikes inoculated with the two isolates, a significant up-regulation of only *pal1* was registered at 24 hours post inoculation (hpi). To check if a systemic defence response was modulated, the expression of the genes was analysed in leaves collected at seven and 14 days post inoculation (dpi) of roots, resulting in an up-regulation of the *sod* gene at seven dpi. Even if induction of resistance is probably not the main mode of action of the two isolates, when applied on spikes at anthesis in the presence of the pathogens, a significant reduction of the DI, comparable with the fungicide, was observed for ICC012 and ICC080, alone and co-inoculated, and Remedier®. Finally, the amount of *F. graminearum* was measured (as DNA and number of perithecia) on wheat straw pieces where usually the pathogen overwinters, resulting - after six months - in a significant reduction when in the presence of the two isolates, alone and together. Results here reported support the possible use of Remedier® for the management of FHB on wheat, whose registration is now ongoing.

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Exploring the genomic basis of host specificity in *Stemphylium vesicarium*

XQ. Chen, F. Negrini, M. Karas, E.G. Kabeto, M. Menghini, M. Collina, R. Baroncelli, E. Baraldi

Department of Agricultural and Food Sciences, University of Bologna, Viale G. Fanin 42, 40127 Bologna, Italy. E-mail: xueqin.chen2@unibo.it, riccardo.baroncelli@unibo.it, elena.baraldi@unibo.it

Pear, a widely cultivated fruit, faces a significant challenge in Italy, particularly in the Emilia Romagna region. This region was once a leader in European pear production, averaging 1 million tonnes annually until 2011 and around 0.7 million tonnes from 2012 to 2018. However, recent years have witnessed a dramatic decline, with production plummeting to 0.42 million tonnes in 2019 and a mere 0.25 million tonnes in 2023. The culprit behind this alarming decline is the worsening of pear brown spot disease caused by the fungus *Stemphylium vesicarium* (Sv). Intriguingly, while Sv as a species can infect various plants like asparagus, onion, and tomato, it exhibits a strong host specificity at the strain level with only specific Sv strains able to infect pear. This suggests a unique genetic adaptation in these pear-pathogenic strains. To unravel the molecular basis of this host specificity, we embarked on a comprehensive comparative genomic analysis. We collected, sequenced, and annotated the genomes of 22 Sv strains isolated from diverse hosts, including pear, onion, apple, asparagus, and hazelnut. A comparative genomic analysis is ongoing and aims to achieve three objectives: 1) Investigate the overall genetic variation within the Sv population; 2) Uncover the evolutionary relationships between different Sv strains pathogenic to different hosts; 3) Identify the specific genetic elements within the pear-pathogenic Sv strains that enable them to specifically infect this host. This presentation will delve into the bioinformatics pipeline we employed for genome analysis and share the initial findings of our research.

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Aureobasidium pullulans **formulations: evaluation of the effectiveness against grey mould of table grape**

R. Cignola[1], G. Firrao[1], G. Freschi[2], A. Di Francesco[1]

[1]Department of Agriculture, Food, Environmental and Animal Sciences, University of Udine, Italy; [2]Cleverbioscience, Campospinoso (Pavia), Italy. E-mail: alessandra.difrancesco@uniud.it

Antagonism against *Botrytis cinerea* is often carried out using yeast as direct antagonists. *Aureobasidium pullulans* strain AP1 was tested in two different formulations: wettable powder (WP) and oil dispersion (OD). By *in vitro* assays, the viability of the strain cells was constantly evaluated for seven months, and the OD formulation ensured the highest cell viability. The efficacy of the formulations was assayed by assessing the production of volatile and non-volatile metabolites. Results showed that the formulation affected the non-volatile less than the volatile metabolites. Both AP1 WP and AP1 OD non-volatile metabolites displayed almost 50% of mycelial pathogen inhibition. Comparing the two products, the lowest EC_{50} value (518.15 mg L^{-1}) was detected for the AP1 OD formulation that was thus chosen for postharvest *in vivo* assays. The preventative treatments (200, 400, 800 mg L^{-1}) were active in reducing the pathogen incidence on table grape by 52% on average. Instead, in the curative application assay, the highest concentration (800 mg L^{-1}) reduced grey mold incidence by 86%. The present study reported the potential of two new formulations to use against the postharvest grey mold of table grape for a possible further commercial product development.

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Beneficial microbial consortia: alternative solutions for ecosystem health and plant protection

C. Cimminella[1,2], R. Isticato[1,2], S. Castaldi[1], M. Papaianni[3], G. Manganiello[2], S.L. Woo[3,4]

[1]Department of Biology, University of Naples Federico II, 80126 Naples, Italy; [2]National Biodiversity Future Center (NBFC), 90133 Palermo, Italy; [3]Department of Agricultural Sciences, University of Naples Federico II, 80055, Portici, Italy; [4]Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy. E-mail: carmen.cimminella@unina.it

Research has increasingly focused on identifying effective biological control agents (BCAs) to combat plant pathogens. Microorganisms are crucial for preserving environmental resources and consumer health. Their interactions with soil, plants, and other organisms are essential for ecosystems and agricultural production. This work aims to develop an innovative formulation using selected *Bacillus* and *Trichoderma* species, applied individually or in consortia, to control *Pseudomonas syringae* pv. *tomato* (*Pst*), a plant pathogen causing severe tomato crop losses. *Bacillus* species were isolated from extreme environments, sand samples in salt-pans, and were identified as *Bacillus gibsonii* RHF15, *B. vallismortis* RHFS10 and *B. amyloliquefaciens* RHFS18. These bacteria were tested with *Trichoderma* genera known for plant growth-promoting and biocontrol properties, particularly *T. asperellum* T25 and *T. virens* GV41. The potential of these microorganisms, individually and in consortia, was assessed through *in vitro* and *in vivo* assays. *In vitro* compatibility tests confirmed their suitability for consortia use. *In vivo* tests on tomato plants assessed their plant growth-promoting capacity by measuring biometric indices. Germination rate (%) and plant wet mass were increased compared to the untreated control. In the biocontrol test against *Pst*, with disease incidence (%DI) of about 70% in the untreated control, %DI was reduced by about 10% in the *B. vallismortis* and T25+*B. vallismortis* treatments compared to the untreated control. There was also a reduction of approximately 10% in disease severity (%DS) for *B. vallismortis*, T25+*B. gibsonii* and T25+*B. vallismortis* treatments. Using beneficial microbial consortia improves the biocontrol efficacy of these microorganisms.

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Specialized metabolites as stimulants of suicidal germination or inhibitors of seed germination and seedling growth for sustainable development of parasitic weeds management

A. Cimmino[1], M. Masi[1], M. Fernández-Aparicio[2]

[1]Department of Chemical Sciences, University of Naples Federico II, Naples, Italy; [2]Department of Crop Protection, Institute for Sustainable Agriculture (IAS), CSIC, Avenida Menéndez Pidal s/n, 14004 Córdoba, Spain. E-mail: alessio.cimmino@unina.it

Parasitic weeds represent a tremendous threat to agriculture, and despite many attempts to contain them through the use of various traditional management approaches, they persistently adapt and spread, posing growing challenges in effectively minimizing their impact on crops. Broomrapes (*Orobanche* and *Phelipanche* spp.) and dodders (*Cuscuta* spp.) are particularly problematic because they compete with a large number of important cultivated crops by penetrating their roots and stems for nutrient and water absorption. The broomrapes produce many tiny seeds which can remain dormant in the soil for decades until the germination is induced by a root-derived chemical signal exuded by the host crop. The isolation of allelochemicals from root exudates and extracts of plants, with the potential to stimulate suicidal germination of broomrape seeds in the absence of a susceptible host or inhibit seed germination and seedling development of broomrape and dodders in the presence of susceptible hosts, opens the door to the design new herbicides based on natural sources with parasitic weed-specific mechanisms of action being potentially safe for the rest of the agroecosystem. In this communication, the isolation and chemical characterization of specialized metabolites from the most promising allelopathic plants selected as *Bellardia trixago, Centaurea cineraria, Convolvulus arvensis, Conyza bonariensis*, and *Retama raetam*, as well as the bioassays against four root holoparasitic plants: *O. crenata, O. cumana, O. minor, P. ramosa* and one stem parasitic plant: *Cuscuta campestris*, will be reported. Furthermore, hemisynthesis, ecotoxicological and SAR studies of selected bioactive specialized metabolites will be discussed.

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Occurrence of citrus yellow vein clearing virus (CYVCV) in Campania: a preliminary survey

M. Cinque[1], M. Minutolo[1], C. Pugliese[1], R.V. Griffo[2], F. Di Serio[3], B. Navarro[3], D. Alioto[1]

[1]Dipartimento di Agraria, Università degli Studi di Napoli Federico II, 80055 Portici, Italy; [2]Servizio Fitosanitario Regione Campania, Centro Direzionale is. A6 - 80143 Napoli; [3] Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, 70126 Bari, Italy. E-mail: maria.cinque@unina.it

Citrus yellow vein clearing virus (CYVCV), a member of the genus *Mandarivirus*, is the causal agent of yellow vein clearing, a devastating citrus disease first reported in Pakistan and then in India, China, Iran, Turkey, and the USA. Since CYVCV causes relevant economic losses by affecting fruit quality and productivity, it has been included in EPPO's Alert list since 2022. Recently, the natural occurrence of CYVCV was reported in Palma Campania (Naples Province) on six lemon trees with symptoms of leaf yellow vein clearing and in one sweet orange without symptoms, representing the first report of the virus in Italy and in the European Union territory. Following this detection, a follow-up survey was started to determine the extent of CYVCV spread and the citrus species involved. The presence of CYVCV was assessed in RT-PCR with specific primer pairs and targeting the viral CP gene. By May 2024, several private gardens and orchards in the provinces of Napoli, Caserta, and Salerno (Campania region) have been inspected, allowing to identify CYVCV in symptomatic lemons and asymptomatic sweet orange, mandarin, and clementine trees. Altogether, these data support the idea that CYVCV is more widespread in Campania than initially thought. The next step will be to extend monitoring to other citrus growing areas of the region, and especially to other wood or herbaceous species such as grapevine, *Malva sylvestris, Ranunculus arvensis, Sinapis arvensis*, and *Solanum nigrum,* which have been reported as hosts of the virus.

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Assessment of the effectiveness of natural and biological means to control phytopathogenic *Fusarium* spp. on wheat

T. Conte, M.G. Morea, A. Libutti, A. Carlucci

Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia, Foggia, Italy. E-mail: antonia.carlucci@unifg.it

The environmental, economic, and ethical issues linked to traditional management enforce the need to find sustainable alternatives to the conventional management of plant fungal diseases. Since agricultural wastes (AWs) and biocontrol agents (BCAs) represent promising tools for new bio-sustainable field management, research activities have been carried out in *in vitro* and *in vivo* conditions. The first consisted of performing dual cultures with six AWs (bergamot, eucalypt, laurel, pomegranate, sage) and three BCAs strains against eight fungal pathogens (*Athelia rolfsii, Fusarium graminearum* [F30], *F. oxysporum* [F1B], *F. solani* [F18], *Plectosphaerella ramiseptata, Rhizoctonia solani, Sclerotina sclerotiorum, Verticillium dahliae*). The effectiveness of AWs essayed was determined by an inhibition index (%) based on the radius of mycelial growth inhibited with respect to the radius achieved from the same fungal colony without the AW. Based on results obtained in *in vitro* conditions, two plant wastes (bergamot and pomegranate peel), one organic product available on the market (EP5), and one microorganism (*Streptomyces* sp. Strep22), able to inhibit the mycelial growth, were selected to perform the *in vivo* experiment. This was carried out in a greenhouse to control three *Fusarium* species (F1B, F18, F30) on wheat by setting 80 different treatments. The results confirmed that AWs and BCA used protected significantly the Fusarium infections on wheat roots and collars. Moreover, the *Streptomyces* strain also promoted the growth of the wheat plants.

Exploring the biocontrol potential of *Candida oleophila* strain O against citrus green mold: molecular insights into plant defense activation

S. Conti Taguali[1,2], F. La Spada[1], E.I. Rovetto[1], S. El boumlasy[1,3], M. Riolo[1], C. Bua[1], R. Parlascino[1], A. Pane[1], M.G. Li Destri[2], S.O. Cacciola[1]

[1]Department of Agriculture, Food and Environment, University of Catania, 95123 Catania, Italy; [2]Department of Agricultural Science, Mediterranea University of Reggio Calabria, 89122 Reggio Calabria, Italy; [3]Laboratory of Materials-Catalysis, Department of Chemistry, Faculty of Science, Université Abdelmalek Essaadi, 93000 Tetouan, Morocco. E-mail: olga.cacciola@unict.it

Green mold by *Penicillium digitatum* is a major post-harvest challenge for citrus fruit, responsible for about 90% of total losses. This study evaluates the effectiveness of the biological control agent *Candida oleophila* strain O in managing green mold in clementine tangerines, oranges, and lemons at various post-harvest stages of the supply chain. Three infection scenarios were simulated: inoculation before, during, and after treatment, followed by storage under refrigerated (6° C) and shelf-life (18 °C) conditions. Results showed that *C. oleophila* significantly reduced disease incidence across all citrus types and scenarios. Disease incidence was much lower in treated fruits than in untreated controls, comparable to those treated with the synthetic fungicide imazalil (IMZ). Clementine treated with *C. oleophila* had a 5% disease incidence after 10 days, similar to IMZ-treated fruits. After 14 days, *C. oleophila*-treated fruits had a disease incidence (45%) lower than IMZ-treated ones (50%), indicating longer-term efficacy. Additionally, *C. oleophila* treatment upregulated defense-related genes encoding β-1,3-glucanases, phenylalanine ammonia-lyase (PAL), and peroxidase (PEROX). In clementines, significant upregulation was observed at 0, 24, and 48 hours post-treatment, with the highest expression for β-1,3-glucanase and PEROX at 48 hours. Oranges showed increased gene expression at 24 and 48 hours, especially for β-1,3-glucanase and PAL, while lemons exhibited significant upregulation at 48 hours. This stronger, prolonged defense response in clementines suggests *C. oleophila*'s potential as an eco-friendly alternative to synthetic fungicides for controlling citrus green mold. These findings support its use in sustainable postharvest management, potentially reducing postharvest losses in citrus supply chains.

This study was supported by the project "Smart and innovative packaging, postharvest rot management, and shipping of organic citrus fruit (BiOrangePack)" under Partnership for Research and Innovation in the Mediterranean Area (PRIMA)– H2020 (E69C20000130001), the "Italie–Tunisie Cooperation Program 2014–2020" project "PROMETEO «Un village transfrontalierpour prot´eger les cultures arboricoles m´editerran´eennes enpartageant les connaissances» cod. C-5–2.1–36,CUP 453E25F2100118000 and the European Union (NextGeneration EU), through the MUR-PNRR project SAMOTHRACE (ECS00000022) and the "Investigation of phytopathological problems of the main Sicilian productive contexts and eco-sustainable defense strategies (MEDITECO)"- PiaCeRi-PIAno di inCEntivi per la Ricerca di Ateneo 2020–22 linea 2" "5A722192155" funded by the University of Catania, Italy.

Impact of light and dark conditions on secondary metabolite production and biocontrol efficacy in *Clonostachys rosea*

N. Conti[1], V. Lazazzara[1], A. Frascella[2], C. Brunetti[1,3], G. Della Rocca[1]

[1]Institute for Sustainable Plant Protection (IPSP), National Research Council, Sesto Fiorentino, 50019 Italy; [2]Institute of Biosciences and Bioresources (IBBR), National Research Council, Sesto Fiorentino, 50019 Italy; [3]Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, 50144 Florence. E-mail: valentina.lazazzara@ipsp.cnr.it

The ascomycete *Clonostachys rosea* has been considered an important biocontrol agent due to its ability to produce various secondary metabolites with antagonistic effects towards numerous plant pathogens. *Clonostachys* fungi are abundant in many classes of secondary metabolites, including mainly nitrogen-containing compounds, polyketides, and terpenoids. However, the spectrum of secondary metabolites produced by *C. rosea* has yet to be studied. Moreover, sensitivity to light is one of the main limitations for applying biocontrol agents in the field, and the biocontrol efficacy of *C. rosea* exposed to light or dark conditions has received little attention so far. This study aims to elucidate the metabolic profile of *C. rosea* grown under dark and light conditions using high-performance liquid chromatography coupled with diode array detector and quadrupole-time of flight mass spectrometry (HPLC-DAD-QTOF). The biocontrol activity of *C. rosea* grown in both light and dark conditions was investigated against the telluric pathogenic oomycetes *Phytophthora cambivora* and *P. cinnamomi*, and ongoing *in vitro* inhibition tests are revealing different inhibitory abilities of *C. rosea* when grown in light or dark conditions against both *Phytophthora* species. Moreover, HPLC-DAD-QTOF and tandem mass spectrometry (MS/MS) analyses revealed a significant increase of secondary metabolites with antimicrobial activities (*i.e.*, a tetronic acid derivative and a polyketide derivative) in *C. rosea* isolates grown in dark compared to light conditions. These results contribute to a deeper understanding of the biocontrol potential of *C. rosea* against *Phytophthora* spp.

Portable solutions for on-site rapid assessment of the storability and safety of agrifoods

L. Convertini[1], O. Incerti[1], G. Liso[1], A. Abdallah[2], A. Ippolito[1], E. Santovito[2], S.M. Sanzani[1]

[1]Department of Soil, Plant, and Food Sciences, University of Bari Aldo Moro, Bari, Italy; [2]National Research Council of Italy, Institute of Sciences of Food Productions (CNR-ISPA), Italy. E-mail: simonamarianna.sanzani@uniba.it

The FOODsens project aims to demonstrate the use of sensor-based rapid testing systems for the monitoring of fungal plant pathogens and their metabolites, particularly the mycotoxin Ochratoxin A (OTA), which might affect the shelf-life and safety of plant-based minimally processed foods (PBMPFs). Advanced portable optical sensor platforms will be fine-tuned and validated for on-site assessment of: i) microorganism contaminations by an autonomous handheld reader and sensor coatings into sample vials or bags; ii) the mycotoxin OTA by an autonomous handheld reader and a colorimetric reaction-based on an OTA-aptamer sandwich system. Such portable platforms will be combined in an integrated workflow for fast on-site detection of mycotoxigenic fungi and OTA. The proposed sensors will be further validated for their use in laboratory conditions and then demonstrated in semi-industrial settings with different PBMPFs (fresh fruits/vegetables, dry nuts/grains, liquid juices/purees, etc.), with the view of replacing the costly, slow, and laborious conventional techniques for assessing microbiological quality (*i.e.*, colony counting tests) and mycotoxin contamination (*i.e.*, HPLC-MS/FLD). Adoption of the FOODsens approach might provide more accessible, faster, and more comprehensive microbial and mycotoxin testing; significant improvement of batch release timing (same shift/day to result vs current 48–72 h); reduced product losses due to microbial or mycotoxin contaminations; better control of production processes, and more frequent assessments of safety and hygiene of commodities and working environments.

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A continuous microwave system for the management of plant pathogens and fungal contamination on almond in postharvest

D. Cornacchia, D. Gerin, G. Incampo, A. Tamborrino, A. Berardi, A. Leone, S. Pollastro, F. Faretra

Department of Soil, Plant and Food Sciences, University of Bari, Bari, Italy; E-mail: stefania.pollastro@uniba.it; antonia.tamburrini@uniba.it

This study investigated the potential of a pilot system developed for a continuous microwave treatment (MW) against fungal plant pathogens and mycotoxin contamination and for improving the quality and safety of dried almond seeds. Conidial suspensions of *Alternaria alternata, Aspergillus carbonarius, Aspergillus niger, Botrytis* *cinerea, Cylindrocarpon destructans, Fusarium oxysporum* f. sp. *radicis-lycopersici, Monilia fructicola*, and *Phaeomoniella chlamydospora*, selected as representative of different conidial sizes, cell numbers and pigmentation, were subjected to MW treatment using six different electric motor frequency (30–80 Hz) resulting in 18.1–7.3 kJ kg^{-1} of specific energy and 65.1–42.8 °C of almond surface temperature. The conidial germination of all fungal species was already totally inhibited at 80 Hz, corresponding to 7.3 kJ kg^{-1} of specific energy and 42.8 °C of temperature. The MW was also applied on almonds cv Tuono artificially inoculated with $1x10^6$ conidia ml^{-1} of *A. carbonarius* and its 6-day-old culture filtrate in minimal medium containing 330 µg L^{-1} of Ochratoxin A (OTA). MW at 30 Hz (18.1 kJ kg^{-1} and 65.1 °C) inhibited up to 91.5% of the *A. carbonarius* conidia germination and reduced by 25.5% the OTA contamination. The 45 Hz MW determined as effective against the moth *Ephestia kuehniella*, was also applied on 'Tuono' seeds that proved contaminated with different fungal species mainly belonging to the genera *Aspergillus, Penicillium* and *Rhizopus* (from 7.3×10^3 to 2.0×10^6 UFC $seed^{-1}$). Also in this case, the MW totally or strongly inhibited the fungal germination confirming the good MW effectiveness and their detoxicant action.

This research was carried out in the framework of the project Misura 16 Cooperazione– Sottomisura 16.2 "Sostegno a progetti pilota e allo sviluppo di nuovi prodotti, pratiche, processi e tecnologie" - Progetto "Almond Management Innovations (Approcci per una Mandorlicoltura biologica Innovativa)" AMÌ CUP: B99J20000110009

Investigating plant-microbe interactions to mitigate frost stress in crop plants

S. Cremonesi[1], M.K.M. Malek[1], M. Perazzolli[1,2]

[1]Center Agriculture Food Environment (C3A), University of Trento, Via E. Mach 1, 38098 San Michele all'Adige, Trento, Italy; [2]Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38098 San Michele all'Adige, Trento, Italy. E-mail: michele.perazzolli@unitn.it

Climate change is responsible for mild winters and warm spring periods that can induce premature plant development, increasing the risk of exposure of vulnerable plant tissues to chilling and frost stress. Plants are associated with complex microbial communities that confer fitness advantages to the plant host, including growth promotion, nutrient uptake, abiotic stress tolerance, and resistance to pathogens. However, scarce information is available on the role of plant-associated bacteria in mitigating frost stress. This project aimed to taxonomically and functionally characterize the endophytic bacterial communities associated with cold-adapted Rosaceae plants and assess their mechanism of action in protecting apple plants against spring frosts. The bacterial community structure associated with flowers, leaves, and roots of wild alpine Rosaceae plants differed according to the plant tissue and plant species, but it was scarcely affected by the collection site. Some cold-tolerant bacterial isolates reduced frost damage on Rosaceae crops, limiting the electrolyte leakage of leaf tissues. Physiological analyses are in progress to further characterize the efficacy of three cold-tolerant bacterial isolates to mitigate frost damage on potted apple plants exposed to simulated frost. These results will provide better information on the function of cold-tolerant bacterial endophytes associated with alpine plants.

The project has received funding from the Fondazione CARITRO, Cassa di Risparmio di Trento e Rovereto (project MITICLIMA).

Long-reads Nanopore amplicon-sequencing for fast and precise identification of multiple microorganisms

V. Crosara[1,2], G. Tatulli[2], V. Scala[2], M. Reverberi[1], N. Pucci[2], S. Loreti[2], L. Faino[1]

[1]Department of Environmental Biology, Sapienza University of Rome, 00185 Rome, Italy. [2]Research Centre for Plant Protection and Certification, Council for Agricultural Research and Economics, Via C.G. Bertero 22, 00156 Rome, Italy. E-mail: valeria.crosara@uniroma1.it

The microbial species described to date represent only a small fraction of Earth's total biodiversity. To expand this knowledge, DNA barcoding is a powerful method for multiple species identification. It is based on the amplification and sequencing of conserved regions. Unfortunately, conventional short-reads sequencing leads to limited taxonomic resolution due to fragmentary sequences, but the emergence of new long-reads sequencing techniques, such as Nanopore sequencing, improves this aspect. Indeed, long-reads, by including a more significant part of the standardized marker sequence, enhance the accuracy of species identification and increase the possibility of identifying low-abundance species. However, compared to earlier technologies, bioinformatic tools specifically designed for Nanopore sequences' analysis are lacking. Therefore, in our work, we focused on setting an amplicon-Nanopore sequencing workflow and developed a pipeline able to process long-noisy reads. To test and compare our pipeline with the existing ones, the 16S rDNA and ITS of several known microorganisms, with different taxonomic similarities were amplified and sequenced using Nanopore technology. The produced sequences were then analyzed using our pipeline and the available software. The results showed a precision and a recall close to 1, comparable to the Emu software. Then, we tested our method's flexibility and reliability by applying it using other selected genetic markers to achieve deep identification at the pathovar/subspecies level for quarantine bacterial pathogens. Our approach proved promising for the precise identification of microorganisms at the genus-species level for broader metabarcoding studies and at a deeper taxonomic resolution: pathovar or sequence type.

Impact of climate change-related factors on the effectiveness of *Aspergillus flavus* biocontrol

M. Crosta[1], C. Cervini[2], A. Medina[2], P. Battilani[1]

[1]Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, Piacenza, Italy; [2]Applied Mycology Group, Environment and AgriFood Theme, Cranfield University, Cranfield, UK. E-mail: paola.battilani@unicatt.it

Aflatoxin (AFs) contamination is one of the most serious problems for peanuts worldwide due to their high susceptibility to *Aspergillus flavus* infection. The use of non-aflatoxigenic strains as biocontrol agents is one of the most effective practices exploited to reduce this issue. However, the decrease in precipitation together with the increase in temperature and CO_2, caused by climate change, promotes the spreading of *A. flavus* and may influence the effectiveness of this strategy. Therefore, this work was aimed at assessing *in situ* the efficacy of a non-aflatoxigenic strain to reduce the mycotoxin production of an aflatoxigenic one, under simulated climate change (CC) scenarios. Three *A. flavus* spore suspensions with different ratios (100% aflatoxigenic, 100% non-aflatoxigenic, 50/50% aflatoxigenic:non-aflatoxigenic; 10^5 spores ml^{-1}) were inoculated on peanut seeds, which were incubated for 10 days at different conditions of temperature (T, 25, 30, 35 °C), water activity (a_w, 0.85, 0.90, 0.95), and CO_2 (400, 1000 ppm). In the whole study, the application of the biocontrol agent led to a significant reduction of AFB1 production, over 70%. Considering the extreme T, $a_{w,}$ and CO_2 conditions fixed in this study, the results showed that the AFB1 reduction increased when T raised up to 35°C, and decreased when a_w and CO_2 moved from the optimal conditions (a_w=0.90, CO_2=400 ppm). These findings provided evidence for the first time that the effectiveness of the biocontrol strategy using an *A. flavus* non-aflatoxigenic strain is expected to be confirmed, with eventual slight reductions, in climate change conditions.

Matteo Crosta carried out this work within the PhD School "Agrisystem" of the Università Cattolica del Sacro Cuore (Italy). The PhD project "Enhancement of Italian peanut supply chain" is co-financed by the Italian Ministry of Education and Merit, through the PON program, and the Università Cattolica del Sacro Cuore.

Characterization of the chloromonilicin biosynthetic gene cluster in the brown rot fungus *Monilinia fructicola*

M. Crudele, N.F. Barnaba, D. Di Cosmo, T. Mascia, P.R. Rotondo, F. Faretra, R.M. De Miccolis Angelini

Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy. E-mail: ritamilvia.demiccolisangelini@uniba.it

Monilinia fructicola is a major plant pathogen responsible for brown rot leading to blossom and twig blight and pre- and post-harvest fruit rot that cause significant yield losses, especially on stone fruit. Analysis of the *M. fructicola* genome sequence (GenBank accession number GCA_008692225.1) revealed a type I polyketide synthase gene cluster, named BGC-10, putatively involved in chloromonilicin biosynthesis. This gene cluster, ~55 Kb in length, consists of twenty-four genes, including one polyketide synthase (*pks1*), one flavin-dependent halogenase, two N-acetyltransferases and three methyltransferases, six oxidoreduction-related genes, one scytalone dehydratase and two NAD-dependent epimerases, one lactamase-like protein gene, two transcription factors, and three transporter protein genes. Chloromonilicin has been described as a nonphytotoxic compound showing a relevant antimicrobial activity against bacteria, yeasts, and plant pathogenic fungi. Functional analysis of BGC-10 was performed by CRISPR/Cas9-mediated targeted gene disruption. Mutants targeting the *pks1* gene were characterized by sequence analysis at the target cleavage site, ddPCR assays for copy number determination of hygromicine insertion, and gene expression analysis. There were no statistical differences between mutants and the wild-type strain in conidia production and germination rate, colony growth, and virulence on artificially inoculated fruits. Moreover, metabolomic analysis using UHPLC-MS/MS showed that chloromonilicin production and other metabolites putatively associated with BGC-10 (*i.e.*, chloromonilinic acid A and B, and 4-chloropinselin) significantly increased in wild-type strains co-cultured with *Penicillium expansum* compared with monoculture and was very low after inoculation on fruits. Mutants did not produce detectable amounts of these metabolites in all tested growing conditions.

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)– MISSIONE 4

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Focus on biodiversity: looking for sustainable approaches to manage tomato leaf curl New Delhi virus on cucurbits

M. Crudele, M. Marashi, N.F. Barnaba, R.M. De Miccolis Angelini, M. Mastrochirico, R. Spanò, T. Mascia

Università degli Studi di Bari "Aldo Moro", Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, via Amendola 165/A, 70125 Bari, Italy. E-mail: tiziana.mascia@uniba.it

Tomato leaf curl New Delhi virus (ToLCNDV) is an emerging begomovirus listed in the EPPO Alert-list 2 and reported in 2015 for the first time in southern Italy on Sicilian courgette. The virus is persistently transmitted by *Bemisia tabaci*, and it is particularly harmful on cucurbits, causing 100% production losses. A sustainable and environmentally friendly approach is needed to counteract the viral disease. Local cucurbit ecotypes were screened to assess tolerance levels, and *Cucumis melo* cv. Barattiere was found to be the most tolerant, showing no detectable disease symptoms and very low levels of viral DNA accumulation using a time-course quantitative dot-blot assay. This local variety was then used as a potential rootstock to achieve levels of tolerance in commercial cucurbit varieties. Different graft combinations of susceptible and moderately susceptible cucurbit genotypes onto *C. melo* cv. Barattiere plants showed a generalized delay in viral symptom appearance and less severe disease than those observed in non-grafted counterparts. Consistently, the viral DNA accumulation in all grafted combinations showed a significant reduction between 11 and 28 days after inoculation. RNA-Seq analyses at 11 dpi were performed to investigate the transcriptome profile of *C. melo* cv. Retato Standard F1 grafted onto *C. melo* cv. Barattiere used as rootstock. Experimental open fields have been established in areas exposed to high viral inoculum to evaluate the response of grafted plants to counteract ToLCNDV infection. Furthermore, the setup of a portable real-time PCR-based diagnostic assay is in progress to provide a rapid and quantitative tool.

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Effect of ozone exposure on postharvest decay of peach fruits in cold storage and its potential environmental savings from shelf life extension

A.L. D'Ortenzio, S.M. Makau, K.A. Boakye-Yiadom, V. Mancini, S. Piancatelli, A. Ilari, M. Moumni, S. Murolo, L. Landi, D. Duca, G. Romanazzi

Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche, 60131 Ancona, Italy. E-mail: g.romanazzi@univpm.it

Fungal infections are one of the main problems that cause losses and fruit waste in postharvest. Peach is susceptible to brown rot, caused by *Monilinia* spp. We evaluated the effects of two sprayers, traditional and innovative with flow control, to rationalize preharvest treatments and postharvest application of gaseous ozone for the control of brown rot. The experiment was carried out on peach cv. Royal Summer and Extreme 486, sprayed with fungicides (tebuconazole in 2019 and 2020 and boscalid+pyraclostrobin in 2021) in the field. Immediately after harvest, fruits were stored at 4 °C without or with ozone treatment at 45–50 ppb and 45–200 ppb concentrations during the day and night, respectively, for 10 and 20 days. Fruits were removed from cold storage, and decay was measured daily in shelf life. A life cycle assessment was conducted to quantify the potential environmental impacts of using ozone, considering the energy use of the ozone treatment system and the potential savings from waste avoided. Concentration of 50 ppb day/200 ppb night for 10 and 20 days on peach cv Extreme 486 effectively controlled *Monilinia* spp. but induced phytotoxic effects. There were no significant differences between the two sprayers tested for preharvest treatment. Ozone-treated peaches had better environmental performance than the control. The application of ozone, following optimisation of concentration, can contribute to the postharvest management of brown rot in peaches.

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Predictive models as decision support systems for grape downy mildew, powdery mildew and ocratoxin A wine contamination in South Italy

F. Dalena[1], A. Agnusdei[1], R. Coronelli[1], R.M. De Miccolis Angelini[1], F. Spataro[1], D. Gerin[1], S. Pollastro[1], G. Perrone[2], K. Gialluisi[2], C. Dongiovanni[3], M. Carrieri[3], M. Di Carolo[3], E. Del Prete[4], V. Buono[4,5], M. Corsi[5], P. Battilani[6], M. Camardo Leggieri[6], F. Faretra[1]

[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Bari, Italy; [2]Istituto di Scienze delle Produzioni Alimentari del CNR, Bari, Italy; [3]Centro di Ricerca, Sperimentazione e Formazione in Agricoltura «Basile Caramia», Locorotondo, Italy; [4]Sysman Progetti e Servizi S.r.l., Bari, Italy; [5]AGRIS soc. coop., Bari, Italy; [6]Dipartimento di Scienze delle produzioni vegetali sostenibili, Università Cattolica del Sacro Cuore, Piacenza, Italy. E-mail: angelo.agnusdei@uniba.it

According to Sustainable Development Goals, new and more sustainable control strategies are highly desirable. Predictive models can improve crop protection management and rationalize the use of plant protection products. In this work, a three-year evaluation of forecasting models for the onset of primary infections of downy mildew (EPI and DMCast), powdery mildew (Pmaxacc), and the risk of Ochratoxin A (OTA) contamination (OTA-Grapes) was conducted. The reliability of the models was assessed through the application of Bayesian statistics, in comparison with data collected from over 160 table and wine grape vineyards, representative of the mesoclimatic heterogeneity of the Apulian and Calabrian grape growing areas. The limited occurrence of winter rainy events influenced the mechanistic model DMCast, which correctly predicted the absence of downy mildew, but showed a high rate of false negative predictions. Oppositely, the empiric model EPI consistently rightly predicted the onset of disease, although resulting in a high rate of false positive predictions. Based exclusively on temperatures, Pmaxacc frequently predicted the appearance of powdery mildew in areas with high temperatures during the growing season, even when the disease was undetected. The capability of the OTA-Grapes model to predict the risk of OTA contamination was poor under the assayed conditions; however, the numerous data collected will allow a calibration of the model, improving its reliability and may be helpful in developing maps of risk. These results highlight the need for calibration of forecasting models in different grape growing conditions, especially for empirical models.

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Gene expression dynamics in *Monilinia fructicola* and *Prunus persica* during the battle for infection revealed by dual RNA-Seq analysis

R.M. De Miccolis Angelini[1], D. Di Cosmo[1], M. Crudele[1], P.R. Rotondo[1], M. Romito[1], S. Pollastro[1], L. Landi[2], F. Faretra[1]

[1]Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy; [2]Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Ancona, Italy. E-mail: francesco.faretra@uniba.it

Monilinia fructicola is the most destructive pathogen among *Monilinia* species, causing significant yield losses mostly on stone fruits. In this study, the infection dynamics on *Prunus persica* fruits was explored by dual RNA-Seq. A total of 1,003,651,968 next-generation sequencing reads (2 x 100 bp, Illumina technology) were generated from peach fruits, inoculated or not with the pathogen, at 0, 12 and 24 hours post-inoculation and mapped to the reference genomes of *P. persica* and *M. fructicola*. By comparing inoculated *vs* uninoculated samples at different sampling times, 1,996 differentially expressed genes (DEGs) of *P. persica* and 5,063 DEGs of *M. fructicola* were identified (FC $\geq|2|$, FDR $\leq$ 0.05). Functional analysis of peach DEGs showed induction of defence responses, phytohormone signalling, glucosinolate and glutathione metabolism, cell wall macromolecule catabolism, and fungal polysaccharide degradation. Photosynthesis light reaction, starch/sucrose metabolism, and photorespiration were repressed. The mevalonate pathway related to sterol and terpenoid biosynthesis was activated, while the non-mevalonate pathway associated with photosynthetic isoprenoids was repressed. Defence-related genes identified included berberine bridge enzyme-like proteins, pathogenesis-related proteins, key genes for biosynthesis of antimicrobial compounds, hydrolytic enzymes, transcription factors, receptor-like and disease-resistance proteins. In *M. fructicola*, infection-induced toxin biosynthesis, peptidase activity, cell-wall degrading enzymes, monoxygenases,

dioxygenases, and oxidoreductases were upregulated, while biosynthetic and mitosis-related processes were downregulated. Candidate effectors, cell-death elicitors, fungal-specific transcription factors, stress response proteins, and other putative virulence factors were identified. This study provides insights into molecular interactions between *M. fructicola* and *P. persica*, highlighting potential targets for brown rot control.

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Multi-omics approaches to explore phenotypic and genetic diversity in the brown rot fungal pathogens *Monilinia fructicola, Monilinia laxa* and *Monilinia fructigena*

R.M. De Miccolis Angelini[1], L. Landi[2], S. Pollastro[1], D. Di Cosmo[1], M. Crudele[1], P.R. Rotondo[1], G. Romanazzi[2], F. Faretra[1]

[1]Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy; [2]Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Ancona, Italy. E-mail: francesco.faretra@uniba.it; g.romanazzi@univpm.it

Monilinia fructicola, Monilinia fructigena and *Monilinia laxa* are the main causal agents of brown rot and blossom blight, among the most critical diseases on pome and stone fruits, responsible for heavy losses both in the field and in postharvest. Following the introduction of *M. fructicola* into Europe, the plant pathogen spread widely and rapidly and became prevalent over the endogenous species *M. laxa* and *M. fructigena*. To improve knowledge of biology, evolutionary history, and diversity of these important plant pathogens, high-quality reference genomes and transcriptomes of *M. fructicola* Mfrc123, *M. fructigena* Mfrg269 and *M. laxa* Mlax316 were *de novo* assembled and compared. The study was focused on several key areas: (i) phylogenomic and synthenic relationships between the *Monilinia* genomes and those of the closely related species *Botrytis cinerea* and *Sclerotinia sclerotiorum*; (ii) the abundance and evolutionary dynamics of transposable elements; (iii) the genetic basis of mating type and the genome-wide occurrence and extent of Repeat-Induced Point (RIP) mutations; (iv) common and species-specific effectors, carbohydrate-active enzymes and secondary metabolite gene clusters; and v) gene expression profiling under different cultural conditions. The results highlighted several differences that might explain host plants and organ preferences distinguishing the three *Monilinia* species. Moreover, a metagenomic approach was used to investigate the mycovirome of *M. fructicola* in a worldwide collection of isolates from different hosts, which revealed a great abundance and diversity of mycoviruses infecting the fungus. A total of 32 positive-sense single-stranded (ss)RNA viruses and a new putative ssDNA mycovirus were identified and characterized.

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Development of a qPCR assay for ready-to-use in-field detection and quantification of resistance to SBI-class III fungicides in *Botrytis cinerea* using a portable real-time-PCR thermocycler

R.M. De Miccolis Angelini, C. Rotolo, S. Pollastro, F. Faretra

Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy. E-mail: stefania.pollastro@uniba.it

Botrytis cinerea Pers. is the fungal pathogen causing grey mould, one of the major diseases affecting yield and quality on numerous fruit and vegetable crops worldwide. The fungus is recognised as a high-risk plant pathogen for developing fungicide resistance, also due to intensive usage of fungicides with a single-site mode of action. For several fungicides, resistance development led to reduced effectiveness of grey mould control, and then continuous monitoring and the adoption of appropriate anti-resistance strategies should be implemented in disease management. In this study, a real-time quantitative polymerase chain reaction (qPCR) assay was developed for rapid in-field detection and quantification of *B. cinerea* isolates resistant to fenhexamid, a sterol biosynthesis inhibitor (SBI-III), using a Franklin® Three9 portable thermocycler for real-time PCR (Biomeme, Inc., Philadelphia, PA, USA). DNA was extracted from mycelium and conidia of wild-type strains, usually sensitive to SBI-III, and several mutants of the fungus showing high resistance (HydR3$^+$), carrying different point mutations in the *erg27* gene responsible for several amino acid changes at position 412 of the encoded 3-keto reductase enzyme (F412I, F412V, F412S, and F412C). Different DNA extraction protocols and rapid sample preparation methods were tested and compared. The qPCR assay proved to be sensitive (up to 10–50 pg of target DNA) and specific for quantitative detection of field resistance to SBI-III fungicides in *B. cinerea*. It provides a novel tool for fast and accurate in-field monitoring of plant pathogen populations, reducing time and labour requirements compared to traditional laboratory detection methods.

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)– MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4– D.D. 1032 17/06/2022, CN00000022).

Fungal endophytes in grapevines affected by trunk diseases in Georgia (Caucasus)

G. Del Frari[1,2], A. Sadallah[1], L. Ujmajuridze[3], M. Morgante[1,4]

[1]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via delle Scienze 206, 33100 Udine, Italy; [2]LEAF-Linking Landscape, Environment, Agriculture and Food-Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349–017 Lisboa, Portugal; [3]LEPL Scientific Research Center of Agriculture, 36B Marshal Gelovani Ave., 0159 Tbilisi, Georgia; [4]Istituto di Genomica Applicata, via Jacopo Linussio 51, I-33100 Udine, Italy. E-mail: giovanni.delfrari@uniud.it

All viticultural regions of the world host vineyards that experience symptoms associated with grapevine trunk diseases. The most evident symptoms include apoplectic events (sudden wilting of grapevine leaves often followed by plant death), tiger stripes in leaves, and a general loss of plant vigor and yield. These symptoms are increasingly reported in the Kakheti province of Georgia, the country's most important vine/wine region. In October 2023, three symptomatic grapevines (cv. Saperavi) were uprooted, sectioned, and wood fragments were used to isolate fungal endophytes. Examination of the perennial wood sections revealed wood discoloration, brown wood streaking, necrotic lesions, and white rot, while the annual wood displayed only wood discoloration and brown wood streaking. Using molecular tools, specifically sequencing the ITS1-ITS4 region, we identified several known wood pathogens. These included fungi associated with (i) the esca complex of diseases: *Phaeomoniella chlamydospora, Phaeoacremonium* sp., and *Fomitiporia mediterranea*; (ii) Botryosphaeria dieback: *Botryosphaeria dothidea* and *Diplodia seriata*; (iii) Phomopsis dieback: *Diaporthe ampelina*; as well as others like *Cadophora luteo-olivacea, Schizophyllum commune*, and *Stereum hirsutum*. Additionally, we identified ubiquitous genera such as *Aureobasidium* sp., *Alternaria* sp., *Cladosporium* sp., and *Penicillium* sp., and rare species such as *Xylogone sphaerospora* and *Biscogniauxia nummularia*. This is the first scientific report of grapevine trunk diseases and associated fungi in Georgia.

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Emerging fungal threats involved in forest decline in southern Italy: *Diplodia corticola, Diplodia quercivora* and *Neofusicoccum vitifusiforme* associated with cankers and necrosis of holm oak

C. Del Grosso[1,2], D. Palmieri[1], L. Marchese[1], L. Melissano[3], G. Lima[1]

[1]Department of Agricultural, Environmental and Food Sciences, University of Molise, 86100 Campobasso, Italy; [2]Institute for Sustainable Plant Protection, National Research Council (CNR), 70126 Bari, Italy; [3]Department of Agriculture, Environment and Rural Development, Sustainable Management and Protection of Natural and Forest Resources, Apulia Region, 70100 Bari, Italy. E-mail: carmine.delgrosso@ipsp.cnr.it; lima@unimol.it

The present study investigated the emergence of new plant diseases, focusing on the sudden declines of holm oak (*Quercus ilex* L.) in the Salento Peninsula (Apulia Region, Italy) observed since 2016. Extensive and periodic sampling, followed by laboratory analysis, revealed the presence of dangerous emerging phytopathogenic fungi associated with these declines. Morphological and molecular characterization, with ITS and TEF-1α sequencing, identified *Diplodia corticola, D. quercivora,* and *Neofusicoccum vitifusiforme* as the most frequently recovered fungal isolates. Among these fungal species belonging to the *Botryosphaeriaceae* family, *N. vitifusiforme* has never been reported as a pathogen on oak trees. Fungal species isolated with a lower frequency were *D. mutila, D. gallae,* and *N. parvum*. For phylogenetic analysis and pathogenicity tests, a representative morphotype was selected for each of the most frequently isolated species (*D. corticola, D. quercivora,* and *N. vitifusiforme*). In artificially inoculated holm oak plants, the tested fungal species confirmed their aggressiveness, causing severe cankers, extensive necrosis, and plant decline. Understanding the ecological roles of the recovered fungal species is essential for effective control and landscape conservation. It is crucial to emphasize preventive measures against environmental stressors and insect pests, as well as enhance forest resilience and monitor global trade for the spread of pathogens. Early detection of emerging plant pathogens using advanced molecular tools and promoting collaboration between scientists, policymakers, and stakeholders are crucial for the sustainable management of oak forests and their health safeguard.

Yeast volatile organic compounds: antifungal and nutraceutical effects on cultivated mushroom species

A. Di Francesco[1], E. Moret[1], M. Di Foggia[2]

[1]Department of Agriculture, Food, Environmental and Animal Sciences, University of Udine, Italy; [2]Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy. E-mail: alessandra.difrancesco@uniud.it

Yeast volatile organic compounds (VOCs) were investigated comprehensively for their effective biological control against plant fungal pathogens. In most cases, VOCs produced by yeasts positively affect crops, acting as antifungals or biostimulants. According to results obtained from *in vitro* assays, VOCs produced by *Aureobasidium pullulans* and *Metschnikowia pulcherrima* strains limited green mold without hindering the growth of *Pleurotus ostreatus, Lentinula edodes,* and *Cyclocybe cylindracea*, thus indicating beneficial effects of these VOCs on the growth and biochemical composition of mushroom crops. In specific tests of mycelial growth, VOCs produced by the antagonistic yeasts significantly inhibited the growth of *Trichoderma* spp. but did not affect mushroom mycelial growth. Conversely, *M. pulcherrima* VOCs significantly stimulated *L. edodes* mycelial growth. FT-IR spectroscopy on mushroom mycelia exposed to yeast VOCs allowed us to correlate stimulation of their mycelial growth with an increased protein and lipid content, determining interesting nutraceutical differences. Moreover, compounds emitted by *A. pullulans* and *M. pulcherrima* strains were identified by SPME-GC-MS, revealing that the alcohol class dominated the volatilomes, particularly in *A. pullulans*. In contrast, *M. pulcherrima* was the only yeast producing isobutyl acetate, often recognized as a plant growth promoter. Using different yeasts to create a composite volatilome could be advantageous for cultivating edible fungi because a variety of compounds could hinder fungal pathogens present in natural habitats. Further studies will be necessary to understand better the potential application of VOCs and their effect in the context of large-scale mushroom cultivation.

Fungal postharvest pathogens of apple in a climate change scenario: PRediction models, Epidemiological studies and sustainable control STrategies (PREST.APPLES)

A. Di Francesco[1], A. Sadallah[1], S. Mosca[2], A. Malacrinò[2], I. Martino[3], V. Guarnaccia[3,4], D. Spadaro[3,4], M. Camardo Leggieri[5], P. Battilani[5]

[1]Department of Agriculture, Food, Environmental and Animal Sciences, University of Udine, Italy; [2]Department of Agriculture, University of Reggio Calabria, Italy; [3]Department of Agricultural, Forest and Food Sciences, University of Torino, Italy; [4]Interdepartmental Centre for the Innovation in the Agro-Environmental Sector AGROINNOVA, University of Torino, Italy; [5] Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Piacenza, Italy. E-mail: alessandra.difrancesco@uniud.it

Apple is an economically important fruit crop worldwide, highly appreciated by consumers, and cultivated in various areas of northern Italy. Postharvest diseases of apple determine significant economic losses during storage. Even if fungicides applied before harvest can be effective against wound pathogens, several other plant pathogens with a long latency can remain quiescent on symptomless fruits, causing rots only after different months of storage. They include two major postharvest apple diseases: bull's eye rot (BER), caused by *Neofabraea vagabunda,* and white haze (WH), by *Tilletiopsis*-like basidiomycetes, a complex of fungal species belonging to the genera *Entyloma, Golubevia, Tilletiopsis.* PREST.APPLES project is based on an interdisciplinary consortium with the aim to characterize *N. vagabunda* and *Tilletiopsis*-like basidiomycetes epidemiology and to develop sustainable strategies to reduce food losses. A successful control of latent postharvest diseases requires a deep understanding of the causal agent infection process and its interaction with the host crop to define the proper disease management plan. The project will focus on the support of prediction modelling and biological tools to develop a sustainable approach to managing apple diseases.

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Fluorescent *Pseudomonas* in the tomato pith necrosis, biological activity and genomics

G. Dimaria[1], M.E. Massimino[1], A. Mosca[1], D. Nicotra[1], L.C. Miceli[1], C. Gaetani[1], F. Sansone[1], P. Bella[2], V. Catara[1]

[1]Department of Agriculture, Food, and Environment, University of Catania, Catania, Italy; [2]Department of Agricultural, Food and Forest Sciences, University of Palermo, Palermo, Italy. E-mail: vcatara@unict.it

Tomato pith necrosis (TPN) is a complex disease caused by different *Pseudomonas* species that have been reported in individual or mixed infections. In this study, we investigated four fluorescent *Pseudomonas* strains isolated from tomato plants affected by the disease (FPTPN strains) in different years and countries with a not yet resolved taxonomy. Based on partial *16S rRNA* and *rpoD* gene sequences analysis, all strains were members of *P. corrugata* subgroup within *P. fluorescens* complex, three of them having the highest similarities to *P. brassicacearum* and one to *P. viciae*. The co-inoculation of FPTPN strains with either or both *P. corrugata* and *P. mediterranea* increased, in some cases, the extension of pith necrosis. All but one FPTPN culture filtrates putatively contained cyclic lipopeptides (CLPs), the main virulence factors in *P. corrugata*, that exhibited antimicrobial activity against *Rhodotorula pilimanae* and *Bacillus megaterium*, sensitive to these compounds. In addition, the ability to produce *N*-acyl homoserine lactones (AHLs), quorum sensing (QS) signal molecules, was assessed for the three CLP-producing strains by observing violacein production in the biosensor *Chromobacterium violaceum* CV026, sensitive to short- and medium-chain AHLs. Three FPTPN strains determined the activation of *P. corrugata* AHL-QS system PcoI-PcoR, measured using *pcoI* (*luxI* homologue) mutant strain harbouring *pcoI::lacZ* promoter probe reporter construct as biosensor, thus suggesting an interspecies cross-talk mediated by AHL signal molecules. Draft genome sequences were obtained for insight into mechanisms putatively involved in virulence. Genome BLAST distance phylogeny analysis suggested that two strains belong to a novel species.

Evaluation of a *Lysobacter* biocontrol strain for the eco-friendly management of basil downy mildew

A. Dinesh Kothari[1,2], G. Puopolo[1,2]

[1]Center of Agriculture, Food, Environment, University of Trento, San Michele all'Adige, Italy; [2]Research and Innovation Centre, Department of Sustainable Agro-Ecosystems and Bioresources, Fondazione Edmund Mach, San Michele all'Adige, Italy. E-mail: amulya.dineshkothari@unitn.it

Sweet basil is an economically important annual herb cultivated in greenhouses and open fields under monoculture conditions. Basil downy mildew (BDM), caused by the oomycete *Peronospora belbahrii*, has been a devastating disease of sweet basil since its first report in Uganda in 1932. By the early 2000s, BDM had become the most significant basil disease worldwide, resulting in losses amounting to millions of dollars. Current BDM management strategies include developing resistant varieties, chemical control, and climate-management techniques, yet biological control remains underexplored. In this study, we evaluated the efficacy of *Lysobacter capsici* AZ78 (AZ78) in controlling BDM. AZ78 (10^9 CFU mL^{-1}) was sprayed manually to the plants until runoff. After 24 hours, the plants were inoculated with the pathogen (1×10^5 sporangia mL^{-1}) and incubated under suitable greenhouse conditions. Disease severity was assessed seven days later by calculating the leaf area covered by sporulation across all treatments. While untreated plants showed 85–90% disease severity, those treated with AZ78 demonstrated significantly lower disease severity, with control efficacy ranging from 85–95%. The study also investigated the induction of resistance mechanisms in plants. The untreated control showed very minimal defence induction. Unlike the positive control (mandipropamid), which showed no induction of defence mechanisms, AZ78-treated plants exhibited significant activation of plant defences, indicating an enhanced resistance response. In conclusion, AZ78 holds great potential as an effective biocontrol agent, offering a sustainable alternative to chemical treatments. Its ability to induce plant resistance further enhances its value in integrated plant protection strategies.

Comparison and development of an improved TaqMan qPCR assay for the detection and quantification of *Phytophthora cinnamomi* from soil samples

M.I. Drais[1], S. Turco[1], C. Morales Rodriguez[2], A. Vannini[2], A. Mazzaglia[1]

[1]Dipartimento di Scienze Agrarie e Forestali, Università degli Studi della Tuscia, Viterbo, Italy; [2]Dipartimento per l'innovazione nei sistemi biologici, agroalimentari e forestali, Università degli Studi della Tuscia, Viterbo, Italy. E-mail: drais@unitus.it

Alien Invasive Forest Phytophthoras (AIFPs) pose significant threats to the evergreen oak and chestnut ecosystems in southern Europe and the Mediterranean basin. These invasive agents, notably *Phytophthora cinnamomi,* are particularly destructive, causing root and stem cankers that lead to widespread decline and mortality of broadleaf species. *Phytophthora cinnamomi* is one of the world's most invasive plant pathogens, and it is very important to diagnose its presence in plants and soil using molecular tools. This study compared the performances of published qPCR assays to our newly developed assays targeting the Internal Transcribed Spacer (ITS) gene. Few of the existing primers were found to discriminate between *P. cinnamomi* and several newly described species, and for those that could do so, the sensitivity was inadequate. To accurately detect the presence and quantify *P. cinnamomi* in soil, we designed new primers and species-specific probes that allow reliable ITS gene amplification. When tested, the assay was able to detect the plant pathogen consistently at a limit of detection of 10 fg. The assay was also effective in identifying the pathogen in artificially infected samples, providing rapid detection in all symptomatic specimens. The developed qPCR assay represents a significant advancement in the detection of *P. cinnamomi* from soil samples, surpassing existing assays. This diagnostic tool ensures precise identification, offering enhanced capabilities for accurate diagnosis.

Effect of biocontrol agent applications on kiwifruit plants under Kiwifruit Vine Decline Syndrome infection pressure

P. Ermacora[1], S. Saro[2], C. Bernardini[1], S. Tomada[2], M. Fabro[2], M. Martini[1]

[1]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via delle Scienze 206, 33100 Udine, Italy; [2]Agenzia Regionale per lo Sviluppo Rurale del Friuli Venezia Giulia - ERSA Via Sabbatini 5, 33050 Pozzuolo del Friuli, Italy. E-mail: chiara.bernardini@uniud.it

Kiwifruit Vine Decline Syndrome (KVDS) has significantly impacted the Italian kiwifruit industry since 2012; although the aetiology remains uncertain, the oomycetes genera *Phytophthora* and *Phytopythium* have been frequently associated with KVDS. While waterlogging prevention and resistant rootstocks are effective strategies against KVDS in new plantations, existing plantations lack management strategies. To evaluate the impact of biocontrol agents on KVDS, an experimental trial was conducted on potted *Actinidia deliciosa* cv. Hayward plants transplanted in soil from KVDS symptomatic orchard. The following commercial products were tested: Amylo-X® LC (*Bacillus amyloliquefaciens* D747), Remedier (*Trichoderma gamsii* ICC 080 and *T. harzianum* ICC 012) and Micosat F® (a microbiological consortium). Additionally, the trial assessed the isolate Pa4A7 (*Pseudomonas asplenii*) from the University of Udine microbiological collection, along with vermicompost soil amendment and potassium phosphite (PK) foliar applications. All microbiological products enhanced the aerial growth of the plants in the following order: vermicompost > Amylo-X® = Remedier = Micosat F® > Pa4A7> untreated plants = PK. The experiment was stopped when 80% of the most affected groups of plants exhibited typical KVDS symptoms on the canopy. Irreversible wilting occurred in 88% of Amylo-X®-treated plants, 75% of Micosat F® and Remedier, and 50% of vermicompost-treated and untreated plants. Interestingly, Pa4A7- and PK-treated plants displayed no KVDS symptoms on the canopy and showed the highest protection as measured by root system expansion and integrity. Overall, the tested commercial biocontrol agents did not outperform the untreated control, highlighting the need to identify biocontrol agents with enhanced specificity and effectiveness for the KVDS/kiwifruit pathosystem.

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The use of microbial based inoculants for enhancing the salinity tolerance of tomato plants: preliminary results

A. Esposito[1], F. Vitali[1], M. Aragona[2], V. Scala[2], S. Mocali[1]

[1]Research Centre for Agriculture and Environment, Council for Agricultural Research and Economics (CREA), Florence, Italy. [2]Research Centre for Plant Protection and Certification, Council for Agricultural Research and the Analysis of Agricultural Economics (CREA), Rome, Italy E-mail: stefano.mocali@crea.gov.it; valeria.scala@crea.gov.it

The use of beneficial microorganisms as biostimulants and biopesticides is a potential environmentally friendly input to minimize biotic/abiotic stresses in horticulture. However, the efficacy of these products depends significantly on soil type and the native soil microbial communities. This work aims to promote salinity tolerance of tomato plants through fungal inoculants (*Trichoderma harzianum, Penicillium bilaie, Penicillium variabile, Aspergillus niger*) available from EXCALIBUR H2020 project. A preliminary trial was conducted to evaluate the salinity tolerance at different time points from the initial application by characterizing the soil microbiome associated with plants and assessing the level of plant response to salinity stress through oxylipins, a secondary metabolite produced in response to these stress conditions. Results indicated that salinity stress reduced the soil microbiome alpha biodiversity at 7 and 14 days following salinization. The response to stress simultaneously resulted in a shift in community structures between experimental conditions. Furthermore, oxylipins analysis showed an accumulation of salicylic acid and metabolites derived from 9-lipoxygenase pathway in stressed plants at 7 and 14 days after the start of the salinity treatment. The broth and agar dilution methods were employed to measure the *in vitro* antimicrobial activity of these four fungal inoculants against eight phytopathogenic bacteria and three phytopathogenic fungi of tomato plants. The agar plug diffusion method revealed that *T. harzianum* can inhibit the growth of all tested fungi. Whereas the growth of *Xanthomonas vesicatoria* and *Ralstonia solanacearum* was inhibited only by the culture filtrate of the fungal bioinoculum *P. variabile*.

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New findings on the etiology and epidemiology of Phomopsis cane and leaf spot disease on grapevine

G. Fedele[1], J. Armengol[2], T. Caffi[1], L. Languasco[1], N. Latinovic[3], J. Latinovic[3], M. León[2], G. Marchi[4], L. Mugnai[4], V. Rossi[1]

[1]Dipartimento di Produzioni vegetali sostenibili (DiProVeS), Università Cattolica del Sacro Cuore, Via E. Parmense 84, 20199 Piacenza, Italy; [2]Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022-Valencia, Spain; [3]Biotechnical Faculty, University of Montenegro, M. Lalica 15, 810000 Podgorica, Montenegro; [4]Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DiSPAA), Sezione di Patologia Vegetale ed Entomologia, Università di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: giorgia.fedele@unicatt.it

Phomopsis cane and leaf spot (PCLS) disease affecting grapevines (*Vitis vinifera* and *Vitis* spp.) has been historically associated with *Diaporthe ampelina*. Typical disease symptoms, with bleaching and black pycnidia, have also been associated with other *Diaporthe* spp. To evaluate this possible association, a molecular identification of *Diaporthe* strains isolated from grapevine canes showing PCLS symptoms in different geographic areas of southern Europe was performed. Their morphological characters, such as mycelium growth and production of pycnidia, alpha, and beta conidia, were studied in response to temperature. Finally, artificial inoculations on grapevine shoots and leaves were carried out. Based on the obtained results, the etiology of PCLS should be reconsidered; even though *D. ampelina* has been confirmed to be the most important causal agent of PCLS, *D. foeniculina* and *D. eres* were pathogenic, while *D. rudis* was not. Since *D. foeniculina* and *D. eres* produce both pycnidia and alpha conidia at lower temperatures than *D. ampelina*, the range of environmental conditions favourable for PCLS development should be then enlarged. Whether these differences in temperature requirements among species are also valid for the infection by conidia should be confirmed.

Insights on the biome composition of the microbial complex causing sour rot of grapes

G. Fedele, C. Brischetto, V. Rossi

Research Center on Plant Health Modelling (PHeM), Department of Sustainable Crop Production (DI.PRO.VE.S.), Università Cattolica del Sacro Cuore, Piacenza, Italy. E-mail: giorgia.fedele@unicatt.it

Sour rot (SR) is one of the main diseases affecting grapevine berries during ripening. It can cause severe yield losses and deterioration of wine quality. SR is caused by an etiologic complex of microorganisms that includes yeasts, bacteria, and filamentous fungi. A first literature systematic review on SR, considering 74 papers published between 1986 and 2023, identified 146 microorganisms isolated from SR-affected grapes (44.5% for yeasts, 34.3% for bacteria, and 21.2% for filamentous fungi). However, the published papers did not definitively clarify which species are primarily involved in the etiology of the disease and how environmental conditions affect disease development. In a second study, the fungal and bacterial microbiota of healthy and rotten ripe bunches were characterized across 47 epidemics (39 vineyards in five Italian grape-growing areas) over three years. Based on a linear discriminant analysis, the genera strongly associated with bunches showing SR symptoms are the bacteria *Orbus, Gluconobacter, Komagataeibacter, Gluconacetobacter*, and *Wolbachia* and the yeast *Zygosaccharomyces, Zygoascus, Saccharomycopsis, Issatchenkia*, and *Pichia*. Finally, a third study on the effect of environmental conditions on the composition of the microbiome associated with SR-affected grapes highlighted possible changes in SR-related microbiome in relation to the bioclimatic conditions of the grape-growing area. Further studies are needed to better understand the ecological requirements of the different microorganisms, defining their ecological niches to be used for understanding their geographical distribution and epidemiology, with the ultimate aim of improving SR control.

Multifunctional valorization of pigmented wheat genotypes for high-polyphenolic food production, Fusarium Head Blight resistance studies, and waste reuse for crop protection

L. Felici[1], C. Miccoli[1], S. Palombieri[1], F. Castellani[2], Pasquale de Vita[3], S. Francesconi[1], F. Sestili[1], M. Vitali[2], G.M. Balestra[1]

[1]University of Tuscia, Department of Agriculture and Forest Sciences (DAFNE), Viterbo, Italy; [2]Department of Public Health and Infectious Diseases, University of Rome "La Sapienza", p.le Aldo Moro 5, I-00185 Rome, Italy; [3]Council for Agricultural Research and Economics (CREA), Research Centre for Cereal and Industrial Crops (CREA-CI), S.S. 673, Km 25,200, 71122 Foggia, Italy. E-mail: balestra@unitus.it

In the last years, rising demand for healthy foods has focused attention on foods naturally rich in antioxidants, such as pigmented wheat genotypes, which accumulate anthocyanins in the grains. Purple wheat contains anthocyanins in the pericarp layer, while blue wheat in the aleurone layer. In this work, the results of a three year long project, focused on the multifunctional valorisation of these genotypes, are presented. Three purple pericarp and two blue aleurone bread wheat lines were tested for their milling quality features. Different milling processes were compared, obtaining flour with poor technological qualities but maintaining the antioxidants capacity from the grain to the final product. The same genotypes were used as a model to study the role of flavonoids in plant-pathogen interaction, considering the pathosystem wheat-*Fusarium graminearum*. For this purpose, different phytopathological assays were considered, including transcriptomics and metabolomics analysis. The results suggested the lack of a direct relationship between grain colour and resistance. However, pigmented genotypes showed a more activated flavonoids biosynthetic pathway in response to *F. graminearum* infection. Then, wheat milling process wastes (bran) were considered for the extraction of cellulose and the synthesis of cellulose nano-crystals (CNC). This nanomaterial was used as a carrier to improve the application of a known bio-based antimicrobial (chitosan hydrochloride). The obtained formulation was tested *in vitro*, showing high antifungal activity, and *in vivo*, both in controlled condition and in open field, where investigations are in progress.

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Evaluating fungal antagonists and natural active molecules towards the eco-friendly management of bacterial tomato pathogens

R. Fiorani[1], N. Pucci[1], E. Lahoz[2], V. Battaglia[2], C. Pane[3], S. Esposito[4], D. Naviglio[4], C.V.L. Giosafatto[4], V. Scala[1]

[1]Council for Agricultural Research and Economics (CREA)-Research Centre for Plant Protection and Certification, Rome, Italy; [2]Council for Agricultural Research and Economics (CREA)-Research Center for Cereal and Industrial Crops, Caserta, Italy; [3]Council for Agricultural Research and Economics (CREA)-Research Center for Vegetable and Ornamental Crops, Pontecagnano - Faiano, Italy; [4]Department of Chemical Sciences, University of Naples Federico II, Naples, Italy. E-mail: valeria.scala@crea.gov.it

The infections of bacterial diseases are increasing due to the lack of effective control measures, the concerns regarding both the impact on environmental and human health caused by copper-based products, and the outbreak of resistance. Alternative methods such as biocontrol agents, their secondary metabolites accumulated in the culture filtrate, and natural extracts to reduce the impact of chemical pollution and residues in soil and plants should be studied. The present study aimed to evaluate the *in vitro* effects of 12 isolates of *Trichoderma* spp., an isolate of *Clonostachys rosea,* and natural active molecules (AM) from orange wastes against the most relevant bacterial pathogens of tomato. The agar well diffusion technique and the broth microdilution assay were carried out as preliminary tests to evaluate the ability of the biocontrol agents and AM to inhibit bacterial growth at different times and concentrations. The diffusion method demonstrated that 5 *Trichoderma* strains inhibited the growth of *Xanthomonas euvesicatoria, X. perforans,* and *Ralstonia pseudosolanacearum* at different time points post inoculation. Preliminary results from the broth microdilution assay indicate that the dilution of culture filtrates and AM showed antibacterial activity against some of the bacteria tested. For instance, *T. atroviride* TAT3 and TAIC12, and *T. asperellum* TPE3 are effective against one of the Xanthomonadaceae and the Burkholderiaceae. The inhibitory antagonistic fungi and the effective AM will be evaluated *in vitro* to test their antibiofilm activity.

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European Commission can be considered responsible for them.

The genomic landscape of repetitive elements in *Colletotrichum*: implications for evolution and virulence

C. Fiorenzani[1], A. Menicucci[1], I. Martino[2], S. Iacono[1], M. Thon[3], S. Sukno[3], A. Vogan[4], C. Ratti[1], R. Baroncelli[1]

[1]Department of Agricultural and Food Sciences, University of Bologna, Viale G. Fanin 42, 40127 Bologna, Italy; [2]Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo P. Braccini 2, 10095 Grugliasco, TO, Italy; [3]Universidad de Salamanca, Instituto de Investigación en Agrobiotecnología (CIALE), Villamayor, Castilla y León, Spain; [4]Department of Organismal Biology, Uppsala University, Norbyv. 18D 75236 Uppsala, Sweden. E-mail: riccardo.baroncelli@unibo.it

Repetitive elements (REs), especially transposable elements (TEs), are key players in fungal genome evolution. They contribute to genetic diversity through mutations and rearrangements, leading to new traits such as drug resistance and altered virulence in pathogens. REs can also affect gene expression and genome architecture through amplification, deletion, and epigenetic modifications. The two-speed genome model explains how RE-rich regions evolve rapidly, harbouring virulence-related genes and driving adaptation in the co-evolutionary arms race with hosts.

This study investigated the distribution of REs across *Colletotrichum* species and their correlation with lifestyle, adaptation, host range, and virulence. Analysing RE distribution and dynamics can aid in elucidating species boundaries and unveiling the connections between biological features and phylogenetic relationships within this diverse group of plant pathogens. We collected high-quality genomes from 50 isolates across 10 of 20 *Colletotrichum* species complexes. Using an integrated bioinformatic approach, we analysed REs and characterized gene families crucial for host adaptation (candidate effectors, biosynthetic gene clusters, etc.). The results revealed a highly variable landscape of RE abundances and distributions within the same species complex. Additionally, dispensable chromosomes influenced the number of retroelements and DNA transposons across isolates. This project aimed to explore the diversity of REs across *Colletotrichum* genomes and propose an automated, standardized pipeline applicable to other fungal genera.

Effect of different agronomical management strategies on Fusarium head blight species complex composition: testing of novel bio-based agrochemicals for sustainable control strategies

S. Francesconi[1], A. Marocchini[1], P. Colombatto[2], S. Maruccia[2], M. Blandino[2], T. Bertuzzi[3], G.M. Balestra[1]

[1]Dipartimento di Scienze Agrarie e Forestali (DAFNE), Università della Tuscia, Via San Camillo de Lellis snc, 01100 Viterbo, Italy; [2]Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Università degli Studi di Torino, Largo Braccini 2, 10095 Grugliasco, Italy; [3]Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti (DIANA), Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122, Piacenza, Italy. E-mail: francesconi.s@unitus.it, balestra@unitus.it, massimo.blandino@unito.it

Fusarium head blight (FHB) is one of the most devastating fungal diseases for wheat, caused by diverse *Fusarium* spp. FHB is managed by the application of synthetic fungicides, which are under the spotlight, because of their environmental concern, broad range toxicity for non-target organisms, and selection of resistant *Fusarium* strains. FHB severity and mycotoxin accumulation are influenced by the composition of *Fusarium* populations, which change in relation to climatic regions and agronomical practices. We performed a survey of *Fusarium* spp. from experimental fields located in two areas of the Piedmont Region, where the application at wheat heading of five biological agrochemicals, based on chitosan, basalt flour and beneficial microorganisms, have been compared to the treatment with prothioconazole and an untreated control. We isolated 486 fungal colonies, morphologically grouped into 194 morphotypes. The molecular analysis of the TEF sequence individuated 172 *Fusarium* isolates, where: 108 were *F. graminearum*, 37 were *F. avenaceum*, 11 were *F. proliferatum*, 11 were *F. verticillioides*, two were *F. incarnatum*, two were *F. subglutinans*, one was *F. temperatum*. FHB severity and DON content have been reduced in the plots treated with the biological agrochemicals compared to the untreated control but not as much as to prothioconazole, highlighting their ability to be used as management strategies. Ongoing analyses are focused on correlating the *Fusarium* populations to the agronomical strategies, establish a Real-Time qPCR assay to quantify the total *Fusarium* spp. and the single species, characterize the isolated *Fusarium* spp. for their pathogenicity and putative resistance to synthetic fungicides.

Farina di Basalto® - based formulations act as multi-site agrochemicals against three impacting plant pathogens while displaying biocompatibility on wheat, olive and grapevine plants: a story from *in vitro* to in field applications

S. Francesconi[1], A. Riccini[2], T. Salvati[1], F. Primavera[2], G. Pizzuti[2], F. Ruggeri[2], G.M. Balestra[1]

[1]Dipartimento di Scienze Agrarie e Forestali (DAFNE), Università della Tuscia, Via San Camillo de Lellis, snc, 01100, Viterbo, Italy; [2]Basalti Orvieto s.r.l., Località il Cornale snc, 05014 Castel Viscardo, Italy. E-mail: francesconi.s@unitus.it, balestra@unitus.it

Farina di Basalto®-based formulations are corroborants admitted in organic agriculture and demonstrated fortifying properties on several crops. We evaluated two formulations: Farina di Basalto® XF, a micronized flour of volcanic origin rich in silicon, potassium, iron, calcium, and magnesium, and Micobas®, in a liquid and in a lyophilized form, a product based on a combination between Farina di Basalto® and a consortium of beneficial microorganisms. This combination restores nutrients for soil fertility and can also potentially protect crops respect to different plant pathogens. We tested the formulations *in vitro, in vivo* and in field 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 on olive tree. *In vitro* assays demonstrated that Farina di Basalto® XF acts as a multi-site agrochemical, since it showed both direct growth inhibition and indirect interactions with conidia and bacterial cells, suggesting its ability to block the first step of the infection stages by favouring cells flocculation and decreasing their adhesion on the host surface. Micobas® inhibited Bc by producing soluble antimicrobial compounds. On plants (*in vivo* and in field tests), both formulations demonstrated to act as elicitor-like molecules, by boosting innate immunity, especially in grapevine, and great biocompatibility on the three different plant species. Ongoing and future experiments will be focused on evaluating the impact of the treatment on final products (grains, grapes and olives), with a focus on yield and quality parameters.

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Plant microbiome and plant health: toward a predictive model based on machine learning

E. Francomano, M.M. Aci, L. Schena, A. Malacrinò

Department of Agriculture, Università degli Studi Mediterranea di Reggio Calabria, Loc. Feo di Vito, 89124 Reggio Calabria, Italy. E-mail: edda.francomano@unirc.it

Plants are associated with diverse microorganisms, which play crucial roles in plant nutrition and defense against biotic and abiotic stresses, thus significantly influencing plant growth and survival. However, plants are constantly exposed to the risk of pathogen attacks, which can reduce their viability and negatively impact agricultural production. Considering that the plant microbiome is crucial to plant health and productivity, its management and the development of microbiome-based products are emerging as promising alternatives to chemical interventions in agriculture. In this context, we studied how the plant microbiome changes in response to different pathogens. Our goal is to develop a Machine Learning model capable of detecting hidden patterns in microbiome dynamics and predicting the presence of communities detrimental to plant health. To achieve this goal, we exposed tomato plants (cultivar Moneymaker) to five pathogens: one oomycete (*Phytophthora nicotianae*), three fungi (*Alternaria alternata, Fusarium oxysporum* f. sp. *lycopersici*, and *Rhizoctonia solani*) and one bacterium (*Pseudomonas syringae* pv. *tomato*). Results indicate that exposure to these plant pathogens induces significant changes in the composition of the plant microbiome. Data analysis suggests the possibility of identifying specific microbial indicators associated with the presence of the pathogens. These alterations could be exploited to develop new computational strategies for integrated plant health management, monitoring and managing them more sustainably and effectively, reducing dependence on chemical pesticides.

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Control of potato late blight by fungal secretome of *Trametes versicolor* as a biopesticide against *Phytophthora infestans*

R.S. Fratini[1], M. Beccaccioli[1], V. Cecchetti[1], M. Verni[1], A. Brannetti[1], M. Brus-Szkalej[2], C.G. Rizzello[1], L.J. Grenville-Briggs[2], L. Faino[1], M. Reverberi[1]

[1]Department of Environmental Biology, Sapienza University of Rome, 00185 Rome, Italy; [2]Department of Plant Protection Biology, Swedish University of Agricultural Sciences, 230 53 Alnarp, Sweden. E-mail: rositasilvana.fratini@uniroma1.it

Potato late blight caused by the oomycete *Phytophthora infestans* is one of the most destructive diseases affecting potato crops worldwide. Managing potato late blight primarily through chemical control is indeed effective but leads to significant health and environmental impacts. For this reason and according to EU directives, sustainable alternatives, including biocontrol agents, are needed. In this work, we studied the effect of the cultural filtrate (CF) obtained from the *in vitro* culture of the ligninolytic basidiomycete *Trametes versicolor* as a biopesticide candidate against *P. infestans*. CF, the fungus's exo-secretome, consists of bioactive molecules with antimicrobial properties that can be tested in crude form or by separating its components by protein precipitation and polysaccharide purification. By performing *in vitro* assays, we evaluated the sporangia germination and mycelium growth of *P. infestans* when CF, protein, or polysaccharide fractions were added to the media. Moreover, to test the effect of CF against late blight symptoms, we used a detached leaf assay on potatoes by spraying the plants with CF, infecting them with *P. infestans,* and evaluating the lesions. Interestingly, we show that crude CF of *T. versicolor* can affect the growth of *P. infestans*, by inhibiting both sporangia germination and mycelium growth, suggesting that some secreted proteins can have antimicrobial activity. Further investigation on the compounds' modes of action and in a potato-*P. infestans* pathosystem will help to assess how to apply crude CF or only bioactive components as biopesticides for an integrated pest management strategy for potato disease.

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Exploring TAL effectors diversity in common bacterial blight causative agents as a tool to identify targets for improving resistance in common bean

A. Gaiti[1], E. Avite[2], C. Pizzatti[1], M. Saracchi[1], D. Bulgari[1], P. Cortesi[1], C.M. Pozzi[3], A. Kunova[1], G.M. Balestra[4], M. Pasquali[1]

[1]Department of Food, Environmental and Nutritional Sciences. University of Milan, Italy; [2]Blumen Group Spa, Piacenza, Italy; [3]Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, University of Milan, Italy; [4]Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy. E-mail: angelo.gaiti@unimi.it

Common bacterial blight (CBB), caused by *Xanthomonas* spp., is responsible for significant yield losses in common bean (*Phaseolus vulgaris*). Transcription Activator-Like (TAL) effectors produced by these pathogens are pivotal in disease development by modulating host plant gene expression. This study explores the diversity of TAL effectors in *Xanthomonas* spp. strains responsible for CBB, aiming at identifying potential targets for developing resistant common bean cultivars. We sequenced a strain isolated in Italy using Nanopore platform and predicted its TAL effector genes. Moreover, we predicted TAL effector genes using all NCBI available genome assemblies obtained through long-read sequencing technologies. Our preliminary results led to the discovery of several new TAL classes in *X. phaseoli* pv. *phaseoli*, indicating a high diversity of TAL effectors among the strains. These new TAL classes are putatively targeting key genes involved in plant defence responses. Future validation of the targets identified in this study will provide crucial insights for breeding varieties with enhanced resistance. Target host genes will be explored in the future for their function in a mutagenized population recently developed on a commercial variety.

In vitro activity of essential oils against *Verticillium dahliae*

M. Gallo[1], V. Lahoud[1], M. Carlucci[2], F. Valentini[1], A.M. D'Onghia[1], F. Nigro[2]

[1]Centre International de Hautes Etudes Agronomiques Méditerranéennes Bari (CIHEAM Bari), Via Ceglie 9, 70010 Valenzano, Bari, Italy; [2]Department of Soil, Plant, and Food Sciences (Di.S.S.P.A.), University of Bari - Aldo Moro 70126, Bari, Italy. E-mail: gallo@iamb.it

Verticillium dahliae is a significant threat to many economically important crops and is challenging to manage.

Biological and sustainable control methods, like essential oils (EOs) from thyme, oregano, rosemary, and sage, show promise against this plant pathogen. This study examined the antifungal effects of these EOs on *V. dahliae* by testing their ability to inhibit mycelial growth and reduce the viability and germination of conidia and microsclerotia (MS) *in vitro*. EOs were evaluated at concentrations of 3%, 0.6%, and 0.3%. Mycelial growth inhibition was assessed after 5 and 7 days by measuring the inhibition halos around the EOs. Conidial germination was observed at 4, 8, and 48 hours of incubation. MS germination at 4, 10, 24, and 72 hours post-treatment (hpt). The results showed that thyme and oregano EOs at 3% concentration were highly effective, completely stopping mycelial growth after 5 and 7 days. Additionally, thyme and oregano EOs at 3% reduced conidial germination by 77.8% and 74.5%, respectively. Rosemary and sage EOs at 3% reduced MS germination by 41.47% and 49.03% at 4 hpt, while thyme at 0.3% and oregano at 0.6% reduced MS germination by 42.74% and 33.52% at 24 hpt. These findings indicate that thyme and oregano EOs have significant fungicidal and fungistatic properties. Future studies are needed to confirm these effects in plants and understand their impact on MS. Plant EOs offer a promising natural alternative to chemical fungicides, providing a safer, environmentally friendly, and user-friendly solution for plant protection and the development of sustainable agricultural practices.

Impacts of *Scaphoideus titanus* disturbance vibrational signal on grapevine defence mechanisms

S. Galvagni[1,2], R. Nieri[1,3], V. Mazzoni[2], M. Perazzolli[1,2]

[1]Center Agriculture Food Environment (C3A), University of Trento, Via E. Mach 1, 38098 San Michele all'Adige, Trento, Italy; [2]Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38098 San Michele all'Adige, Trento, Italy; [3]Department of Mathematics, University of Trento, Via Sommarive 14, 38123 Povo, Italy. E-mail: stefano.galvagni-1@unitn.it

Plants react to mechanical stimuli, which can induce defence mechanisms. For example, substrate-borne vibrations emitted during chewing by caterpillars of *Pieris rapae* can induce defence responses in *Arabidopsis thaliana*. A new environmentally-friendly approach, called vibrational mating disruption, is under development against the leafhopper *Scaphoideus titanus* on grapevine. This method involves the transmission to plants of a disturbance vibrational signal to interfere with the mating communication of the insects. This study aims to analyse the effects of the disturbance vibrational signal on defence mechanisms and physiological processes of grapevine plants. The disturbance vibrational signal was transmitted to potted grapevine plants cv Pinot Noir for 53 days without interruption under controlled conditions. As a control, plants of the same cultivar were left untreated. To simulate the field setting of disturbance noise transmission, treated plants were in contact with a scaled trellis system, where metallic wires were vibrated using a shaker. Stomatal conductance, chlorophyll content, photosynthetic activity, and growth parameters were monitored during the treatment. Leaf samples were collected at four time points (1 day, 16 days, 28 days, and 50 days), and RNA was extracted from leaves of treated and control plants. Following reverse transcription, the expression analysis of defence-related genes was carried out by quantitative real-time PCR. The results will provide better insights into plant responses to substrate-borne vibrations and the modulation of genes associated with defence mechanisms against biotic stress.

Severity of *Puccinia triticina* in wheat varieties and its management in northern Sinaloa, Mexico

E. García-León[1], R. Hortelano-Santa Rosa[2], J. Huerta-Espino[1], H.E. Villaseñor-Mir[1]

[1]Campo Experimental Valle del Fuerte, INIFAP, Juan José Ríos, Sinaloa, México; [2]Campo Experimental Valle de Mexico-INIFAP, Texcoco, Mexico. E-mail: garcia.elizabeth@inifap.gob.mx

Wheat (*Triticum* spp.) is one of the three major cereals produced worldwide, and Mexico ranks third as an exporter of durum wheat. However, leaf rust caused by *Puccinia triticina* is the most significant disease in all wheat-growing areas, particularly in the northwest region of Mexico. During the autumn-winter 2023–2024 cycle, 66 differential lines and commercial wheat varieties, constituting the Rust Trap Nursery, were established in experimental plots of 2 rows of 1 meter per genotype in two locations (Guasave and Ahome, Sinaloa, Mexico). The genotype `Morocco' was planted around the periphery of the trials as a naturally susceptible rust spreader. Disease severity was assessed in the field using the modified Cobb scale every 7 days from tillering to flowering, and the resistance/susceptibility response was recorded. The differentials *Yr33, Yr37*, and the varieties 'Ravi F2021', 'Noroeste C2021', 'Noreste F2018', 'Rio Bravo C2018', 'Don Goyo C2019', 'Quetchehueca Oro C2013', 'Baroyeca Oro C2013', and 'Barobampo C2015' maintained their resistance in both locations. Meanwhile, the three varieties with the largest cultivated area in northern Sinaloa, Mexico, such as 'Ciano M2018' and 'Cirno

C2008’, reached percentages of 2% and 5% in Ahome, and 2% and 10% in Guasave. In contrast, the variety ‘Borlaug 100’ remained resistant in Guasave but showed moderate resistance (2% severity) in Ahome. The establishment of trap nursery technology provides an opportunity to identify early the ideal conditions for rusts in susceptible varieties. This proactive approach allows for timely interventions and better management practices for mitigating the impact of *Puccinia triticina*.

Chemical control of wheat leaf rust caused by *Puccinia triticina* in northwestern Mexico

E. García-León[1], R. Hortelano-Santa Rosa[2], H.E. Villaseñor-Mir[1], H.G. Palafox-Castro[3], F. Rodriguez-Novoa[1]

[1]Campo Experimental Valle del Fuerte, INIFAP, Juan José Ríos, Sinaloa, México; [2]Campo Experimental Valle de Mexico-INIFAP, Texcoco, Mexico; [3]Universidad Autónoma de Occidente, Unidad Regional Los Mochis, Sinaloa, Mexico. E-mail: garcia.elizabeth@inifap.gob.mx

The wheat (*Triticum* spp.) production area in northwestern Mexico faces phytosanitary issues that, combined with a limited varietal catalog, make the crop susceptible to epidemics, primarily caused by leaf rust (*Puccinia triticina*). Consequently, fungicide applications are necessary to minimize losses due to leaf rust. The objective was to evaluate the biological effectiveness of eight fungicides (alone and in combination) during the 2022–2023 autumn-winter cycle on the susceptible variety ‘Morocco’ at the flowering stage, which reached 100% severity under natural conditions. The study was conducted using a randomized complete block design with four replications. Treatments were applied using a 20 L capacity motorized backpack sprayer. Each treatment included the adjuvant Break Thru® at 0.05%. Rust severity was evaluated using the modified Cobb scale at 7, 14, 21, 28, and 35 days after application, and the efficacy percentage of the products was calculated using Abbott’s formula. The treatments with the highest effectiveness percentages were: Propiconazole + Benzovindiflupyr, Azoxystrobin + Cyproconazole, and Azoxystrobin + Propiconazole with 94%, 90%, and 85% control, respectively. On the other hand, the fungicides Azoxystrobin+Flutriafol and tebuconazole showed the lowest effectiveness in controlling leaf rust, with 57 and 53% control, respectively. These results highlight how it is important to evaluate the effectiveness of chemical products every year to make rational use of them and avoid losses due to drift and excessive environmental pollution.

Analysis of the biofilm metabolome of *Xylella fastidiosa*

A. Gaspari[1], A. Staropoli[2], E. Santilli[3], V. Scala[4], L. Di Costanzo[2], M. Lorito[2], F. Vinale[1]

[1]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, 80138 Naples, Italy; [2]Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; [3]Council for Agricultural Research and Agricultural Economy Analysis, CREA · Research Centre for Olive Growing and Olive Oil Industry, 87036 Rende, Italy; [4]Council for Agricultural Research and Agricultural Economy Analysis, CREA Plant Defense and Crop Protection, 00184 Roma, Italy. E-mail: anna.gaspari@unina.it

Xylella fastidiosa is a Gram-negative xylem-limited bacterium responsible for significant economic losses in agriculture due to its destructive impact on crops. Initially identified on the American continent, this plant pathogen has recently spread to Europe, causing severe outbreaks, particularly in olive trees in the Mediterranean region. The aim of the study is to characterize biofilms produced by *X. fastidiosa* subsp. *pauca* to define this self-produced matrix attached to an inert or living surface. The experimental approach involves the combined use of liquid/solid and liquid/liquid extractions of microbial molecules, the metabolome analysis through both liquid and gas chromatography-mass spectrometry (LC/MS and GC-MS), and scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS). The metabolites present on biofilm mainly belong to the following class of natural compounds: i) fatty acid (*i.e.*, hexadecanoic acid, octadecanoic acid); ii) fatty acid derivatives (*i.e.*, hexadecanamide, octadecanamide, octadecenamide); iii) carbohydrates (*i.e.*, D-pinitol, inositol, D-fructose, glucose, sucrose); iv) and diketopiperazines (*i.e.*, cyclo(L-prolyl-L-valine), cyclo-(L-Arg-D-Pro)). The identified molecules on biofilm are present in different amounts and are involved in several interaction processes with other microbes or plants. Moreover, to assess surface analysis and elemental chemical composition, SEM combined with EDS is ongoing on dried particles of *X. fastidiosa* subsp. *pauca* biofilm. Characterization and developing strategies to control or exploit biofilms are important areas of research with broad applications in agriculture.

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Characterization of cattle manure vermicompost microbial communities and biocontrol potential of associated bacterial isolates

D. Gerin, G. Di Cuia, R.M. De Miccolis Angelini, V. Montilon, A. Agnusdei, P. Filannino, S. Pollastro, F. Faretra

Department of Soil, Plant and Food Sciences, University of Bari, Bari, Italy; E-mail: stefania.pollastro@uniba.it

The microbial composition of cattle manure vermicompost (CMV) obtained using *Eisenia fetida* and *E. andrei*, was determined by microbiological, molecular, and metabolic analysis. The CMV biocontrol potential and the CMV-associated microorganisms were evaluated against different fungal pathogens by dual culture assay. Fungi were poorly represented conversely to bacteria. On nutrient agar (NA), the bacterial community was determined in 1.37×10^7 CFU/g of dried CMV, and it was represented by 19 different morphotypes that, according to the BLASTn analysis of the partial 16S rDNA sequence and metabolic profiling, belonged to the classes Actinomycetes (69.5%), Bacilli (21.3%), Gamma proteobacteria (7.8%) and Flavobacteria (1.4%). On starch casein agar (SCA), the bacteria were 5.09×10^7 CFU/g, and nine different morphotypes belonging to the classes Gamma proteobacteria (91.7%) and Actinomycetes (8.3%) were identified. According to metagenomic analysis, bacteria (95.0%) were confirmed to be the most predominant kingdom, followed by Archaea (3%), Eukaryota (0.22%), and Viruses (0.04%). Preliminary *in vitro* experiments against *Botrytis cinerea* suggest a possible biocontrol potential of CMV due to its associated microorganisms. In dual culture, out of the 28 bacteria isolated from NA and SCA, were effective against 13 isolates of *Cylindrocarpon destructans*, 8 of *Fusarium oxysporum* f. sp. *radicis-lycopersici*, 5 of *Rosellinia necatrix,* 3 of *Sclerotinia sclerotiorum* and 25 of *Verticillium dahliae. Pseudomonas aeruginosa* DiSSPA_PA1 showed the best biocontrol potential included in the range 41% (*F. oxysporum* f. sp. *radicis-lycopersici*) to 81% (*V. dahliae*). Results achieved in this work suggest that vermicompost can be an additional tool in integrated and organic pest management.

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Antifungal effect of stilbene deoxyrhapontigenin against rice blast pathogen

S. Ghosh, C. Pinna, D. Dozio, C. Pizzatti, S. Princiotto, P. Cortesi, S. Dallavalle, A. Pinto, A. Kunova

[1]Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, 20133 Milan, Italy. E-mail: sharmila.ghosh@unimi.it

Among fungal plant diseases, *Pyricularia oryzae* is considered one of the most relevant threats due to its economic and scientific impact. Indeed, *P. oryzae* causes blast disease in many important crop plants, including rice, and it is responsible for the annual loss of more than 30% of the rice harvest. Management of rice blast disease in Europe relies primarily on strobilurin fungicides. The situation became alarming recently as strobilurin-resistant populations emerged in Italy, and no valid alternatives to strobilurins are currently available. Stilbenes are well known for their ability to act as phytoalexins, low molecular weight compounds that are synthesized *de novo* in plants in response to microbial plant pathogens and rapidly accumulate at the infection sites. We evaluated a variety of stilbenes against strobilurin-sensitive and -resistant strains of *P. oryzae* for their ability to inhibit mycelium growth and spore germination. Deoxyrhapontigenin (DRHAP), a monomeric methoxylated stilbene, inhibits mycelium growth of both strobilurin-sensitive and -resistant strains up to 80% at 200 μM concentration, while no inhibition was observed for its demethylated analogue resveratrol. The 50% inhibition of mycelial growth (ED_{50}) was obtained at a concentration range 122.3–237.9 μM for tested strains. Moreover, DRHAP completely inhibited spore germination and appressorium formation at 100 μM concentration. Investigations about the molecular mechanism involved in DRHAP activity are currently ongoing.

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Comparative genomics analysis of Flavescence Dorée phytoplasma strains in grapevines from the Trentino province

Z. Golestani Hotkani[1,2], G. Calia[2,3,4], D. Micheletti[2], A. Cestaro[5], M. Brilli[1], M. Moser[2]

[1]Department of Bioscience, University of Milan, Milan, Italy; [2]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy; [3]Faculty of Agricultural, Environmental and Food Sciences, Free University of Bolzano, Bolzano, Italy; [4]INRAE, Institut Sophia Agrobiotech, Université Côte d'Azur, CNRS, Sophia-Antipolis,

France; [5]Technological Transfer Centre, Edmund Mach Foundatio, San Michele all'Adige, Italy. E-mail: zahra.golestani@fmach.it

Phytoplasmas are phytopathogenic, phloem-inhabiting bacteria transmitted by phloem-feeding insects, causing significant economic losses in crop plants. Flavescence dorée (FD) is a severe epidemic disease of grapevines in Europe caused by FD phytoplasma (FDp) transmitted by the leafhopper *Scaphoideus titanus*. Control of FD relies only on insecticides against *S. titanus* and the uprooting of infected plants. No fully resistant grapevines exist, thus understanding phytoplasmas pathogenesis and genomics can be crucial for developing alternative defense strategies. Over the past decade, whole genome sequencing of multiple phytoplasma strains enhanced our understanding of their biology. Here, we reconstructed the genomes of four FDp subtypes from symptomatic Chardonnay, Pinot gris, and Pinot Noir varieties in the Trentino province using Oxford Nanopore long-read sequencing. These subtypes belong to the 16SrV-C and FD2 phytoplasma strain clusters. Comparative genomics with all available phytoplasma genomes (NCBI) enabled us to analyse gene content dynamics, cluster strains by gene similarity, and construct phylogenetic trees using core gene concatenation. The assembled genomes were collinear and consistent in function compared to the available reference genome. However, all strains had SNPs and indels with non-random SNP distribution. A 365,541 bp region inversion was found in the Chardonnay strain, consistent in gene orientation and function with homologous regions in other strains. Key Mollicutes marker genes and potential mobile units (PMUs) were absent, indicating minimal genomic rearrangements. Our genomic analyses of four FDp subtypes highlight both conserved features among phytoplasmas and specific characteristics unique to the FDp strains in Trentino Province.

Study of the mechanism of action of Tramesan, an exo-polisaccharide from *Trametes versicolor*, as plant resistance inducer in *Arabidopsis thaliana*

G. Gramegna, M. Rizzato, E. Romanelli, V. Cecchetti, M. Reverberi

Department of Environmental Biology, Sapienza University of Rome, Italy. E-mail: massimo.reverberi@uniroma1.it

The intensive use of agrochemicals to counteract plant pests leads to the emergence of resistant plant pathogen populations and poses several risks to the environment and human health. The European Regulation 128/2009 governing the use of pesticides has removed several products from the market, promoting the employment of non-synthetic compounds in agriculture. In this context, Tramesan, an exo-polysaccharide derived from the basidiomycete *Trametes versicolor*, is emerging as a natural alternative to protect crops. In fact, in addition to strongly inhibiting aflatoxin biosynthesis by the mycotoxigenic fungus *Aspergillus flavus*, it has been previously demonstrated that Tramesan enhances the resistance of wheat against Septoria Leaf Blotch Complex (SLBC) through the induction of plant defence responses. However, the mechanism by which Tramesan elicits plant defences remains unclear. Here, we explored the mechanisms underlying the action of Tramesan in the model plant *Arabidopsis thaliana*. The results obtained indicate that treatment with Tramesan enhances the resistance to *Botrytis cinerea* in Arabidopsis leaves. Tramesan, other than slightly inhibiting the growth of *B. cinerea*, has the ability to induce Arabidopsis defence responses, such as induction of defence genes and H_2O_2 production. Moreover, our results suggest that Tramesan acts as a priming agent by enhancing chitin-induced gene expression in Arabidopsis seedlings. In conclusion, this work provides a starting point for further molecular analysis that will allow us to clarify the mode of action of Tramesan and therefore to develop sustainable strategies for crop protection.

Shifts in soil microbiota induced by biofumigation with *Brassica* spp. in melon cultivation

A. Grottoli[1], L. Orzali[1], M.T. Valente[1], L. Pirone[1], D. Maini[2], C. Papalini[2], A. Haegi[1], A. Infantino[1]

[1]Research Center for Plant Protection and Certification, Via C.G. Bertero 22, 00156 Rome, Italy; [2]Regional Agency for the Development and Innovation of Agriculture in Lazio. E-mail: alessandro.grottoli@crea.gov.it

In terrestrial ecosystems, soil microorganisms are crucial for managing and recycling organic matter and the nutrient flow between different trophic levels. Their activities significantly affect plant growth and soil vitality. Within the framework of the Regional Project EcoDif, two years of biofumigation with green manure and pellets of *Brassica* spp. has been used in the greenhouse as an efficient and sustainable control method against soil-borne pathogens of melon. To assess changes in soil microbiota, the fungal microbial community has been evaluated by the massive and parallel amplicon sequencing of the fungal ITS region using the PacBio platform. A protocol for eDNA extraction was developed to secure high-quality DNA required for further molecular processes leading to amplicon sequencing. Over 2400 Amplicon Sequence Variants (ASV) were identified after blasting on the UNITE database. Although no diseases were observed in the field, the beta diversity clearly evidenced a substantial shift in the soil microbiota over time, clearly differentiating between biofumigated and control soils, showcasing the significant potential of the technique. Ascomycota was the most represented phylum with a % of ~70–80%, while *Aspergillus, Acremonium* and *Wallemia* were the most reduced genera. Long-term experiments are needed to monitor further changes in soil fungal populations and define a good microbiota within the framework of integrated pest management strategies.

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Epidemiological studies of monosporic strains of *Venturia asperata* from Trentino and South Tirol region

V. Gualandri[1,2]

[1]Centre for Technology Transfer, Fondazione Edmund Mach, via E. Mach 1, 38098 San Michele all'Adige, Italy; [2]Center of Agriculture, Food, Environment, University of Trento, 38098 San Michele all'Adige, Italy. E-mail: valeria.gualandri@fmach.it

After its first appearance in Italy in 2012 (Emilia-Romagna), *Venturia asperata* was recently discovered in the Province of Trento (2018) and in South Tyrol (2019), in northern Italy. *Venturia asperata* has progressively spread in several apple-growing areas of Trentino and South Tyrol, affecting mainly resistant apple cultivars, but not only and also under fungicide control. Limited information is available on the epidemiology of *V. asperata*. To contain the increase and spread of this disease, it is necessary to further investigate the biology of *V. asperata*. During 2018–2023, several monosporic strains of *V. asperata* were obtained from symptomatic apple fruits and leaves of different origins in terms of both cultivar and growing area. These monosporic strains were studied *in vitro* to investigate their behavior compared to the temperature compare to *Venturia inaequalis* to better understand the biological and epidemiological parameters and improve possible control strategies.

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Polysome profiling is a powerful method to study plant gene translational regulation

L. Guan[1], **M. Moser**[2], **G. Sharma**[3], **G. Viero**[3], **C. Moser**[4], **E. Baraldi**[1]

[1]Department of Agricultural and Food Science, DISTAL, Alma Mater Studiorum - University of Bologna, Bologna, Italy; [2]Genomics and Biology of Fruit Crops Department, Research and Innovation Centre, Fondazione Edmund Mach, via Mach 1, 38098 San Michele all'Adige (TN), Italy; [3]Institute of Biophysics, CNR Unit at Trento, Trento, Italy; [4]Functional Genomics, Department of Genomics and Biology of Fruit Crops, Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all'Adige, Italy. E-mail: lubin.guan2@unibo.it

Translation, the process of converting mRNAs into proteins, is crucial for living organisms. Increasing evidence shows that more mRNAs do not necessarily lead to more proteins, highlighting the importance of translational regulation. Polysome profiling allows the separation and purification of ribosomes associated with mRNAs undergoing active translation. By RNA-Seq or qPCR analysis of purified polysomal mRNAs, it is possible to study the translational status of mRNAs and unravel differences between transcriptome and proteome. In this work we analysed published transcriptome and proteome data from 7-day-old *Arabidopsis thaliana* cotyledons and hypocotyls to identify genes with uncoupled transcription and translation levels, indicating post-transcriptional regulations. We found several uncoupled genes, suggesting differential translational activities in these tissues. We thus chose 4-day-old *A. thaliana* where the real leaves are just about to sprout as the example to dissect the post-transcriptional regulations. Our results showed higher overall translational activities in cotyledons, while transcript abundance was higher in hypocotyls. Additionally, the translational activities of individual genes varied, with some genes shifting from polysome fractions to ribosome-free fractions, indicating possible reasons for the uncoupling of mRNA and protein levels. Furthermore, we applied the same method on 6-day-old and 10-day-old seedlings, observing highly dynamic translational reorganization during *Arabidopsis* growth. Polysome profiling can also reveal how plants manage infections in plant-pathogen interactions shedding light on the translation of specific mRNAs during the immune response. Future work should develop polysome profiling protocols for crop plants during pathogen interactions characterizing the functional state of key genes in plant responses to pathogens.

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Mechanical stress drives hyphal penetration in the fungal pathogen *Fusarium oxysporum*

V.M. Guastaferro[1], **S. Vitale**[1], **E. Russo**[1], **N. Minc**[2], **M. Lorito**[1], **D. Turrà**[1]

[1]Department of Agricultural Sciences, University of Naples "Federico II", Via Università 100, 80055, Portici, Italy; [2]Institute Jacques Monod - Cnrs University Paris City, 15 Rue Hélène Brion, 75013 Paris, France. E-mail: stefania.vitale@unina.it

All biological systems, from single-celled bacteria to higher eukaryotes, exist in a three-dimensional context and are subjected to various physical forces (*e.g.*, tensile, compressive, or shear stress). The fungal cell wall is a dynamic structure that gives each cell a unique shape and protects it from various stresses. Additionally, it acts as a physiochemical regulator, transmitting external signals through numerous cell wall-anchored proteins. *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), a ubiquitous soil-dwelling fungus, is known to cause parenchymal or vascular diseases in many plant hosts, leading to significant yield losses. While penetrating and colonizing the host tissues, *Fol* hyphae are subjected to high compressive forces as the fungus explores both the spaces between (apoplast) and within host cells (symplast). In both cases, hyphae should readapt their shape and size to fit into the sub-micrometric spaces they encounter on their way to the plant's vascular system. Which external signals are sensed by *Fol* within plant tissues and how these modulate *Fol* penetration remain largely unknown. Here, by using Polydimethylsiloxane (PDMS) microchannels, 3D-printed devices, and fluorescence microscopy, we investigated how *Fol* responds to physical pressure. Our findings show that compressive stress, generated by forced hyphal growth in micro-spaces or in between a cellophane layer and a pressurizing agent, triggers *Fol* invasive growth. Additionally, fluorescence microscopy analysis revealed that when exposed to compressive stress, *Fol* can form enlarged, appressoria-like cells with an increased distribution of actin filaments. Such preliminary results highlight potential mechanisms that *Fol* may use to penetrate and colonize plant tissues.

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Anthracnose of strawberry caused by *Colletotrichum fioriniae* in Mexico

M.G. Hernández-Deheza[1], A.R. Solano-Báez[2], A.D. Victoria-Arellano[3], G. Márquez-Licona[2]

[1]Plant and Soil Health Supervisor BSF, BerryMex, Baja California, México; [2]Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional (CEPROBI-IPN), Morelos, México; [3]Universidad Interserrana del Estado de Puebla, México. E-mail: gmarquezl@ipn.mx

Mexico ranks fourth worldwide in strawberry production, with an annual output of 578,142 tons. The state of Baja California stands out as the country's third-largest producer. During the summer of 2022, anthracnose symptoms were observed on fruits, petioles, and stolons of strawberries in this state. Affected plants exhibited a distinctive reddish discoloration, particularly when the pathogen affected the crown. The disease incidence was 15%. The objective of this research was to identify the causal agent of the disease. Intense pink fungal isolates were recovered from symptomatic plants, producing abundant salmon-coloured conidial masses containing characteristic conidia and appressoria typical of *Colletotrichum acutatum* complex species. For further identification, genomic DNA extraction was performed from a representative morphotype, and the internal transcribed spacer (ITS) region, as well as partial sequences of actin (*act*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), and β-tubulin (*tub2*), were amplified and sequenced. Sequence analysis using the nBlast tool showed 100% similarity with *Colletotrichum fioriniae*. Pathogenicity tests were conducted by inoculating crowns and leaves of 2-month-old plants, revealing characteristic disease symptoms five days post-inoculation, while control plants remained healthy. The fungus recovered from symptoms was morphologically identical to the inoculated isolate. To date, *C. fioriniae* has not been reported in Mexico, causing anthracnose in strawberries.

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Advances in the diversity of *Fusarium* spp. in the main producing areas of strawberry (*Fragaria* x *ananassa*) in Mexico

M.A. Hernandez-Hernandez[1], A.R. Solano-Báez[1], G. Márquez-Licona[1], M.J. Velasco-Alvarado[2]

[1]Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional (CEPROBI-IPN), Morelos, México;[2] Strawberry Breeding Program Plantas de Navarra S. A., (PLANASA). E-mail: asolanob@ipn.mx

In Mexico, strawberry cultivation covers a planted area of 13,000 hectares, with an average annual production of 555,000 tons, representing an income of USD 1.139 billion in 2023. This makes the country the fourth-largest producer worldwide. In the main producing areas of the states of Michoacán, Guanajuato, and Baja California, losses ranging from 50% to 100% of production have been recorded, caused by soil-borne pathogens, with *Fusarium* spp. being particularly notable. The objective of this work was to identify the diversity of *Fusarium* species associated with wilt disease in strawberries. In September 2023, plants with initial wilt symptoms were collected from commercial fields. The morphological characteristics of pure cultures corresponded to those of *Fusarium*. Genomic DNA of representative isolates was extracted, and the Internal Transcribed Spacer (ITS) and the second largest subunit of RNA polymerase II (RPB2) partial gene were amplified and sequenced. The nBLAST analysis of the sequences of our isolates showed 100% similarity with species belonging to the *F. oxysporum* and *F. fujikuroi* species complexes. A pathogenicity assay was carried out, and the inoculated strawberry plants showed wilting and root rot five days after inoculation, while the control plants remained symptomless. The recovered fungus from the inoculated plants was morphologically identical to the inoculated ones, thus fulfilling Koch's postulates. A multigene analysis is required to identify the cryptic species involved in this disease. Understanding the biodiversity of pathogens provides pertinent information to plant breeders and growers for designing disease management strategies.

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Biocontrol of Basil Downy Mildew: a study on the efficacy of four different commercial products against *Peronospora belbahrii*

F. Hilaj, L. La Placa, M. Camardo Leggieri, P. Battilani

Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Piacenza, Italy. E-mail: paola.battilani@unicatt.it

Sweet basil (*Ocimum basilicum*) is one of the most important herbs grown worldwide and is mainly used for culinary purposes. Basil downy mildew (*Peronospora belbahrii*) represents the main threat to basil, and the control of the pathogen is poorly effective and relies mainly on the use of chemical pesticides. Reducing the use of chemical pesticides is the primary goal of the European Green Deal; therefore, alternative control approaches must be found. In this study, four different commercial products with four different biocontrol agents as active ingredients were chosen that are already used on plants for fresh consumed leaves: Amylo-x (*Bacillus amyloliquefaciens*), Blossom Protect New (*Aureobasidium pullulans*), Serenade Max (*Bacillus subtilis*) and Trianum-P (*Trichoderma harzianum*). The effectiveness of these products was evaluated on detached basil leaves in Petri dishes and on pot-grown basil plants. The products were sprayed on the leaves, and *P. belbahrii* was inoculated after 24 h and incubated at 19°C in the dark for 24 h to enhance the infection. Subsequently, leaves and plants were maintained at 25°C for 10 days. On detached leaves, Blossom Protect New significantly reduced the disease incidence and severity, while the other products showed no significant difference compared to the inoculated control. The trial on plants showed no significant differences between incidence and severity in the inoculated control and the treated plants. This research remarks that the management of *P. belbahrii* is complex, and valid biological control agents are not available, being poorly effective and only in a controlled environment.

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Isolation of mycotoxin-producing ear rot fungi from maize kernels from Rwanda: a first resume of colourful fungal strains

E. Hotaj[1,2], V. Rafiei[1], A. Hategekimana[3], A. Rutikanga[4], R.R. Vetukuri[1], F. Uwamahoro[3], M. Karlsson[1], M. Dubey[1]

[1]Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden; [2]Department of Agriculture, Food and Environment, University of Pisa, Italy; [3]Rwanda Agriculture and Animal Resources Board, Huye, Rwanda; [4]Ministry of Agriculture and Animal Resources, Kigali, Rwanda. E-mail: e.hotaj@studenti.unipi.it

Maize (*Zea mays*) is considered the most important cereal crop based on the quantity produced thanks to its adaptability in terms of temperatures, altitudes, and latitudes and versatility as food, animal feed, biofuel, and bioproducts. Although maize is crucial for food safety and food security in Rwanda, the average maize production is lower compared to the expected potential. Maize ear rot disease (ER), caused by mycotoxigenic fungal species such as *Aspergillus flavus, Fusarium graminearum,* and *Fusarium verticillioides*, is one of the key factors affecting maize production. Apart from lowering yield, ER reduces grain quality by contaminating it with mycotoxins such as aflatoxin, deoxynivalenol, zearalenone, and fumonisin. If consumed, the mycotoxin-contaminated maize would pose a serious threat to human and animal health. The current study aims to investigate the population structure of ER pathogens and their association with ER disease severity and mycotoxin production in Rwanda. Initially, sixty-six samples from infected maize ears were collected from eleven different areas in Rwanda, covering all provinces. The kernels were placed on a half-strength PDA medium supplemented with kanamycin antibiotics and were incubated at 20 °C for fungal growth. A total of 150 fungal colonies were isolated after five to seven days of incubation. Single-spore purification and single hypha isolation were used to obtain pure cultures. These cultures were used for DNA extraction and molecular identification by using Multilocus DNA barcoding.

Evaluating fungistasis in pathogenic and beneficial fungi: influence of land use intensity and soil microbiome

G. Iacomino[1], S. Gaglione[1], M. Idbella[2], G. Bonanomi[1,3]

[1]Department of Agricultural Sciences, University of Naples Federico II, via Università 100, 80055 Portici (NA), Italy; [2]College for Sustainable Agriculture and Environmental Sciences, Mohammed VI Polytechnic University, Ben Guerir 43150, Morocco; [3]Task Force on Microbiome Studies, University of Naples Federico II, 80138 Naples, Italy. E-mail: giuseppina.iacomino@unina.it

Soilborne plant pathogens significantly impact agroecosystem productivity, highlighting the need for effective control methods for sustainable agriculture. Soil fungistasis, the ability of soil to inhibit fungal spore germination, is key for biological control. This study examines soil fungistasis across various land use intensities, including forests, grasslands, shrublands, and horticultural areas in open fields and greenhouses. Soil properties analysed included organic matter, pH, total nitrogen, C/N ratio, key cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}), enzymatic activities, microbial biomass, and microbiota via high-throughput sequencing of 16S rDNA genes. Fungistasis was tested against *Botrytis cinerea* and *Trichoderma harzianum*. Glucose addition temporarily nullified fungistasis for both *B. cinerea* and *T. harzianum*, with significant fungal growth observed within 48 hours. The relief of fungistasis was proportional to the glucose application rate and varied across soil types. Fungistasis correlated negatively with sand and total nitrogen content, but positively with load, clay, and Mn content. Surprisingly, soil organic carbon and enzymatic activity showed no correlation with fungistasis. Microbiome analysis revealed significant differences along the land use intensity gradient. Soils with higher Basidiomycetes levels exhibited greater fungistasis compared to those with more Ascomycetes. Intensive horticultural soils had more Cyanobacteria and Proteobacteria, alongside reduced fungistasis. This study sheds light on soil fungistasis variability in diverse ecosystems, underscoring the roles of soil texture rather than soil organic matter and microbial biomass to explain the variability of fungistasis across landscapes.

Unveiling microbial community dynamics in response to pear brown spot disease and management strategies

S. Iacono[1], M. Menghini[1], D. Giovanardi[2], L. Solieri[2], M. Collina[1], R. Baroncelli[1]

[1]Department of Agricultural and Food Sciences (DISTAL), University of Bologna, 40127 Bologna, Italy; [2]Department of Life Sciences, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy. E-mail: marina.collina@unibo.it

Pear brown spot, caused by the Ascomycete fungus *Stemphylium vesicarium*, is a major economic threat to pear production in northern Italy. This study investigated the influence of microbial communities on brown spot development and the impact of disease management strategies on these communities. The specific objectives of the project are: A) To compare microbial communities (bacteria and fungi) on pear surfaces (cv. Abate Fétel) in orchards conducted with organic and integrated pest management; B) To investigate potential correlations between *S. vesicarium* and the adopted disease control strategy, *S. vesicarium* abundance and brown spot incidence, specific microbial taxa and *S. vesicarium* abundance. Metabarcoding with high-throughput sequencing was employed to analyse the epiphytic microbial communities on pear surfaces from both orchard types. We targeted the V3-V4 region of the *16S rRNA* gene for prokaryotes and the ITS2 region for fungi. QIIME, a bioinformatic pipeline, was used for data analysis. Our preliminary results revealed significant differences (p-value < 0.05) in fungal and bacterial beta-diversity (community composition) and fungal alpha-diversity (species richness) between organic and integrated orchards. Notably, *Aureobasidium, Vishniacozyma*, and *Rhodotorula* were the most abundant fungal taxa, while *Pseudomonas, Sphingomonas*, and *Pantoea* dominated the bacterial population. Further analyses are needed to confirm correlations between *S. vesicarium* and disease incidence. Investigating potential negative correlations between previously identified abundant taxa (*e.g., Pseudomonas*) and *S. vesicarium* abundance or disease severity is warranted, considering their reported biocontrol potential.

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Comprehensive analysis of pathogen-induced *Pinus mugo* “fairy ring” in the Apennines: soil microbiome and chemistry, dendrochronology, and vegetation assessment

**M. Idbella[1], M. Zotti[2], A. Rita[2], L. Saulino[2], A. Saracino[2],
M.F. Carloni[3], M. Allegrezza[3], G. Tesei[3], S. Murolo[3], L. Landi[3], G. Ciaschetti[4], S. Mazzoleni[2,5], G. Bonanomi[2,5]**

[1]College for Sustainable Agriculture and Environmental Sciences, Mohammed VI Polytechnic University, Ben Guerir 43150, Morocco; [2]Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici (NA), Italy; [3]Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Ancona, Italy; [4]Plant Biodiversity Monitoring and Conservation Office, Maiella National Park, v. Badia 28, 67039 Sulmona, AQ, Italy; [5]Task Force on Microbiome Studies, University of Naples Federico II, Naples, Italy. E-mail: mohamed.idbella@um6p.ma

In the Majella massif, we observed ring-shaped dieback patches in a monodominated stand of *Pinus mugo*. These patches were categorized based on their size as: small, medium, and large. The patches were also divided based on *P. mugo* health status into four positions: OUT, FRONT, DEAD, and IN. In each position, we conducted vegetation and dendrochronological analyses, soil microbiota using next-generation sequencing, soil chemistry, and pathogen isolation from dead roots. We recorded 35 circular dieback patches, ranging from few to 76.1 meters in diameter. Our results show that increased light availability in DEAD belts promotes plant diversity, particularly hemicryptophytes. Conversely, OUT position, unaffected by dieback, had the lowest plant diversity, with the highest cover of phanerophytes and a significant presence of nemoral species. Dendrochronological analyses unveil a temporal sequence in the growth patterns of dead, surviving, and recruited trees. A high mortality rate among mature individuals occurred in a short time frame, followed several years later by a recolonization within the patches where dieback occurred. Morphological and molecular identification of fungal isolates suggested a fungus belonging to *Heterobasidion* genus as the main agent of the dieback. Metabarcoding results indicated higher abundance of Basidiomycota in DEAD position, with significant contributions from *Actinophytolocla, Nitrobacter, Trichoderma, Penicillium, Trechispora*, and *Lepiota* taxa. These findings suggest that the observed dieback is a multifaceted process driven by complex interactions between biotic and abiotic factors. Understanding the interplay between these factors is crucial for developing management strategies aimed at promoting forest resilience in subalpine and alpine ecosystems.

Development of diagnostic tools for quantitative detection of *Apiospora marii* using qPCR and ddPCR technologies

G. Incampo, D. Geri, D. Cornacchia, E. Chiaromonte, F. Dalena, F. Faretra, S. Pollastro

Department of Soil, Plant and Food Sciences, University of Bari, Bari, Italy. E-mail: donato.gerin@uniba.it

Apiospora marii (sin. *Arthrinium marii*) is an ascomycete recently associated with olive tree dieback in Italy and Spain. With this study, quantitative (q)PCR and digital droplet (dd)PCR protocols were developed and validated to detect and quantify *A. marii.* Two primers/probe sets (AM135 and AM158) were designed on the ITS sequences of the fungus. The optimization of the PCR conditions allowed to identify 60 °C as the best annealing temperature and 500/250 nM as the best primers/probe concentration in both qPCR and ddPCR. Under these conditions, up to 1 fg μL^{-1} of DNA of the *A. marii* DiSSPA_A1 was detected in qPCR (Cq=34 for both AM135 and AM158). The same DNA concentration was the lowest one detected in ddPCR after 40 cycles of amplification and corresponded to 0.20 and 0.12 DNA copies μL^{-1}, respectively for AM135 and AM158. The specificity of both primer/probe sets was tested against a panel of microorganisms commonly associated with olive wood of several olive varieties. Untargeted amplifications were observed after 35 cycles, using the DNA of *Armillaria mellea, Pseudophaeomoniella oleae,* and *Verticillium dahliae*, and in accordance with the qPCR–LoD was Cq 35. For the assay validation, healthy and *A. marii* artificially-inoculated olive plants were analysed simultaneously in qPCR assay and on potato dextrose agar medium, confirming a sound performance of the qPCR assay. Results achieved in this work let to improve monitoring and surveillance of this new olive pathogen.

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Application of electrolyzed salt solutions to preserve postharvest safety and quality of fresh fruits and vegetables

O. Incerti[1], C. Hadjila[1,2], G. Celano[1], M. Desopo[1], A. Ippolito[1], S.M. Sanzani[1]

[1]Department of Soil, Plant, and Food Sciences, University of Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy; [2]CIHEAM Bari, Via Ceglie 9, 70010 Valenzano (BA), Italy. E-mail: ornella.incerti@uniba.it; simonamarianna.sanzani@uniba.it

Over the last three decades, the postharvest management of fresh fruits and vegetables (FFVs) has heavily depended on chemicals. This has resulted in the rise of resistant pathogens causing spoilage and the formation of byproducts, which are unhealthy to consumers and detrimental to equipment, causing a progressive restriction in the allowed active substances. As such, the sustainable management of phytopathogens (known and emerging), microbial contaminants, and FFVs' quality is a significant concern for the horticultural industry. The electrolysis process of water and its product, *i.e.*, electrolyzed water (eW), gained considerable interest from researchers due to nonthermal, nontoxic, and broad-spectrum microbial inactivation, as well as for its low cost, portability, and preservation of FFV quality. In the present study, the use of electrolysis in the presence of different electrolytes is reported for its application as a sanitization process of citrus fruit washing water. A consistent reduction of the microbial population of the water was recorded after 1 h of electrolysis in the presence of $NaHCO_3$. Hints on the putative mode of action are also provided. Furthermore, the efficacy of eW and electrolyzed salts (NaCl and $NaHCO_3$) solutions as postharvest antifungal treatment was tested *in vitro* and *in vivo* on several host-pathogen combinations. Overall, eNaCl resulted in the most efficient treatment for preventing spore germination and minimizing fruit rots, followed by eW and $eNaHCO_3$. As such, electrolysed solutions seem promising as dipping treatments for preventing postharvest decay of FFVs.

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Use of a portable electronic nose for rapid diagnosis of fungal pathogens agents of garlic bulb rot

A. Infantino, E. Degli Innocenti[1], P. Di Antonio[1], M.T. Valente[1], F. Tocci[2], C. Costa[2]

[1]CREA Research Centre for Plant Protection and Certification, CREA-DC, Rome, Italy; [2]Research Centre for Engineering and Agro-Food Processing, Rome, Italy. E-mail: alessandro.infantino@crea.gov.it

Garlic (*Allium sativum* L.) is a bulbous herbaceous perennial plant of the family Amaryllidaceae, worldwide cultivated for its organoleptic, nutritional, and therapeutic properties. The presence of outer tunics in the bubs favours the development of the fungus during cultivation and storage and hampers the early diagnosis of the diseases. The analysis of the profiles of Volatile Organic Compounds (VOCs) released by fungal pathogens during infection of garlic with *Fusarium proliferatum* has been recently characterized at CREA by using PTR ToF GC/MS and solid-phase microextraction gas chromatography/mass spectrometry (SPME-GC/MS). In this work, a portable electronic nose was used to compare the VOCs emitted by garlic samples naturally and artificially infected with *F. proliferatum* and *Penicillium allii* as compared to healthy ones. Appropriate statistical analysis of the data obtained has been used to set up a model to be implemented in the instrument for the fast, economical, accurate, and high throughput diagnostic tool of garlic naturally and artificially infected samples.

Turfgrass diseases of golf courts in central Italy: problematic and solutions

A. Infantino[1], M.T. Valente[1], F.S. Modestini[2], S. Vitale[1]

[1]Council for Agricultural Research and Economics - Research Centre for Plant Protection and Certification (CREA - DC), Via C.G. Bertero 22, 00156 Rome, Itayl; [2]Agronomist Consultant. E-mail: alessandro.infantino@crea.gov.it

In Italy, the game of golf is practiced by about 100,000 people on more than 300 courses, of which about 70% are located in northern Italy. The courses are subject to numerous diseases, the control of which is made difficult given their intensive utilization, which requires constant and very specialized maintenance. During 2023, several diseased samples of both green and fairways from golf clubs located in central Italy have been analysed at CREA DC of Rome. Obtained fungal isolates were identified as *Clarireedia jacksonii, Fusarium culmorum, Curvularia* (*Cu.*) *lunata, Pyrenophora dictyoides* (syn. *Drechslera andersenii*) *Ceratobasidium cereale Microdochium bolleyi Magnaporthiopsis cynodontis* by using both morphology and molecular sequencing of Internal Transcribed Spacer and Elongation Factor regions. *In vitro* tests highlighted the resistance of *C. jacksonii* to tebuconazole, also at the double suggested doses, while *F. culmorum* was controlled entirely. The efficacy of one isolate each of *Trichoderma gamsii* and *T. asperellum* of the CREA-DC culture collection, *Saccharomyces cerevisiae* and *Bacillus subtilis* commercial product in controlling *C. jacksonii* and *F. culmorum in vitro* showed variable efficacy depending of the viocontrol agent/pathogen considered interaction. Pathogenicity tests of *C. jacksonii,* *F. culmorum* and *Cu. lunata* in the greenhouse are in progress on *Agrostis stolonifera* var. *palustris, Lolium perenne* and *Cynodon dactylon* × *C. transvaalensis* and *Cynodon dactylon* var. *dactylon*.

The impact of agricultural practices on the contamination of durum wheat kernels by mycotoxigenic fungi and mycotoxins in central Italy

M. Jasarevic[1], C. Morales Rodriguez[1], G. Scialò[2], B. De Santis[2], F. Debegnach[2], A. Palchetti[3], L. D'Ambrosio[3], E. Caprai[4], G. Minkoumba Sonfack[4], R. Mancinelli[5], A. Catalani[1], G. Chilosi[1]

[1]Department of Innovation in Biology, Agrifood and Forest Systems (DIBAF), University of Tuscia, Viterbo; [2]Italian NRL for Mycotoxins and Plant Toxins, Italian National Institute of Health, Rome, Italy; [3]Food Analysis and Product Safety Laboratory, Environmental and Climate Protection Agency of the Autonomous Province of Bolzano, Bolzano, Italy; [4]Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna, Bologna, Italy; [5]Department of Agricultural and Forestry Sciences (DAFNE), University of Tuscia, 01011 Viterbo, Italy. E-mail: chilosi@unitus.it

The appearance of the black point symptoms of wheat grains, associated with *Alternaria* mycotoxigenic fungal species and the already well-known Fusarium head blight (FHB), is becoming a significant threat to disease management. Agronomic practices can influence the extensiveness of the mycotoxigenic fungal community. The study aimed to evaluate the effects of two different fertilization methods (mineral fertilizer versus organic fertilizer) and soil tillage techniques (ploughing, subsoiling, and spading) on the mycotoxigenic fungal community colonizing durum wheat kernels. The study was conducted through a three-year trial in central Italy in a durum wheat field in a rotation with a potato crop. The results show that the kernels harvested in 2020 and 2021 exhibited black point symptoms, while no symptoms were observed in 2022. *Alternaria alternata* and *A. infectoria* resulted in the most frequently isolated species in all three years, with the occasional appearance of *Fusarium* species. Detection of mycotoxin contamination from *Alternaria* spp. was observed, whereas those from *Fusarium* spp. were below the indicative levels established by the European Commission. However, there was no correlation between the frequencies of *Alternaria* and black point symptoms, with a negative correlation between *Alternaria* infection and mycotoxin accumulation. Mycotoxin levels were linked to soil carbon levels after mineral fertilization, while *A. alternata* levels were negatively associated with organic fertilization frequencies. Obtained results demonstrate that contamination of kernels with *Alternaria* mycotoxins could represent a possible food safety risk on a European scale. These findings indicate that organic fertilization could help reduce contamination over an extended period.

Severity scale for the evaluation of crown rot in strawberries caused by *Neopestalotiopsis rosea* in Mexico

K.J. Juárez-Cruz[1], Márquez-Licona[1], M.J. Velasco-Alvarado[2], A.R. Solano-Báez G[1]

[1]Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, Morelos, México; [2]Strawberry Breeding Program Plantas de Navarra S. A., (PLANASA). E-mail: asolanob@ipn.mx

Mexico is the fourth largest strawberry producer in the world, with an annual production of 578,142 tons. Over the past five years, production has been threatened by *Neopestalotiopsis rosea*, which causes crown and root rot, a disease leading to yield losses of up to 50%. This research aimed to develop a validated severity scale for evaluating the disease using a characterized pathogen population. Diseased plant tissue was collected from the states of Michoacán, Baja California, and Guanajuato. For population formation, isolates were made, and 16 representative isolates were selected, whose cultural and conidial morphology indicated they belonged to the genus *Neopestalotiopsis*. The identity of the isolates was confirmed by amplifying the ITS region and partial sequences of the beta-tubulin gene. Sequence analysis using the nBLAST tool showed 100% similarity to *Neopestalotiopsis rosea*. The pathogenicity of the isolates was tested by inoculating strawberry plant crowns (2 x 10^6 conidia/mL). The first symptoms were observed 15 days after inoculation. The proposed severity scale consists of values from 0 to 5 corresponding to 0= healthy plant, 1= yellowing of older leaves, 2= stunted growth or dwarfed plants, 3= wilting of young leaves and leaf necrosis, 4= death of older leaves while central leaves remain green or crowns turn reddish-brown, 5= dead plant. The scale enabled detection that isolates from Michoacán caused the highest disease severity. This scale is a validated tool for the early recognition of symptoms, aiding breeders and growers.

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Application of RNA interference technology and evaluation of biological formulates as alternative methods to control grapevine Flavescence doreé phytoplasma and its vector *Scaphoideus titanus*

E.G. Kabeto[1], F. Negrini[1], M. Karas[1], XQ. Chen[1], M. Dall'Ara[1], C. Lanzoni[1], C. Marzachì[2], L. Galetto[2], D. Bosco[2,3], B. Mezzetti[4], S. Sabbadini[4], L. Capriotti[4], L. Landi[4], R. Musetti[5], S. Favia[5], M. de Zotti[6], E. Baraldi[1], C. Ratti[1]

[1]Lab of Plant Pathology University of Bologna DISTAL, viale Fanin 42, 40127 Bologna, Italy; [2]Institute for Sustainable Plant Protection IPSP-CNR, Strada delle Cacce 73, 10135 Torino, Italy; [3]Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, Italy; [4]Department of Agricultural, Food, and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche 10, 60131 Ancona, Italy; [5]Department of Land, Environment, Agriculture and Forestry (TESAF), University of Padova, viale dell'Università 16, 35020 Legnaro, Italy;[6]Department of Chemistry, University of Padova, Via Marzolo 1, 35131 Padova, Italy. E-mail: elena.baraldi@unibo.it, claudio.ratti@unibo.it, endriyas.kabeto2@unibo.it

Flavescence dorée (FD) is a grapevine disease that has significant consequences for the wine industry in terms of economic losses. The associate pathogen, FD phytoplasma (FDp), is classified as a quarantine pest and a priority pest by the European Union. FDp is a phloem-limited, wall-less pathogen mainly transmitted by sap-sucking vector, *Scaphoideus titanus*. Not many management methods are available to control FD; the only efficient compounds acting directly against the phytoplasmas are the tetracyclines not authorized in agriculture, and disease-resistant varieties for breeding protocols are not available. Therefore, the primary method for limiting FD, at present, is to control the vector through the use of insecticides. However, this method raises social concern for the adverse effects on human health and the environment. Recent progress in utilizing RNA interference to induce gene silencing in target pests has facilitated the development of gene-specific pest management. Similarly, applying biological formulations on plant tissues to directly target pathogens offers a promising alternative to conventional pesticides. The project aims to develop novel protocols and tools to manage the vector *S. titanus* through host-induced gene silencing (HIGS) and to evaluate the efficacy of specific biological formulations against the FD phytoplasma via trunk injection application.

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Susceptibility assessment of chestnut cultivars to *Cryphonectria parasitica* in Friuli Venezia Giulia

A. Kabir[1], S. Tomada[2], L. Poggetti[2], V. Cacitti[2], M. Fabro[2], S. Baric[1,3]

[1]Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Piazza Università 5, 39100 Bozen-Bolzano, Italy; [2]Agenzia Regionale per lo Sviluppo Rurale del Friuli Venezia Giulia– ERSA, Via Sabbatini 5, 33050 Pozzuolo del Friuli, Italy; [3]Competence Centre for Plant Health, Free University of Bozen-Bolzano, Piazza Università 5, 39100 Bozen-Bolzano, Italy. E-mail: Azaz.Kabir@student.unibz.it

Chestnut blight, caused by *Cryphonectria parasitica*, is a major threat to chestnut cultivation across Europe. The fungus produces perennial necrotic lesions, commonly known as cankers, on the bark of susceptible hosts, particularly in young and grafted trees. The high genetic diversity of the fungus and the disease incidence in the Friuli Venezia Giulia region require effective management strategies. Two main strategies to counter chestnut blight are cultivating tolerant genotypes and using biocontrol via virus-induced fungal hypovirulence. This study focuses on evaluating the susceptibility of three local chestnut varieties from Friuli Venezia Giulia, 'Obiacco', 'Marujac' and 'Muron' along with the commercial hybrid cultivar 'Bouche de Betizac' (*Castanea sativa* × *Castanea crenata*). In the first step, fungal strains with the highest degree of virulence were identified, which belonged to the most dominant vegetative compatibility types of *C. parasitica* in the Friuli-Venezia Giulia region, EU-13, EU-17, EU-28, and EU-12. In a second step, mycelium plugs were inoculated on wounded one-year-old detached stems (8 cm in length) and midveins of detached leaves of the investigated chestnut varieties. The inoculated plant material was incubated in humid boxes at 25°C. Necrosis length along the midvein was measured after 7 days, and canker size on detached stems was measured weekly for 28 days. The knowledge of the degree of tolerance or susceptibility of local cultivars to *C. parasitica* in Friuli Venezia Giulia will aid the production of more robust nursery plants and the valorisation of local varieties through the spread and cultivation of tolerant genotypes.

Survey of the abundance, seasonal variation, and localization of *Colletotrichum* on olive trees in Japan

Y. Kageyama, S. Ohsato

Graduate School of Agriculture, Meiji University, Japan. E-mail: kageyama2021@patholab-meiji.jp

In recent years, the area under olive cultivation in Japan has increased. High rainfall in Japan compared with that in the Mediterranean region has caused anthracnose to become a problem. This study aimed to elucidate the process of olive anthracnose infection by examining the life cycle of *Colletotrichum* spp. in Japan, a country with higher precipitation. We sampled olive groves in Kagawa Prefecture, the largest olive-growing region in Japan, and in Kanagawa Prefecture, which has higher rainfall than Kagawa Prefecture, and obtained 41 and 43 strains of *Colletotrichum* sp.-like fungi, respectively. We identified each strain based on its characteristics and nucleotide sequence. The results revealed the composition and proportion of the dominant species in both prefectures. The distribution and localization of *Colletotrichum* spp. in olive trees were studied using seasonal latent infection rates. Endophytic fungi were isolated monthly starting in May 2023. The isolation rate of *Colletotrichum* spp. from leaves and branches decreased with increasing flower bud differentiation, whereas that from fruits increased. The latent infection rate in Japan is higher than that reported in a survey of endophytic fungi in Spain. Scanning electron microscopy of leaf cross-sections revealed the presence of *Colletotrichum* sp.-like microspores on the epidermal lining of the leaves. Thus, *Colletotrichum* spp. are inferred to survive near the epidermis of leaves and branches pre- and post-disease onset, and the disease is caused by the movement of *Colletotrichum* spp. from the surface to the inflorescences and fruits.

Optimization of dsRNA uptake in *Stemphylium vesicarium* for spray-induced gene silencing (SIGS) technology application

M. Karas[1*], F. Negrini[1*], XQ. Chen[1], C. Minnelli[2], G. Mobbili[2], V. Sunico[3], A. Ricci[3], S. Sabbadini[3], B. Mezzetti[3], E. Baraldi[1*]

*[1]Department of Agricultural and Food Sciences, DISTAL, Alma Mater Studiorum -University of Bologna, Viale Fanin 42, 40127, Bologna, Italy; [2]Department of Life and Environmental Sciences (DISVA), Polytechnic University of Marche, Via Brecce Bianche, Montedago, 60131 Ancona, Italy; [3]Department of Agricultural, Food, and Environmental Sciences, Polytechnic University of Marche, Via Brecce Bianche, 60131 Ancona, Italy. E-mail: milan.karas@unibo.it; francesca.negrini6@unibo.it; elena.baraldi@unibo.it. *These authors contributed equally to the work*

Over the past decade, *Stemphylium vesicarium*, the fungal pathogen of brown spot of pears, caused dramatic losses in the pear production of Emilia-Romagna, a region where the cultivation of 'Abate' pear traditionally represents a distinctive hallmark of the local agriculture and has a great significance. Spray Induced Gene Silencing is a novel promising control management strategy inspired by the cell regulatory mechanism of RNA interference (dsRNAi), which relies on the efficient uptake of dsRNA molecules by pests. In line with the urgency to find alternatives to pesticides, this study investigates methods to enhance dsRNA uptake in *S. vesicarium.* We employed fluorescence-labelled dNTPs to measure dsRNA uptake and evaluated the use of nanoparticles to facilitate dsRNA delivery. Fluorescence microscopy and quantitative assays were used to track and quantify dsRNA internalization, then compared traditional dsRNA delivery methods with nanoparticle-mediated ones to assess the impact of different formulations on dsRNA uptake efficiency. Different conditions, mix-ratios and application protocols were assessed in infection tests. Our results emphasize the importance of dsRNA uptake process in the application of the RNAi-based control technology against *S. vesicarium* and pests in general and stress the need to find innovative delivery methods that allow to overcome the bottleneck of dsRNA uptake by target organisms. Further research dedicated to optimising the nanoparticle formulations and delivery techniques will be essential for maximizing the practical applications of this promising technology in crop pest management, especially for complex cases such as the one of *S. vesicarium* in regions like Emilia-Romagna.

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1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Bayesian phylogenetic analysis on plum pox virus-*An* strain revitalizes and sharpens host-specific adaptation, and evolutionary scenarios

S. Kawakubo[1], F. Palmisano[2], M. Chiumenti[2], P. Leonetti[2], T. Candresse[3], C. Masuta[1], V. Pantaleo[2], A. Minafra[2]

[1]Laboratory of Pathogen-Plant Interactions, Graduate School of Agriculture, Hokkaido University, Japan; [2]Institute for Sustainable Plant Protection, Research Unit of Bari, National Research Council, Italy; [3]INRAe Bordeaux, France. E-mail: vitantonio.pantaleo@ipsp.cnr.it

The whole genome sequence of the divergent plum pox virus (PPV) strain denoted as "*An*" (Ancestral) isolated from a plum tree (*Prunus domestica*) in Eastern Albania was determined. *An* strain shows the same genomic organization as the previously sequenced PPV isolates but a quite divergent 5' region of about 1500 bp compared to the widespread D strain. Maximum likelihood tree and Bayesian phylogenetic comparative methods using either full-length polyprotein, as well as selected regions of *An*, were used to allocate the strain into the proper phylogenetic clade, evaluate recombination events and reconstruct a timeline of ancestors, and explore host-adaptive evolution of the strain. The analysis suggests *An* to be a recombinant of a Turkish (T strain) accession and a still-unknown ancestral, non-recombinant PPV strain. Moreover, this 5' end genomic portion shows a slight affinity with a comparable portion of the EA strain. Accordingly, molecular clock analysis has placed the EA and *An* in the same clade, diverging around the 18th century. Characterizing the *An* strain allowed us to extend and re-elaborate the phylogenetic analysis of PPV. *An* is suggested to have originated from the recombination of two ancestors, of which one is not yet found or already extinct by the phenomenon of population replacement with the prevalent European recombinants (*e.g.*, M, Rec, and T), which derived from An in subsequent events. It makes *An* strain a critical reference to clarify the PPV evolution further and consolidate the virus as an ideal model to study the process of host-adaptive evolution and phylogeography.

Iron chelation by natural and nature-inspired catechols inhibits early development of the rice blast fungus, *Pyricularia oryzae*

A. Kunova, C. Pinna, S. Ghosh, S. Princiotto, F. Sacchi, C. Pizzatti, L. Musso, P. Cortesi, A. Pinto, S. Dallavalle, M.S. Christodoulou

Department of Food, Environmental and Nutritional Sciences, University of Milan, Via Celoria 2, 20133 Milan, Italy. E-mail: andrea.kunova@unimi.it

Pyricularia oryzae is a global threat to cereal cultivation, causing a highly destructive blast disease in various cereal crops, including rice and wheat. This plant pathogen is managed mainly by fungicides, whose use is drastically reduced due to strict regulations and increasingly present fungicide resistance. Therefore, identifying new targets for fungicide development and using natural or nature-inspired molecules for plant pathogen control are necessary. Ferroptosis was recently described as a new form of a regulated, iron-dependent cell death, which plays a key role also during *P. oryzae* infection. In particular, the iron-dependent death of conidial cells during the germination and recycling of their content into the appressorium is necessary for the infection of the host plant cells. The uncovered correlation between appressorium development and iron homeostasis evidenced iron sequestration by siderophores as a promising new strategy to prevent *P. oryzae* infection. Siderophores are low molecular weight chelators that can transport the insoluble iron (Fe^{3+}) into the cell, which is then released in Fe^{2+} soluble form. To better understand the role of siderophores and iron homeostasis in the appressorium development and virulence of *P. oryzae*, we prepared a collection of catechol-containing natural siderophores and their derivatives. We observed that the catechol moiety is indispensable for the chelating activity of synthesized molecules. Moreover, a good correlation between the ability to chelate iron and the inhibition of appressorium development in the rice blast fungus was obtained.

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Evaluation of Italian flora biodiversity as a source of metabolites to inhibit *Pseudomonas syringae* virulence

G. Lancia[1,2], G. Beggiato[1], V. Friz[1], S. Negri[1,2], D. Danzi[1], L. Avesani[1,2], F. Guzzo[1,2], E. Vandelle[1,2]

[1]Department of Biotechnology, University of Verona, 37145 Verona, Italy; [2]NBFC, National Biodiversity Future Center, 90133 Palermo, Italy. E-mail: giulia.lancia@univr.it

The extensive use of copper-based products to control plant bacterial diseases, including the bacterial canker of kiwifruit caused by *P. syringae* pv. *actinidiae* (Psa), raises several concerns due to the emergence of bacterial resistance and its persistence in the environment. An alternative strategy is reducing bacterial virulence rather than killing cells using natural compounds. This approach ensures an eco-friendly plant protection mechanism while preventing the occurrence of new resistant strains, aiming for sustainable agriculture. To this aim, plant secondary metabolites represent a valuable reservoir for developing such innovative plant protection products. Psa exploits its type III secretion system (T3SS) as a pivotal virulence mechanism for kiwifruit infection. Thus, T3SS represents a key target for weakening Psa aggressiveness to control the disease. Here, using a reporter system combining the blue fluorescent protein (BFP)-encoding gene under the control of the promoter of the *hrpA1* gene, encoding the major pilus of the T3SS, we screened the activity of more than 80 plant extracts as potential Psa T3SS inhibitors. The first results provided promising outcomes, with a subset of ten extracts showing a significant inhibitory activity of Psa *hrpA1* promoter activity. In particular, the leaf extracts from leaves of *Philadelphus coronarius* (Phco), *Empetrum hermaphroditum* (Emhe), and *Ceratonia siliqua* (Cesi) emerged as the most intriguing for further studies in terms of protection efficiency and deciphering the mechanism of T3SS regulation in Psa.

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Biostimulant activity of microbial enriched vermicompost granules for sustainable tomato production

S. Lanzuise[1], G. Manganiello[1], E. Ingenito[2], L. Izzo[2], C. Scamardella[1], M. Michelino[1], A. Pappalardo[1], S. Marcone[1], C. Cimminella[1], M. Lorito[1], S.L. Woo[2]

[1]Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; [3]Fertilidea S.r.l., Pompei, Italy; [2]Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy. E-mail: stefania.lanzuise@unina.it

The intensive use of agrochemicals can result in a detrimental effect on natural resources and risks to human health. Microorganism-based biofertilizers are effective solutions to preserve biodiversity and improve plant growth and yield by enhancing nutrient mobilization/solubilization and stimulating plant natural defence responses to plant pathogens-pests. Vermicompost is a source of nutrients that can improve soil structure, increase soil water retention capacity and, applied in combination with beneficial microorganisms, may enhance their effectiveness. This study aimed to develop innovative formulations including beneficial microorganisms, applied singularly or in consortia, with or without vermicompost granules, and to evaluate their effects on tomato yield and fruit nutritional quality. The ecological compatibility between bacteria (*Bacillus subtilis, Azospirillum brasilense*) and fungi (*Trichoderma asperellum*) was assessed *in vitro*; and their persistence on the vermicompost granules was evaluated over time. The field applications of granules containing *B. subtilis* and *A. brasilense* was the most effective, increasing tomato yield by 20%, in comparison to other conditions tested. Fruits obtained from plants treated with *B. subtilis*- or *A. brasilense*-enriched vermicompost granules, and combinations based on *T. asperellum-A. brasilense* or *T. asperellum-B. subtilis-A. brasilense* showed a 10% increase in total soluble solids compared to controls. Plants treated with *A. brasilense*-alone or with *T. asperellum-B. subtilis-A. brasilense-* enriched granules showed a significant increase in fruit lycopene, β-carotene, and total carotenoid contents. The application of vermicompost in association with selected microbial consortia represents a sustainable approach to improve soil fertility, yield, and production of functional foods naturally enriched in health-promoting phytonutrients.

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Metabolomics insights for the evaluation of safety, quality, and origin of wheat and selection of bioactive endophytes

L. Lascala, M. Beccaccioli, I. Montaina, F. Colais, C. Manetti, M. Reverberi

Department of Environmental Biology, Sapienza University of Rome, 00185 Rome, Italy. E-mail: leonardo.lascala@uniroma1.it

With a production of about 6.8 million tons in 2023, Italy is one of the most important countries for wheat production. In the context of rising global temperatures and shifting distribution of plant pathogens that will impact wheat yield, it is crucial to develop new technologies and methodologies to rapidly ensure the safety and quality of wheat production. In this context, we are investigating the metabolomic profile and the endophytic mycobiome of twenty-four Italian and two commercial varieties of durum and soft wheat. Extraction methods for polar (e.g., polyphenols, alkaloids, polypeptides, etc.) and apolar (e.g., fatty acids, oxylipins, etc.) compounds have been studied and refined for wheat samples and analysed through an "untargeted" approach using HRAM-Orbitrap and Compound Discoverer^{TM}-Thermo Scientific^{TM}. Results indicate that the proposed method allows clear identification of wheat varieties and suggests potential markers for the quality and safety aspects. Fungal endophytes were selected and divided into "common-to-all-varieties" and "specific-to-one-variety", some of them will be exploited for biological control toward the main wheat pathogens such as *Fusarium langsethiae* and *F. graminearum.* The proposed approaches aim to correlate the plant chemistry and fungal endophytic community to provide insight into the potential correlation between the chemical and fungal side.

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Revealing ambrosia beetles/fungi association: a new species of *Thyridium* (Thyridiaceae, Thyridiales) infecting bay laurel and associated with the invasive wood-boring pest, *Xylosandrus compactus* (Coleoptera: Curculionidae: Scolytinae)

G.R. Leonardi[1], H. Voglmayr[2], M.B. Costanzo[1], A. Gugliuzzo[1], A. Biondi[1], G. Polizzi[1], D. Aiello[1]

[1]Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi di Catania, Via Santa Sofia 100, 95123 Catania, Italy; [2]Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, 1030 Vienna, Austria. E-mail: giuseppa.leonardi@phd.unict.it

Bay laurel plants were occasionally sampled in urban areas and in nurseries of the Catania and Syracuse provinces (Sicily, Italy), starting in 2022. Most plants showed attacks of the invasive wood-boring pest *Xylosandrus compactus* (Coleoptera: Curculionidae: Scolytinae), in association with stem blight and internal necroses. *Thyridium*-like colonies were consistently recovered from symptomatic tissues, while, the main beetle mutualist, *Ambrosiella xylebori*, inhabited mainly the inside of beetle galleries. *Thyridium*-like isolates were identified based on the morphological characteristics and molecular phylogenetic analyses of six genomic loci (ITS, LSU, *act1, rpb2, tef1*, and *tub2*). The isolates were identified as a member of the genus *Thyridium* (Sordariomycetes, Thyridiaceae), forming a clade significantly distinct from all *Thyridium* species for which sequence data are available. Therefore, our isolates have been described as a new species. Pathogenicity tests were performed testing a representative isolate on bay laurel plants maintained in a growth chamber. The isolate was pathogenic and reproduced symptoms identical to those observed in the nurseries. The new species of *Thyridium* was consistently re-isolated from inoculated plants and the identity was confirmed by morphological identification and molecular analysis completed Koch's postulates. The role of *X. compactus* as a vector of the new *Thyridium* species was also successfully demonstrated by fulfilling Leach's postulates. Overall, the present study provides new important knowledge on fungal species associated with stem blight and internal necroses in bay laurel and on the potential epidemiological role of *X. compactus*.

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Progress on cross-protection of citrus trees from Citrus tristeza virus VT strain isolates causing decline

G. Licciardello[1], G. Scuderi[2], M. Russo[2], M. Bazzano[2], M. Bar-Joseph[3], A.F. Catara[4]

[1]Council for Agricultural Research and Economics, Research Centre for Olive, Fruit and Citrus Crops, Acireale, Italy; [2]Agrobiotech soc. coop. Catania, Italy; [3]The S. Tolkowsky Laboratory, Department of Plant Pathology, The Volcani Center, A.R.O. Bet Dagan, Israel; [4]Formerly, Department of Phytosanitary Science and Technologies, University of Catania, Catania, Italy. E-mail: grazia.licciardello@crea.gov.it

Citrus tristeza virus (CTV; genus *Closterovirus*) that may cause quick decline (QD) of citrus grafted on sour orange (SO) rootstock, stem pitting (SP) in grapefruit and sweet orange, and seedling yellow (SY) of sour orange, lemon and grapefruit. SP may be mitigated through cross-protection and QD using alternative rootstocks. Some of them do not perform well in calcareous and heavy soils, are susceptible to biotic factors, or incompatible with some varieties. To overcome the damages induced by QD, extensive molecular assays, bioindexing, and high throughput sequencing (HTS) allowed to select two cross-protective VT no-SY isolates in Sicily. Genome comparative analysis between VT SY and no-SY isolates revealed minor variations on Orf1a, p23 and p33 genes. SO seedlings protected since 12 years and challenged four years later with virulent SY isolates are still asymptomatic, as well Hamlin sweet orange trees grafted on SO, protected and challenged four-years ago. High Resolution Melting (HRM) analysis showed that only the no-SY isolate was colonizing the protected plants. The lack of replication of aggressive SY isolates was confirmed by back inoculation of SO seedlings with bark tissues taken from protected and challenged plants. After 14 months the SO plants are still without any leaf, stem, and root modification similarly to those inoculated with no-SY isolates. Differently, SO plants inoculated with SY isolates died in few months. The results suggest an increase of the defence mechanism in the host plants, that may play an important role in the pathogenesis and evolution of virus population.

Modelling biological invasions of alien plant pathogens through the analysis of spatial gradients

G. Lione[1,2], M. Giraudo[1], P. Gonthier[1,2]

[1]Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Grugliasco, Italy; [2]Interdepartmental Centre for Innovation in the Agro-environmental Sector (AGROINNOVA), University of Torino, Grugliasco, Italy. E-mail: guglielmo.lione@unito.it

Biological invasions caused by alien plant pathogens can heavily impact forest ecosystems and agricultural crops. The moving contour separating areas already infested from those still pathogen-free is defined as "front of invasion". While the front of the invasion moves, the disease incidence and severity may display a spatial pattern resulting in a gradient. Along the main direction of the gradient, the disease impact is expected to decrease, scoring higher where the pathogen established first, lower in the proximity of the front of invasion. Assessing the gradient direction may be pivotal to investigating the epidemiology and dispersal patterns of alien plant pathogens, as well as to implementing effective control strategies. Gradient analysis needed to model the dynamics of the front of invasion requires advanced computational and programming skills, hence the need for user-friendly tools made available to plant pathologists. In this study, we designed an open-source algorithm running on R, one of the most popular statistics software. Starting from the dataset including site coordinates and disease impact data, the user can obtain as output the most likely direction the front of invasion is coming from and moving to, by simply copying/pasting the algorithm code. The algorithm was successfully tested *in silico* and validated on actual cases of invasions by plant pathogens, including *Heterobasidion irregulare* in central Italy and *Ophiostoma novo-ulmi* in the Swedish island of Gotland.

Environmental and anthropic-driven factors associated with ash dieback caused by *Hymenoscyphus fraxineus* in northwestern Italy

G. Lione[1,2], M. Giraudo[1], D. Giunta[1], S. Prencipe[1], A. Ebone[3], P.G. Terzuolo[3], L. Zarantonello[3], P. Gonthier[1,2]

[1]Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Grugliasco, Italy; [2]Interdepartmental Centre for Innovation in the Agro-environmental Sector (AGROINNOVA), University of Torino, Grugliasco, Italy; [3]Institute for Wood Plants and Environment (IPLA), Torino, Italy. E-mail: paolo.gonthier@unito.it

The dieback of European ash (*Fraxinus excelsior*) caused by the fungal pathogen *Hymenoscyphus fraxineus* is currently the major disease of this tree species. From the first outbreak in central Europe during the early 1990s, the pathogen spread rapidly across the continent, severely affecting ash trees in natural forests and plantations, in association with high mortality rates and economic losses. This plant pathogen was first reported in Italy in the eastern Alps in 2009. In northwestern Italy, *H. fraxineus* was first detected in Piedmont in 2016 in a single mixed forest stand, but its current distribution and impact in that geographic area are still largely unknown. This study aimed to assess which environmental and anthropic-driven factors might be associated with the presence, incidence, and distribution of *H. fraxineus* and with the severity of ash dieback in northwestern Italy. Results from phytosanitary surveys and samplings conducted in Aosta Valley and Piedmont since 2019, showed that *H. fraxineus* is widespread in northwestern Italy, affecting its host from plain to mountain areas, in mixed forests as well as in urban sites, with peaks of incidence as high as 100%. Statistical modelling showed that the site location, rainfalls, and temperatures could affect the probability of infection, which tends to increase in high tree forests rather than in coppices, especially where ash basal area attains larger values and in the presence of other tree species, such as maples (*Acer* spp.). Silvicultural strategies could be designed and implemented to mitigate the impact of ash dieback.

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Evaluation of biocontrol agents in tomato/*Botrytis cinerea* and peach/*Monilia* spp. pathosystems for sustainable crop production

A. Lizzio[1,2,3], V. Battaglia[2], M. Petriccione[3], M. Reverberi[1], E. Lahoz[2]

[1]Department of Environmental Biology, "Sapienza" University of Rome, 00185 Rome, Italy; [2]CREA Council for Agricultural Research and Economics, Cereal and Industrial Crops (CREA-CI), Via Torrino 3, 81100 Caserta, Italy; [3]CREA Council for Agricultural Research and Economics, Olive, Fruit and Citrus Crops (CREA-OFA), Via Torrino 3, 81100 Caserta, Italy. E-mail: agata.lizzio@crea.gov.it

Biocontrol agents are tools for sustainable agriculture, offering balanced and efficient solutions for disease management as they generally have low impacts on health and the environment. Tomato and peach are two important crops, and gray mold and brown rot, caused by *Botrytis cinerea* and *Monilinia* spp., respectively, are two of the major fungal diseases affecting these crops. This study aimed to evaluate the efficacy of several formulated candidates as biocontrol agents toward the two plant pathogens. *In vitro* tests were used to assess the antifungal efficacy of the formulations based on *Bacillus* spp. and chitosan against *Monilinia* spp. and *B. cinerea,* while a commercial formulate containing essential oils was only evaluated against *Monilinia* spp.. Furthermore, the concentrations that completely inhibited *in vitro* growth of *Monilinia* spp. and *B. cinerea* were chosen to assess the efficacy *in vivo* in the two pathosystems. Our results showed that these BCAs inhibited the mycelial growth of the two pathogens *in vitro* at different concentrations with relation to the tested pathogen. In particular, the formulated candidates showed a percentage of growth inhibition of *Monilinia* spp. ranging from 50 to 80% and a growth inhibition of *B. cinerea* from 80 to 100% at 1000 $\mu g\ mL^{-1}$. The formulated candidates also confirmed their effectiveness *in vivo* against the two plant pathogens by reducing the disease severity also delaying symptoms on fruits.

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Copper-alternative products for the control of citrus fruit pathogens and their impact on beneficial carpospheric microbial communities

M.F. Lombardo

Dipartimento di Agricoltura Alimentazione e Ambiente, Università degli Studi di Catania, via Santa Sofia 100, 95123 Catania, Italy. E-mail: monia.lombardo@phd.unict.it

Citrus cultivation represents one of the most important industries worldwide, with Mediterranean countries as key producers. *Colletotrichum* spp. and *Alternaria alternata* threaten Mediterranean citriculture, causing severe preharvest fruit symptoms of anthracnose and Alternaria brown spot, respectively, and leading to substantial economic losses. The management of these pathogens is problematic, particularly in organic citrus orchards relying on copper-based antimicrobials. With limitations in the use of Cu compounds imposed by the European Commission due to environmental harm, alternative control strategies are needed. This need is emphasized in Sicilian citrus farming, leader in organic citrus production. Consequently, research for sustainable strategies is advocated, and multiple possibilities should be explored, such as applying alternative formulations or promoting beneficial microbiome members. In this context, the multidisciplinary research activities of the Ph. D. thesis focused on: (i) identifying alternative biological products able to reduce copper-based antimicrobials in citrus farming; (ii) evaluating the effectiveness of alternative products across different citrus orchards in pluriannual trials, (iii) understanding the citrus fruit carposphere microbiome composition; (iv) assessing the impact of alternative and copper treatments on the fruit microbiome; (v) establishing a collection of biocontrol agents from the core microbiome. Overall, the results provide evidence of the effectiveness and sustainability of alternative products, positioning them as promising substitutes to copper-based antimicrobials in managing citrus fungal diseases for large-scale use and as promoters of a beneficial fruit microbiome. Finally, an innovative approach integrating cultivable-dependent and cultivable-independent tools is proposed as an effective method to explore the microbiome composition of the citrus fruit carposphere, providing new insights into the microbiome's potential in disease management.

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Addressing the challenges of monoculture: the case of the Margherita White Onion PGI

F. Lops, M.L. Raimondo, A. Carlucci

Department of Agriculture, Food, Natural Resources, and Engineering (DAFNE), University of Foggia, Italy. E-mail: francesco.lops@unifg.it

The farms specialized in the traditional cultivation of the Margherita White Onion PGI, located in Margherita di Savoia, Zapponeta, and Manfredonia, in the Apulia region (Italy), follow the monoculture system, dedicating the entire cultivable area to a single species, *Allium cepa*. In intensively cultivated fields, plant pathogens, such as soil parasites, abnormally proliferate in the fields, causing significant reductions in yields and quality and, sometimes, complete production loss. The activities began with an initial phase of monitoring the phytosanitary status of the crops. This activity led to the execution of sampling and isolation of all parts of the plants showing symptoms and the isolation of fungal species from the soil. The isolation technique confirmed the presence of three primary pathogens: *Setophoma terrestris, Fusarium* spp., and *Colletotrichum circinans*. The investigations also confirmed the presence of *Ditylenchus dipsaci*, a nematode typical of the aerial parts of the plants. When crop rotations are not possible, soil disinfestation becomes necessary to contain soil-borne plant pathogens. Field trials were conducted to develop management and control strategies that reflect environmental sustainability, ensuring a quality product that emphasizes the typical organoleptic and nutritional characteristics over quantity. Based on the experimental evidence, an intervention plan was developed using natural substances and microorganisms with repressive properties against nematodes and soil pathogenic fungi. The need to restore, maintain, and improve soil health is crucial for intensive horticulture, and the innovative method proposed constitutes a fundamentally important practice for continuing to produce satisfactorily while respecting ecosystems.

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Characterization of a novel endophytic strain of *Epicoccum nigrum* with antagonistic activity against *Fusarium verticillioides* and *Alternaria alternata*

D. Lotito[1], A. Gaspari[1], V. Guastaferro[2], I. Vicente[3], A. Staropoli[2], A. Zoina[2], F. Vinale[1]

[1]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, 80138 Naples, Italy; [2]Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; [3]Department of Microbiology and Genetics, Institute for Agribiotechnology Research (CIALE), University of Salamanca, 37185 Villamayor, Spain. E-mail: frvinale@unina.it

Endophytes are microorganisms that reside within a host plant for at least part of their life cycle without causing any apparent disease. These microorganisms are recognized as a rich and reliable source of bioactive metabolites with significant agricultural and industrial potential. Despite their potential, endophytes remain a relatively unexplored field. In this research, a novel strain of *Epicoccum nigrum* was isolated from oat and was identified through morphological analysis of the colonies on Petri dishes, followed by molecular characterization (ITS4 and ITS5). *In vitro* antagonistic activity was evaluated against *Fusarium verticillioides* and *Alternaria alternata.* The antibiosis assay revealed a 33,86% growth inhibition after 48 h incubation against *F. verticililoides*, weak inhibition was detected against *A. alternata*. Moreover, the production of bioactive secondary metabolites was investigated by cultivating *E. nigrum* on potato dextrose broth (PDB) for 30 days and analysing the culture filtrate by mass spectrometry coupled to liquid chromatography (LC-MS). Several secondary metabolites were identified, such as epicoccamide, epicocconone, diketopiperazines. Interestingly, the most abundant compound was identified as orevactaene (also confirmed by NMR), a brownish pigment with known antioxidant and antibiotic activities. Since the inhibition zone around the actively growing mycelium exhibited the same brownish colour, the pigment has been purified and antibiotic activity was confirmed against *F. verticillioides* and *A. alternata*.

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)– MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4– D.D. 1032 17/06/2022, CN00000022).

Beneficial microbe application on tomato plants significantly improves the accumulation of metabolites with nutraceutical value

D. Lotito[1], M.I. Prigigallo[2], G. Iacomino[3], C. Gigliotti[4], G. Bubici[2], A. Staropoli[3,5], M. Lorito[3], F. Vinale[1]

[1]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, 80137 Naples, Italy; [2]Institute for Sustainable Plant Protection, National Research Council, 70126 Bari, Italy; [3]Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; [4]Institute for Sustainable Plant Protection, National Research Council, 80055 Portici, Italy. E-mail: daria.lotito@unina.it

The use of microorganisms and biostimulants is increasingly supported in agriculture due to their advantageous impact on plant disease management, growth enhancement, and the synthesis of beneficial bioactive secondary metabolites (SMs). Tomato (*Solanum lycopersicum*) is an important crop consumed worldwide since it is an excellent source of natural compounds (*i.e*., b-carotene and flavonoids) and minerals useful for human health. In this study, the improvement of the nutritional value of tomato (*S. lycopersicum* var. Heinz), by using beneficial microorganisms, including selected strains of *Streptomyces microflavus* (S), *Trichoderma harzianum* (M10) and *T. afroharzianum* (T22), has been investigated. These microbes were applied to tomato plants as single inoculants or microbial consortia. The effects were evaluated through statistical analysis of biological parameters. T22 treatments significantly increased plant height compared to control and M10-based treatments. The similarities observed in plant height between S-treated plants and those treated with the combination of S and T22 highlight the potential beneficial effects of microbial consortia. Moreover, the berries were subjected to an untargeted metabolomic analysis by LC-MS qTOF that led to the identification of 18 metabolites, including tomatine and its derivatives solafloridine. Multivariate analysis demonstrated differences in berries metabolic profiles, depending on the treatment. Specifically, T22-based treatment increased the accumulation of most of the identified metabolites compared to untreated plants, while combined treatment S+T22 induced a significant accumulation of solafloridine. In conclusion, field microbial applications significantly induced metabolic profile change in tomatoes and the accumulation of metabolites with nutraceutical value.

A comprehensive approach to investigate LuxR solos protein in *Pseudomonas syringae* pv. *actindiae* elucidates PsaR3 pivotal in upregulating virulence through an auto-regulation mechanism

I. Lucato[1], A. Regaiolo[2], D. Danzi[1], R. Heermann[2,3], A. Polverari[1], E. Vandelle[1]

[1]Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy; [2]Institute of Molecular Physiology (imP), Johannes-Gutenberg-University Mainz, Biocenter II, Microbiology and Biotechnology, Hanns-Dieter-Hüsch-Weg 17, 55128 Mainz, Germany; [3]Institute for Biotechnology and Drug Research gGmbH (IBWF), Hanns-Dieter-Hüsch-Weg 17, 55128 Mainz, Germany. E-mail: ilaria.lucato@univr.it

Pseudomonas syringae pv. *actinidiae* (Psa) is the etiological agent of the bacterial canker of kiwifruit (*Actinidia* spp.). The most aggressive biovar Psa3 is characterized by the presence of a plasmid containing a gene encoding for a LuxR solo protein, PsaR3. Although a role of PsaR3 in Psa virulence has been proposed, the autoinducer signal(s) and the targets are still unknown. To elucidate the function(s) of PsaR3, we conducted a transcriptomic analysis of Psa overexpressing PsaR3 in an inducible manner, which revealed the upregulation of genes associated with the type III secretion system, flagellum-related motility, together with a plasmid-borne gene cluster including *psaR3* itself. Interestingly, the intergenic region (IR) separating the two operons of the cluster is a functional promoter positively regulated by PsaR3, as demonstrated with a reporter system. Since no inducer molecule was included in our experimental medium, it suggested that i) the signal(s) may be produced by Psa or, alternatively, ii) the overexpression may lead to an autoactivation of the regulator. To allow a «controlled» post-translational activation of PsaR3, we thus designed and constitutively expressed a chimera protein consisting of the DNA-binding domain of PsaR3 and the auto-inducer-binding domain of the LuxR protein CviR of *Chromobacterium violaceum* that is activated by known AHLs. Preliminary data indicate that the chimera is not responsive to AHLs, thus supporting the hypothesis of sensor auto-activation. Moreover, the first results suggest that the constitutive autoactivation may lead to negative feedback on PsaR3 function, likely to avoid a constant virulence induction.

Susceptibility of almond (*Prunus dulcis*) cultivars to red leaf blotch caused by *Polystigma amygdalinus*

P.G. Lucchese[1,2], E. Chiaromonte[1], D. Gerin[1], D. Cornacchia[1], R. Coronelli[1], G. Incampo[1], F. Faretra[1], S. Pollastro[1,] F. Nigro[1,2]

[1]Department of Soil, Plant and Food Sciences, University of Bari, Bari, Italy; [2]Centro di Ricerca, Sperimentazione e Formazione in Agricoltura Basile Caramia, Locorotndo, Bari, Italy. E-mail: donato.gerin@uniba.it

Polystigma amygdalinum is a biotrophic ascomycete responsible for the red leaf blotch (RLB) affecting almond worldwide. Small leaf blotches, developing into necrosis throughout the season, are reported to be associated with the disease. The work was aimed at studying the RLB susceptibility of varieties representing most of Apulian almond biodiversity and setting molecular protocol for pathogen detection. Field-observations on 237 varieties grown in an *ex-situ* biodiversity almond collection field located at the Centro di Ricerca, Sperimentazione e Formazione in Agricoltura "Basile Caramia" in Locorotondo, and on six varieties grown in an orchard located in Ruvo countryside, were conducted for at least two years. RLB infections occurred at the end of spring to summer and were particularly severe in the year 2022, reaching up to 46.7% of incidence on 'Guara'. 'Guara' and 'Lauranne Avijor' were highly susceptible to the disease (Tukey test: $p<0.01$), while 'Belona', 'Centopezze', 'Ficarazza', 'Genco' and 'Rachele piccola' were the most tolerant (RLB never exceeding 6%). In 2023, in both the experimental fields, symptomatic leaves showing the typical RLB symptoms, or small red spots bordered by yellow halo (bacterial-like) or small necrotic spots, were singularly sampled from 33 varieties and used in molecular assays. A qPCR-based method was improved and used for *P. amygdalinum* detection in leaves. Our results confirmed the presence of the plant pathogen in the area, suggested different levels of susceptibility among *P. dulcis* varieties, and the mandatory use of suitable diagnostic tests to ascertain the presence of the pathogen when symptoms are not clearly attributable to RLB.

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Evaluating the potential of extreme environments for the isolation of biocontrol agents

M. Lucci, G. Firrao, A. Di Francesco

Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, 33100, Italy. E-mail: alessandra.difrancesco@uniud.it

Extreme environments are a largely unexplored reservoir of microbial diversity, with a remarkable exploitation potential for application in agriculture. Accordingly, the aim of this study was to isolate and characterize *Aureobasidium* spp. strains from different extreme ecosystems ranging from glaciers to deserts. A total of 117 strains were isolated and molecularly identified by internal transcribed spacer (ITS) region sequencing. The most represented yeast genera were *Aureobasidium* (57%), *Cryptococcus* (12%), *Vishniacozyma* (7%) and *Naganishia* (6%). For *Aureobasidium* spp. strains, a phylogenetic analysis was performed using multi-locus sequence typing (MLST), based on ITS, elongase (ELO), and elongation factor (EF-1alpha). To investigate *Aureobasidium* extremophilic or extreme-tolerant behaviour, five different temperatures (0 °C, 5 °C, 10 °C, 25 °C, and 45 °C) were assayed for the colony growth and conidiation on two different media (NYDA and Czapek). The growth rate was measured on a medium with different salt concentrations (5%, 10%, 15% NaCl) and different pH (2, 4, 6, 8, 10, 12). Preliminary results displayed a significant variability for growth and conidial production by *Aureobasidium* strains depending on culture conditions, and a remarkable thermotolerance aptitude. The displayed adaptation and versatility of *Aureobasidium* strains represent a promising starting point for investigating the role of genes and metabolites in microbial antagonism in a climate change scenario.

Presence and spread of grapevine yellows and esca disease complexes in two contiguous vineyards (cv Pinot b. and Nosiola) in Trentino

U. Malossini

Technology Transfer Center, Fondazione Edmund Mach, San Michele all'Adige, Italy. E-mail: umberto.malossini@fmach.it

Sanitary clonal selection activities (*Vitis*) are an international reference (IPPC, EPPO) for controlling and containing many phytosanitary problems. In a complex regulatory context, we summarize and simplify the experimental results of four-year evaluations on plants symptomatic of “grapevine yellow” (GY) and Esca complex (ESCA) in two adjacent Pinot blanc (Pb) and Nosiola (Nos) vineyards. The findings, on 5,371 pergola-grown vines with integrated production protocols, are reported to the individual plant. The activities (*e.g*., sampling and/or elimination of symptomatic shoots, uprooting, and replacements) highlight how the manifestation of GY symptoms varies between years and, above all, between cv. Nos, without a doubt and unlike Pb, is less subject to GY manifestations but particularly susceptible to ESCA. The diagnostic tests confirm the presence of samples positive for FD or BN (*Flavescence dorée* and *Bois noir*) in both cv and differentially in the shoots of the same vine (presence/absence). The symptom maps overlay concerning the original vines allows to support the epidemiological interpretation of the GY phenomenon, *e.g*., regarding the nursery materials used. Attention is being paid to the distance from possible alternative hosts such as inoculum sources known (shrubs, trees, or herbaceous essences) present on the margins or in the inter-rows (grass cover) of the vineyards. Future activities intend to verify the distribution of phytoplasmas in the plant according to the symptoms and their conservation and/or transmissibility with propagation materials, investigating what is improperly defined as the “recovery” phenomenon.

Combinations of beneficial microbes and lignin-copper nanoparticles for sustainable control of *Plasmopara viticola*

G. Manganiello[1], C. Pane[2], S. Lanzuise[1], R. Marra[1], N. Lombardi[1], C. Cimminella[3], M. Crimaldi[1], S. Cinti[4], A. Raucci[4], M. Lorito[1], S.L. Woo[4]

[1]Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; [2]Council for Agricultural Research and Economics (CREA), Research Centre for Vegetables and Ornamental Crops, Via Cavalleggeri 25, 84089 Pontecagnano Faiano, Italy; [3]Department of Biology, University of Naples Federico II, 80126 Naples, Italy; [4]Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy. E-mail: gelsomina.manganiello@unina.it

Copper-based pesticides are essential for disease management in both conventional and organic agriculture, being the most effective active ingredients against different phytopathogens, including *Plasmopara viticola*– grapevine downy mildew. However, their massive use produces negative long-term implications on the environment, soil biota, crop and human health due to soil and groundwater contamination. This study aims to reduce copper doses by applying biological control agents (BCAs), belonging to *Trichoderma* (*T. virens - T. asperellum*) and *Bacillus* (*B. subtilis - B. amyloliquefaciens*) genera, alone and in combination with lignin-copper nanoparticles, hybrid materials with reduced metal concentration compared to the commercial formulations on the market. Continuous experiments were conducted (2022 to present) on Falanghina grafted vine, treated with 22 different formulations. Treatments were foliar sprays, except for *Trichodema* spp., which were applied as a soil watering. Natural *P. viticola* infections, disease incidence, and severity indices were monitored under field conditions. Multi- and hyper-spectral technologies were applied to monitor grapevine health in the field and identify informative spectral bands and high-performing vegetative indices to discriminate between healthy and infected plants. Among the BCAs tested, *B. subtilis* resulted as the best performer; the application of *B. subtilis* in association with lignin-copper nanoparticles reduced disease parameters on both leaves and grape clusters with effects comparable to the commercial fungicide (tribasic copper sulphate). Furthermore, in the first year of production (2024), the application of *B. subtilis* and *B. subtilis* in combination with lignin-copper nanoparticles determined a positive effect on grape production compared to controls.

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Nematicidal activity of *Lippia graveolens* essential oil against *Meloidogyne enterolobii* and *Rotylenchulus reniformis* in cucumber

J.P. Manjarrez-Quintero, J.A. Carrillo-Fasio, J.B. Heredia, H. Bayardo-Rosales, O. Valdez-Baro, J.M. Tovar-Pedraza

Centro de Investigación en Alimentación y Desarrollo, Coordinación Regional Culiacán, Laboratorio de Fitopatología, Culiacán, 80110, México. E-mail: hbayardo123@estudiantes.ciad.mx

Mexico ranks fifth in cucumber (*Cucumis sativus* L.) production worldwide. However, the nematodes *Meloidogyne enterolobii* and *Rotylenchulus reniformis* cause severe losses in this crop. Therefore, this study evaluated the nematicidal activity of *Lippia graveolens* essential oil, obtained by hydrodistillation, against *M. enterolobii* and *R. reniformis* in cucumber plants grown in pots. Cucumber plants cv. Estrada (30 days old) grown in 3 kg pots containing a sterilized soil mixture, were used. Three treatments were applied in a completely randomized design, evaluating three concentrations of essential oil (1000, 1500, 2000 ppm) with three applications via drench (100 mL per pot) every seven days. Thirty days after inoculation, the number of nematodes per 100 g of soil for both nematodes was quantified. In addition, for *M. enterolobii*, the number of galls per root was determined, while for *R. reniformis*, the number of females per gram of root and the percentage of necrosis were evaluated. The results showed that all concentrations of *L. graveolens* essential oil significantly decreased the number of galls on roots induced by *M. enterolobii*. In the case of *R. reniformis*, a significant decrease was observed in the number of females and the percentage of root necrosis. Furthermore, a decrease in the number of nematodes per 100 g of soil for both species was observed. The results of this study indicate that *L. graveolens* essential oil could be a biorational alternative for use in the integrated management of *M. enterolobii* and *R. reniformis* in cucumber.

Effects of plant growth-promoting and phosphate-solubilizing microorganisms on wheat (*Triticum aestivum* L.) under controlled conditions

S. Marcone[1], A. Gentile[1], E. Ingenito[2], V. Cozzolino[1], S. Lanzuise[1], R. Marra[1]

[1]Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy; [2]Fertilidea S.r.l., Pompei, Italy. E-mail: sonia.marcone@unina.it

Rock phosphate is an inorganic phosphorus (P) source and represents one of the alternatives to synthetic plant fertilizers. However, rock phosphate-based fertilizers are non-renewable sources of P and can precipitate after the application, forming no bioavailable complexes. Phosphate-solubilizing bacteria (PSB) have been reported to increase phosphate content and plant growth. This study aimed to evaluate the ability of *Bacillus megaterium, B. pumilus,* and *Pseudomonas putida* to promote the growth and improve P absorption of wheat (*Triticum aestivum* L.) in the presence of different P sources. Surface-sterilized wheat seeds were soaked in bacterial suspensions and planted in pots containing vermiculite in the presence/absence of inorganic insoluble or soluble P sources, *e.g.*, phosphorite or monopotassium phosphate (KH_2PO_4), respectively. Seeds soaked in sterilized distilled water served as control. The experiment was carried out under controlled conditions (15–25° C, 16h light/8h dark). Germination was assessed on the fifth day after sowing, and plants were harvested after 30 days. Growth parameters, *i.e.*, root and shoot lengths and weights, as well as chlorophyll and P contents, were recorded at harvesting. Plants inoculated with bacteria and phosphorite significantly increased shoot length and weight. In particular, *B. megaterium* in the presence of phosphorite determined a +39% and +120% increase in shoot and fresh weights, respectively, compared to uninoculated control, while the chlorophyll content increased up to 50%. Moreover, all bacterial isolates increased P absorption in treated plants. Further experiments investigating the beneficial role of these PSB under field conditions are currently in progress.

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Mycobiota associated with sclerotia of *Claviceps gigantea* in Mexico

G. Márquez-Licona[1], A.R. Solano-Báez[1], M. Kolařík[2], C. De León[3]

[1]Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional (CEPROBI-IPN), Morelos, México; [2]Institute of Microbiology, Czech Academy of Sciences, Prague, Czechia. [3]Instito de Fitosanidad Fitopatología, Colegio de Posgraduados, Texcoco, México. E-mail: asolanob@ipn.mx

Claviceps gigantea infects the female inflorescence of maize, causing the plant to produce sclerotia instead of grains. In Mexico, the disease occurs with incidences of up to 90% and severities of 80% in susceptible hybrids. This research aimed to identify potential biocontrol agents present in the sclerotia. To achieve this, sclerotia were collected from intensively managed fields in the State of Mexico and Hidalgo and organically managed plots in Puebla. From the collected sclerotia, 140 isolates of the asexual state of *C. gigantea* were obtained, along with *Clonostachys* and *Trichoderma*. The sclerotia from organically managed fields exhibited five times more isolates of *Clonostachys* and three times more isolates of *Trichoderma*. The fungi were separated using monosporic cultures. The Internal Transcribed Spacer (ITS) region was amplified and sequenced to complement the identification of the fungi. A comparison of the sequences using the nBlast tool showed 100% similarity with *Clonostachys rosea* and *Trichoderma harzianum*.

The combination of conidia of *C. gigantea* and *C. rosea* in PDA culture medium demonstrated parasitism by *C. rosea*, inhibiting conidial production by 90%, and changes in the colony morphology were observed. In contrast, pure cultures of *C. gigantea* maintained abundant production of micro- and macroconidia with conserved cultural morphology. In the case of *T. harzianum*, this interaction did not occur. There are reports that *C. rosea* can degrade sclerotia up to 80% due to its parasitic capability. The isolates of *C. rosea* recovered in this study represent a biological management alternative for *C. gigantea*.

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Biocontrol activity of natural compounds and *Bacillus* antagonists against different phytopathogens

R. Marra[1], M. Papapaianni[1], D. De Rosa[1], M. Sinno[2], G. Sapatiello[1], N. Lombardi[1], S. Woo[2]

[1]Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; [2]Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy. E-mail: robmarra@unina.it

Applications of microbial antagonists and natural bioactive molecules have been proposed as sustainable alternatives to synthetic pesticides for plant disease control. In this study, the biocontrol activity of different natural compounds: essential oils (EOs), *Trichoderma* secondary metabolite 6-pentyl-α-pyrone (6PP), or isolates of *Bacillus* sp., were evaluated both *in vitro* and *in vivo*. Double-layer agar assay of *Pseudomonas syringae* pv. *tomato* (Pst) and *Xanthomonas campestris* pv. *campestris* (Xcc) showed total growth inhibition by cinnamon (*Cinnamomum zeylanicum*) and clove (*Syzygium aromaticum* L.) EOs at concentrations up to 0.25% (v/v). Conversely, 6PP at 10^{-3}–10^{-5}M was partially effective, and *Bacillus* spp. showed antibacterial activity according to the isolate/combination used. Cinnamon and clove EOs significantly inhibited bacterial biofilm formation when applied at 0.5–1% (v/v). EOs and 6PP also inhibited the growth of fungal pathogens (*Alternaria alternata, Fusarium oxysporum* f. sp. *lycopersici, Botrytis cinerea*) when applied in antibiosis assays at concentrations $\leq$ 0.05% (v/v). Spray applications to tomato (*Solanum lycopersici* L.) or kohlrabi (*Brassica oleracea*) with natural compounds showed that cinnamon and clove EOs and 6PP significantly reduced disease incidence and severity by Pst or Xcc, respectively, compared to untreated control; whereas *Bacillus* sp. reduced *in planta* symptoms but were less effective in controlling infection by both pathogens. Selected treatments will also be tested on olive (*Olea europaea*) infected with *Xylella fastidiosa* subsp. *pauca* in Apulia (Italy). These findings confirm that microbial antagonists and EOs represent interesting tools for plant bacterial diseases for which effective control measures are lacking.

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Genome-assisted fermentation strategies: enhancing *Lysobacter capsici* AZ78 resistance to UV radiations

A. Martini[1,2], G. Puopolo[1,2]

[1]Center of Agriculture, Food, Environment, University of Trento, San Michele all'Adige, Italy; [2]Research and Innovation Centre, Department of Sustainable Agro-Ecosystems and Bioresources, Fondazione Edmund Mach, San Michele all'Adige, Italy. E-mail: gerardo.puopolo@unitn.it

With conventional pesticide use restricted, microbial biocontrol agents (mBCAs) have become crucial for sustainable pest management. Despite success in laboratories and greenhouses, field efficacy remains inconsistent, primarily due to UV radiation's germicidal effects. However, microbes have evolved molecular mechanisms to protect themselves against UV. For instance, *Lysobacter* spp. produce xanthomonadins, pigments conferring resistance to UV, and its biosynthesis is positively regulated by the signal molecule 3-hydroxybenzoic acid (3-HBA). Based on this, this study aimed at enhancing the survival of *Lysobacter capsici* (AZ78) exposed to UV by increasing the production of xanthomonadins. The AZ78 genome has an operon consisting of seven genes with a high identity to the genes responsible for the production of xanthomonadins in *L. enzymogenes* OH11. Moreover, AZ78 genome mining showed the presence of an orthologue of *xanB2* responsible for 3-HBA biosynthesis in *Xanthomonas* spp., indicating that AZ78 can synthesize this signal molecule. The exposure of AZ78 colonies grown on LBA amended with 3-HBA produced a higher amount of xanthomonadins and, consequently, showed improved resistance to UV radiation. Furthermore, a greater resistance to UV radiations was observed when

AZ78 cells grown on LBA amended with 3-HBA were mixed with the co-formulant titanium oxide. The results achieved in this work clearly show that increasing pigment production during the fermentation of a Gram-negative bacterium can be a sound strategy to make its cells more resistant UV radiations, paving the way for a more effective application in the field.

"Mal dello stacco": a serious threat to hazelnut and preliminary attempts to develop biological control

I. Martino[1], V. Piattino[1], M. Maspero[2], T. De Gregorio[2], D. Spadaro[1,3], V. Guarnaccia[1,3]

[1]Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Braccini 2, 10095 Grugliasco (TO), Italy; [2]Hazelnuts company division, Ferrero Trading Luxembourg, 16 Rue de Trèves– L– 2633 Senningerberg, Luxembourg; [3]Centre for Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Torino, Largo Braccini 2, 10095 Grugliasco (TO), Italy. E-mail: ilaria.martino@unito.it

Italy is the second largest hazelnut producer with 105,048 tons in 2023. In Piedmont, hazelnut is one of the major crops and the disease incidence and the threat posed by wood diseases, such as "mal dello stacco", are increasing, causing serious concerns for producers. To identify the main fungal trunk pathogens affecting this crop, a polyphasic approach was conducted and demonstrated the role of *Anthostoma decipiens* as the predominant causal agent. Reddish conidial masses on infected branches represent the main inoculum source for the pathogen spread, which could be enhanced by climate change. The morphological similarity of this fungus with *Cytospora* spp. has often led to misidentification. Currently, the use of chemical fungicides on hazelnuts is highly limited in Italy. Considering the progressive restrictions on authorized active ingredients, different management strategies need to be developed. Preliminary trials were conducted on detached shoots treated with commercial products containing *Trichoderma* spp. showing promising results. A 60–70% reduction in lesion lengths was observed compared to untreated shoots. The results obtained from ANOVA followed by Tukey's post hoc test (p-value > 0.05) were comparable to those from shoots treated with a chemical fungicide as a positive control. *In planta* trials are ongoing to explore potential biological control tools to be applied in the field to implement effective management strategies to protect hazelnut cultivation and to ensure its sustainability.

The research was conducted with the cooperation and contribution of the Hazelnut company division of Ferrero Group.

Glechoma hederacea as source of specialized metabolites with suppressive activity against the phytopathogenic oomycete *Plasmopara viticola*

M. Masi[1], J.G. Zorrilla[1,2], O. Giovannini[3], S. Nadalini[3,4], A. Zanini[4], M.T. Russo[1], G. Puopolo[3,4], A. Cimmino[1]

[1]Department of Chemical Sciences, University of Naples Federico II, Naples, Italy; [2]Allelopathy Group, Department of Organic Chemistry, Facultad de Ciencias, Institute of Biomolecules (INBIO), University of Cadiz, Puerto Real, Spain; [3]Center Agriculture Food Environment (C3A), University of Trento, San Michele all'Adige, Italy; [4]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy. E-mail: marco.masi@unina.it

Grapevine (*Vitis vinifera* L.) is affected by many different phytopathogenic microorganisms causing significant economic losses worldwide. Among them, the phytopathogenic oomycete *Plasmopara viticola* is the causal agent of grapevine downy mildew, one of the most devastating diseases in viticulture. Copper-based fungicides are the oldest and the most common plant protection products used to control *P. viticola.* However, to reduce the use of copper, which has a negative impact on the environment and on human health, the scientific community is looking for alternative protection products. Based on this, the plant *Glechoma hederacea* was studied to evaluate the potential production of biofungicides able to control *P. viticola*. From its aqueous extract, causing a significant reduction of *P. viticola* infection, six metabolites have been purified by chromatography techniques and identified using spectroscopic methods as three aromatic compounds (carvacrol, caffeic acid and methyl caffeate), two flavonoids (cirsimaritin and apigenin), and a polyphenolic acid (rosmarinic acid). When tested against *P. viticola*, caffeic acid and methyl caffeate exhibited the highest disease severity reduction, while carvacrol, cirsimaritin, and apigenin showed moderate activity. Furthermore, the inhibitory activity of the aqueous extract could suggest synergetic or additive action of these specialized metabolites with other compounds contained in the extract. To our knowledge, this is the first report related to the effective control of *P. viticola* through the application *G. hederacea* aqueous extract, and the isolation and identification of the metabolites responsible for their biological activity.

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Investigations on the wide-spread occurrence of *Pectobacterium* stem-rot in greenhouse grown tomato in Sicily

M.E. Massimino[1], D. Nicotra[1], G. Dimaria[1], A. Mosca[1], D. Caponnetto[1], S. Iudicelli[1], J.M. van der Wolf [2], V. Catara[1]

[1]Department of Agriculture, Food, and Environment, University of Catania, Catania, Italy; [2]Bionteractions and Plant Health, Wageningen University & Research, Wageningen, The Netherlands. E-mail: vcatara@unict.it

Surveys conducted in South-East Sicily (Italy) showed the presence of plants affected by stem-rot. Externally, symptoms appeared as water-soaked, dark, longitudinal discolorations on the stem and at the petiole juncture. Longitudinal sections of the stem reveal either soft-rotted pith tissue or hollowing and/or browning. Xylem discoloration through the stem was also observed. Stem-rot caused by *Pectobacterium* spp. has been reported in different Italian regions on Solanaceous crops, including Sicily. Due to the presence of infected plants on several farms, in which tomatoes were grown in soil and soilless substrates, further investigation was conducted to determine the disease's etiology. Bacterial isolates showing pectolytic activity and testing positive by polymerase chain reaction (PCR) with *Pectobacterium* genus-specific primers Y1/Y2 were obtained from seven affected farms. Further PCR analysis confirmed that all isolates were positive for *P. brasiliense* using primers BR1f/L1r. Analysis of the *16S rRNA* gene and *dnaX* sequences further supported this identification. A pathogenicity test on tomato 'Moneymaker' seedlings successfully reproduced the disease symptoms. Bacterial community analysis using amplicon-based metagenomics on stem pith and roots revealed the presence of *Pectobacterium* amplicon sequence variants (ASV) both in symptomatic and asymptomatic plants present in the same greenhouse. An increased relative abundance of *Pectobacterium* ASV was correlated to the presence of symptoms. *P. brasiliense* is of increasing importance due to its spread and high virulence in potato blackleg, where it is transmitted via seed potatoes. Further investigations are needed to define its epidemiology in tomatoes and identify potential inoculum sources.

Sensitivity of causal agents of Brown Apical Necrosis and new anthracnose of walnut to fungicides and biological control agents

A. Matere[1], L. Luongo[1], I. Garaguso[1], D. Sansone[1], S. Panzardi[1], A. Fiorin[2], A. Signori[3], S. Vitale[1]

[1]CREA DC Research Centre for Plant Protection and Certification, Via C.G. Bertero 22, 00156 Roma, Italy; [2]NOCETO S.C.A. Via Chiusurata, n. 30/A, 31040 Chiarano, Italy; [3]NOGALBASoc. Cons. Agr. a r.l., via Pietro Nenni– Area Ind. G. Palatucci 250, 45025 Fratta Polesine, Italy. E-mail: salvatore.vitale@crea.gov.it

Brown Apical Necrosis (BAN) is important walnut hull fungal disease. In the last six years, a recurrence of BAN and a new walnut anthracnose by *Colletotrichum* spp. have been observed in northeastern Italy orchards. The use of chemicals control as tebuconazole and copper is necessary but may not be enough to control these new diseases. Thus, it is necessary to evaluate the behavior of new chemical and biological active ingredients. In this regard, the "*in vitro*" effect of seven fungicides - diphenoconazole, fludiuxonil, dodine, captan, mefentrifluconazole, potassium phosphonate, dithianon, and two biological control agents (BCAs) - *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae* - were evaluated for their potential to inhibit the mycelial growth of the causative agents of BAN and walnut anthracnose. Three treatments were evaluated for each compound: full application dose, half dose, and 1/10 dose. The active compounds used in these assays showed different efficacy on the growth of the tested fungi. Most of the tested fungicides show no inhibitory effect against the growth of the fungi. Diphenoconazole and two BCAs in application dose showed a fungistatic effect against *Phomopsis* and *Colletotrichum* spp., while *B. amyloliquefaciens* showed a fungistatic effect against most isolates in the application and at half dose. Regarding the variability of response within each fungal species toward the fungicides and BCAs tested, the assays performed are intended to provide preliminary indications for setting up targeted field trials.

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Trichoderma gamsii T6085 as potential biocontrol agent of *Fusarium verticillioides* on maize

D. Mencarini[1], F. Laddaga[1], M. Cesarini[1], A. Petrucci[1,2], S. Sarrocco[1]

[1]Department of Agriculture, Foos and Environment, University of Pisa, Pisa, Italy; [2]Department of Plant and Environmental Sciences and Copenhagen Plant Science Centre, University of Copenhagen, Denmark. E-mail: d.mencarini1@studenti.unipi.it

Fusarium verticillioides (*Fv*) is one of the major pathogens threatening maize, an important crop worldwide. Besides reducing its yield and quality, the plant pathogen produces fumonisins, mycotoxins harmful to humans and animals. Among environment-friendly tools, biological control is a valid approach to reduce *F. verticillioides*-caused maize disease. This study aims to evaluate the possible use of *Trichoderma gamsii* (T6085) as biological control agent in this patho-system. In the first part of the work (T6085 *vs. Fv*), T6085 significantly reduced plant pathogen growth and spore germination through diffusible compounds and showed signs of mycoparasitism. No effects of volatile substances (VOCs) were observed. When the molecular cross-talk occurring between the two isolates was investigated, T6085 significantly up-regulated the expression of its *thy* (putative hydrophobin) and *mdr* (major-facilitator superfamily membrane transporter) genes, with no modulation of *tri5* (terpene synthase). This behaviour could be linked to *T. gamsii* defence response after perceiving the pathogen. In the second part of the study, gene expression analysis was performed during T6085-maize interaction as consequence of T6085 endophytic root colonization. From T6085 perspective, *thy, tri5*, and *epol* (endo-polygalacturonase) gene expression was investigated at three days post inoculation of T6085 on roots, resulting in an up-regulation only of *thy*. From maize perspective, the expression of the defence-related genes *lox10* (lipoxygenase), *chit2* (chitinase), *hpl* (hydroperoxide lyase), *myc7* (myc transcription factor7), and *pal3* (phenylalanine ammonia-lyase) was analyzed. Results are still under elaboration. This work represents a promising starting point to deeply investigate the T6085 application to control *F. verticillioides* on maize.

Genomic insights into host adaptation and virulence mechanisms of *Colletotrichum lupini*

A. Menicucci[1], D. Da Lio[2,3], S. Becerra[4], C. Fiorenzani[1], A. Prodi[1], M.R. Thon[4], S.A. Sukno[4], G. Vannacci[2], G. Le Floch[3], S. Sarrocco[2], R. Baroncelli[1,5]

[1]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), Bologna, Italy; [2]University of Pisa, Department of Agriculture, Food and Environment, Pisa, Italy; [3]Université de Brest, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, Plouzané, France; [4]Universidad de Salamanca, Instituto de Investigación en Agrobiotecnología (CIALE), Salamanca, Spain; [5]University of Naples Federico II, Center for Studies on Bioinspired Agro-Environmental Technology, Portici, Italy. E-mail: riccardo.baroncelli@unibo.it

Understanding the genomic determinants underlying host specificity and pathogenicity in plant pathogens is crucial for devising effective disease management strategies. *Colletotrichum lupini* is a well-known plant pathogen, both as a model study and as an economic threat. Using an integrated approach based on biological evidence and bioinformatics analysis, we aimed to elucidate the genomic factors driving this interaction. After conducting a thorough pathogenicity assay to define pathogenicity boundaries, we employed two parallel strategies to identify candidate pathogenicity-related genes. The first approach was based on clustering predicted proteins based on sequence similarity comparing proteomes of pathogenic and non-pathogenic isolates. The second analysis focused on identifying genomic Lineage-Specific Regions (LSRs) in plant pathogenic strains. These regions are hypothesized to harbour horizontally transferred genes (HGT) and others that may be genomic footprints of plant-pathogen coevolution. Through these methods, we identified pathogen-specific genes linked to candidate effectors, secondary metabolism, post-transcriptional modification, and other essential biological functions. Hence, our analysis revealed several candidate genes associated with pathogenicity, suggesting their roles in host specificity and virulence. Further studies are ongoing to validate the biological function through targeted gene knockout experiments and functional characterization assays. Our study has the potential to improve disease management and crop protection. By providing novel insights into the genomic determinants driving host specificity and pathogenicity, we have paved the way for developing targeted strategies. Uncovering these candidate pathogenicity genes is a significant step forward, offering the possibility to develop more effective and sustainable approaches to disease management and crop protection.

Investigating the nature and role of leaf-associated fungi in the specialization of *Zymoseptoria* spp. in wild grasses

L. Merani[1,2], V. M. Flores-Núñez[1], E. Holtgrewe-Stukenbrock[1,3]

[1]Environmental Genomics, Christian-Albrechts University of Kiel, Germany; [2]Department of Agriculture, Food and Environment, University of Pisa, Italy; [3]Max Planck Institute for Evolutionary Biology, Plon, Germany. E-mail: l.merani@studenti.unipi.it

Zymoseptoria spp. are leaf hemibiotrophic pathogens specialized to distinct grass hosts. During the biotrophic colonization, the pathogen invades the leaf tissue through opening stomata. Since plants are considered metaorganisms and leaf tissue is inhabited by a plethora of microbes, our hypothesis is that the specialized pathogen could have evolved a mechanism of interaction with microbial members and that may be drivers in host specialization. In this study, we tested lineages of *Zymoseptoria tritici* and *Zymoseptoria passerinii* that infect wild relative species of wheat and barley, respectively. To understand if they have evolved microbial preference, we created a culture collection from the leaf-associated fungi of wild *Aegylops cylindrica* and *Hordeum murinum* during the biotrophic phase infection and we tested their interaction *in vitro*. Based on ITS sequencing, *Penicillium* is the most abundant genus and for their species identification, we performed a morphological description and molecular analysis based on β-tubulin gene and ITS sequences. The phylogenetic tree suggests a clade that shares common strains and another clade that contains strains exclusively from *A. cylindrica.* For characterizing the interaction mechanism, we performed an *in vitro* assay for the putative effect of volatile and diffusible metabolites on fungi and pathogen growth and we assessed *in planta* colonization by leaf inoculation. In general, diffusible metabolites negatively influence the growth of *Zymoseptoria* and *Penicillium* by *in vitro* assay, which likely suggests a competitive relationship. Instead, further analysis has to be performed on the plant host leaves to clarify the role of *Penicillium* on the disease progression.

Microbiota on almonds, cherry and peach trees in Apulia region

N. Mezgueldi[1], O. Farahy[1], K. El Handi[1], D. Gerin[2], A.M. D'Onghia[1], S. Pollastro[2], F. Faretra[2], F. Valentini[1]

[1]CIHEAM Bari, Centre International des Hautes Etudes Agronomiques Méditerranéennes, via Ceglie, 9–70010 Valenzano, Italy; [2]Department of Soil, Plant and Food Sciences, (DiSSPA), University of Bari Aldo Moro, Bari, Italy. E-mail: donato.gerin@uniba.it

Stone fruits are economically important crops in Italy, particularly in the southern regions. Different diseases can affect them, and fungal and bacterial diseases cause significant economic losses by reducing yield and stone fruit production. A two-year study aimed to evaluate the epiphytic microorganisms present in buds, twigs, leaves, and fruits of almond, cherry, and peach trees from different mesoclimatic Apulian areas. Samples were collected during the growing seasons 2022–2023 and 2023–2024, from the end of March to June, and over 250 bacterial and yeast isolates were isolated. All were grouped into different morpho-molecular types. Considering the presence of all morphotypes for each sampling time, according to the multivariate analysis, significant differences ($p>0.05$) were observed between morphotypes, orchards, and locations. Based on the biochemical characterization (KOH, fluorescence, LOPAT, and urease tests) performed on 52 isolates representing 14 morphotypes, 16 biochemical profiles were identified, generally corresponding to non-pathogenic bacteria and yeasts. The molecular analysis of the partial 16S rDNA sequences proved that *Pseudomonas* was the most represented genus (six morphotypes), followed by *Curtobacterium, Sphingomonas, Rhodotorula* (two morphotypes each), and *Pantoea* and *Hannaella* (one morphotype each). All of them are well known to include species with antimicrobial activity. In conclusion, the data provide valuable information on the epiphytic bacteria living on the aerial organs of Puglia's main stone fruit species. Furthermore, the isolated species can provide a useful collection of bacteria and yeasts to study for their antagonistic effects towards other organisms harmful to stone fruit.

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Investigating plant-pathogen interactions in pigmented wheat lines for Fusarium Head Blight (FHB) resistance using metabolomics and a targeted trichothecene analysis

C. Miccoli[1], L. Narduzzi[2], L. Felici[1], S. Francesconi[1], A.M. Garcia- Campaña[2], S. Palombieri[1], F. Sestili[1], G.M. Balestra[1]

[1]University of Tuscia, Department of Agriculture and Forest Sciences (DAFNE), San Camillo De Lellis Street snc, 01100 Viterbo, Italy; [2]Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Av. Fuentenueva s/n, Granada, E-18071, Spain. E-mail: cecilia.miccoli@unitus.it

Fusarium Head Blight (FHB) is one of the most destructive diseases affecting cereal crops. Secondary metabolites, particularly polyphenols, are known for their protective properties against environmental stress and pathogenic microorganisms. Therefore, pigmented wheat genotypes, which naturally contain high levels of polyphenols in their pericarp and aleurone layers, are of significant interest for their potential resistance to FHB. This study examines the plant-pathogen interactions between five pigmented bread wheat lines, which exhibit different levels of resistance to Fusarium head blight (FHB). The analysed genotypes include two blue-aleurone bread wheat varieties (Purendo and Skorpion) and three purple-pericarp varieties (Rosso, Vanilnoir, and Indigo) which were artificially treated with the fungal pathogen *Fusarium graminearum* on spikes *via* tip inoculation. The aim of this research is to identify secondary metabolites of interest related to disease resistance, particularly focusing on the role of polyphenols. Advanced analytical techniques were used: a triple quadrupole instrument, coupled with liquid chromatography through electrospray ionization (LC-ESI-QqQ-MS/MS) was used to detect trichothecenes in the samples. Additionally, two metabolomic methods with a hybrid quadrupole time-of-flight mass spectrometer coupled to liquid chromatography through ESI (LC-ESI-Q-ToF-MS) were performed to analyze the metabolic responses induced by infection. The study observed specific metabolic and trichothecenes profiles across the different varieties in response to fungal infection. These findings highlight the potential of certain metabolic responses to serve as biomarkers for FHB resistance.

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Eco-friendly strategies for enhanced crop growth: The synergistic effects of *Trichoderma virens* and lignin extracts

M. Michelino[1], S. Lanzuise[2], V. Macchia[3], C. Scamardella[1], C. Cimminella[4], S. Marcone[2], A. Pappalardo[1], S.L. Woo[2,5]

[1]Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy; Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; [3]Green Innovation GMBH, Grabenweg 68, 6020 Innsbruck, Austria; [4]Department of Biology, University of Naples Federico II, 80126 Naples, Italy; [5]BAT Center—Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, University of Naples "Federico II", 80055 Portici, Italy. E-mail: mari.michelino@unina.it

The need to increase crop production and, at the same time, reduce the use of synthetic fertilizers are the main goals of modern agriculture. Among the possible eco-sustainable strategies is the use of biological formulations based on beneficial microorganisms and natural extracts, which are known to stimulate plant development and protect from biotic and abiotic stress. The present study investigated biotreatment effects on seed germination and growth promotion of important horticultural crops such as tomato, onion, lettuce, pepper, carrot, and beet. Treatments were based on applications with the plant-beneficial fungus *Trichoderma virens* Gv41 and the commercial extract of lignin (Lign; Solargo™ - a plant biostimulant), used singly and in combination in greenhouse assays under controlled conditions. Results showed that a combined treatment of Lign+Gv41, significantly increased seed germination of onion and pepper compared to the untreated control (and the individual treatments61% and 26%, respectively). Furthermore, the Lign+Gv41 formulation was the most effective in terms of growth promotion of tomato, increasing plant fresh weight by 19% compared to the water control. Nonetheless, Gv41 single treatment was the most effective on onion, lettuce, pepper, carrot, and beet, significantly increasing the stem's fresh and dry weight. Bioassays are currently ongoing to evaluate the effects of these biotreatments on plant diseases caused by plant pathogens *Fusarium oxysporum* f. sp. *lycopersici, Rhizoctonia solani*, and *Sclerotinia sclerotiorum.* These results suggest that beneficial microorganisms and lignin extracts can positively contribute to an eco-friendly strategy to increase crop production.

Functional characterization of cold-tolerant endophytic bacteria isolated from alpine Rosaceae plants

I. Milanese[1,2], M.K.M. Marian[1], S. Nigro[3], V. Lefebvre du Prey[3], N. Campo[3], M. Perazzolli[1,2]

[1]Center Agriculture Food Environment (C3A), University of Trento, Via E. Mach 1, 38098 San Michele all'Adige, Trento, Italy; [2]Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38098 San Michele all'Adige, Trento, Italy; [3]ITAKA Crop Solution, 20121 Milano, Italy. E-mail: michele.perazzolli@unitn.it

Microbial communities associated with plants growing in cold environments can promote plant growth at low temperatures and improve plant tolerance to cold stress. Thus, the use of psychrotolerant endophytic bacteria could be a promising approach to protect crop plants from cold stress. This work aims to investigate the effect of bacterial endophytes from cold-adapted alpine plants on tomato tolerance against cold stress. A total of 41 cold-tolerant bacterial isolates collected from the roots of three alpine Rosaceae plants were tested for their growth-promoting activity on tomato plants. Seedlings were grown at 25 °C under controlled conditions and the most promising isolates were selected according to their ability to increase dry and fresh plant biomass. Ten bacterial isolates were then tested for their ability to promote tomato growth at 10 °C, and two of them improved shoot growth under cold-stressed conditions, reducing the content of H_2O_2 and modulating the expression of cold-stress-related genes. These two bacterial isolates improved the root growth of tomato plants under commercial greenhouse conditions. Thus, plant-growth-promoting bacteria isolated from cold environments could represent a valid approach to enhance plant growth and mitigate abiotic stress.

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The role of multifaceted characterization of Chilean *Xanthomonas arboricola* strains in the management of the hazelnut bacterial blight

P. Minardi[1], E. Biondi[1], N. Cortez[2], A. Checcucci[3], N. Biondo[1], F. Quaiotto[1], D. Giovanardi[4], J. Guerrero[5], S. Perez[2]

[1]Department of Agricultural and Food Sciences (DISTAL), Alma Mater Studiorum - University of Bologna, V. le G. Fanin 42, 40127 Bologna, Italy; [2]Institute of Agri-Food, Animal and Environmental Sciences (ICA3), Universidad de O'Higgins, Ruta 90 km. 3, 3070000 San Fernando, Chile; [3]Department of Agriculture, Food, Environment and Forestry (DAGRI) - Genetics Section, University of Florence, Via Maragliano 77, 50144 Firenze, Italy; [4]Department of Life Sciences (DLS), University of Modena and Reggio Emilia, via Amendola 2, 42122 Reggio Emilia, Italy; [5]Facultad de Ciencias Agropecuarias y Forestales, Universidad de La Frontera, 4811230 Temuco, Chile. E-mail: paola.minardi@unibo.it

The *Xanthomonas arboricola* (Xa) species has a high genetic variability and the pv. *corylina* (Xac) is the etiological agent of the bacterial blight of hazelnut which is one of the main bacterial diseases in areas where hazelnut is cultivated. In this study, 19 Xa and 19 Xac strains isolated in Chile from hazelnut symptomatic plants, were characterised through Rep-PCR analysis. Several Xa pathovar reference strains (*e.g. celebensis*, *pruni*, and *juglandis*) were used as controls, while *X. axonopodis* pv. *vitians* DISTAL 9081 was used as outgroup. Then, multiplex end-point PCR on T3SS/T3Es/T3SPs genes, Biolog GenIII^{TM} analysis, hypersensitivity and pathogenicity tests were carried out. The UPGMA analysis of Rep-PCR data discriminated the 38 Xa strains in statistically significant groups corresponding to the relative pathovars: *pruni, corylina, juglandis*, and *celebensis*. The Xac strains showed a high genetic variability (approx. 45%). Similarly, the Rep-PCR data of the Xa strains from symptomatic shoots, leaves, buds and fruits gave results with a similarity of 50% among strains included in the *corylina* pathovar. As for virulence genes, the presence of *hpaA, hrpD5, hrpF*, and *xopAQ* was confirmed in all strains, while *xopZ* was absent. The HR on French bean resulted positive for all strains, and the selected Xa/Xac strains resulted pathogenic on hazelnut plants. Finally, most of metabolic activity confirmed the molecular characterization of Xac/Xa strains, pooled in the same group. Moreover, the Xa strains were here combined in a sub-group of the Xac cluster. The epidemiological role of multiple characterization of Xa strains is discussed.

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Identification and characterization of citrus bark cracking viroid isolates infecting citrus in southern Italy

M. Minutolo[1], M. Cinque[1], P. Nicoletti[1], B. Navarro[2], M. Chiumenti[2], M. Eiras[3], L. Assis da Silva[4], A. Levi Rodrigues Chaves[3], D. Alioto[1], F. Di Serio[5]

[1]Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Portici, Italy; [2]Istituto per la Protezione Sostenibile delle Piante, CNR, Bari, Italy;[3]Laboratório de Fitovirologia e Fisiopatologia, Centro de Pesquisa de Sanidade Vegetal, Instituto Biológico, São Paulo, SP, Brazil; [4]Laboratório de Baculovirus, Departamento de Biologia Celular, Instituto de Ciências Biológicas, Universidade de Brasília, DF, Brazil; [5]Istituto per la Protezione Sostenibile delle Piante, CNR, Torino, Italy. E-mail: minutolm@unina.it

Citrus bark cracking viroid (CBCVd; species *Cocadviroid rimocitri*), initially reported in citrus, is currently considered a major hop pest due to the severe symptoms this viroid may induce in this host. Although CBCVd is generally considered a minor pest of citrus grafted onto sour orange rootstock, it may cause severe bark cracking associated with characteristic green streaks in some citrus species grafted onto trifoliate orange or carrizo rootstocks. In Italy, this viroid has been reported in sweet orange, lemon, clementine, and sour orange, which are generally infected with more than one viroid species and do not show any symptoms on the bark. However, Italian CBCVd isolates have yet to be molecularly characterized, and the relationships between these isolates and those distributed elsewhere are unknown. In a preliminary survey aimed to further assess the distribution of this viroid in Italy and Brazil as part of a joint CNR/FAPESP research project, additional isolates of CBCVd were identified in southern Italy, including symptomless lemon trees grafted on sour orange rootstocks and sweet orange trees grafted onto carrizo-citrange. The latter plants showed reduced vegetative growth, and although bark cracking was absent, characteristic green streaks were easily observable after scraping the bark. In these trees, CBCVd was identified by RT-PCR using specific primers followed by direct sequencing of the amplification products. Here, the results of the molecular characterization of these CBCVd isolates will be presented, and the relationships concerning other CBCVd isolates reported previously from different geographic areas, including Brazil, and from other hosts will be discussed.

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Biocontrol of fungal disease affecting hops: green and sustainable alternatives for the Italian cultivation

F. Modica[1], M. Cortiello[1], F. Bellameche[1], L. Prodi[1], E. Costi[1], C. Riccioni[3], F. De Marchis[3], A. Rubini[3], L. Brilli[4], E. Nunez Carmona[2], F. De Canio[1], E. Stefani[1], D. Giovanardi[1]

[1]Department of Life Science, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy; [2]Institute of Bioscience and Bioresources, CNR-IBBR, 50019 Sesto Fiorentino (FI), Italy; [3]Institute of Bioscience and Bioresources, CNR-IBBR, 06128 Perugia, Italy; [4] Institute of Bioeconomy, CNR-IBE, 50145 Firenze, Italy. E-mail: francesco.modica@unimore.it

The Italian craft beer market is supporting the demand for local hops. However, hop cultivation and pest management are still challenging. *Verticillium* spp. (VW), *Podosphaera macularis* (PM), and *Botrytis cinerea* (BC) are the main fungi affecting hops, whose current management relies on resistant cultivars and extensive use of fungicides.

In 2023, hop roots and rhizomes were sampled and analysed to isolate root-associated bacteria (from the rhizosphere and the endosphere) and bacteria harboured on rhizomes. Plant samples were obtained from cultivated hop, cv. Cascade, and from wild accessions available in the Province of Modena (Italy). A total of 207 isolates were obtained and screened for their ability to trigger a Hypersensitive Reaction (HR) on tobacco and tomato and, for further characterisation, only HR-negative isolates were considered. Such isolates were then tested *in vitro* for their ability to inhibit the growth of VW, BC, and PM. Twelve isolates showing significant antifungal activity were further evaluated for possible plant growth-promoting traits such as saline stress tolerance, phosphate solubilization, siderophores, ammonia, and hydrogen cyanide production. The best-performing isolates were identified through partial sequencing and NCBI blast analysis of the *16S rRNA* gene, revealing them as members of the genera *Erwinia, Agrobacterium,* and *Pantoea*. Additional experiments for assessing bacterial ability to colonize hop plants and their effectiveness against fungal pathogens *in planta* are ongoing. Our study identified hop root and rhizome-associated bacteria that are potential candidates as biostimulant and biocontrol agents for "green" applications aimed to increase hop quality and productivity.

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Lettuce seedlings rapidly assemble their microbiome from the environment through deterministic processes

N.Z. Mohamed[1,2], L. Schena[1], A. Malacrinò[1]

[1]Department of Agriculture, Università degli Studi Mediterranea di Reggio Calabria, Loc. Feo di Vito, 89124 Reggio Calabria, Italy; [2]Agronomy Department, Faculty of Agriculture, Cairo University, Egypt. E-mail: nesma.mohamed@unirc.it

Plant-associated microorganisms have significant impacts on plant biology, ecology, and evolution. Although several studies have examined the factors driving variations in plant microbiomes, the mechanisms underlying the assembly of the plant microbiome still need to be better understood. In this study, we used gnotobiotic plants to test (i) whether seedlings create a selective environment and drive the assembly of root and leaf microbiomes through deterministic or stochastic processes and (ii) whether seedlings structure the microbiome that is transferred through seeds using deterministic processes and whether this pattern changes when seedlings are exposed to the environmental microbiome. In this study, we grew lettuce (*Lactuca sativa* L.) plants under gnotobiotic conditions and exposed them to 21 soil microbial communities. After one week, we collected root and shoot samples for amplicon metagenomics. Our results show that the microbiome of gnotobiotic plants (*i.e.*, inherited through seeds) is not under the selective influence of the host plant but changes quickly when plants are exposed to soil microbiomes. Within one week, plants could select microorganisms from the inocula, assemble the root microbiome, and assemble the shoot microbiome. This study supports the hypothesis that plants at early developmental stages might exert strong selective activity on their microbiomes and contribute to clarifying the mechanisms of plant microbiome assembly.

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Investigating the mode of action of a new sustainable fungicide against *Botrytis cinerea* and *Phytophthora infestans*

S. Montanari[1,2], F. Trenti[1], A. Nesler[3], C.M.O. Longa[2], L. Navazio[4,5], B. Baldan[4,5], M. Perazzolli[1,2]

[1]Centre Agriculture Food Environment (C3A), University of Trento, Via Mach 1, 38098 San Michele all'Adige, Trento, Italy; [2]Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38098 San Michele all'Adige, Trento, Italy; [3]Bi-PA nv (Biological Products for Agriculture), Londerzeel, Belgium; [4]Department of Biology, University of Padova, Via U. Bassi 58/B, 35131 Padova, Italy; [5]Botanical Garden, University of Padova, Via Orto Botanico 15, 35123 Padova, Italy E-mail: sofia.montanari@unitn.it

Botrytis cinerea and *Phytophthora infestans* are major plant pathogens causing threats to many crops and severe losses worldwide. Disease management involves the frequent use of synthetic fungicides, with potential negative impacts on human health and the environment. This study aimed to investigate the mechanism of action of a new natural substance-based fungicide based on choline pelargonate (CP, patent BE1026779B1). CP efficacy was tested *in vitro* against *B. cinerea* and *P. infestans*, and the inhibitory effects were assessed on mycelium growth and spore germination to identify minimum inhibitory concentration and minimum fungicidal concentration. Structural alterations of CP-treated *B. cinerea* and *P. infestans* cells were investigated by transmission electron microscopy to corroborate the negative impacts of CP on mycelial cells. Overall, this study demonstrated that CP is a promising broad-spectrum fungicide that can inhibit plant pathogen growth. Further metabolomic studies will provide better information on the precise target sites of CP at the cellular level and on possible response mechanisms by the pathogens.

The role of the endobacterium *Candidatus* Glomeribacter gigasporarum in *Gigaspora margarita*-tomato roots interaction

M.M. Monti[1], M. Novero[1,2], V. Bianciotto[1], S. Ghignone[1], F. Palomba[1], E. Lumini[1], A. Salvioli[1,2], P. Bonfante[2], M. Ruocco[1]

[1]Istituto per la protezione Sostenibile delle Piante, Strada delle Cacce 73, Torino, Italy. [2]Dipartimento Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, V.le P.A. Mattioli 25, 10125 Turin, Italy. E-mail: michelina.ruocco@cnr.it

The widespread association between plants and arbuscular mycorrhizal fungi (AMF) positively affects the growth performance of many crops, acting as biofertilisers and bioprotectors. Recent studies have identified AMF-associated bacteria as cooperative partners participating in AMF symbiosis. The research aims to determine whether i) endobacteria identified as *Candidatus* Glomeribacter gigasporarum (*Ca*Gg) and living inside the AMF *Gigaspora margarita* (BEG 34, B+) positively stimulate the growth of a tomato cultivar (San Marzano Nano) and ii) they offer protection against a tomato pathogen, *Botrytis cinerea* (isolate SC1). The availability of a cured line (without the endobacterium, B-) allowed to perform a direct comparison of the impact of the two isogenic lines on the tomato plants. Both lines did not lead to a significant improvement in plant growth when considering physiological parameters (photosynthesis and stomatal conductance). The level of AM colonization of our tomato cultivar is low, according to previous data collected on colonization on a different tomato cultivar, M82. The success of colonization on non-infected healthy plants was higher when plants were colonized by the B+ line compared with infection by the B- line. On the contrary, in plants infected with *B. cinerea*, the cured line B- resulted in more efficient root colonization. Symptom analysis highlighted a lower severity of leaf damage in plants colonized by the B+ line. The AMF protective effect against plant pathogens is well acknowledged, but the synergy between the symbiotic fungus and an endobacterium is a novel result, even if the molecular basis of such three-kingdom interactions is still unknown.

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Identification and characterisation of microbial isolates from pear carposphere and their possible role in brown spot disease management

A. Montorsi[1], A. Fochesato[1], C. Nasuti[1], A. Barigazzi[1], G. Agolino[3], M. Menghini[2], M. Cortiello[1], F. Bellameche[1], F. Modica[1], R. Baroncelli[2], M. Collina[2], L. Solieri[1], E. Stefani[1], D. Giovanardi[1]

[1]Department of Life Science, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy; [2]Department of Agricultural and Food Sciences (DISTAL), University of Bologna, 40127 Bologna, Italy; [3]Department of Agriculture, Food and Environment (Di3A), University of Catania, 95123 Catania, Italy. E-mail: alessandro.montorsi@unimore.it

Brown spot of pear (BSP) caused by *Stemphylium vesicarium* (*Sv*) is one of the most important fungal diseases in Europe, as it may cause more than 90% yield losses. In Abate Fétel pear orchards, located in five Emilia Romagna provinces (Modena, Bologna, Ferrara, Ravenna and Forlì-Cesena) and under different pest management strategies (integrated or organic), studies on the carposphere microbiota helped us to identify bacterial and yeast epiphytes suitable to be exploited as microbial biocontrol agents (mBCAs) against *Sv.* During the summer of 2023, a total of 359 bacterial and 233 yeast strains were isolated and tested *in vitro* against *Sv* DLS2021 (UNIMORE collection). The dual-plate assay showed a reduction of *Sv* mycelial growth higher than 65% for nine bacteria and nine yeasts, later taxonomically identified. Based on ITS regions (spanning ITS1, 5.8S rRNA and ITS2) sequencing, antagonistic yeasts were attributed to the species *Aureobasidium pullulans, Metschnikowia pulcherrima, Rhodotorula babjevae, Rhodotorula glutinis*, and *Sporobolomyces patagonicus*. For bacterial isolates, the partial sequencing and NCBI blast of the *16s rRNA* gene confirmed their identity as members of the genera *Bacillus, Pseudomonas, Pantoea*, and *Erwinia*. Putative mBCAs were checked for their biocontrol activity *in vivo* on detached fruits, under laboratory conditions. The tested bacterial and yeast isolates were able to reduce the BSP lesion severity on fruits by up to 55%. Our study may help to understand the epidemiological importance of culturable microbiota residing on the pear carposphere and the selection of effective mBCAs suitable for implementing sustainable management of BSP.

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Integrated analyses of the plant and soil microbiome identify *Phytopythium vexans* as agent of the Kiwifruit Vine Decline Syndrome

S. Mosca, M.M. Aci, G. Procopio, V. Vadalà, G. Vizzari, E. Francomano, N.Z. Mohamed, M.G. Li Destri Nicosia, G.E. Agosteo, L. Schena, A. Malacrinò

Department of Agriculture, Università degli Studi Mediterranea di Reggio Calabria, Reggio Calabria, Italy. E-mail: saveria.mosca@unirc.it

Kiwifruit Vine Decline Syndrome (KVDS) is a disease that is currently challenging kiwifruit production in Italy and is spreading to new production areas. However, the causal agent of this syndrome has yet to be clearly identified, and we still need to learn more about the overall effects of KVDS on the interactions between the host plant and its microbiome. In this study, we combined amplicon metagenomics and targeted isolation (leaf baiting) to characterize the changes in the rhizosphere and root microbiomes associated with symptoms of KVDS. Our results suggest that KVDS has little impact on the bacterial, fungal, and oomycete communities associated with soil and roots, and we detected weak signatures of potential dysbiosis. On the other hand, we found a consistent association of the oomycete *Phytopythium vexans* with samples from plants symptomatic to KVDS, which matches the nucleotide sequences of the isolates obtained through baiting and, partially, the isolates from previous studies. While our results support the idea that *P. vexans* might be the major candidate agent of KVDS, there are still several unanswered questions that need to be addressed before being able to provide effective solutions to this emerging challenge in kiwifruit production.

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Protein hydrolysates: a sustainable solution for mitigating biotic stress in horticultural and non-horticultural crops

A. Mostacci[1], F. Rivardo[2], O. Incerti[1], M. Desopo[1], L. Convertini[1], A. Ippolito[1], R. Sartori[2], S.M. Sanzani[1]

[1]*Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, via Amendola 165/A, 70126 Bari, Italy;* [2]*A. Costantino & C. SpA, Via Francesco Romana, 11, 10083 Favria, Italy. E-mail: simonamarianna.sanzani@uniba.it*

By 2030, as part of the Green Deal Farm to Fork Strategy, the European Commission intends to achieve a 50% reduction in the overall chemical pesticide use. An alternative approach to meet the objective is to empower plant natural defence responses by using biostimulants, which can positively act on plant biological processes, including the defence against stresses. In the present investigation, a formulate (AgriD) based on a protein hydrolysate

(PH) derived from soybean (*Glycine max*) and one of its main peptides (Pep) were tested for their activity against plant fungal pathogens. *In vitro* interaction assays were carried out between PH and pathogens of horticultural (lettuce - *Alternaria alternata* and *Botrytis cinerea*) and non-horticultural crops (wheat– *A. alternata, Fusarium oxysporum and Fusarium culmorum*), using plate microdilution assays and growth on PH/Pep amended solid media. At the label doses of 2.5 ml/L, AgriD did not present antifungal activity and contrariwise seemed to stimulate *F. oxysporum* and *B. cinerea* growth. However, the lowest concentrations (≤ 0.0025 ml/L) showed a slight antifungal activity (−20%) against *A. alternata*. Considering the peptide, the dose of 5 g/L proved to have antifungal activity against *A. alternata* (−37%) and *F. oxysporum* (−16%), while it seemed to stimulate the growth of *B. cinerea*. *In vivo* interaction assays are ongoing in the presence of biotic/abiotic stresses in traditional and hydroponic cultivation systems.

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Effectiveness of chitosan and low-risk fungicides for the control of fungal seedborne pathogens on cabbage

M. Moumni, S. Piancatelli, S.M. Makau, M. Tunç, A.L. D'Ortenzio, G. Romanazzi

Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, via Brecce Bianche, 60131 Ancona, Italy. E-mail: g.romanazzi@univpm.it

Pesticide restrictions and requests for high-quality seeds highlight the need for eco-friendly solutions against seedborne pathogens. This work aimed to test chitosan, COS-OGA, terpenes, and *Bacillus amyloliquefaciens*, in stand-alone application (-NT) or together with standard fungicide treatments (-T) in controlling fungal diseases and improving seed quality of seed-bearing cabbage. Trials were run in 2021, in two fields, located in Monterado and Montefiore dell'Aso, Marche region, central-easter Italy. In both fields, all the strategies showed variable levels of plant protection. In -NT, terpenes and chitosan generally showed a lower incidence of leaf necrosis due to fungal infections. COS-OGA was more effective when applied together with a standard program. For stems and siliquae, *B. amyloliquefaciens* and terpenes showed promising results. Seeds produced from both fields were analyzed using the blotter test method. *Alternaria brassicicola, Alternaria alternata, Cladosporium* spp., and *Stemphylium botryosum* were identified from the seeds of Monterado. From the -NT part, seeds treated with chitosan showed the lowest level of *A. alternata* incidence, with no differences from copper. Terpenes and COS-OGA reduced the incidence of *A. brassicicola* compared to the untreated control. In -T, the infection level was lower, and differences emerged only for *A. alternata*, where COS-OGA -T and *B. amyloliquefaciens* -T provided significant reductions. From the seeds of Montefiore dell'Aso, *A. brassicicola, A. alternata*, and *Cladosporium* spp. were identified. All the strategies reduced *A. brassicicola* and *A. alternata* incidence compared to the untreated control. These results open the way for the use of low-impact compounds in the protection of seed-bearing cabbage.

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Stemphylium vesicarium on onion: activity of synthetic fungicides and natural compounds

I. Muratore[1], D. Palmieri[1], F. Di Virgilio[1], M.T. Scarano[1,2], G. Lima[1], F. De Curtis[1]

[1]Dipartimento di Agricoltura, Ambiente e Alimenti, Università del Molise, via F. De Sanctis snc, 86100 Campobasso, Italy; [2]U.O.T-C.V.R., INAIL direzione regionale MOLISE, via Insorti d'Ungheria, 70, 86100 Campobasso, Italy. E-mail: decurtis@unimol.it

Stemphylium vesicarium (Cooke) Wint., the causal agent of leaf blight, is a severe worldwide disease in onion cultivation. The lack of registered fungicides to effectively control the disease represents a serious problem. Furthermore, in other crops, this fungus has been shown to be resistant to some classes of fungicides, such as quinone outside inhibitors (QoIs) and succinate dehydrogenase inhibitors (SDHIs). Consequently, very few tools are available for farmers to contain this harmful pathogen. In this scenario, natural compounds, including biomolecules and microelements, with side effects on both plants and pathogens, can represent interesting alternative tools. Some of these products, carried by chelators of different chemical nature, can improve natural defence and plant health. Preliminarily, we assessed the pathogenicity of a strain of *S. vesicarium* on onion plant, hybrid F1 'Borneo', demonstrating Koch's postulates. We tested *in vitro* and *in vivo* on onion plants five synthetic fungicides: azoxystrobin, boscalid, difenoconazole, tebuconazole, and trifloxystrobin and two natural compounds, such as copper and zinc-based products, compared to difenoconazole as a standard fungicide. Synthetic fungicides showed the best activity against the pathogen, reducing by

70–95% the pathogen growth/disease in plants compared to the untreated controls. Encouraging results have also been obtained by the tested natural compounds. Further research is underway to identify the best control application strategy against such an insidious plant pathogen.

Innovative strategies to produce dsRNAs as a sustainable grapevine protection strategy against *Botrytis cinerea*

A. Narduzzo[1,2], F. Favaretto[1], W. Chitarra[1,3], L. Nerva[1,3]

[1]Research Centre for Viticulture and Enology (CREA-VE), Council for Agricultural Research and Economics, Via XXVIII Aprile 26, 31015 Conegliano, Italy; [2]Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padua, Agripolis, Viale dell'Università 16, 35020 Legnaro (PD); [3]Institute for Sustainable Plant Protection (IPSP-CNR), National Research Council of Italy, Strada delle Cacce 73, 10135 Torino, Italy. E-mail: anna.narduzzo@crea.gov.it

Grapevine is one of the most important and globally widespread fruit crops, significantly impacting the economies of many countries. Concomitantly, viticulture exerts considerable adverse environmental effects due to the massive use of agrochemicals. Therefore, developing new sustainable defence strategies against fungal pathogens is crucial to achieving sustainable agriculture. RNAi strategies, which involve the direct application of exogenous dsRNAs/siRNAs on plants, are among the most innovative approaches developed to overcome the limitations of GMO restrictions. Several studies have been successfully applied to synthetize dsRNAs *in vitro* and *in vivo* against plant pathogenic fungi, such as *Botrytis cinerea, Fusarium graminearum,* etc.. However, these methods face significant limitations, such as the high costs of dsRNAs production and low stability of naked dsRNAs in the environment. To address these issues, our study aims to develop several plant molecular farming strategies to produce and stabilize dsRNAs through protein combination. We developed a specific plasmid that binds the dsRNA sequence to the CDS of the viral P0 capsid protein. The latter has been shown to encapsulate dsRNA molecules, protecting them from degradation. Using this plasmid, we are selecting different platforms for the dsRNA-P0 expression such as *Nicotiana benthamiana*, BY-2 and MM2d cell lines as well as ligninolytic fungi. Our efforts are directed towards producing high-specific sequences capable of controlling *Botrytis cinerea*. The final goal will be to scale up dsRNAs production and enhance their stability, providing a valuable alternative to agrochemicals in plant pest management strategies.

Selection of bacterial strains for interaction studies with microbial biocontrol agents in the rhizosphere

D. Nicotra[1,2], G. Dimaria[1], A. Mosca[1], M.E. Massimino[1], K. Di Miceli[1], F. Valenti[1], M. Sinno[3], A. Martini[4,5], R. Marra[6], I. Pertot[4,5], V. Catara[1]

[1]Department of Agriculture, Food, and Environment, University of Catania, Catania, Italy; [2]Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy; [3]Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy; [4]Center Agriculture Food Environment, University of Trento, 38098 San Michele all'Adige, Italy; [5]Research and Innovation Center, Fondazione Edmund Mach, 38098 San Michele all'Adige, Italy; [6]Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy. E-mail: vcatara@unict.it

MIND project aims to enhance our understanding of microbe-microbe interactions within the plant holobiont with the goal of developing more effective microbial biocontrol agents (mBCAs) for managing tomato pathogens. mBCAs, when introduced into the plant environment, engage with resident microbiota that, in turn, can affect the mBCAs' establishment and effectiveness. To set up studies to investigate these interactions, a collection of bacteria was assembled based on the response of the resident bacterial communities to mBCA treatments. Model mBCAs belonging to the genera *Lysobacter*, *Trichoderma, Bacillus,* and *Pseudomonas* were applied singly or as small consortia by soil drenching to one-month-old tomato cv. Moneymaker. Experiments were performed under controlled conditions in pots filled with agricultural soil collected from a greenhouse with a tomato cultivation history. The effect of the treatments on the microbial communities of the tomato rhizosphere was evaluated one-month post application. DNA was extracted from rhizosphere samples and was analysed by amplicon-based metagenomics. Differences in community diversity, structure, and complexity were analysed with the Amplicon Sequence Variant (ASV) method. Bacterial isolates were obtained from the agricultural soil by dilution plating on general and semi-selective media to select bacterial taxa that belong to enriched, depleted, and not modulated bacteria as identified by metagenomics. Purified selected bacteria were identified by *16S rRNA* gene sequencing. These bacteria will be used in further project steps to evaluate their impact and interaction on mBCAs and tomato plant pathogens.

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Genomic insights into ten plant growth promoting bacteria isolated from tomato endorhizosphere

D. Nicotra[1,2], S. Ghosh[3], A. Mosca[1], F. Ghadamgahi[3], A. Anzalone[1], G. Dimaria[1], M.E. Massimino[1], R.R. Vetukuri[3], V. Catara[1]

[1]Department of Agriculture, Food and Environment, University of Catania, Catania, Italy; [2]Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy; [3]Department of Plant Breeding, Swedish University of Agricultural Sciences, Alnarp, Sweden. E-mail: vcatara@unict.it

Research into plant-associated microbial communities has rapidly expanded with high-throughput sequencing techniques, unveiling new ways to investigate plant-microbiome and microbe-microbe interactions. A microbiome-guided top-down approach was used to select ten bacterial strains from different taxa within the core microbiome of tomato plants in the production chain for potential use as bioinoculants. This selection included well-known biofertilizers and biocontrol agents like *Pseudomonas* and *Bacillus*, as well as less-studied genera such as *Leclercia, Chryseobacterium, Glutamicibacter*, and *Paenarthrobacter*. Inoculating these strains into the tomato rhizosphere promoted plant growth and reduced the severity of Fusarium Crown and Root Rot and Bacterial Spot infections, regardless of their *in vitro* antagonistic activity. We assessed these strains' plant growth-promoting (PGP) potential in silico, identifying genes associated directly and indirectly with plant growth. In all strains, the highest proportion of genes were linked to colonization, stress control, and biofertilization. Annotation of the genomes against the 'plant bacterial only interaction factors (proteins) (PIFAR)' dataset revealed two distinct clusters according to antimicrobial activity. Pseudomonads had the highest percentages of toxin-related factors. The lowest toxin content was detected in the Micrococcaceae strains, which in turn showed the greatest abundance of hormone-related factors. In *Pseudomonas* and *Bacillus*, the highest number of Biosynthetic Gene Clusters (BGCs) was predicted compared to the other strains, explaining their potent antimicrobial activity. This genomic information will aid in developing strategies to use these bacteria individually or in consortia, coupling strains with complementary traits, effects, and mechanisms of action to enhance plant growth and resilience.

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Identification and pathogenicity of fungi in the Botryosphaeriaceae family associated with dieback of blueberry (*Vaccinium corymbosum* L.) in Sinaloa, Mexico

P.R. Nuñez-Garcia[1], A. Rebollar-Alviter[2], J.G. Ramírez-Gil[3], K.C. Correia[4], R.S. García-Estrada[1], J. León-Félix[1], J.M. Tovar-Pedraza[1]

[1]Laboratorio de Fitopatología, Centro de Investigación y Desarrollo, Coordinación Regional Culiacán, Culiacán, Sinaloa, Mexico; [2]Centro Regional Morelia, Universidad Autónoma de Chapingo, Morelia, Michoacán, Mexico; [3]Departamento de Agronomía, Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Sede Bogotá, Bogotá, Colombia; [4]Centro de Ciências Agrárias e da Biodiversidade, Universidade Federal do Cariri, Crato, Ceará, Brazil. E-mail: pnunez222@estudiantes.ciad.mx

Mexico is currently the fifth largest producer of blueberries worldwide. However, dieback is the most harmful disease, particularly in northwest Mexico. The aim of this study was to identify the fungi associated with blueberry dieback in commercial fields in the state of Sinaloa, Mexico, using morphological and molecular approaches. To achieve this, symptomatic blueberry branches were sampled and collected from August 2022 to May 2023 in 17 commercial fields located in the municipalities of El Fuerte, Ahome, Guasave, Culiacán, and Navolato, in Sinaloa, Mexico. From the samples of 11 blueberry cultivars established in Sinaloa, 896 fungal colonies belonging to the family Botryosphaeriaceae were isolated. For morphological and molecular identification as well as pathogenicity tests, 24 isolates were selected and identified to the genus level through a combination of morphological analysis and ITS rDNA sequencing. In total, 17 isolates of *Lasiodiplodia*, three of *Neofusicoccum*, three of *Cophinforma*, and one isolate of *Pseudofusicoccum* were identified. In the pathogenicity test, three-year-old blueberry plants were used, inoculating three branches with mycelial discs of each fungal isolate. The evaluation was carried out 14 days after inoculation, recording the length of external and internal necrosis. The test was carried out twice with similar results. The results indicated that all fungal isolates were pathogenic but with different levels of virulence. Overall, *Lasiodiplodia* isolates were the most virulent, followed by *Pseudofusicoccum, Cophinforma*, and *Neofusicoccum* isolates. Subsequently, *Tef1* and *Btub* gene sequences will be amplified to distinguish the isolates at the species level.

Identification and pathogenicity of *Phytopythium helicoides* causing root and crown rot of blueberry in Mexico

P.R. Nuñez-García[1], W.A. Vieira[2], I. Cruz-Lachica[1], R.S. García-Estrada[1], J.M. Tovar-Pedraza[1]

[1]Laboratorio de Fitopatologia, Centro de Investigación en Alimentación y Desarrollo, Coordinación Regional Culiacán, 80110, Sinaloa, México; [2]Departamento de Fitopatologia, Universidade de Brasília, Brasília, 70910-900, Brazil. E-mail: pnunez222@estudiantes.ciad.mx

In August 2023, blueberry (*Vaccinium corymbosum* L.) plants showing foliar symptoms of wilting and root rot were observed in a field in Sinaloa, Mexico. Disease incidence was approximately 20%. For isolation, symptomatic root pieces were surface sterilized with 2% NaClO for 2 min, rinsed in sterilized distilled water two times and placed on potato dextrose agar (PDA), corn meal agar (CMA), and V8 agar media. Colonies with oomycete morphology were obtained and two purified isolates were grown on PDA, CMA, and V8. Colonies were white and cottony in all three media used. The morphology of colonies and reproductive structures (oogonia, antheridia, oospores, and sporangia) coincided with those reported for *Phytopythium helicoides*. For molecular identification, genomic DNA was extracted, and the internal transcribed spacer (ITS) region and partial sequences of large subunit (LSU) gene were amplified and sequenced with the primer sets ITS5/ITS4 and NL1/NL4, respectively. A phylogenetic tree including published ITS and LSU sequence data of *Phytopythium* spp. was inferred using the maximum likelihood approach. The phylogenetic analysis placed the two isolates in the same clade as *P. helicoides*. Healthy blueberry plants (2-year-old) cultivated in 10 kg pots were inoculated for the pathogenicity assay. The pathogenicity test was performed twice. Wilting symptoms were observed 30 days after inoculation, whereas no symptom was observed in control plants. To the best of our knowledge, this is the first report of *P. helicoides* causing root and crown rot in blueberries in Mexico and worldwide.

Alternative control strategies for *Plasmopara viticola* on grapevine: efficacy of volatile organic compounds

S. Oberhofer[1], S. Avesani[2,3,4], M. Perazzolli[3,4], P. Robatscher[2], U. Spitaler[1]

[1]Institute for Plant Health, Laimburg Research Centre, Auer, Italy; [2]Laboratory for Flavours and Metabolites, Laimburg Research Centre, Auer, Italy; [3]Center for Agriculture Food Environment (C3A), University of Trento, San Michele all'Adige, Italy; [4]Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38098 San Michele all'Adige, Italy; E-mail: sabine.oberhofer@laimburg.it

Plasmopara viticola, the causal agent of downy mildew, is a widespread obligate biotroph pathogen in viticulture. The oomycete can overwinter as oospores in leaf litter, shoots, and soil. Infection occurs by the release of zoospores from sporangia and their subsequent leaf infection through stomata. Application of synthetic chemical fungicides and copper formulations in viticulture will be limited, and the efficient control of *P. viticola* poses new challenges for grape cultivation. Possible alternatives to chemical fungicides include novel natural substances, like volatile organic compounds (VOCs), and the development of sustainable approaches for future pest control, such as RNA interference (RNAi). By using sequence-specific short interfering RNA (siRNA), target genes of the pathogen can be silenced and further growth of the organism can be prevented. In our experiment, the efficacy of three VOCs against downy mildew was evaluated in greenhouse assays. Volatile application was conducted on potted grapevines of the cultivar Vernatsch/Schiava. Treated plants were artificially inoculated with a *P. viticola* spore suspension and disease severity was evaluated six days post-infection by calculating the diseased leaf area with an image processing software. Preliminary results showed good efficacy of linalool, and low efficacy of two other VOCs against *P. viticola*. The results of VOC-application on entire plants under greenhouse conditions will provide further insides into their applicability as active substances in future control strategies.

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Fusarium pseudograminearum, a causal agent of Fusarium crown and root rot on durum wheat in Sardinia

S. Oufensou[1,2], V. Balmas[1,2], G. Porqueddu[1], Q. Migheli[1,2]

[1]Dipartimento di Agraria, Università degli Studi di Sassari, Viale Italia 39A, 07100 Sassari, Italy; [2]Nucleo di Ricerca sulla Desertificazione, Università degli Studi di Sassari, Viale Italia 39A, 07100 Sassari, Italy. E-mail: soufensou@uniss.it

Fusarium Root Rot (FRR) and Crown Rot (FCR) are devastating diseases, mainly caused by *Fusarium culmorum*, in wheat-producing areas in Sardinia. Few reports on *Fusarium pseudograminearum* have been so far reported in Europe: Italy, and Spain, and EFSA considers it as a potential quarantine pest. Between 2015 and 2022, seven strains of *F. pseudograminearum* were isolated from plants affected by FCR collected from four different areas in Sardinia. Samples were identified molecularly using species-specific primers. Pathogenicity assays in the greenhouse were conducted to confirm their pathogenicity on a susceptible variety of durum wheat (Saragolla). A pathogenic strain of *F. pseudograminearum* isolated in Foggia (Apulia) in 1992 was used as a reference. After 21 days, the disease index was calculated. All strains proved highly pathogenic. A re-isolation from the experimentally inoculated host was performed and identified as *F. pseudograminearum* satisfying Koch's criteria. These results indicate that *F. pseudograminearum* is an additional responsible agent of FCR and FRR diseases in Italy.

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Biocontrol activity of yeast strains against *Aspergillus ochraceus* on maize products

S. Oufensou[1,2], V. Balmas[1,2], G. Porqueddu[1], Q. Migheli[1,2]

[1]Dipartimento di Agraria, Università degli Studi di Sassari, Viale Italia 39A, 07100 Sassari, Italy; [2]Nucleo di Ricerca sulla Desertificazione, Università degli Studi di Sassari, Viale Italia 39A, 07100 Sassari, Italy. E-mail: soufensou@uniss.it

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

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Identification of QTLs associated with fire blight resistance in European pear genotypes

F. Pacini, L. Bergonzoni, L. Dondini, S. Tartarini, A. Bertaccini

Department of Agricultural and Food Sciences, Alma Mater Studiorum-University of Bologna, 40127 Bologna, Italy. E-mail: francesco.pacini2@unibo.it

Among pear infectious diseases, the fire blight, caused by *Erwinia amylovora,* is the most devastating. The restricted tools available and the limitations to antibiotic usage in EU regulation make the use of resistant/tolerant varieties the basis of a sustainable disease management approach. The quantitative nature of the fire blight resistance trait makes it difficult to identify candidate genes. Sources of fire blight resistance are available in pear (*Pyrus communis* L.) germplasm. As such, the cultivar ‘Old Home’ has been employed in cultivar and rootstock breeding. The crossing population ‘Abbé Fetel’ x ‘Old Home’ was developed to determine the genomic regions associated with fire blight resistance. The *E. amylovora* virulent strain EaFP621 was used for phenotyping 132 genotypes of this progeny through experimental inoculation in 2022 and 2023, via the bisected-leaves method under greenhouse conditions. A wide susceptibility range has been detected, and a few genotypes will be further evaluated to confirm their lower susceptibility and determine their agronomic performance. SPET (Single Primer Enrichment Technology) genotyping was conducted to obtain high-density genetic maps for each parental genotype. The QTL analysis of ‘Old Home’ allowed the identification of a major QTL in LG2 and a minor QTL at the end of LG4. Results confirmed the QTL’s major role in LG2 for fire blight resistance as reported by studies on other pear varieties such as Harrow Sweet, Moonglow, and Potomac. RNA-Seq in ‘Old Home’ leaves is planned to highlight the role of candidate genes in the QTLs regions.

Leeks extracts from horticulture by-products, possible applications for plant protection

S. Pagnoni, L. Bedrengan, A. Passera, M. Esposito, P. Casati, B. Scaglia

Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, University of Milan, via Celoria 2, 20133 Milan, Italy. E-mail: saul.pagnoni@unimi.it

Allium ampeloprasum L. is a monocotyledonous species that includes common leeks, edible vegetables rich in bioactive organosulfur compounds (OC), which are appreciated for their nutritional and pharmaceutical properties. Leeks are typically commercialized as first-range or fresh-cut products (*i.e.*, non- or minimally processed and packed stems), but their production generates a conspicuous amount of residues from non-edible green leaves. Due to the presence of OC with anti-microbial activity, these residues cannot be utilized as feedstock for fermentation or biorefinery purposes and are typically left on soil or buried. In our research, we devised a low-impact extraction method to obtain leek extracts enriched in OC. After a preliminary quali-quantitative characterization of these extracts by GC-MS, we tested their potential bioactivity against common phytopathogenic fungi and bacteria *in vitro* and *in vivo*. Initial *in vitro* results using disk diffusion tests and optical density evaluation do not suggest a direct biocidal or biostatic effect of the extracts. Nevertheless, *in vivo* pot trials with lettuce indicate a mitigation of symptoms caused by *Rhizoctonia solani*, increasing seed germination and biomass accumulation in soil inoculated with the pathogen. Seeds sown in soil treated with extracts and inoculated with the pathogen showed germination percentages and dry biomass at 20 days after sowing comparable to those registered in the untreated, uninoculated control. Further experiments will aim to assess the reproducibility of these effects, using extracts obtained from different leek batches as starting material.

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Importance of endo-xylanase encoding genes in *Fusarium graminearum* enzymatic activity and pathogenicity

A. Panichi, S. Tundo, S. Odorizzi, C. Castiglioni, R. Musetti, F. Favaron, L. Sella

Department of Land, Environment, Agriculture and Forestry (TESAF), University of Padova, 35020 Legnaro, Italy. E-mail: arianna.panichi@phd.unipd.it

Fusarium graminearum is a plant pathogen responsible for the Fusarium head blight disease in cereals, which reduces grain yield and quality. During the infection process, the fungus produces several cell wall-degrading enzymes to penetrate the host tissues. Among these enzymes, xylanases are crucial for the degradation of xylan, a major component of monocot plant cell walls. In this study, we focused on *FGSG_03624* and *FGSG_10999*, two endo-xylanase encoding genes highly expressed during wheat spike infection, which were knocked-out by inserting an antibiotic resistance gene. Pathogenicity assays on *Triticum aestivum* spikes and soybean seeds, performed with both single and double mutants, highlighted that only the deletion of *FGSG_10999* significantly reduces fungal virulence on both plant hosts compared to the wild-type and KO_03624 strains. However, the double knock-out mutant exhibited virulence like the single *FGSG_03624* mutant. Additionally, we compared the xylanase, cellulase, and pectinase activities produced by the mutant strains with those of the wild-type and we evaluated the genetic expression of other endo-xylanase encoding genes during wheat spike infection at three days post-inoculation to assess any compensatory effects resulting from the absence of the two knocked-out genes. Results showed that both the single KO_10999 strain and the double knock-out mutant, were affected in the secretion of total xylanase activity. Expression analysis highlighted that two endo-xylanase genes are over-expressed in the mutants with the *FGSG_10999* gene deleted. Further experiments are needed to clarify the lower virulence of the single *FGSG_10999* mutant compared to the double mutant.

Pseudomonas putida V01 inhibits the growth of fungal pathogens through the production of volatile organic compounds (VOCs)

A. Pappalardo[1], A. Gaspari[2], S. Parlanti[3], S.L. Woo[4], F. Vinale[2]

[1]Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; [2]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy; [3]Versalis S.p.A., 20097 San Donato Milanese, Italy; [4]Department of Pharmacy, University of Naples Federico II, Naples, Italy. E-mail: annabella.pappalardo@unina.it

Rhizospheric bacteria are essential for plant's health and productivity as they can increase nutrient uptake, degrade toxic compounds, and produce growth regulators. These microbes also benefit plant protection through competition for nutrients and the production of metabolites with antibiotic activity, including volatile organic compounds (VOCs). VOCs are lipophilic substances with high vapor pressure (0.01 kPa) and low molecular weight, able to act over distances thanks to soil diffusion. *Pseudomonas putida* V01 was isolated from the rhizosphere of eggplants (*Solanum melongena*) and identified by analysis of the 16S rDNA region. *In vitro* assays demonstrated antagonistic activity to *Athelia rolfsii* and the ability to solubilize nutrients. Moreover, V01 positively affected shoot length, weight, and chlorophyll content of *Triticum aestivum* seedling *in vivo*. Sealed plate test to assess the antifungal effect of *P. putida* VOCs, was performed on different plant pathogens. VOCs were also absorbed on active charcoal, desorbed with dichloromethane and analysed by chromatography coupled with mass spectrometry (GC-MS) for chemical characterization. A significant inhibition of *Botrytis cinerea* (30%) was detected, and a morphological change of *A. rolfsii, Alternaria alternata, Fusarium proliferatum,* and *Rhizoctonia solani* was highlighted. We demonstrated that antibiotic activity can be related to the production of the main metabolites, 2- undecanone and 2- undecanol. Further studies are needed to test the *in vivo* antifungal activity of the selected *P. putida* VOCs.

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Short-chain fatty acid combined with beneficial fungi as a tool for new formulations in plant protection

A. Pappalardo[1], C. Scamardella[1], S. Parlanti[2], S.L. Woo[3], F. Vinale[4]

[1]Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; [2]Versalis S.p.A., 20097 San Donato Milanese, Italy; [3]Department of Pharmacy, University of Naples Federico II, Naples, Italy; [4]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy. E-mail: annabella.pappalardo@unina.it

Pelargonic acid is a saturated nine-carbon fatty acid with antimicrobial activity, approved for use in food, personal care products, and transdermal drug delivery. Soil-beneficial microorganisms, such as fungi of the genus *Trichoderma*, are well known for their role in protecting plants and promoting growth. In this study, we tested the biocontrol activity against post-harvest fungal pathogens of fatty acids products (short and medium chain) in combination with beneficial fungi. Biocontrol assays of short and medium-chain fatty acids, at different concentrations (from 0 to 0.1%) against *Alternaria alternata, Botrytis cinerea, Fusarium incarnatum, F. proliferatum,* and *F. verticilloides* were performed. Sodium salts were also obtained and tested (from 0 to 1%). Moreover, the capability of selected *Trichoderma* strains to grow and survive in the presence of short and medium-chain fatty acids and their salts was investigated. All pathogens were inhibited at the minimum doses of short and medium-chain fatty acids. In particular, *B. cinerea* was inhibited entirely at tested doses; meanwhile *A. alternaria* was totally inhibited only at 0.05%. *Trichoderma afroharzianum* and *T. virens* showed significant compatibility with minimum doses of all the tested compounds, with no inhibition of *T. afroharzianum* for all substances tested and of *T. virens* for both salts. Conversely, at high concentration, any of the selected strains tolerated the molecules. In conclusion, we can hypothesize that a combination of *Trichoderma* with short and medium-chain fatty acids and its salt, at moderate doses, could be applied for a biocontrol strategy of plant pathogens.

This research was funded by Versalis S.p.A. ("Convenzione per il finanziamento di una borsa di studio da destinarsi al corso di dottorato di ricerca in "SUSTAINABLE AGRICULTURAL AND FORESTRY SYSTEMS AND FOOD SECURITY"– XXXVIII° ciclo, con sede amministrativa presso l'Università degli Studi di Napoli Federico II").

Recombinase polymerase amplification assay for the detection of Mal secco disease by *Plenodomus tracheiphilus*

R. Parlascino[1], E.I. Rovetto[1], S. Conti Taguali[1,2], M. Riolo[1], A. Pane[1], C. Bua[1], S. Moricca[3], M. Garbelotto[4], M. Amato[5], D. Ezra[6], F. La Spada[1], S.O. Cacciola[1]

[1]Department of Agriculture, Food and Environment (Di3A), University of Catania, Catania, Italy; [2]Department of Agricultural Science, Mediterranean University of Reggio Calabria, Reggio Calabria, Italy; [3]Department of Agri-food Production and Environmental Sciences, Plant Pathology and Entomology Division, University of Florence, Florence, Italy; [4]Department of Environmental Science, Policy and Management, University of California, Berkeley, CA, USA; [5]Agdia EMEA, Soisy-sur-Seine, France; [6]Department of Plant Pathology and Weed Research, Agricultural Research Organization, Rishon LeZion, Israel. E-mail: federico.laspada@unict.it

This study presents the development of a novel diagnostic assay, based on recombinase polymerase amplification (RPA) technology, for the detection of *Plenodomus tracheiphilus*, the causative agent of mal secco of lemon. To establish the specificity of the assay, a 142 bp RPA-compatible barcode was identified within the ITS regions and *P. tracheiphilus* specificity was confirmed by using BLAST in the NCBI database. Then, RPA primers and probes were designed. The inclusivity and specificity of the RPA assay were assessed by testing genomic DNA isolated from 29 strains of *P. tracheiphilus* and non-target fungal and oomycete plant pathogens commonly associated with citrus trees. The RPA assay had a detection threshold of 1.0 pg of genomic DNA. Notably, the RPA assay exhibited greater sensitivity than SYBR® Green I Real Time-PCR in tests with non-purified rapidly extracted DNA. Preliminary field applicability tests indicated the potential of the RPA assay for rapid, user-friendly, and cost-effective in-field diagnosis of Mal secco. This highlights its promising role as an efficient tool for the practical detection of *P. tracheiphilus* in citrus trees.

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Analysis of virome by high-throughput sequencing disclosed double infection in a symptomatic plant of *Hydrangea macrophylla*

G. Parrella[1], L. Sigillo[2], G. Serratore[2], A. De Stradis[3], A. Marais[4], C. Faure[4], T. Candresse[4]

[1]Institute for Sustainable Plant Protection of the National Research Council (IPSP-CNR), 80055 Portici, Italy; [2]Council for Agricultural Research and Economics, Research Centre for Vegetable and Ornamental Crops, 84098 Pontecagnano, Italy; [3]Institute for Sustainable Plant Protection of the National Research Council (IPSP-CNR), 70126 Bari, Italy; [4]Univ. Bordeaux, INRAE, UMR BFP, CS 20032, 33882 Villenave d'Ornon CEDEX, France. E-mail: giuseppe.parrella@ipsp.cnr.it

Among phytosanitary problems of ornamental plants, viruses stand out for their capacity to reduce quality and yield. However, detecting and identifying viral infections in ornamental plants can sometimes be challenging. This study performed a high throughput sequencing (HTS) of the viral pathogens present in a French hydrangea (*Hydrangea macrophylla*) showing virus-like symptoms to elucidate the etiology. Analysis of the RNA-seq data from a plant with leaf yellowing and vein clearing identified the presence of two distinct nucleorhabdoviruses, eggplant mottle dwarf virus (EMDV; genus *Alphanucleorhabdovirus*) and tomato betanucleorhabdovirus 2 (TBRV2; genus *Betanucleorhabdovirus*) which were probably implicated in the observed symptoms. Electron microscopic examinations of symptomatic leaf samples confirmed the presence of rhabdovirus-like particles. The complete genome of the EMDV hydrangea isolate showed high genetic similarity (*ca*. 95.0% nt identity) with previously characterized isolates of this virus. TBRV2 was identified as several small contigs that do not cover the whole genome. The largest contig obtained (2230 nt in the 3' terminal genome region) showed *ca*. 95.7% nucleotide identity with previously described TBRV2 isolates. These results obtained by RNA-Seq of total RNAs parallel those obtained in a first round of siRNA-based HTS analysis which also identified these two viruses. They suggest that TBRV2 accumulates in *H. macrophylla* at much lower levels than EMDV. This is the first time that EMDV and TBRV2 have been reported in mixed infection in *H. macrophylla*.

The reported HTS analysis of the French hydrangea samples was carried out as a transnational service access in the frame of the European Union-funded EVA-Global infrastructure project (H2020 - grant agreement n° 871029-EVA-GLOBAL).

A new rhabdovirus found in *Jasminum sambac* and survey for possible insect vectors in Italy

G. Parrella[1], E. Troiano[1], T. Candresse[2], C. Faure[2], A. Marais[2], P.A. Pedata[1]

[1]Institute for Sustainable Plant Protection of the National Research Council (IPSP-CNR), 80055 Portici (NA), Italy; [2]Univ. Bordeaux, INRAE, UMR BFP, CS 20032, 33882 Villenave d'Ornon CEDEX, France. E-mail: giuseppe.parrella@ipsp.cnr.it

In Spring 2023, a plant of *Jasminum sambac* showing virus-like symptoms consisting of bright yellow mosaic and deformation of the leaves was noticed in a nursery located in the Latium region. Similar symptoms were noticed again in Spring 2024 on another plant of *J. sambac* in the same nursery. Leaf samples collected from the plant identified in 2023 were subjected to high-throughput sequencing (HTS) of ribodepleted total RNAs. The results indicate that this plant was infected by two viruses: jasminum virus H and a new tentative rhabdovirus. Near complete genomes were assembled *de novo* for both viruses. The new rhabdovirus was also detected by RT-PCR, using specific primers designed on the sequence obtained by HTS, in the plant sampled in Spring 2024. Sanger sequencing confirmed amplicons specificity. Following the ICTV rules for the nomenclature of viruses, this new virus species has been tentatively named *Alphanucleorhabdovirus jasmini.* Possible insect vectors were identified by collecting specimens from several species of *Jasminum,* including *J. sambac*. In particular, two species of leafhopper were identified by morphological analysis and molecular characterization of the partial cytochrome oxidase gene (COI), as *Hishimonus diffractus* and *Synophropsis lauri*. These two species are under evaluation as potential vectors of the new rhabdovirus. The presence of infected plants coming from nurseries represents a serious problem for the spread of diseases, especially for new, incompletely characterized diseases. This situation requires more stringent controls that only the application of diagnostic systems such as HTS can guarantee.

The reported HTS analysis of the Jasminum sambac samples was carried out as a transnational service access in the frame of the European Union-funded EVA-Global infrastructure project (H2020 - grant agreement n° 871029-EVA-GLOBAL).

Citrus yellow vein clearing virus in symptomatic plants of *Citrus lemon* identified in a nursery of Latium

G. Parrella[1], E. Troiano[1], A. De Stradis[2]

[1]Institute for Sustainable Plant Protection of the National Research Council (IPSP-CNR), 80055 Portici (NA), Italy; [2]Institute for Sustainable Plant Protection of the National Research Council (IPSP-CNR), 70126 Bari, Italy. E-mail: giuseppe.parrella@ipsp.cnr.it

In spring 2024, during a visual inspection in a nursery in Lazio, some lemon plants were identified with suspicious symptoms, consisting of vein clearing and deformations of the leaves. Symptoms were mostly concentrated in the leaves of the distal portion of the branches. Symptomatic leaves were collected from different plants and taken to the laboratory for further analysis. Examination of leaf dip extracts by electron microscopy showed the presence of filamentous virions resembling those of potexviruses and of spherical to pleomorphic particles of about 20–35 nm in diameter. The potexvirus was identified as an isolate of citrus yellow vein clearing virus (CYVCV) by RT-PCR and Sanger sequencing using two primer pairs designed on two different genomic regions of CYVCV. Attempts to identify the other virus by serological and molecular methods failed; thus, samples will be subjected to HTS analysis to identify this second virus. CYVCV has been reported in some citrus-growing regions in countries in Eurasia, including Pakistan, India, Türkiye, Iran, China, South Korea, and recently in North America, in California's citrus-growing region. CYVCV has been reported to be spread by aphid and whitefly vectors and is graft and mechanically transmitted. Hence, it is an invasive pathogen that could represent a significant threat to the Italian citrus cultivations, especially lemons, which are highly symptomatic to CYVCV. Further investigations are needed to determine the CYVCV diffusion in the Italian citrus cultivations.

Revealing wild rocket priming by compost as a suppressing mechanism to *Fusarium oxysporum* f. sp. *raphani*

A. Pasqualini[1], G. Manganiello[2], R. Spaccini[2], M. Verrillo[2], C. Pane[1]

[1]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA), Centro di ricerca Orticoltura e Florovivaismo, via Cavalleggeri 51, 84098 Pontecagnano Faiano, Italy; [2]Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy. E-mail: catello.pane@crea.gov.it

Wilting caused by the soil-borne phytopathogenic fungus *Fusarium oxysporum* f. sp. *raphani* restricts the yield of fresh cut wild rocket (*Diplotaxis tenuifolia*). The pathogen enters host plants at the root level and colonizes xylem vessels, causing their occlusion. Thus, diseased young plants show symptoms evolving from stunting growth to desiccation. The endophytic course of pathogenesis could be contained by inducing systemic resistance (ISR) in plants. In this view, the potential of compost suppressing wilting by priming plant defence responses could be exploited to face and prevent outbreaks. To elucidate this, two parallel investigations were conducted by treating plants before inoculation with: i) a compost obtained from olive processing residues and its organic fractions such as humic substances (HS), compost tea, dissolved organic matter (DOM) and lipid fraction; ii) a compost obtained by olive tree pruning and vegetable residues also biofortified with *Trichoderma harzianum* TH23. Two distinct cases were conducted, respectively, with two molecular approaches: organic components fractionation and ISR marker gene expression analyses. The evaluation of the indirect ability of the compost-derived fractions to reduce infection showed that the preventive seedling root treatments with the compost suspension was the most suppressive, followed by HS and DOM, reducing, on average, 20–40% disease severity. On the other hand, plants grown in compost- and compost/TH23-enriched peat showed a reduction of disease severity by around 45% and overexpression of lipoxygenase 2 and pathogenesis-related protein 1 genes, suggesting the activation of jasmonic and salicylic acid pathways, key regulators of plant defence responses.

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From apple flowers to control fire blight: new potential biocontrol agents of *Erwinia amylovora*?

A. Pedroncelli[1,2], L. Franceschi[1,3], M.K.M. Marian[1,2], G. Puopolo[1,2]

[1]Center Agriculture Food Environment (C3A), University of Trento, Via E. Mach 1, 38098 San Michele all'Adige, Italy; [2]Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38098 San Michele all'Adige, Italy [3]Department of Life Science, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy. E-mail: gerardo.puopolo@unitn.it

The fire blight caused by *Erwinia amylovora* (*Ea*) is a significant threat to worldwide apple and pear production. It is now widely accepted that apple flowers harbour bacterial taxa that can hinder *Ea* ability to colonize apple flowers. Based on this, we aimed to select new potential biocontrol agents active against *Ea* from apple flower microbiota. *Malus domestica* cv. Golden Delicious flowers from Trentino apple orchards were sampled at the 'Baloon stage' and surface sterilised to isolate only bacteria within the flowers. According to the *16S rRNA* gene sequencing, the bacterial isolates mainly belonged to the *Enterobacteriaceae*, *Pseudomonadaceae*, and *Microbacteriaceae* families. One member of each bacterial family was selected and tested against *Ea*, both on newly open apple flowers and pear slices. Among the tested bacterial strains, *Pantoea agglomerans* AFF2001 showed the highest efficacy in controlling *Ea* in both conditions. To characterize its mode of action, AFF2001 was grown in a specific medium mimicking the apple stigma nutrient conditions, and the cultural filtrates were tested to evaluate their impact on *Ea*'s growth and virulence. Based on the results, we investigated the molecular mechanisms involved in the AFF2001 biocontrol activity by generating knockout mutants. Mutants impaired in reducing *Ea*'s growth were subsequently characterized *in vitro* and *in vivo*. Results indicated changes in motility, biofilm production, and siderophore release between the knockouts and the wildtype. These results suggest the involvement of these mechanisms in the ability of *P. agglomerans* AFF2001 to control *Ea* on apple flowers and pear slices.

Can the combined use of the beneficial isolates *Trichoderma gamsii* T6085 and *Clonostachys rosea* IK726 improve the control of *Fusarium graminearum* on wheat?

A. Petrucci[1,2], M. Cesarini[1], I. Vicente[3], L. Merani[1], B. Jensen[2], D. B. Collinge[2], S. Sarrocco[1]

[1]Department of Agriculture, Food and Environment, University of Pisa, Italy; [2]Department of Plant and Environmental Sciences and Copenhagen Plant Science Centre, University of Copenhagen, Denmark; [3]Department of Microbiology and Genetics, Institute for Agribiotechnology Research (CIALE), University of Salamanca, Salamanca, Spain. E-mail: arianna.petrucci@phd.unipi.it; arpe@plen.ku.dk

Wheat is an important staple food often affected by FHB mainly caused by *Fusarium graminearum*. This threatens food safety due to the risk of mycotoxin contamination. We are testing the two beneficial fungal isolates *Trichoderma gamsii* T6085 and *Clonostachys rosea* IK726, for combined use to enhance FHB management. In confrontation assays, we found no evidence of mycoparasitism or inhibition due to diffusible metabolites. However, VOCs produced by T6085 inhibited IK726 growth slightly. Furthermore, IK726 reduced spore germination of T6085 in liquid culture but not on wheat spikes. At seven days post inoculation, a minor up-regulation of the defence-related (DR) gene *pr1* was induced by root-applied IK726 alone, and when co-inoculated with T6085, whilst *pgip2* was induced only by IK726 treatment in roots. Moreover, systemic induction was not evident as these genes were not up-regulated in leaves. In spikes, DR-genes *pal1, pr1,* and *lox1* were up-regulated by IK726 alone and in combination with T6085 at 96 hours post inoculation (hpi). When inoculated with *F. graminearum, pgip2* was up-regulated after 24 h, mainly in co-inoculation, while at 72 hpi, only T6085 induced this response in the host. However, the co-inoculum reduced the disease incidence to less than 10%. Finally, *F. graminearum* inoculated wheat straw treated with the two beneficial isolates, alone and co-inoculated, showed a significantly reduced number of perithecia developed after six months of incubation. The results support the idea that combining the use of these two isolates improves the management of FHB.

Basic substances and other natural fungicides for the management of downy and powdery mildew in organic viticulture

S. Piancatelli[1], M. Moumni[1], S.M. Makau[1], M. Tunç[1], A.L. D'Ortenzio[1], Y. Davillerd[2], E.P. Pérez-Álvarez[3], T. Garde-Cerdán[3], D. Animali[4], G. Romanazzi[1]

[1]Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, via Brecce Bianche, 60131 Ancona, Italy; [2]Institut de l'Agriculture et de l'Alimentation Biologiques (ITAB), 149 Rue de Bercy, Paris, F-75012, France; [3]Instituto de Ciencias de la Vid y del Vino (CSIC, Universidad de La Rioja, Gobierno de La Rioja), Finca La Grajera, Ctra. de Burgos km. 6 (LO-20, Salida 13), 26007, Logroño, La Rioja, Spain; [4]Terre Cortesi Moncaro SCA, Via Piandole, 7/A 60036 Montecarotto, Italy. E-mail: g.romanazzi@univpm.it

Consumer and retailer demands, public policies, and organic agriculture growth drive research toward alternatives to pesticides for grapevine disease management. Chitosan, whey, *Equisetum arvense, Urtica* spp. extracts, sweet orange essential oil (EO), and *Ampelomyces quisqualis* were applied weekly from May to August in a cv. Montepulciano vineyard in central-eastern Italy. Trials were conducted in 2022, under high powdery mildew (PM) pressure, and in 2023, under high downy mildew (DM) pressure. Basic substances and natural fungicides effectiveness were compared with copper, sulphur, farm application, untreated, and water controls. All the treatments showed reductions in DM and PM infections compared to untreated and water controls. Chitosan and sweet orange EO provided the highest protection levels from both diseases, and whey showed promising results against PM. In a representative assessment of 2023, chitosan and the EO reduced the DM McKinney Index by 76% and 40% on leaves, and by 72% and 47% on bunches, respectively, compared to untreated plants. For PM, in 2022, the reduction was 89% and 83% on leaves, 40% and 37% on bunches, respectively. Chitosan was not significantly different from copper in controlling DM on both leaves and bunches, under high disease pressure. Chitosan, sweet orange EO, and whey can represent potential alternatives to copper and sulphur for DM and PM management. These products are also waste material of the food industry, and demonstrated effectiveness in viticulture could contribute towards their valorization in a circular economy perspective. Large-scale validation trials in different environments are needed to scale up such findings.

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Eucalyptus leaf aqueous extract: a biopesticide for bacterial spot disease in tomato plants

M. Pinto[1,2,3,4], C. Soares[1], T. Andreani[1], F. Fidalgo[1], F. Tavares[2,3,4]

[1]GreenUPorto - Sustainable Agrifood Production Research Centre/ INOV4AGRO, Biology Department, Faculty of Sciences of University of Porto, Rua do Campo Alegre, s/n, 4169-007, Porto, Portugal; [2]CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661, Vairão, Portugal; [3]Biology Department, Faculty of Sciences of University of Porto, Rua do Campo Alegre, s/n, 4169-007, Porto, Portugal; [4]BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661, Vairão, Portugal. E-mail: mafalda.pinto@fc.up.pt

Plant diseases threaten plant biodiversity and food production worldwide and are expected to emerge due to climate change. To counteract this, farmers depend on pesticide applications that cause environmental contamination. Therefore, eco-friendly alternatives to synthetic pesticides must be found. We have recently shown that aqueous extracts from dried leaves (DLE) of young *Eucalyptus globulus* Labill. have promising antibacterial activities against crop-related pathogens. In fact, the growth of *Xanthomonas euvesicatoria* (*Xeu*), responsible for the bacterial spot disease in tomato plants, was severely repressed by DLE (15 g/L). However, the effectiveness of this strategy for the treatment of infected tomato plants remains unclear. The main goal of this study was to evaluate the *in vivo* efficiency of DLE against *Xeu*. For this, one-month-old tomato plants (*Solanum lycopersicum* cv. Roma) were foliar-infected with a suspension of *Xeu* and, upon 48 h, treated by foliar-spraying with DLE (0, 15, and 30 g/L). Symptoms were monitored weekly over three weeks. Upon DLE application, disease symptoms decreased over time in a dose-dependent manner. Indeed, after 21 days, DLE at both concentrations significantly and equally reduced (about 12%) leaf bacterial population compared to water-treated infected plants. To understand DLE effects, oxidative stress markers, photosynthetic pigments, phenols, and antioxidant enzymes' activity were evaluated in tomato leaves. Statistically significant differences were found for catalase activity, which increased upon DLE application, compared to water-treated plants, regardless of the infection. Overall, one can conclude that DLE can be used as a tool against *Xeu* and, thus, contribute to reducing pesticide application.

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Streptomyces sp. IPV2742: solid-state fermentation on defatted wheat bran and application as a candidate biocontrol agent

L. Pisoni, M. Saracch, C. Pizzatti, A. Kunova, P. Cortesi, M. Pasquali, D. Bulgari

Department of Food, Environmental and Nutritional Sciences, University of Milan, Milan, Italy. E-mail: daniela.bulgari@unimi.it

Fungal pathogens represent a major threat to global crop production. Generally, they are controlled by fungicides. However, fungicides can have negative effects on the biosphere, posing risks to the environment and human health. The sustainability of the agricultural sector is strongly bound to the development of new biological approaches as an alternative to chemicals. *Streptomyces* species play a key role in the biocontrol of plant pathogens thanks to the high number of secondary metabolites with antimicrobial activity produced. Moreover, growing *Streptomyces* spp. on unconventional substrates like agri-food waste can both re-valorise agricultural waste and, at the same time, offer new biocontrol strategies to reduce crop losses. In this study, the spore morphology of *Streptomyces* sp. IPV2742, isolated from *Vitis vinifera*, was characterized by scanning electron microscope (SEM). It was observed that IPV2742 strain produces rugose ornamented spores. Moreover, the growth of the strain in solid-state fermentation (SSF) was evaluated to produce bioactive molecules against plant pathogens. *Streptomyces* sp. IPV2742 grew well on defatted wheat bran, reaching the maximum concentration of 10^8 CFU/g seven days after the inoculation. The crude extracts obtained from fermented substrates were tested against *Botrytis cinerea, Fusarium culmorum, Fusarium oxysporum* f. sp. *basilici*, and *Sclerotinia sclerotiorum*. The crude extract showed stronger antimicrobial activity against *F. culmorum* (35%), while the activity against *F. oxysporum* f. sp. *basilici* was less significant (10%).

These results suggest that IPV2742 strain can be considered a possible biocontrol agent against plant pathogens and a source of new bioactive molecules.

Dry lenticel rot– an emerging postharvest disease on apples in Italy: reproduction of symptoms and cultivation methods of *Ramularia mali*

S. Primisser[1,2], E. Deltedesco[1], S. Öttl[1]

[1]Laimburg Research Centre, Laimburg 6 - Pfatten/Vadena, 39040 Auer (Ora), Italy. [2]Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Turin, 10095 Grugliasco, Italy. E-mail: Sabine.Oettl@laimburg.it

Ramularia mali Videira & Crous, an emerging postharvest pathogen on apple in Italy and other apple-growing regions in Austria and France presents a growing concern due to the increasing incidence of dry lenticel rot since 2017. Unlike in the field where the fungal infection remains asymptomatic, the disease has been observed on apples after long-term conservation in cold storage. This study aimed to reproduce symptoms and confirm the pathogenicity of *R. mali* through the fulfilment of Koch's postulates, as well as to evaluate several various cultivation methods for optimal fungal growth and spore production. Five different cultivation media were tested to determine their effectiveness in supporting fungal growth and sporulation, including an optimized method for liquid cultivation. The findings of this study provide insights into the pathogen's behaviour and improved cultivation conditions for the first time and, thus, represent the basis for in-depth research into this newly emerging postharvest disease of apples. Thereby aiding to mitigate the impact of dry lenticel rot on apple production and storage.

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Biodegradation capabilities and microbial interactions of *Ramularia mali*, an emerging post-harvest pathogen of apples

S. Primisser[1,2], M. Hoyer[3], L. Becker[3], A. Schneider[3], K. Russ[3], S. Öttl[1], S. Neuhauser[3]

[1]Laimburg Research Centre, Laimburg 6 - Pfatten/Vadena, 39040 Auer (Ora), Italy; [2]Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Turin, 10095 Grugliasco, Italy;[3] Institute of Microbiology, Universty of Innsbruck, Innsbruck, Austria. E-mail: sigrid.neuhauser@uibk.ac.at

Dry lenticle rot caused by the fungus *Ramularia mali* is an emerging post-harvest pathogen of apples. *Ramularia mali* causes brown to black spots on the apple peels. Symptoms manifest only after prolonged cold storage. The main objective of this study was to analyse the degradation of cell wall components and the interaction of *R. mali* with common plant-associated microorganisms. Different *R. mali* strains were tested for their abilities to use cellulose, pectin, and starch as sole carbon source at various temperatures (4 °C, 10 °C, 25 °C, 37 °C). Furthermore, we examined how light affected the growth and physiology of these fungi. To get a first insight into their interaction with the apple microbiome, selected *R. mali* strains were confronted with bacteria (*Bacillus* spp.) and yeasts (*Rhodothorula* spp.) in agar-plate experiments. *Ramularia mali* can degrade cellulose, pectin, and starch, with the fastest growth at 25 °C and the slowest at 4 °C. The introduction of short light pulses slowed growth, while darkness increased biodegradation in cultures incubated at 4 °C for four weeks. Two *Bacillus* spp. were found to have a clear inhibitory effect on most, but not all tested *R. mali* strains. The tested *Rodothorula* spp. yeasts did not result in any inhibitory effect. Overall, it can be concluded that *R. mali* strains demonstrate a broad physiological spectrum with higher degradation of apple peel components at low temperatures. However, initial tests exploring their interaction with selected microbes yielded varied outcomes. Certain strains appeared to be more susceptible to the chosen microorganisms, while others remained relatively unaffected.

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Sustainable control of white rot on red onion "Cipolla Rossa di Tropea"

G. Procopio, G. Pelle, A. Malacrinò, L. Schena, M. Li Destri Nicosia

Department of Agriculture, Università degli Studi Mediterranea di Reggio Calabria, Loc. Feo di Vito, Reggio Calabria, Italy. E-mail: giusy.procopio@unirc.it

Sclerotium cepivorum, the causal agent of white rot, is a plant pathogen that severely affects the production of the red onion "Cipolla Rossa di Tropea", a certified product that plays an essential role in the local economy of Calabria (Italy). *Sclerotium cepivorum* produces overwintering sclerotia, which can remain alive in soil for many years. We conducted field trials to evaluate the efficacy of solarization and bio-solarization in controlling the disease. The soil was covered with transparent plastic sheets for 53 days starting from August 2023, and a litter of mushroom cultivation was used for bio-solarization. We found that treatments had a significant impact on the viability of sclerotia and the soil microbiome. Treated and untreated plots were utilized to seed a commercial onion cultivar (November 2023), and the incidence and gravity of the disease were evaluated at the harvesting time (April 2024). Solarization and bio-solarization proved very effective in reducing the disease incidence by 62% in the solarized plots and 73% in the bio-solarized ones. In addition, the average plant biomass was higher in the solarized and bio-solarized plots compared to the control. We also isolated several bacteria showing a strong inhibitory activity against *S. cepivorum* from sclerotia of naturally infected onions. In dual culture assays, these isolates reduced the growth of fungal colonies by up to 50%. Interestingly, the inhibitory activity of bacteria was stronger against the strain from which they were isolated. Overall, our results can contribute to developing effective and sustainable control methods against the white rot of onions.

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Release of *Venturia inaequalis* ascospores induced by sprinkler irrigation on sunny days results in a lower risk of primary infections

D. Prodorutti[1,2], R. Bugiani[3], C. Rizzi[1], E. Coller[1], N. Vendrame[2], D. Zardi[2,4], A. Stensvand[5], V. Philion[6], I. Pertot[1,2]

[1]Fondazione Edmund Mach, via Mach 1, 38098 San Michele all'Adige, Italy; [2]Center Agriculture Food Environment, University of Trento, via Mach 1, 38098 San Michele all'Adige, Italy; [3]Servizio Fitosanitario-Plant Protection Service, Regione Emilia-Romagna, Via Andrea da Formigine 3, 40128 Bologna, Italy; [4]Department of Civil, Environmental and Mechanical Engineering, University of Trento, Via Mesiano 77, 38123 Trento, Italy; [5]Norwegian Institute of Bioeconomy Research (NIBIO), P.O. Box 115, NO-1431 Ås, Norway; [6]Institut de Recherche et de Développement en Agroenvironnement, IRDA, Saint-Bruno-de-Montarville, Québec, J3V 0G7 Canada. E-mail: daniele.prodorutti@fmach.it

Apple scab, caused by the ascomycete *Venturia inaequalis*, is one of the most significant diseases affecting apples worldwide. Experiments show how sprinkler irrigation, performed on sunny days before forecasted rainfall, can promote the release of ascospores and reduce the pathogen's inoculum potential. In spring, volumetric spore traps were placed above apple leaf litter containing pseudothecia with ascospores of *V. inaequalis*. The impact of irrigation was evaluated by comparing irrigated and non-irrigated leaf litter. Irrigation resulted in a significant ascospore discharge (more than 50% of spores released during the entire primary season), and fewer spores were counted on rainy days following irrigation. Field trials were carried out to evaluate the efficacy of sprinkler irrigation in controlling disease development. By the end of the primary season, the overall incidence of infected leaves and fruit had decreased by more than 50%. To better understand the aerial dispersal of *V. inaequalis* ascospores with under-canopy sprinkler irrigation, rotating-arm spore traps were placed at heights ranging from 0.3 m to 3.0 m above the ground. Irrigation was carried out 0.3 m above overwintered apple leaves infected with scab in two different situations, *i.e.*, in a wind-protected enclosure and under real orchard conditions. Ascospores were found to disperse above the irrigated layer, with more spores detected above the sprinklers than below. Based on these results, since ascospores can settle on susceptible apple tissues, it is essential to ensure a rain-free period of at least 24 hours post-irrigation to prevent scab infections.

The tour of the world in eight wood rot species: enzymatic and non-enzymatic degradation in Esca Complex of Diseases (ECD) white-rot agents

A. Puca[1,2], S. Moretti[2], S. Farine[2], C. Bertsch[2], L. Mugnai[1]

[1]Department of Agricultural, Food, Environmental, and Forestry Science and Technology (DAGRI), Plant Pathology and Entomology section, University of Florence, P.le delle Cascine 28, 50144 Firenze, Italy; [2]Laboratoire Vigne, Biotechnologies et Environnement UPR-3991, Université de Haute-Alsace, 33 rue de Herrlisheim, 68000 Colmar, France. E-mail: alessandro.puca@unifi.it

Recent research has shown that the presence of white-rot (WR) degraded tissue in the grapevine dramatically increases the possibility of developing ECD foliar symptoms, and some effective strategies for foliar symptom control mainly affect the presence of the WR agent *Fomitiporia mediterranea* in the wood of the vines. This plant pathogen is the most well-investigated WR agent associated with ECD, showing the ability to degrade wood using both enzymatic and non-enzymatic mechanisms, but it is hardly the only one. This study aims to investigate the degradation capabilities of other ECD-associated WR agents, such as *F. capensis, F. langloisii, F. polymorpha, F. australiensis, Inonotus vitis, Stereum hirsutum*, and *Tropicoporus texanus*. The production and activity of lignocellulosic enzymes (*i.e.*, laccases, class-II peroxidases, and lignin peroxidases) were evaluated using enzymatic assays from 1- and 2-week-old secretomes harvested from liquid cultures supplemented with grapevine sawdust. The non-enzymatic degradation capabilities of ECD-WR species were assessed by investigating if the low-molecular-weight fractions of fungal liquid culture grown on iron-deprived medium could: i) reduce Fe^{3+}, ii) produce H_2O_2 during ferrous iron oxidation, iii) generate hydroxyl radicals, all the steps of the Chelator-Mediated-Fenton pathway (CMF). Preliminary results reveal differences in the secretome of ECD-associated WR agents in enzyme production. In addition, this study confirms that the CMF pathway, classically associated with brown-rot degradation, is widespread among ECD-associated WR agents. Overall, the research provides insights into ECD-associated WR agents' ability to degrade grapevine woody tissues, leading to new paths into ECD symptomatology and possible control strategy development.

Optimizing the protocol for precise inoculation of single lenticel of apple fruit

H. Qayyum[1], S. Bharti[1], S. Baric[1,2]

[1]Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Bozen-Bolzano, Italy; [2]Competence Centre for Plant Health, Free University of Bozen-Bolzano, Bozen-Bolzano, Italy. E-mail: Humdah.Qayyum@student.unibz.it

Apple is an economically important fruit crop that can experience substantial losses because of different post-harvest pathogens. Latent post-harvest pathogens, such as *Phlyctema vagabunda*, species of the *Colletotrichum acutatum* complex, and *Cadophora luteo-olivacea*, causing bull's eye rot, bitter rot, and side rot of apple, respectively, infect the apple fruit through lenticels in the field but remain dormant and symptoms become apparent after an extended period of storage. Controlled infection of single lenticels is crucial for studying host-pathogen interactions at the molecular level during the latent infection phase and during disease progression. However, the existing procedures for in vitro inoculation of apple fruit are primarily based on dipping the entire fruit in conidial suspensions or by making wounds. Nevertheless, dipping is not seen as a highly effective technique as it is impossible to predict which exact lenticels would be affected by the pathogen. Wounding, in contrast, may trigger fruit responses to mechanical injury, thus, hindering the distinction of genes expressed as part of the disease response. We report here a non-invasive inoculation protocol using small adhesive spot plasters soaked with concentrated conidia suspensions targeting specific lenticels. The optimization of the protocol is based on investigating the interaction between apple cultivar susceptibility and fungal pathogenicity by assessing disease incidence across varying incubation periods. The development of a reliable inoculation procedure for single lenticels shall contribute to a comprehensive understanding of the pathogen behaviour during the infection process and help in improving disease control strategies.

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Sustainable management of *Coniella granati* on pomegranate in southern Italy

M.L. Raimondo, F. Lops, A. Carlucci

Department of Agriculture, Food, Natural resources and Engineering (DAFNE), University of Foggia, Italy. E-mail: marialuisa.raimondo@unifg.it

Pomegranate production is threatened by various problems, primarily due to fungal infections and, to a lesser extent, by wounds and other physical damages. Among phytopathogenic fungi causing severe diseases on pomegranate, *Coniella granati*, also known as *Pilidiella granati*, is one of the main reasons for the yield decrease and deterioration of the quality of trees and fruits. To date, no fungicides are fully registered for pomegranate diseases in the major producer countries of Mediterranean basin, including Italy, where the use of some fungicides can be temporarily authorized for a specific period through an emergency registration process. Moreover, the Farm to Fork goals want to minimize, by 2025, the impact of pesticides on human health and the environment by reduced dependency and through the increased use of low-risk and non-chemical pesticides. Based on this, we assessed the ability of biocontrol agents such as *Streptomyces albidoflavus* CARA17 and *Trichoderma harzianum,* and a resistance inducer such as chitosan to control the infection by *Coniella granati* and to preserve the quality of fruits by using the biocontrol agents alone and/or in combination with each other. Experimental trials were performed in the open field on eight years-old pomegranate trees cv "Wonderful" to compare the response of biocontrol agents with the employ of three conventional fungicides as Tebuconazole, Dithianon, and Fosetyl-Aluminium. Results showed that the biocontrol agents could preserve fruit production and quality, although with less efficiency than conventional fungicides.

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Secondary metabolites from *Beauveria bassiana* modulate root exudate composition and recognition

M. Ranesi[1], S. Vitale[1], A. Staropoli[1], L. Di Costanzo[1], M. Lorito[1], S. Woo[2], F. Vinale[3], D. Turrà[1]

[1]Department of Agricultural Sciences, University of Naples "Federico II", Via Università 100, 80055 Portici, Italy; [2]Department of Pharmacy, University of Naples "Federico II", Via Domenico Montesano 49, 80131 Naples, Italy; [3]Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", 80137 Naples, Italy. E-mail: marta.ranesi@unina.it

Filamentous fungi and other microorganisms secrete various classes of organic acids (OAs) into their surroundings. These low-molecular weight compounds have multiple ecological functions and potential for biotechnological applications. For instance, microbial OAs are known to modulate environmental pH and ion availability, facilitate mineral acquisition by plants, and mediate microbial communication. In this study, we analyzed the ability of different *Beauveria bassiana* (*Bb*) isolates to produce bioactive-secreted OAs. Among them, we identified the metabolite Bb1993, which can both lower environmental pH at high concentrations and inhibit germ tube formation and growth of the fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) at very low concentrations. Interestingly, when plant roots were inoculated with *Bb* spores or treated with the Bb1993 metabolite, their root exudates became repellent to *Fol* germ tubes despite accumulating higher concentrations of peroxidases, the major fungal chemoattractant produced by plant roots. Furthermore, dual confrontation assays in which *Fol* and *Bb* colonies were grown opposite to each other, or direct application of the Bb1993 metabolite, enhanced *Fol*'s invasive growth on cellophane membranes. Altogether, our results suggest that Bb1993 exerts both direct and indirect (*i.e.*, plant-mediated) biological effects on *Fol* by interfering with spore germination, plant recognition, and penetration, key virulence steps in this class of soil-borne fungal pathogens.

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Specific QTLs combination offer broad-spectrum resistance in pepper against *Phytophthora* blight

A. Reggiani[1], P. Riccardi[1], C. Wijnen[2], M. Mammella[1]

[1]BASF Nunhems Italy, via Ghiarone 2, 40019 Sant Agata Bolognese, Italy; [2]BASF Nunhems Netherlands, Napoleonsweg 152, 6083 AB Nunhem, The Netherlands. E-mail: alberto.reggiani@basf.com

Phytophthora blight is one of the main diseases of pepper (*Capsicum annuum*) globally. The disease is commonly associated with the soilborne oomycete *Phytophthora capsici*. The pathogen can infect multiple parts of the plant (stems, roots, fruits, and leaves); the most destructive symptom is the basal rot of the plant with constriction and browning of the stem at the soil level, which easily leads to the death of the plant. For several years, BASF-Nunhems has been studying the resistance to *Phytophthora* blight in pepper testing the combination of 3 different resistance QTLs (PC-CRR-Pc5-1, Pc6-1, and Pc10-1) known in the literature against different *P. capsici* physiological races (from Italy, Mexico, India, and China). It is confirmed that several combined resistant genes are required for broad-spectrum and durable resistance. To support Italian breeding programs, pathogens causing *Phytophthora* blight were collected in different areas of pepper production, with a particular focus on Campania. Genetic characterization of isolates reports the presence of *P. capsici* and *Phytophthora nicotianae*. This is an interesting information since *P. capsici* has long been considered the only *Phytophthora* species pathogenic to pepper in Italy. The specialization of the pathogen races emerged in the same areas of investigation, for *P. nicotianae* colonies isolated from tomatoes are not pathogenic for peppers. First preliminary studies show that specific combinations of resistance QTLs for *P. capsici* are also performing against *P. nicotianae.*

Efficacy of antagonistic yeasts in reducing grey mould of grapes and bioprotection activity in winemaking

G. Remolif[1,2], M. Garello[1,2], S. Prencipe[1], S. Giacosa[1], V. Englezos[1], D. Spadaro[1,2]

[1]Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Italy; [2]Interdepartmental Centre for Innovation in the Agro-Environmental Sector-AGROINNOVA, University of Turin, Italy. E-mail: gi.remolif@unito.it

Biocontrol agents (BCAs) represent a promising alternative to synthetic fungicides for disease management. This study evaluated the efficacy of antagonistic yeasts in controlling grey mould of grape, caused by *Botrytis cinerea*, in postharvest, while assessing the impact of the treatments on the fruit microbiome. Additionally, their bioprotection activity against *Hanseniaspora uvarum*, which negatively affects wine quality, was assessed during winemaking. Experiments were conducted over two years on grapes cv. Nebbiolo. Trials were set up by treating grape bunches, inoculated with *B. cinerea*, with strains of *Aureobasidium pullulans, Metschnikowia pulcherrima, Pichia kluyveri, Starmerella bacillaris,* and *Torulaspora delbrueckii*. All treatments significantly reduced rot incidence seven and 14 days post-inoculation compared to the inoculated control. Treatments with *M. pulcherrima* and *T. delbrueckii* were the most effective. Microbiome analysis showed a good development of the yeast BCAs on the treated bunches, along with a reduction of *Botrytis* spp. abundance compared to the control. Moreover, the abundance of other fungal genera was modified. All treatments had a positive effect in reducing the population of *H. uvarum* on the bunches two days after their application. During alcoholic fermentations, the decrease of *H. uvarum* was treatment-dependent, but a complete reduction after four days was observed in treatments with *S. bacillaris* and *T. delbruekii* in spontaneous fermentations. Furthermore, all the yeasts applied did not significantly affect the population of *Saccaromyces cerevisiae*. Results showed the potential of antagonistic yeasts in managing grey mould of grape and improving wine quality.

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Selection and evaluation of antagonistic yeasts in the control of strawberry postharvest rots and effect on the fruit microbiome

G. Remolif[1,2], M. Garello[1,2], D. Spadaro[1,2]

[1]Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Italy; [2]Interdepartmental Centre for Innovation in the Agro-Environmental Sector-AGROINNOVA, University of Turin, Italy. E-mail: gi.remolif@unito.it

Strawberries are highly susceptible to fungal decay. The use of yeasts as biocontrol agents is a promising tool to control postharvest diseases of fruits. In this study, we selected some antagonistic yeasts to control postharvest rots of strawberries by assessing their efficacy, the effect on the fruit quality, and the fruit microbiome. A protocol was developed to isolate endophytic yeasts from healthy strawberries. Isolated endophytes were tested for their potential antagonistic activity, together with yeast strains present in the collection of the University of Turin. All trials were performed *in vivo* using naturally infected strawberries. The most effective strains in the screening trials, identified as *Metschnikowia pulcherrima* and *Aureobasidium pullulans*, were selected to set up efficacy trials. All the tested yeasts showed a significantly lower rot incidence and severity compared to the untreated control both after 10 days of storage at 1 ± 1 °C and after 2 days of shelf-life at 19 ± 1 °C. Additionally, results were comparable to those obtained for the treatment with a commercial biofungicide based on a strain of *Metschnikowia fructicola*. None of the tested yeasts significantly affected fruit firmness, total soluble solids content, and titratable acidity. Metabarcoding analysis of the strawberry microbiome, sampled during the efficacy trials, highlighted significant modifications in the microbial community in response to the treatments. The findings of this work provide new insights for the development of sustainable strategies for reducing postharvest losses while preserving fruit quality.

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On the composition of the apples' Sooty Blotch pathobiome

F. Rey[1,2], S. Öttl[1], H. Schuler[2]

[1]Laimburg Research Centre, Auer/Ora, Italy; [2]Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Bolzano, Italy. E-mail: Hannes.Schuler@unibz.it

Fungal causal agents of the Sooty Blotch (SB) complex live on the outer waxy layer of apple peels. Although the representatives of the SB complex are generally not classic pathogens on apples, after their mycelial growth, symptoms appear as blemishes and smudges on the surface of apples, hindering the commercialization of fresh fruits. Currently, forecasting models are inadequate to reliably predict the development of SB symptoms due to SB's heterogeneous composition in different growing areas. Furthermore, it is unknown whether infections can be affected by biotic factors, such as interactions with the microbial species of the apple's microbiome. This study aims to reveal whether SB fungi change the composition of the apple microbiome, if certain compositions of the apple microbiome favour or hinder SB growth, and if so, which bacteria or fungi within the microbiome actively antagonize SB growth. Two experiments are envisaged to answer these questions. The first experiment, metabarcoding of the apple pathobiome, by applying next-generation sequencing technologies (Oxford Nanopore technology and Illumina sequencing) with organism barcoding, shall extract, identify, and compare the fungal and bacterial species present in the microbiomes of healthy and infected apples. The second experiment focuses on the inhibition of putative antagonistic microbe species identified from the microbiome of healthy apples with SB fungi by co-culturing. Thus, identification of the SB complex composition is essential for a better understanding of symptom expression and might contribute to the development of targeted and environmentally friendly management strategies.

Characterization of secondary metabolites produced by *Alternaria alternata, Colletotrichum gloeosporioides*, and *Penicillium digitatum* in blood oranges: implications for mycotoxin contamination and quality assurance in citrus production

M. Riolo[1], C. Luz[2], F. La Spada[1], R. Parlascino[1], S. Conti Taguali[1,3], C. Bua[1], M.C. Tambè[1], A. Pane[1], G. Meca[2], S.O. Cacciola[1]

[1]Department of Agriculture, Food and Environment, University of Catania, 95123 Catania, Italy; [2]Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Burjassot, Spain; [3]Department of Agricultural Science, Mediterranean University of Reggio Calabria, 89122 Reggio Calabria, Italy. E-mail: olga.cacciola@unict.it

Citrus fruits are susceptible to infections of several fungal pathogens responsible for pre- and post-harvest rots. In this study, secondary metabolites produced by *Alternaria alternata, Colletotrichum gloeosporioides*, and *Penicillium digitatum* were identified in two blood orange cultivars ('Tarocco Lempso' and 'Tarocco Tapi') from eastern Sicily. Fruits were selected as asymptomatic, hail-injured, or mummified based on external symptoms. Analysis was performed using UHPLC–Q-TOF-MS on separate extracts from peel and juice. Among 47 secondary metabolites identified, 16 were attributed to *A. alternata*, 18 to *C. gloeosporioides*, and 13 to *P. digitatum,* respectively. The metabolic profiles of peels from hail-injured and asymptomatic fruits showed no substantial differences, indicating the presence of these fungi even in asymptomatic fruits. Significant differences were observed in the juice profiles of hail-injured and mummified fruits, with higher presence of 5,4-dihydroxy-3,7,8-trimethoxy-6C-methylflavone and Atrovenetin, particularly in the juice of mummified 'Tarocco Lempso' fruits. Mycotoxins Patulin and Rubratoxin B were detected exclusively in mummified fruits; Patulin was found in both peel and juice, while Rubratoxin B was detected only in juice. This research provides crucial data for evaluating and preventing mycotoxin contamination in the citrus supply chain, highlighting food safety and agricultural implications due to environmental stressors, such as hail, which are increasingly influenced by climate change. These findings also underscore the importance of robust analytical methods, using a specifically designed database for identification through UHPLC-Q-TOF-MS, for monitoring and ensuring the safety of citrus products.

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Induced resistance as a sustainable tool to manage postharvest decay of fresh fruit and vegetables and reduce food loss and waste

G. Romanazzi[1], D. Prusky[2]

[1]Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche, 60131 Ancona, Italy; [2]Department of Postharvest Science, Agricultural Research Organization, The Volcani Institute, Rishon LeZion, Israel. E-mail: g.romanazzi@univpm.it

Harvested fresh fruit and vegetables are perishable, subject to desiccation, show increased respiration during ripening, and can be infected and colonized by postharvest fungal pathogens, leading to loss and waste. Induced resistance is a strategy to control diseases by eliciting biochemical processes in fresh fruit and vegetables. This occurs by modulating the progress of ripening and senescence, which maintains the fresh produce in a state of heightened resistance to decay-causing fungi. The application of induced resistance to protect fresh fruit and vegetables has been improved thanks to increased knowledge and scientific tools that better characterize physiological changes in plants. Induced resistance slows the decline of innate immunity after harvest and increases the production of defensive responses that directly inhibit plant pathogens. This increase in defence response in fresh fruit and vegetables contributes to higher amounts of phenols and antioxidant compounds, including nutraceutical compounds, improving both the quality and appearance of the produce. Mechanisms and treatments that induce resistance in harvested fresh fruit and vegetables to suppress fungal colonization will be covered. Moreover, the importance of timing of application, host maturity, and stage of ripening as limiting conditions for the improved expression of induced-resistance processes will be stressed. Decreasing the incidence of infected fresh fruit and vegetables can reduce food loss and waste, which we all are involved in as consumers.

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Application of Plasma-Activated Fog (PAF) in postharvest treatment to reduce spoilage by fungal pathogens and pesticide residues in fruits

P.R. Rotondo[1], S. Distante[1], D. Aceto[2], S. Laera[1], C. Dongiovanni[3], M. Ambrico[2], M. Di Carolo[3], G. Dilecce[2], F. Faretra[1], R.M. De Miccolis Angelini[1], P.F. Ambrico[2]

[1]Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, 70126, Bari, Italy; [2]Institute for Plasma Science and Technology, National Research Council, 70126, Bari, Italy; [3]Centre of Research, Experimentation and Training in Agriculture (CRSFA) Basile Caramia, 70010 Locorotondo (BA), Italy. E-mail: ritamilvia.demiccolisangelini@uniba.it; paolofrancesco.ambrico@cnr.it

During storage, fruits and vegetables are notably susceptible to significant pathogens responsible for postharvest decay and are affected by the presence of undesirable pesticide residues. Non-thermal plasma is currently proposed as a novel, promising technology against microbial and chemical contamination of produce. Atmospheric air plasma consists of electrons, ions, radicals, stable and short-living products, such as reactive oxygen and nitrogen species (RONS), and ultraviolet radiation. It can be applied either directly on product surfaces or indirectly through a plasma-activated medium, *i.e.*, plasma-activated water (PAW) and plasma-activated mist (PAMi) or fog (PAF). These latter are rich in RONS and can be applied during postharvest stages. In this study, PAF was generated using a device developed at CNR-ISTP (Bari, Italy) and applied to evaluate the effect on conidial germination of major fungal postharvest pathogens like *Alternaria alternata, Aspergillus carbonarius, Botrytis cinerea, Cladosporium* sp., *Monilinia fructicola, Penicillium italicum* and *Penicillium expansum, Rhizopus* sp.. Differences in fungal sensitivity to PAF were recorded, with *A. alternata* showing the lowest sensitivity to treatments. For most of the species, the complete spore inactivation was obtained after 3–5 min of exposure. The efficacy of PAF against fungal rots was assessed on table grape and strawberry, revealing a significant reduction in the percentage of symptomatic fruits exposed to 10 min of treatment (up to

80% of efficacy). PAF treatments also reduced pesticide residues on fruits contaminated by acetamiprid (–40%), abamectina (–90%), bifenazate, and spinetoram (–68%) and several fungicides with various results (up to 38% of reduction).

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A different way of looking at phytopathogenic bacteria: handheld Raman spectrometer coupled with machine learning

C. Sacco Botto[1], C. D'Errico[1], C. Orecchio[2], M. Vincenti[2], M. Ciuffo[1], E. Noris[1]

[1]Institute for Sustainable Plant Protection, National Research Council of Italy (CNR), 10135 Torino, Italy; [2]Department of Chemistry, University of Turin, Via P. Giuria 7, 10125 Torino, Italy. E-mail: emanuela.noris@ipsp.cnr.it

Efficient, accurate, and early identification of plant pathogens is crucial for reducing disease spread and ensuring food security. The development of rapid diagnostic methods based on Raman spectroscopy (RS) coupled with machine learning holds great potential to enable prompt and targeted responses. To enhance the practical applicability of RS for the identification of plant pathogenic bacteria, we investigated the use of a handheld RS instrument coupled with several machine-learning approaches to differentiate isolates belonging to the *Pseudomonas*, *Xanthomonas*, and *Erwinia* genera. A total of 332 Raman spectra were acquired directly on bacterial colonies grown on a solid medium. Following the comparison of different algorithm performances, PLS-DA models were built, enabling us to identify isolates at the genus, species, and pathovar levels, with accuracies ranging from 81 to 100%. This easy and fast approach offers the opportunity to develop a widely accessible database of bacterial spectra, thereby facilitating non-destructive and rapid microorganisms' identification, ultimately aiding in the prevention and management of crop diseases.

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Insight the postharvest antifungal potentials of hydroalcoholic propolis extracts

A. Sadallah[1], E. Aprea[2], A. Caratti[3], C. Cordero[3], A. Angeli[4], S. Martens[4], A. Di Francesco[1]

[1]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via delle Scienze 206, 33100 Udine, Italy; [2]Center Agriculture Food Environment, University of Trento, Italy; [3]Department of Drug Science and Technology, University of Turin, Torino, Italy; [4]Research and Innovation Centre (CRI), Fondazione Edmund Mach, Italy. E-mail: alessandra.difrancesco@uniud.it

In light of the harmful effects of chemical plant protection products (PPPs) on the environment and human health, eco-friendly alternatives need to be explored. This study focuses on the control of *Botrytis cinerea*, one of the most important postharvest pathogens of blueberry and table grape, by the application of different propolis extracts from various Italian regions (Tuscany, Veneto, Friuli Venezia Giulia, and Umbria). In the study, different extraction methods were tested to obtain active hydroalcoholic propolis extracts (HPEs). The study demonstrated that propolis extracted with 90% ethanol for 24 hours, previously sonicated for 20 min, showed the best efficacy against gray mold. The efficacy of non-volatile and volatile metabolites of HPEs at different concentrations (0, 3.12, 6.25, 12.5, 25, 50, 100, 150, 200 ppm) was tested *in vitro* against *B. cinerea* conidial germination displaying an inhibition ranging between 56.51% to 98.83% and 40.3% to 75.6% on average, respectively. By the previously obtained results, the EC50 values of each HPE in relation to the assayed mechanisms of action were determined. Both metabolites were tested on blueberry and table grape to validate their efficacy. By HPLC and SPME-GC×GC-ToF MS analysis, HPEs diversity composition was confirmed.

A new diagnostic tool for simultaneous detection of *Erwinia amylovora* and *Ralstonia solanacearum*

D. Salamone, P.R. Rotondo, E. Chiaromonte, D. Gerin, V. Montilon, S. Pollastro, F. Faretra, R.M. De Miccolis Angelini

Department of Soil, Plant and Food Sciences, University of Bari, Via G. Amendola 165/A, 70126 Bari, Italy. E-mail: stefania.pollastro@uniba.it

Erwinia amylovora and *Ralstonia solanacearum* are polyphagous plant pathogenic bacteria included in the EPPO A2 list. To prevent their introduction and spread in new areas, their monitoring is crucial. This study aimed to develop a rapid and sensitive diagnostic assay for the simultaneous quantification of the two bacteria. Two primers/probe sets reported in the EPPO guidelines and targeting the *amsC* gene of *E. amylovora* and the 16S rRNA region of *R. solanacearum* were marked with two different fluorophores (FAM and HEX) and assessed in duplex qPCR and ddPCR assays. The specificity of both qPCR and ddPCR was confirmed against a panel of six bacteria and fifteen fungi mainly responsible for common diseases in nurseries. The detection limit for the duplex qPCR assay was 100 fg of DNA for both the bacterial species, while ddPCR resulted 10-fold more sensitive, quantifying < 1 copy μL^{-1} of each *E. amylovora* and *R. solanacearum*. Three different protocols for DNA extraction from wood of *Lantana camara* were tested in qPCR assays for in-field diagnostic tests, using A) Sample Homogenization and M1 Sample Prep Cartridge kit for DNA (Biomeme); B) QuickPick SML Plant DNA Kit (BN Products & Services); and C) REDExtract-N-Amp Plant PCR Kit (Sigma-Aldrich). Detection limits of five and ten cells for reaction were obtained with protocols C and B, respectively, while protocol A was less sensitive (> 20 cells per reaction). Protocols A and C were more accessible and more rapid (5 min and 15 min, respectively) than protocol B (50–60 min).

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Morphology, phylogeny, and pathogenicity of *Meloidogyne karsseni* parasitizing lisianthus (*Eustoma grandiflorum*) in Mexico

R.J. Salazar-Mesta[1], J. Torres-López[2], G. Marquez-Licona[3], I. Cruz-Lachica[1], J.A. Carrillo-Fasio[1], R.S. García-Estrada[1], J.M. Tovar-Pedraza[1]

[1]Centro de Investigación en Alimentación y Desarrollo, Coordinación Regional Culiacán, Laboratorio de Fitopatología, Sinaloa, México; [2]Colegio de Postgraduados, Campus Montecillo, Posgrado en Fitopatología, Texcoco, 56230, Estado de México, México; [3]Instituto Politécnico Nacional, Centro de Desarrollo de Productos Bióticos, Yautepec, 62731, Morelos, México. E-mail: juan.tovar@ciad.mx

Lisianthus (*Eustoma grandiflorum*) is an important ornamental plant cultivated in Mexico for cut flower production. In November 2023, lisianthus plants exhibiting root-knot, stunted growth, and yellowing were detected in a greenhouse in Puebla, Mexico. Disease incidence was approximately 70%. Roots were washed with tap water and dissected. Females and egg masses were obtained by dissecting galls. Microscopic examination of the perineal pattern of mature females showed an oval to rounded shape, with coarse striae on the lateral sides around the anus, a low dorsal arch with finer striations, and distinctly lateral lines. Morphological characteristics of the females and juveniles were consistent with those reported for *Meloidogyne karsseni*. For molecular identification, total DNA was extracted from individual females, and the ITS region and *coxI* (mtDNA) gene were amplified by PCR and sequenced. Two phylogenetic trees, including published ITS and *coxI* sequences for *Meloidogyne* spp., were constructed based on Maximum Likelihood and Bayesian Inference methods. The phylogenetic analyses placed the two isolates in the same clade as *Meloidogyne karsseni.* A pathogenicity test was performed by inoculating 4000 eggs of a pure population of *M. karsseni* on 10 healthy lisianthus plants grown in pots. Five uninoculated lisianthus plants were used as controls. Yellowing and root-galling symptoms appeared on inoculated plants after 50 days, whereas control plants remained symptomless. The nematodes on the inoculated roots were morphologically identical to those observed on the original diseased roots. To our knowledge, this is the first record of *M. karsseni* parasitizing lisianthus in Mexico and worldwide.

Deep inside *Alternaria*–tomato pathosystem: matching literature information and ecological trials to develop a mechanistic, weather-driven model

I. Salotti, P. Giorni, P. Battilani

Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy. E-mail: paola.battilani@unicatt.it

Fungal species belonging to the genus *Alternaria* can infect tomatoes and originate destructive epidemics in the field, notably early blight and rots, and post-harvest decay. *Alternaria* spp. is well-known for its ability to produce a wide range of secondary metabolites, including plant-pathogenic phytotoxins and mycotoxins that can contaminate food products with health risks for humans. Although proper regulation and legal limits on the maximum concentration of *Alternaria* toxins in tomato-derived products are still missing, *Alternaria* is gaining attention in research programs and risk assessment studies. Guiding the regulatory framework and developing innovative control tools raises the need for comprehensive and detailed information on the biology, ecology, and epidemiology of the main species associated with tomato. To retrieve and analyse available data on these species, a systematic literature review was conducted focusing on the main biological processes included in the pathogen life cycle and mycotoxin production. Several knowledge gaps emerged in the relationship between *Alternaria* spp., tomato, and the environment, with mycotoxin production the poorest studied process. Retrieved information was also evaluated for applications in building a mechanistic, weather-driven model that incorporates the key steps of the pathogen life cycle and risk of mycotoxin contamination. Existing models, in fact, are data-based models primarily voted at scheduling fungicides. The strong empiricism in the modeling approach and the identified knowledge gaps suggest paths for further research, especially for recently isolated species. *Alternaria tenuissima* was then used as a case study to address missing information and provide new insights on the *Alternaria*–tomato pathosystem.

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Extracellular pH regulates asexual reproduction and exo-metabolome composition in the biocontrol fungus *Trichoderma harzianum*

F. Salzano[1], A. Staropoli[1,2], D. Turrà[1], S. Vitale[1], M. Lorito[1], F. Vinale[2,3]

[1]Department of Agricultural Sciences, University of Naples "Federico II", Via Università 100, 80055 Portici (NA), Italy; [2]National Research Council, Institute for Sustainable Plant Protection (IPP-CNR), Piazzale Enrico Fermi 1, 80055 Portici (NA), Italy; [3]Department of Veterinary Medicine and Animal Productions, University of Naples "Federico II", Via Delpino 1, 80137 Naples, Italy. E-mail: felice.salzano@unina.it; stefania.vitale@unina.it

Trichoderma species are widely used for disease control and plant growth enhancement, as well as as a source of bioactive compounds. Despite this, little is known about *Trichoderma*'s ability to alter environmental pH and how this process is related to secondary metabolism. In our study, we investigated how different nitrogen sources (nitrate and ammonium) affect extracellular pH (pHex) modification and the production of exometabolites in *T. harzianum*, a renowned producer of the bioactive compound Harzianic Acid (HA) and in the biocontrol agent *T. asperellum*. Our findings revealed that both *Trichoderma* species are able to acidify the extracellular milieu and increase biomass production when grown in the presence of the preferred nitrogen source, ammonium. Conversely, the presence of nitrate induced extracellular alkalinization and increased conidia production. In accordance with the finding that different nitrogen sources induce opposite physiological processes in *Trichoderma*, we found that both pHex and the nitrogen source differentially regulate the fungal exo-metabolome. For instance, HA production is strongly increased in acidic conditions and is dependent on the nitrogen source used, with nitrate completely repressing its production. However, by buffering a nitrate-containing medium to acidic pH, HA production was fully restored, suggesting that pHex rather than the nitrogen source regulates HA production. Overall, our results indicate that conidia, biomass, and secondary metabolite production are influenced more by pH levels than by the nutritional source, a phenomenon conserved across different *Trichoderma* species.

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The plant immune system: an unexplored target to fight herbicide-resistant weeds

L. Scarabel[1], A. Milani[1], S. Fodil[2], M. De Zotti[3], S. Luti[4], I. Baccelli[2]

[1]Institute for Sustainable Plant Protection, National Research Council of Italy (CNR-IPSP), Legnaro, Italy; [2]CNR-IPSP, Sesto Fiorentino, Italy: [3]Department of Chemistry, University of Padova, Padova, Italy; [4]Department of Experimental and Clinical Biomedical Sciences, University of Florence, Firenze, Italy. E-mail: ivan.baccelli@ipsp.cnr.it

The European Green Deal aims at a 50% reduction in the use and risk of chemical pesticides by 2030, but there are serious concerns by the stakeholders on the potential implications of a pesticide reduction on food and feed security in Europe. To match agricultural productivity and sustainability, new eco-friendly herbicidal solutions are urgently needed. Herbicides include a very broad array of molecules that hit, by their mechanism of action, relatively few molecular targets in plants. Weeds have evolved resistance to most existing mechanisms of herbicidal action, but, surprisingly, new herbicidal mechanisms have yet to be developed in the last 35 years. To fight herbicide-resistant weeds, our project envisages the plant's innate immune system as a totally novel target in weeds and biocompatible peptides, derived from the immunogenic epitopes of eliciting proteins, as a new class of herbicidal molecules. To achieve this goal, the PEP-HERB project has started investigating a fungal protein named cerato-platanin (CP), which is long known for its ability to trigger plant defence. Our preliminary results show that applying CP to seeds effectively inhibits root and/or shoot development of important weeds such as *Lolium multiflorum* and *Amaranthus hybridus*, as well as inhibiting seed germination of *Digitaria sanguinalis*. Interestingly, weeds showed differences in the sensitivity to the protein elicitor, with some species showing complete insensitivity. We are currently synthesizing short peptides derived from the CP protein and testing their efficacy on weed populations of *Lolium* sp. resistant to commercially available herbicides.

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First insights on the biological properties of chitosan-lignin microparticles to control the agent of kiwifruit bacterial canker

D. Schiavi[1], S. Tacconi[2], L. Dini[2], S. Giovagnoli[3], E. Camaioni[3], G.M. Balestra[1]

[1]Department of Agriculture and Forest Sciences, University of Tuscia, Viterbo, Italy; [2]Department of Biology, University La Sapienza, Rome, Italy; [3]Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy. E-mail: schiavi@unitus.it

Bacterial canker, provoked by the bacterium *Pseudomonas syringae* pv. *actinidiae* (Psa), still represents the major threat to kiwifruit cultivation worldwide. Its control is achieved by observing good agronomical practices and mainly by using cupric salts. The need to find alternative compounds to copper is becoming everyday more urgent, since the appearance of tolerant strains and the growing awareness of the side effects of copper on the environment. Several attempts have been made to use natural substances due to their abundance and affordability, even if the lack of chemical stability makes them challenging to use. In this work, we have proposed a combination of chitosan hydrochloride, a notable plant elicitor, sulfonated lignin, a powerful antioxidant, and starch, an inexpensive excipient, in microparticles (MP) of 20–40 µm in diameter by spray-drying. Obtained MP were chemically characterized and tested for their antimicrobial properties on Psa (strain CFBP 7287), revealing a nearly total inhibition after 24 hours when used at 0.1% w/v. MP tested on kiwifruit seedlings (cv. Hayward) showed no negative effects on leaf area and chlorophyll metabolism, highlighting their biocompatibility. Furthermore, nanotomography of the leaf petioles, showed interesting changes in vessels, which could potentially be explained as an increased deposit of callose due to the elicitor activity of the MP. Control assay in greenhouse confirmed the antibacterial activity of the studied compound on artificially inoculated plants, by a reduced severity of leaf symptoms. These evidences suggest an effective sustainable protectant against Psa, addressing further experiments in the open field.

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Exploring grapevine genetic resistance to Flavescence dorée

M. Schillaci[1], S. Palmano[1], G. Gambino[1], A. Delliri[1], D. Danzi[2], E. Vandelle[2], N. Mori[2], A. Polverari[2], C. Marzachì[1]

[1]CNR-Institute for Sustainable Plant Protection (CNR-IPSP), Torino, Italy; [2]Department of Biotechnology, University of Verona, Verona, Italy. E-mail: martino.schillaci@ipsp.cnr.it

European viticulture is threatened by Flavescence dorée (FD), a disease caused by phytoplasma strains within the 16SrV ribosomal group. Current control strategies are based on roguing of symptomatic plants, production of phytoplasma-free propagation material, and insecticide treatments against the FD vector, *Scaphoideus titanus*. No natural genetic resistance to FD is known, although different *Vitis vinifera* cultivars show varying tolerance to the disease. Two approaches were pursued to reduce the burden of insecticide in FD-affected areas. At first, the response of microvines to FD inoculation under controlled conditions was analysed, by evaluating the ability of *S. titanus* to transmit the phytoplasma and the pathogen multiplication rate in inoculated plants. Although microvines represent a convenient model plant for grapevine genetics, they have not been characterized yet for their susceptibility to FD. Secondly, the response to FD infection was evaluated in transgenic vines overexpressing an ATL member of E3-ubiquitine ligases. Previous knowledge has indicated that this gene is downregulated in FD-infected and symptomatic grapevines. Moreover, E3 ligases of the protein degradation pathway are newly identified targets of phytoplasma effectors, at least in the case of another

phytoplasma species, '*Candidatus* Phytoplasma asteris'. For both experiments, FD inoculation was conducted under controlled conditions and a modified protocol was developed to improve the homogeneity, stability, and processivity of the phytoplasma inoculation phases. Flavescence dorée prevalence and loads were evaluated at eight to 10 weeks post-inoculation. The study aims to explore the possible role of selected candidate genes on FD resistance in *V. vinifera*.

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Plant defence via bio-stimulant or antimicrobial peptides application

L. Sella[1], S. Tundo[1,2], A. Panichi[1], A. Bolzonello[1], A.G. Ilesanmi[1], O.C. Viscardo[1], S. Favia[1], V. Artico[1], K. Konate[1], P. Freoni[1], L. Vivarelli[1], C. Gonzalez-Dehennault[1], I. Busato[1], M. Lucchetta[1], I. Baccelli[3], M. De Zotti[4], F. Favaron[1], R. Musetti[1]

[1]Department of Land, Environment, Agriculture, and Forestry (TESAF), University of Padova, Viale dell' Università 16, 35020 Legnaro, Italy; [2]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell' Università 16, 35020 Legnaro, Italy; [3]Institute for Sustainable Plant Protection, National Research Council of Italy, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy; [4]Department of Chemistry, University of Padova, Via Marzolo 1, 35131 Padova, Italy. E-mail: luca.sella@unipd.it

The management of fungal and bacterial plant diseases mainly relies on chemical control and specifically on applying copper-based products and/or synthetic fungicides that can determine adverse effects on the environment and the health of growers and consumers. Therefore, searching for new sustainable alternatives to chemical control is crucial. A promising low-impact method for controlling plant diseases is activating plant defence mechanisms by treating plants with natural elicitors or antimicrobial compounds. For example, yeasts contain molecules perceived by plant cell receptors as elicitors and, thus, able to activate the plant immune response. In this research, we evaluated and compared the efficacy of different extracts from the yeast *Saccharomyces cerevisiae* for their capacity to induce grapevine tolerance to *Botrytis cinerea*, the causal agent of grey mould disease. Experiments included the combined application of drought stress and *B. cinerea* infection, and the expression analysis of molecular markers related to water stress and pathogenesis. Trichogin GA IV is a non-ribosomal antimicrobial short peptide naturally produced by the fungus *Trichoderma longibrachiatum*. Its antimicrobial activity resides in the ability to insert into phospholipidic membranes and form water-filled pores, thus perturbing membrane integrity and permeability. To develop new sustainable biopesticides, we designed modified trichogin analogs containing one to three Gly-to-Lys substitutions, to increase water-solubility and antimicrobial activity against plant pathogens. By *in vitro* and *in vivo* bioassays, we identified at least two trichogin peptide analogs effective in reducing Fusarium Head Blight and powdery mildew of wheat and tomato bacterial speck disease under controlled conditions.

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The plant microbiome: from rhizosphere to seeds and applications to improve crop performance

A. Sessitsch[1], F. Trognitz[1], C.R. Escobar[1], H. Faist[1], N. Pfaffenbichler[2], B. Mitter[2]

[1]Austrian Institute of Technology (AIT), Austria; [2]ensemo GmbH, Austria. E-mail: Angela.Sessitsch@ait.ac.at

Complex microbiota are found in association with all different plant tissues, from the below-ground organs like roots/rhizosphere to above-ground tissues and reproductive organs. Depending on the tissues, vegetation stage, and many other environmental factors, microbial communities are differently composed, driven by different parameters, and are also responsible for multiple holobiont functions. This talk will address the ecology and functioning of microbiota in different plant tissues, *e.g.*, the ecology and functioning of seed microbiota of *Setaria viridis*, or the potential role of potato root and rhizosphere microbiota in regard to drought stress mitigation and linkage of microbiome and plant phenotypic traits. This talk will also address microbial applications, their potential and limitations as well as new approaches.

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Deciphering the impact of temperature on the conversion capacity and the mycoviral load of conidia of hypovirulent isolates of *Cryphonectria parasitica*

G. Shahzad[1], A. Kabir[2], G. Maresi[3], S. Baric[1,2]

[1]Competence Centre for Plant Health, Free University of Bozen-Bolzano, Piazza Università, 5, 39100 Bozen-Bolzano, Italy; [2]Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Piazza Università, 5, 39100 Bozen-Bolzano, Italy; [3]Unità Protezione delle piante agroforestali e apicoltura, Fondazione Edmund Mach, Via Edmund Mach, 1, 38098 San Michele all'Adige, Italy. E-mail: GullRayna.Shahzad@unibz.it

The European chestnut (*Castanea sativa*) is an economically important tree for its nut production, timber, and ornamental value. Its cultivation has been significantly challenged by the invasive fungal pathogen *Cryphonectria parasitica,* which causes chestnut blight. One of the most important disease control strategies relies on hypovirulence, mediated by the infection of the fungal host by the mycovirus Cryphonectria hypovirus 1 (CHV-1). CHV-1 was shown to be horizontally transmitted by hyphal anastomosis among vegetatively compatible fungal individuals and vertically transmitted through conidia. However, little is known about the impact of temperature on the conversion capacity of hypovirulent strains and the viability of conidia and the mycoviral load, which were addressed in the present study. In the first experiment, hypovirulent isolates of *C. parasitica* were grown at different temperatures to assess the quantity of produced pycnidia and conidia and the viability and proportion of spores carrying CHV-1. In a second experiment, the conversion capacity of virus-containing strains was investigated by co-culturing them with compatible virulent strains at different temperatures. In a third experiment, conidial suspensions of determined concentrations were exposed to different temperature ranges for varying periods of time to investigate the impact on the viability of discharged spores and the dissemination of CHV-1. Considering the warming effect due to climate change, knowledge about the temperature resilience of the *Cryphonectria-Hypovirus* pathosystem is essential to improve disease management strategies based on biological control with hypovirulence.

Microbial consortium with *Trichoderma* and *Beauveria* for dual biocontrol of pathogens and pests, and plant growth enhancement

M. Sinno[1], I. Di Lelio[2], G. Manganiello[2], G.S. Falconieri[3], A. Pascale[4], F. Pennacchio[2], M. Lorito[2], S. Proietti[3], S.L. Woo[1]

[1]Department of Pharmacy, University of Naples Federico II, Naples, Italy; [2]Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy; [3]Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy; [4]Department of Biology, Utrecht University, The Netherlands. E-mail: martina.sinno@unina.it

The future agenda of sustainable agriculture requires increased plant productivity without relying on chemical pesticides and fertilizers. Significant crop losses caused by pests and pathogens demand alternative, efficient, and eco-sustainable strategies for their management. Individual microbial biocontrol agents (mBCAs) and their combinations in consortia offer valuable and diverse benefits to crops for protection and production. This study explores the multifaceted impact of applications with a *Trichoderma afroharzianum* biofungicide and a *Beauveria bassiana* bioinsecticide on tomato. The mBCAs were administered to plants via root irrigation, singly or combined in a microbial consortium, and then their effects were assessed on the biocontrol of an aphid pest, *Macrosiphum euphorbiae*, and a foliar fungal pathogen, *Botrytis cinerea*, as well as on overall plant growth. Significant biocontrol activity against the pathogen was observed with the treatments, mainly with *Trichoderma,* either alone or in combination, as indicated by the reduced infection rates and symptom severity on the leaves. All mBCA negatively affected the survival and fertility of aphids feeding on the treated plants. Moreover, plant growth was notably increased by the mBCAs, particularly with *B. bassiana*. The concurrent applications of *Trichoderma* and/or *Beauveria* produced differential molecular signalling responses in tomato's hormonal and proteomic pathways to the interactions with the microorganisms that resulted in complimentary biocontrol and biostimulant effects. Thus, these results provide insights into the potential applications of microbial consortia as comprehensive solutions to contrast plant damaging agents and improve plant growth, as effective multipurpose biological products for modern agriculture.

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Microbial biocontrol agents boost tomato growth and resilience to water stress and plant pathogens

M. Sinno[1], M. Lettieri[2], A. Dinesh Kothari[3,4], G. Dimaria[5], G. Puopolo[3,4], V. Catara[5], S. Woo[1], R. Marra[2]

[1]*Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy;* [2]*Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy;* [3]*Center Agriculture Food Environment, University of Trento, 38098 San Michele all'Adige, Italy;* [4]*Research and Innovation Center, Fondazione Edmund Mach, 38098 San Michele all'Adige, Italy;* [5]*Department of Agriculture, Food, and Environment, University of Catania, Catania, Italy. E-mail: robmarra@unina.it*

The use of microbial biocontrol agents (mBCAs) in agriculture is increasingly acknowledged as a sustainable strategy to enhance crop growth and resilience to biotic and abiotic stresses. In this study, we investigated the effects of root treatments with mBCAs on tomato (*Solanum lycopersicum* L. cv. Moneymaker), focusing on growth promotion, resistance to water stress, and biocontrol of two plant pathogens: *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lycopersici*. Model mBCAs belonging to the fungal genus *Trichoderma,* and three bacterial strains (*Lysobacter capsici* AZ78, *Bacillus* sp. PSE31B, and *Pseudomonas* sp.) were applied individually and in combinations. In a first pot bioassay, plants were subjected to full or reduced irrigation regimes to evaluate water stress tolerance. Assessment of biometric parameters revealed that treatments containing *Bacillus* sp. PSE31B, either alone or combined with *Trichoderma* strains, significantly increased plant growth and chlorophyll content. Enhanced plant development was also observed under water stress condition, proving that mBCA treatments can counteract the negative effect of reduced water availability. Additionally, these microorganisms effectively reduced mortality and disease symptoms caused by *R. solani* and *F. oxysporum* f. sp. *lycopersici* on tomato plants. *In vivo* experiments monitoring the response of tomato plants to the mBCA application increase plant productivity, and qualitative parameters are currently in progress. These findings highlight the potential of selected mBCAs to mitigate both biotic and abiotic stresses and increase our understanding of the complex plant-microbe interactions occurring within the plant holobiont.

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Comparative rainfastness assessment of different copper-based fungicides

A. Slaviero

Manica Spa, Rovereto Italy; E-mail: a.slaviero@manica.com

Copper antimicrobial properties have been well known since the times of ancient Egyptians, who used it to sterilize water and wounds. In the modern era, after the fungicidal properties of the Bordeaux mixture were discovered by Millardet in 1885, several fungicides were developed using different copper compounds as active substances. The long-term success of these products is linked to their high efficacy at affordable costs and the low risk of developing resistance in the target plant pathogens. However, they can be used only as contact fungicides, and most of the active substances can be washed out from the crop canopy by rainfall. Modern agrochemistry allowed the development of new formulations with increasing rainfastness. Therefore, the old assumption that copper fungicides are not helpful in the context of frequent and heavy rainfalls is no longer valid. This study assessed the rainfastness of seven products containing different copper compounds and formulation technologies after applying a simulated rain of 80 mm on grapevine potted plants. Fungicides implementing the newest formulation technology indicated an increase of metallic copper available on the leaf surface of 18.3–76.7% to the standard Bordeaux mixture and 77.5–158.5% to copper sulphate pentahydrated. Parallel efficacy trials done on *Plasmopara viticola* using the same products revealed a higher efficacy than the reference in heavy rainfalls, consuming less metallic copper per hectare. These results demonstrated that new copper-based fungicides withstand the challenges of extreme weather events and are essential tools for organic farming and IPM strategies.

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Analysis of *Salvia* spp. extracts to control grapevine downy mildew

A. Smaldone[1,2,3], S. Micheloni[1], O. Giovannini[2], M. Oberhuber[3], P. Robatscher[3], M. Perazzolli[1,3]

[1]*Center Agriculture Food Environment (C3A), University of Trento, Via E. Mach 1, 38098 San Michele all'Adige, Trento, Italy;* [2]*Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38098 San Michele all'Adige, Trento, Italy;* [3]*Laboratory for Flavours and Metabolites, Laimburg Research Centre, Laimburg 6, Pfatten (Vadena), 39040 Auer (Ora), Italy. E-mail: anna.smaldone@unitn.it*

Downy mildew, caused by *Plasmopara viticola*, is one of the major threats to grapevine, especially in areas with warm and humid climate conditions. Downy mildew control is mainly based on the use of copper and synthetic fungicides, with possible negative impacts on human health and the environment. Thus, alternative products are required to develop a sustainable viticulture. In particular, extracts of medicinal plants and herbs contain bioactive metabolites for possible applications in plant protection. For example, *Salvia* spp. extracts showed strong inhibitory activity against *P. viticola*. This study aims to characterize the activity of *Salvia* spp. extracts against *P. viticola* and identify bioactive compounds

responsible for the inhibitory activity. Plant, leaf, and flower extracts showed strong disease reduction in grapevine leaf-disk assays, while stem extract was only partially active against *P. viticola*. Moreover, fractions of plant extracts were obtained using high-performance liquid chromatography, and the most efficient fractions were selected by inhibitory activity tests on leaf disks. Active fractions will be subjected to untargeted metabolomic analysis by liquid chromatography-high-resolution mass spectrometry to annotate active compounds for downy mildew control. The identification of *Salvia* spp. bioactive compounds will pave the way for the development of new sustainable alternatives for grapevine protection.

Biocontrol strategies against Brown Spot of Pear (BSP): harnessing bacterial diversity for sustainable orchard management

E. Soriato[1], M. Gatta[1], M. Casagrande[1], D. Danzi[1], A. Cerrato[2], E. Vandelle[1]

[1]Dipartimento di Biotecnologie, Università degli Studi di Verona, Strada Le Grazie, 15, 37134 Verona, Italy; [2]Symbiagro S.R.L., Via dell'artigianato 1/A, 25030 Roncadelle, Italy. E-mail: elia.soriato@univr.it

Stemphylium vesicarium (Cooke) Wint. is one of the most dangerous pathogens that affect pear production worldwide, causing the so-called brown spot of pears (BSP). This fungus can attack several plant species and switch between necrotrophic and saprophytic lifestyles. In the last few years, the economic losses due to the presence of this pathogen in northern Italy led to the replacement of several pear orchards. Disease management still relies on fungicide application in the field, although some resistant pathogen genotypes have emerged in the last few years. In this study, we isolated and evaluated three different potential bacterial biological control agents (BCA) against a local isolate of *Stemphylium vesicarium*. First, we evaluated the biocontrol potential of the bacterial isolates on *S. vesicarium* growth *in vitro,* as direct antagonism or through the emission of volatile organic compounds (VOCs), as well as the possible antimicrobial activity of bacterial cell-free supernatants. Finally, bacterial BCAs were applied to pear fruits to assess their protective capacity against the disease. Furthermore, we sequenced the genomes of the selected isolates to unravel the genomic features related to their biocontrol activities. Overall, these results provide valuable information for developing a promising bio-based alternative or integrative to fungicides to control a major disease and improve the sustainability of pear production.

Unrevealing the potential of bacterial isolates obtained from grapevine rhizosphere

E. Soriato[1], C. Tezza[1], M. Casagrande[1], D. Danzi[1], R. Shmuleviz[1], G.B. Tornielli[2], F. Spinelli[3], M.C. Santos[4], E. Vandelle[1]

[1]Dipartimento di Biotecnologie, Università degli Studi di Verona, Strada Le Grazie 15, 37134 Verona, Italy; [2]Dipartimento di Agronomia, Animali, Alimenti, Risorse naturali e Ambiente, Università di Padova, Via dell'Università 16, 35020 Legnaro, Italy; [3]Dipartimento di Scienze e Tecnologie Agro-Alimentari, Università di Bologna, viale G. Fanin 44, 40127 Bologna, Italy; [4]Department of Biology, LAQV-REQUIMTE, Faculty of Sciences, University of Porto, Porto, Portugal. E-mail: elia.soriato@univr.it

Grapevine is one of the most significant crops in Italy, particularly in the Veneto region. Until now, grape cultivation still largely relies on the extensive use of fungicides and chemical fertilizers to face various fungal diseases and improve yields. Therefore, alternative, sustainable, and eco-friendly approaches should be investigated. This study aims to identify and characterize microbial strains isolated from local vineyards, to be applied as alternatives to chemical products to improve the resilience of vineyards to biotic and abiotic stresses. Out of around 105 bacterial isolates, obtained from the rhizosphere of three different vineyards located in the Verona province, we selected isolates for their significant activities related to the biological control of grapevine fungal pathogens (three strains) and/or plant growth promotion (four strains). These isolates were further subjected to whole-genome sequencing to unravel the genomic features related to their activities. Among them, three isolates resulted as new species of the *Pseudomonas* genera. In particular, the *Pseudomonas* sp. 714A isolate shows a promising plant growth-promoting behavior and a significant biological control capacity, especially through the secretion of volatile organic compounds. These results indicate that vineyard microbial biodiversity represents an important reservoir of endogenous microbe-based potential alternatives to chemicals to improve viticulture sustainability.

Antimicrobial products from a hazelnut endophytic isolate of *Cladosporium perangustum*

A. Staropoli[1], E. Ferrara[2], M.M. Salvatore[3], D. Cice[2], M. Petriccione[2], F. Vinale[4], A. Andolfi[3], R. Nicoletti[1,2]

[1]Department of Agricultural Sciences, University of Naples 'Federico II', Portici, Italy; [2]Council for Agricultural Research and Economics, Research Center for Olive, Fruit and Citrus Crops, Caserta, Italy; [3]Department of Chemical Sciences, University of Naples 'Federico

II', Naples, Italy; [4]*Department of Veterinary Medicine and Animal Production, University of Naples 'Federico II', Naples, Italy. E-mail: rosario.nicoletti@crea.gov.it*

The symbiotic relationships established between endophytic fungi and their host plants may entail involvement in defensive mutualism, which can be related to the production of bioactive secondary metabolites. In this respect, we are currently investigating the role of endophytic fungi in protection against *Pseudomonas avellanae* and *Xanthomonas arboricola* pv. *corylina*, the causal agents of hazelnut bacterial diseases. The culture filtrate of isolate HN01C, identified as *Cladosporium* (*C.*) *perangustum* based on sequencing of DNA markers, strongly inhibited both bacterial pathogens *in vitro*. The culture filtrate was extracted with ethyl acetate and the crude extract was submitted to column chromatography. The fractions obtained were analyzed by LC-MS, and a sterol (cladosporisteroid B), an alkaloid (8-hydroxyerginine), and a series of diketopiperazines, namely cyclo(prolyl-valyl), cyclo(leucyl-prolyl), cyclo(isoleucyl-prolyl) and cyclo(phenylalanyl-prolyl) were detected. While the latter has been reported from many fungi, the other compounds are infrequent. Indeed, 8-hydroxyerginine was only identified from the ergot fungus *Claviceps paspali*, while cladosporisteroid B has been previously reported from two marine-derived strains of *C. halotolerans* and *C. cladosporioides.* However, considering that these species belong to different species complexes, it is likely that cladosporisteroid production is widespread within the genus *Cladosporium*. So far, the antibiotic properties of diketopiperazines have been widely investigated with the notable remark that these products may act in synergism, which could also be effective in the case of our finding. Conversely, the antibacterial properties of cladosporisteroid B and 8-hydroxyerginine require to be more thoroughly assessed in a dedicated study.

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Effect of selected *Trichoderma* strain and 6-pentyl-α-pyrone on rhizosphere environment of grapevines

A. Staropoli[1,2], A. Vassetti[2], C. Gigliotti[2], G. d'Errico[1], E. Troiano[2], F. d'Errico[1], M. Lorito[1], F. Vinale[2,3]

[1]*Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy;* [2]*Institute for Sustainable Plant Protection, National Research Council, 80055 Portici, Italy;* [3]*Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, 80138 Naples, Italy. E-mail: alessia.staropoli@unina.it*

Viticulture plays a crucial role in Italy's agricultural sector, contributing significantly to the economy and the global wine industry. Rhizosphere microflora and microfauna are considered important factors for soil quality, to improve grapevine growth and resistance to biotic and abiotic stresses. In this study, soil characterization of selected vineyards located in Avellino province (Campania, southern Italy), was carried out before and after the application of two beneficial *Trichoderma* strains (M10 and T22) or 6-pentyl-α-pyrone (6PP, a bioactive *Trichoderma atroviride* P1 secondary metabolite). Moreover, physiological change in treated plants was also investigated through untargeted metabolomic analysis. Soil before microbial or 6PP treatments showed a low amount of plant-parasitic nematodes (i.e. *Helicotylenchus* spp., *Rotylenchulus* spp., and *Xiphinema pachtaicum*) and pathogens (i.e. *Agrobacterium tumefaciens, Fusarium* spp. and *Neonectria* spp) and a high level of beneficial microbes (i.e. *Clonostachys rosea, Pseudomonas* spp., *Pseudaminobacter* spp.). Metabolomic analysis revealed that treatments significantly affected the accumulation of metabolites related to beneficial effects on plants (i.e. sugars, antioxidants, organic acids) in comparison to untreated plants. Finally, soil microbiome (metagenomic analysis) was significantly affected by the treatments; microbial-based T22 treatment and 6PP particularly altered bacterial population with *Proteobacteria* superphylum as the most represented (60% T22 and 61% 6PP compared to 47% for the untreated plants). Interestingly, T22 treatment significantly impacts the sugar concentration of grapes. Overall, these findings underscore the potential of using microbial and metabolite-based treatments to enhance soil quality, plant physiology, and microbiome composition, leading to healthier grapevines and more resilient agricultural ecosystems.

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Diversity of vegetative compatibility types of the chestnut blight fungus in a Lake Garda (northern Italy) population

D.H. Tabet[1], A. Kabir[1,] A. Brighenti[2], A. Esposito[3], S. Baric[1,4]

[1]Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Bozen-Bolzano, Italy; [2]Consorzio di Tutela del Marrone di San Zeno DOP, San Zeno di Montagna, Italy; [3]Faculty Università degli Studi di Enna "Kore", Enna, Italy; [4]Competence Centre for Plant Health, Free University of Bozen-Bolzano, Bozen-Bolzano, Italy. E-mail: DaniaHanna.Tabet@unibz.it

Chestnut blight, caused by *Cryphonectria parasitica*, is one of the most important diseases of sweet chestnut trees (*Castanea sativa*) in Europe. The control of the disease largely depends on the natural or human-mediated spread of hypovirulent strains of the fungus, which are infected with Cryphonectria hypovirus 1 (CHV-1). CHV-1 has proven a successful biocontrol agent in many European chestnut growing areas. Still, the transmission of the mycovirus from hypovirulent to virulent strains occurs through hyphal anastomosis between the same vegetative compatibility (VC) types. Therefore, high VC type diversity of the fungal host within a region may limit CHV-1 transmission, and studying VC type diversity is of practical interest to assess the biocontrol potential of hypovirulent strains. This study was conducted in the San Zeno area (Province of Verona) in northern Italy, where bark samples from chestnut blight cankers were collected in several representative chestnut stands. Pure fungal isolates were obtained from the bark samples, from which DNA was isolated, and a PCR analysis of the six known vegetative incompatibility (*vic*) loci was performed. The analysis of a first batch of 40 samples revealed the presence of nine VC types: EU-2, EU-1, EU-13, EU-17, EU-5, EU-12, EU-14, EU-15, and EU-28. VC types EU-2, EU-1, and EU-13 attributed to 60% of the isolates. The main VC types observed in the *C. parasitica* population in the San Zeno area of Lago di Garda reflect the diversity seen in northern Italy and other European populations.

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Real-time RT-PCR High Resolution Melting curve analysis to differentiate variants of Citrus tristeza virus in trees

G. Testai[1], G. Scuderi[2], M. Russo[2], M. Bazzano[2], C. Licciardello[3], A. F. Catara[4], V. Catara[1], G. Licciardello[3]

[1]Department of Agriculture, Food and Environment, University of Catania, 95121 Catania, Italy; [2] Agrobiotech soc. coop. ZI Blocco Palma I, Str.le V. Lancia 57, 95121 Catania, Italy; [3]CREA - Council for Agricultural Research and Economics, Research Centre for Olive, Citrus and Tree crop. 95024 Acireale (CT), Italy; [4]Formerly, Department of Phytosanitary Science and Technologies, University of Catania, Italy. E-mail: grazia.licciardello@crea.gov.it

Citrus tristeza virus (CTV), the causal agent of Tristeza disease, is a complex virus consisting of different isolates molecularly grouped in several genotypes that can produce different phenotypes in the same host. The real-time PCR assay followed by High-Resolution Melting analysis (HRM) has been widely employed for mutational scanning, repeat typing, genetic variant scanning, and genotype differentiation of the infected virus in human, plant, and veterinary medicine. Simple, fast, inexpensive, and effective, HRM in this study aimed to establish a concurrent diagnosis and discrimination of VT seedling yellows (SY) and no-SY isolates of CTV, differing for only eight nucleotides along the entire genome. The assay was developed using the A/G mutation located in the 161 nt of the p23 gene as the target, which is strictly involved in the no-SY/SY phenotype reaction in sour orange (SO). The HRM curve analysis allowed to distinguish no-SY and SY VT variants in artificially inoculated SO plants. The method's robustness has been evaluated by testing ornamental rutaceous plants closely related to the *Citrus* genus and Hamlin sweet oranges grafted on SO inoculated with M39. Moreover, when SO and Hamlin/SO plants, protected and challenged, were analysed by HRM, only the profile associated with the no-SY isolate was revealed, indicating that it is actively replicating in the plant and that the SY challenged isolate has been excluded. The method has also been validated testing SO seedlings, completely asymptomatic, inoculated with bark tissues taken from protected plants, demonstrating the exclusive replication of the no-SY isolate.

Exploring the vegetative compatibility type diversity of the *Cryphonectria parasitica* population in Friuli Venezia Giulia (northern Italy)

S. Tomada[1], A. Kabir[2], L. Poggetti[1], V. Cacitti[1], M. Fabro[1], S. Baric[2,3]

[1]Agenzia Regionale per lo Sviluppo Rurale del Friuli Venezia Giulia– ERSA, Via Sabbatini 5, 33050 Pozzuolo del Friuli, Italy; [2]Free

University of Bozen-Bolzano, Piazza Università 1, 39100 Bolzano, Italy; [3]Competence Centre for Plant Health, Free University of Bozen-Bolzano, Piazza Università 5, 39100 Bolzano, Italy. E-mail: selena.tomada@ersa.fvg.it

Chestnut blight, caused by the fungus *Cryphonectria parasitica*, represents a threat for the sustainability of chestnut cultivation in many countries. The natural and human-mediated spread of hypovirulent isolates infected with Cryphonectria hypovirus 1 (CHV-1) can reduce the virulence of the fungus and is used as a biocontrol practice. CHV-1 is naturally transmitted through hyphal anastomosis formed among vegetatively compatible (VC) *C. parasitica* strains, a feature that is genetically determined and governed by six loci. Consequently, the effectiveness of CHV-1 transmission might be compromised by vegetative incompatibility, especially in populations with high VC type diversity. Therefore, molecular genetic characterization of *C. parasitica* populations is essential to evaluate the implementation of a biological control strategy in the area of concern. This study was conducted in the Friuli Venezia Giulia region, where 122 *C. parasitica* isolates from six sampling zones were obtained from chestnut blight cankers, and 105 of them analysed at the vegetative incompatibility loci and the mating type locus. This study identified 22 VC types, with EU-13, EU-17, EU-28, and EU-12 being the most prevalent, constituting 13.2%, 11.2%, 10.9%, and 9.9% of the fungal isolates, respectively. Both fungus mating types were found to have a nearly equal ratio, pointing out the possibility of further increasing VC-type diversity due to sexual reproduction. Thus, the high genetic variability of the fungal population could impair the biocontrol of *C. parasitica* in the region. Further molecular genetic analyses shall be performed to comprehensively understand the fungal population's genetics.

In vitro evaluation and phytotoxicity assessment of essential oils for controlling lettuce drop by *Sclerotinia* spp.

M. Tunç, S. Piancatelli, S.M. Makau, G. Cantalamessa, A.L. D'Ortenzio, M. Moumni, G. Romanazzi

Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche, 60131 Ancona, Italy. E-mail: g.romanazzi@univpm.it

Lettuce drop, caused by *Sclerotinia* spp., is one of the most economically damaging diseases of lettuce (*Lactuca sativa*) worldwide. The reliance on synthetic fungicides to control this disease has become increasingly unsustainable due to concerns about chemical residue levels. This has spurred significant interest in exploring alternative, sustainable control measures. Essential oils (EOs) have emerged as promising alternatives to chemical pesticides. The antimicrobial activity of *Rosmarinus officinalis, Rosmarinus officinalis* var. *verbenone, Lavandula hybrida, Origanum majorana*, and *Thymus vulgaris* EOs toward *Sclerotinia sclerotiorum* isolated from lettuce was assessed *in vitro* at concentrations of 0.1%, 1%, and 10% in Petri dishes containing amended PDA. The phytotoxicity of these EOs was evaluated at 0.1% and 1% concentrations on lettuce, tomato, and chard. *T. vulgaris* EO exhibited complete inhibition of *Sclerotinia* spp. mycelial growth at all concentrations. At 1% and 10%, *R. officinalis, L. hybrida*, and *O. majorana* were able to inhibit completely the mycelia growth of *S. sclerotiorum*, while *R. officinalis* var. *verbenone* demonstrated substantial mycelial growth inhibition of 80% and 100% at 1% and 10% concentrations, respectively. Minimal phytotoxicity was exhibited by *R. officinalis*, with no adverse effects observed except at a 1% concentration in chard cultures. Lower concentrations demonstrated negligible phytotoxicity. Further field applications are necessary to evaluate the practical implications of EOs for sustainable agriculture.

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Improving management of emerging and re-emerging pests and diseases of *Prunus persica* (L.) Batsch)

S. Turco[1], F. Brugneti[1], L. Rossini[2], M. Cirilli[3], A. Mazzaglia[1]

[1]Dipartimento di Scienze Agrarie e Forestali, Università degli Studi della Tuscia, 01100 Viterbo, Italy; [2]Service d'Automatique et d'Analyse des Systèmes, Université Libre de Bruxelles, Av. F.D. Roosevelt 50, 1050 Brussels, Belgium; [3]Dipartimento di Scienze Agrarie ed Ambientali, Università degli Studi di Milano, 20133 Milano, Italy. E-mail: silvia.turco@unitus.it

Prunus persica (peach) is among the most important fruit tree species, renowned for its nutritional value and significant economic impact. Italy, as the second-largest peach producer in the European Union, is currently facing unprecedented challenges due to the spread of invasive pests such as *Halyomorpha halys* (*HH*), which has recently caused economic losses nearing 100 million euros, and the emergence or resurgence of harmful pathogens such as those associated with the Twig Cankers and Shoot Blight disease (TCSB). With the most effective traditional agrochemicals no longer available, preventive actions based on new Model-based Decision Support Systems (DSS) coupled with germplasm-derived genetic resistance and/or biocontrol agents seem promising solutions to curb these infestations and infections. In the framework of the IMPEACHMENT project (PRIN 2022), a series of experiments aimed at formulating a deterministic model to describe the biology of HH and the epidemiology of TCSB disease are in progress. Additionally, different antagonistic fungi and bacteria, mainly belonging to *Trichoderma* and *Bacillus* genera, as well as entomopathogenic microorganisms, are currently screened and tested as biological control agents (BCAs) against *D. amygdali*, the fungus deemed as the principal causal agent of TCSB, and *H. halys.* The results of this study will be integrated into a pilot study to establish a control strategy involving three key components: plant genetics, monitoring/modeling, and biocontrol agents.

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In vitro fungicidal activity of hydrophilic and lipophilic extracts of Mexican oregano (*Lippia graveolens*) against *Podosphaera xanthii*

O. Valdez-Baro, J.P. Manjarrez-Quintero, J.B. Heredia, J.M. Tovar-Pedraza, R.S. García-Estrada

Laboratorio de Fitopatología, Coordinación Regional Culiacán, Centro de Investigación en Alimentación y Desarrollo, Sinaloa, Mexico. E-mail: ovaldez222@students.ciad.mx

Podosphaera xanthii is a plant pathogenic fungus that affects cucurbit crops, causing a symptom known as powdery mildew. Botanical extracts are an alternative that act similarly to chemical fungicides but do not present resistance, as they exhibit multiple modes of action. Among botanical extracts, hydrophilic and lipophilic fractions from *Lippia graveolens* have demonstrated their high effectiveness. Spores of *P. xanthii* were collected at a concentration of 10^6 spores/mL which were exposed to different concentrations of essential oil and ethanolic extract (100 to 1,000 ppm) at multiple times to observe the minimum fungicidal concentration (MFC) and the effective concentration at 50% (EC_{50}). To determine cell viability, the MTT method was used, in which the MTT reagent reacts with the enzymes of the mitochondria when an organism is alive, forming violet formazan crystals, which are read on a microplate reader at 590 nm. The results indicate that both the ethanolic extract and the essential oil have fungicidal potential, with the effect being up to four times greater in the essential oil with an EC_{50} of 110 ppm for the essential oil and 436 ppm for the ethanolic extract; obtaining, after 18 hours of exposure, a minimum fungicidal concentration of 200 ppm for the essential oil and 1,000 ppm for the ethanolic extract. Regarding time, it is observed that the longer the exposure time, the greater the fungicidal effect. These results suggest that *L. graveolens* extracts can be employed to control powdery mildew on cucurbits to reduce the chemical fungicides.

In vitro fungicidal activity of hydrophilic and lipophilic extracts of Mexican oregano (*Lippia graveolens*) against sclerotia of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*

O. Valdez-Baro, J.P. Manjarrez-Quintero, J.M. Tovar-Pedraza, R.S. García-Estrada

Laboratorio de Fitopatología, Coordinación Regional Culiacán, Centro de Investigación en Alimentación y Desarrollo, Sinaloa, México. E-mail: ovaldez222@students.ciad.mx

Sclerotinia sclerotiorum and *Sclerotium rolfsii* are plant pathogenic fungi that cause severe losses in agricultural crops due to their wide host range, rapid infection, as well as their high production of sclerotia (dense mycelium masses) necessary to disseminate and survive adverse environmental conditions. Managing sclerotia is difficult, with chemical fungicides as the main solution. To mitigate the environmental impact of chemical fungicides, the implementation of non-toxic, effective alternatives is essential. Botanical extracts, particularly hydrophilic and lipophilic fractions from *Lippia graveolens*, offer a sustainable solution. In this study, sclerotia from *S. sclerotiorum* and *S. rolfsii* were treated with various concentrations of *L. graveolens* essential oil and ethanolic extract for 24 hours. Afterward, the sclerotia were bisected and cultured on PDA, enabling the assessment of mycelium growth and the determination of minimum fungicidal concentrations (MFC) and the effective concentration inhibiting 50% mycelial growth (EC_{50}). The results indicate that both the ethanolic extract and the essential oil have a fungicidal effect on both types of sclerotia, with the MFC and EC_{50} for *S. rolfsii* being 700 and 250 ppm, respectively for the essential oil and 1,500 and 620 ppm respectively for the ethanolic extract. While the MFC and EC_{50} for *S. sclerotiorum* was 400 and 175 ppm respectively for the essential oil and 4,000 and 1,650 ppm respectively for the ethanolic extract. These results demonstrate that extracts from *L. graveolens* could be used as a potential botanical fungicide in crops infected with *S. sclerotiorum* and *S. rolfsii* to reduce the chemical fungicides.

Microsatellite characterization for genotyping *Plenodomus tracheiphilus*, the causal agent of "Mal secco" disease of citrus in Italy

M.T. Valente[1], L. Orzali[1], M. Aragona[1], A. Grottoli[1], E. Marinelli[1], S. Vitale[1], V. Battaglia[2], E. Lahoz[2], A. De Iudicibus[1], S. Bechini[1], S. Di Silvestro[3], G. Licciardello[3], V. Vizzarri[4], A. Infantino[1]

[1]CREA Research Centre for Plant Protection and Certification, CREA-DC, via C.G. Bertero 22, 00156 Roma, Italy; [2]CREA Research Centre for Cereal and Industrial Crops, CREA-CI via Torrino 2, 81100 Caserta, Italy; [3]CREA Research Centre for Olive, Citrus and Tree Fruit CREA-OFA, Corso Savoia 190, 95024 Acireale, Italy; [4]CREA Research Centre for Olive, Citrus and Tree Fruit CREA-OFA, 87036 Rende, Italy. E-mail: mariateresa.valente@crea.gov.it

Plenodomus tracheiphilus is a mitosporic plant pathogen affecting particularly lemons, on which it causes a destructive vascular disease named "Mal secco", present in the most citrus-growing countries of the Mediterranean and Black Sea. This research aimed to characterize the genetic structure of *P. tracheiphilus* populations collected in different Italian lemon-producing areas using microsatellite markers. Starting in the spring of 2024, samples from trees showing typical disease symptoms were collected from several lemon orchards. More than 200 isolates of *P. tracheiphilus* were collected on the bases of one isolate per tree. Monohyphal colonies were obtained and identified by morphological and molecular analysis. The *P. tracheiphilus* genome, available at the NCBI Genome Database (273 scaffolds for 34.2 Mb), provided an opportunity to determine the microsatellite distribution and to find polymorphic markers. As a result, 2454 microsatellite loci were detected (0.16% of total sequence length), an excellent starting point for obtaining polymorphic markers. Twenty primer pairs for di-, tri-, tetra-, penta-, and hexanucleotide loci were designed and tested on eight isolates from different sampling areas. Results of the preliminary screening were reported. The next phase of this project will involve long-read sequencing of the *P. tracheiphilus* genome to generate complete chromosome assemblies on which to map selected microsatellites.

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Screening secretomes to limit deoxynivalenol production in *Fusarium graminearum*: a fast *in vitro* assay

I. Valenti, C. Gardana, P. Cortesi, D. Bulgari, A. Kunova, M. Saracchi, M. Pasquali

Department of Food, Environmental and Nutritional Sciences, University of Milan, 20133 Milan, Italy. E-mail: matias.pasquali@unimi.it

Biological control against mycotoxins accumulation is challenging due to the complex interactions on cereal spikes during the Fusarium head blight disease. Identification of novel active compounds able to limit deoxynivalenol (DON) accumulation in cereals is a potentially effective strategy to limit mycotoxin accumulation in food products. Moreover, the control of deoxynivalenol synthesis reduces the incidence of the disease, because DON is a pathogenicity factor in wheat infection. Here, we developed a fast microtiter assay to identify molecules able to reduce DON synthesis by *Fusarium graminearum in vitro*. The assay based on the fluorescent detection of the Tri5-GFP gene fusion protein, showed a good correlation with DON content measured with LC-MS/MS method ($r>0.7$; p-value <0.0001). Using the secretomes of five streptomycetes strains with interesting biological properties against the pathogen, we identified two extracts from two different strains able to reduce DON synthesis effectively up to 99%. Further studies are warranted to characterise the effective molecule(s) from the secretomes of both *Streptomyces* spp. Our assay can screen multiple extracts and molecules with a potential role in deoxynivalenol synthesis inhibition in six days.

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Investigating the enniatin B effect on a promising biocontrol agent active against *Fusarium graminearum*

I. Valenti, C. Pizzatti, D. Bulgari, P. Cortesi, A. Kunova, M. Saracchi, M. Pasquali

Department of Food, Environmental and Nutritional Sciences, University of Milan, 20133 Milan, Italy. E-mail: irene.valenti@unimi.it

Enniatin B (ENB) is among the frequently emerging mycotoxins produced by *Fusarium* species. It is present in all cereals, but its toxicological and biological effects are not completely deciphered. Existing biocontrol strategies include the application of *Streptomyces* spp. as biocontrol agents (BCAs), which produce bioactive compounds that are effective against plant pathogens. Considering the multiple interactions among different microorganisms and molecules on the cereal plants, we investigated the influence of ENB on our promising *Streptomyces* strain, which previously showed the ability to reduce the deoxynivalenol (DON) accumulation on wheat spikes, contrasting the *Fusarium graminearum* disease. Additionally, the ENB ability in modulating drug efflux transporters, arising from literature, is an important factor that could affect the activity of our BCA during field application. In this regard, we investigated the effect of ENB exposure (1 μg/ml) on the metabolome of our *Streptomyces* BCA employing a high-resolution mass spectrometry analysis (UHPLC-HRMS). Overall, a limited number of metabolites shifted upon ENB exposure. Importantly, the metabolome of ENB-exposed-BCA conserved the ability to repress DON biosynthesis in *F. graminearum*, suggesting our bacterial strain can cope with ENB exposure, maintaining its BCA characteristics. Further investigations will be focused on the fate of ENB after the BCA exposure.

Efficacy of biocontrol agents and biostimulants against strawberry crown and root rot caused by *Rhizoctonia solani* under greenhouse conditions

P. Valfrè[1,2], G. Gilardi[1], M. Pugliese[1,2]

[1]AGROINNOVA – Interdepartmental Centre for the Innovation in the Agro-Environmental Sector, University of Turin, Italy; [2]Agricultural, Forestry and Food Sciences Department (DISAFA), University of Turin, Italy. E-mail: paolo.valfre@unito.it

Strawberry crown and root rot caused by *Rhizoctonia solani* is a severe threat to strawberry production worldwide and leads to significant yield losses. The use of biocontrol agents and biostimulants is increasingly investigated in response to the need for sustainable disease control measures. Three trials were conducted on potted strawberry plants cv. Elodì (FC.12.025.01) to evaluate the efficacy of experimental antagonists (*Fusarium oxysporum* MSA35 and *Trichoderma asperellum* FC80), isolated from suppressive soils, and biostimulants (microbial AMF and non-microbial GH), against *R. solani* in the greenhouse. The plants were planted in 12 L plastic pots filled with a peat substrate infested with the pathogen biomass, using four plants/pot, with four replicates (16 plants/treatment). Products applied alone (MSA35 and FC80 $1x10^7$ conidia/mL, GH 0.1%, and AMF 0.5 g/L) or in combination were compared to tolclofos-methyl and to the marketable available products based on *Trichoderma gamsii* + *T. asperellum* and *Glomus* spp. + *Trichoderma* spp. + rhizobacteria. Treatments were first applied by root immersion immediately before transplanting and 15 days later, by soil drench. Plants were monitored for symptom development. Under severe disease pressure in the untreated control plants, disease severity was significantly reduced by FC80 (38% efficacy), FC80+GH (42% efficacy), FC80+MSA35+GH (43% efficacy), and AMF+GH (40% efficacy). Results showed the impact of the type and timing of biocontrol agent and biostimulant application on strawberry crown and root rot. Further studies are required to evaluate the effect of these products used alone or combined under field conditions.

Role and impact of *Xylella fastidiosa* subsp. *pauca* on endophytic communities in resistant and susceptible olive cultivars

M. Vergine[1], F. Vita[2], M. De Pascali[1,3], L. De Bellis[1,3], A. Luvisi[1]

[1]Department of Biological and Environmental Sciences and Technologies, University of Salento, 73100 Lecce, Italy; [2]Department of Biology, University of Bari "Aldo Moro", Bari, Italy; [3]National Biodiversity Future Center, 90133 Palermo, Italy. E-mail: marzia.vergine@unisalento.it

The interaction between endophytic communities and olive trees (*Olea europaea* L.) infected by *Xylella fastidiosa* subsp. *pauca* (*Xfp*), a pathogenic bacterium causing Olive Quick Decline Syndrome (OQDS), was investigated by comparing the composition of endophytic communities in resistant (Leccino) and susceptible (Cellina di Nardò) olive cultivars. Different levels of *Xfp* infection were assessed using samples collected from infected orchards in Apulia (southern Italy), according to a disease severity scale arranged from 0 (no symptoms) to 3 (extensive canopy desiccation). Results allow the identification and quantification of endophytic bacteria and fungi from leaf tissues using culture-independent methods. High-throughput sequencing of the 16S rRNA and ITS regions provided new insights for deciphering the relationship within the holobiont, thus leading to significant differences in the composition of endophytic communities between resistant and susceptible cultivars. The resistant cultivar Leccino showed a higher abundance and diversity of putative beneficial endophytes, including those known to produce antimicrobial compounds and promote plant health. In contrast, the susceptible Cellina di Nardò cultivar had a more compromised endophytic community, which may exacerbate its vulnerability to *Xfp*. These findings suggest that *Xfp* modulates the endophytic community structure by also inducing significant physiological changes in the host plant. These alterations may affect the plant's natural defence mechanisms, highlighting the potential role of specific endophytes in enhancing resistance to *Xfp*. Identifying beneficial endophytes associated with Leccino could provide insights into potential biocontrol agents and strategies to improve the management of OQDS in olive trees.

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Diversification of alkaloid production in *Trichoderma arundinaceum* IBT 40837 acquired by horizontal gene transfer of secondary metabolite biosynthesis cluster

I. Vicente[1], A. Staropoli[2,3], R. Baroncelli[4], D. Lotito[5], L. Di Costanzo[3], S. Vitale[3], R. Hermosa[1], G. Vannacci[6], S. Sarrocco[6], E. Monte[1], F. Vinale[5]

[1]Department of Microbiology and Genetics, Institute for Agribiotechnology Research (CIALE), University of Salamanca, 37185 Villamayor, Spain; [2]Institute for Sustainable Plant Protection, National Research Council, 80055 Portici, Italy; [3]Department of Agricultural Sciences, University of Naples Federico II, Naples, Italy; [4]Department of Agricultural and Food Sciences (DISTAL), University of Bologna, 40127 Bologna, Italy; [5]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137 Naples, Italy; [6]Department of Agriculture, Food and Environment, University of Pisa, 56124 Pisa, Italy. E-mail: i.vicentemu@gmail.com

Trichoderma arundinaceum IBT 40837 (Ta37) can be considered a potential secondary metabolite (SM) superproducer among *Trichoderma* species, as it is characterized by a vast arsenal of SM backbone genes. Here, we analysed nitrogen-containing SMs produced by Ta37, focusing on alkaloids and other compounds with potential bioactive properties. Metabolic profiles were obtained by Gas and Liquid Chromatography-Mass Spectrometry (GC-MS–LC-qTOF-MS) analysis of Ta37 liquid cultures performed at different incubation times in both shaking and stationary conditions. Ta37 produces a wide range of alkaloids, including quinoline, quinolone, β-carboline, indolizidine, and a variety of diketopiperazines. In addition, 3-ethyl-4-hydroxy-6-methyl-2H-pyran-2-one has been isolated and structurally characterized for the first time by X-ray crystallography. Supplementation of L-tryptophan to the Ta37 culture medium increased the diversity of indole alkaloids produced, including ergot alkaloids, whose biosynthesis has not yet been reported in *Trichoderma* species. Analysis of the Ta37 genome determined the presence of a 50 Kb region that has been acquired by horizontal gene transfer from *Xylariaceae* species and encloses an ergot alkaloid biosynthetic cluster. We deleted the dimethylallyl-tryptophan synthase (DMATS)-encoding gene that catalyse the first committed step of the ergot biosynthesis pathway. Deletion of *dmats* reduced fungal growth and spore production in MEA and affected the ability of the fungus to use ammonia and peptone as nitrogen sources. In addition, the *dmats* mutants had reduced tolerance to oxidative stress and lower antagonistic activity against *Botrytis cinerea, Rhizoctonia solani,* and *Pythium irregulare*.

Beyond the tree: a phylogenomic approach to linking species boundaries with functional traits in *Fusarium*

A. Villani[1], A. Susca[1], A. Menicucci[2], A. Prodi[2], L. Faino[3], A. Moretti[1], R. Baroncelli[2,4]

[1]Institute of Sciences of Food Production, National Research Council (ISPA-CNR), 70126 Bari, Italy; [2]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), Bologna, Italy; [3]Environmental Biology, Sapienza University of Rome, Roma, Italy; [4]University of Naples Federico II, Center for Studies on Bioinspired Agro-Enviromental Technology, Portici, Italy. E-mail: alessandra.villani@ispa.cnr.it

The *Fusarium* genus harbours agriculturally and clinically important species with a tangled taxonomic history. Accurate species identification is essential for evaluating mycotoxin risks and developing crop management strategies, especially in the current scenario of the imminent challenges posed by climate change and food security. While next-generation sequencing revolutionizes fungal systematics, challenges persist. Publicly available fungal genomes often suffer from incomplete assemblies and variable quality, hindering research on speciation, evolution, and pathogenesis.

This study addresses this gap by evaluating publicly available genomic data for 1530 fungal strains in the Nectriaceae family. Filtered data constructed a robust phylogenomic workflow to resolve taxonomic ambiguities. Phylogenomic analysis confirmed previously described clades (F1, F2, F3) based on 19 protein-coding genes. Critically, our analyses revealed that nearly 20% of publicly available genomes are not assigned or wrongly assigned, highlighting the importance of establishing solid approaches for the taxonomic designation of genomic data to avoid confounding issues in public databases. These findings pave the way for further research on gene family distribution and evolution within the Nectriaceae genera. Particularly interesting are gene families linked to secondary metabolite biosynthesis, offering insights into the evolutionary forces shaping gene distribution and timing of key evolutionary events. This approach can ultimately contribute to a more comprehensive understanding of fungal diversity and its impact on agriculture and human health.

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Recruitment of biocontrol and plant growth promoting bacterial strains for the sustainable management of tomato cropping systems

C. Vincenzo[1,2], P. Tripodi[1], N. Lombardi[2], C. Pane[1]

[1]Council for Agricultural Research and Economics (CREA), Research Centre for Vegetables and Ornamental Crops, Pontecagnano Faiano, Italy; [2]Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy. E-mail: cono.vincenzo@crea.gov.it; cono.vincenzo@unina.it

Intensive tomato cultivations under soil sickness and climate change scenarios are exposed to the recrudescence of soil-borne pathogens, such as *Fusarium oxysporum* f. sp. *lycopersici* and *Sclerotium rolfsii.* Beneficial microorganisms can be exploited to improve plant production due to their antagonistic and growth-promoting properties. This work is focused on a stepwise selection of endophytic bacterial strains, useful for the tomato sustainable management, isolated from the internal root system of tomato plants survived to the target pathogens in suppressive microcosmos. About 200 bacterial isolates were collected and assayed *in vitro* for their compatibility with reference *Trichoderma harzianum* strains, in the view of future consortium applications. Ten compatible selected bacterial strains were tested *in planta* experiments to evaluate their biocontrol ability against the two above-mentioned pathogens and their plant growth promotion attitude. The trials were performed on potted tomato cv Crovarese (n=264) and treatments with each isolate were applied as seed coating followed by drenching. Performances were assessed using the PlantEye 3D multispectral scanner, an optoelectrical sensor able to detect automatically and accurately twenty morphological and biometric plant traits. Biocontrol activity was evaluated by computing phenomics data and disease index together. Results indicated that four strains of *Peribacillus* were the best isolates in controlling *F. oxysporum* and *S. rolfsii*; while one strain, belonging to *Microbacterium* sp., showed promotion of plant growth as highlighted by digital biomass and plant height indexes. The phenomics approach was a valuable method to screen beneficial microorganisms for biocontrol purposes.

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Involvement of oomycetes in dieback and root rot of *Citrus* spp. in southern Italy

S. Vitale[1], L. Luongo[1], I. Mercuri[1], G. Licciardello[2], M. Caruso[2], M. Cambrea[2], L. Pirone[1], A. Haegi[1]

[1]CREA DC Research Centre for Plant Protection and Certification, Via C.G. Bertero 22, 00156 Roma, Italy; [2]CREA OFA Research Center for Olive, Fruit and Citrus Crops, Corso Savoia 190, 95024 Acireale, Italy. E-mail: anita.haegi@crea.gov.it

Frequent use of rootstocks other than sour orange in citriculture has increased the incidence of root and gummosis diseases in susceptible species. The main cause of these diseases is due to *Phytophthora* spp. *Phytophthora nicotianae* is the most widespread species in world subtropical areas and causes foot and root rot, while *P. citrophthora* causes both gummosis and root rot, and it also attacks the trunk and main branches. Other *Phytophthora* species have also been reported as pathogenic to citrus in various regions worldwide. In experimental orchards and screenhouses in Calabria and Sicily, citrus plants grafted onto Carrizo citrange and seedlings of various rootstocks, were observed to exhibit dieback, wilting, root rot, and poor growth. Causal agents of these symptoms were investigated. Phytophthora and Pythium-like organisms were consistently isolated from symptomatic tissues excised from larger roots of affected trees. The isolates were identified as *Phytophthora nicotianae* and

Pythium (Py.) ultimum (syn. *Globisporangium ultimum*) based on morphological characteristics and confirmed by internal transcribed spacer sequences. From citrus plants in screenhouse, besides *P. nicotianae* and *Py. ultimum, Phytophthora rosacearum,* and *Pythium dissotocum* were identified. The results confirm the occurrence of *P. nicotianae* as causal agent of root rot and poor growth of citrus trees, along with *Py. ultimum* and *Py. dissotocum,* recently reported as causal agents of gummosis in citrus trees in Tunisia. Furthermore, *P. rosacearum,* known as a pathogen of rosaceous fruit trees, has not been reported yet on citrus species. Tests are ongoing to confirm the pathogenicity of the collected isolates.

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Root exudates from stressed plants differentially regulate plant interactions with fungal pathogens and beneficial microorganisms

S. Vitale[1], M. Papaianni[1], M. Ranesi[1], I. Di Lelio[1], F. Pennacchio[1], S.L. Woo[2], M. Lorito[1], N. Lombardi[1]

[1]Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy; [2]Department of Pharmacy, University of Naples Federico II, Naples, Italy. E-mail: nadia.lombardi@unina.it

Root exudates (REs) released by plants play a key role in multitrophic interactions that occur in the rhizosphere and represent the primary means by which plants communicate with the microorganisms, influencing both the diversity and the specificity in the soil-plant microbiome. How stressed plants modulate the chemical composition of their REs, that consequently affect the behaviour of microbial communities, remains poorly understood. In this study, tomato plants were exposed for 24h to different abiotic (wounding) and biotic stresses (foliar pathogen *Botrytis cinerea*, chewing insect pest *Spodoptera littoralis* and sucking insect pest *Macrosiphum euphorbiae*). The REs were collected, then used in chemotropic assays to evaluate their "attractiveness" to the biological control agents (BCAs) *Trichoderma afroharzianum* and *Beauveria bassiana,* and to the plant pathogen *Fusarium oxysporum*. All REs exhibited both anti-germinative and repellent effects on conidia and germ tubes of *F. oxysporum*, but instead attracted germ tubes of both BCAs. Interestingly, REs obtained from plants infested by insects proved to be the most attractive to both fungal biocontrol agents and the most repellent to the fungal pathogen. To further investigate the molecular signals involved in this inter-kingdom communication strategy deployed by plants, we performed a fractionation of the REs, which were then used for an activity-guided chemotropic assay to determine the attractiveness to *F. oxysporum*. Indications are that a low molecular weight proteins may be involved, suggesting that both shared and diverse molecular signatures are associated with plant stress responses influencing their interactions with soil microbial communities.

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The impact of wildfire on soil microbial communities

G. Vizzari, L. Schena, A. Malacrinò

Department of Agriculture, Università degli Studi Mediterranea di Reggio Calabria, Località Feo di Vito, 89124 Reggio Calabria, Italy. E-mail: giuseppina.vizzari@unirc.it

Fires are one of the primary disturbances in forest systems, directly and indirectly affecting the soil microbiome. These effects are particularly important given that plants interact closely with the wide range of organisms that inhabit the soil. In this study, by comparing the microbiome of burnt areas with paired control sites using amplicon metagenomics (targeting bacteria, fungi, oomycetes, invertebrates and arbuscular mycorrhizal fungi), we aimed to test the influence of wildfire on the soil microbial community. Additionally, we tested the hypothesis that fire may alter the microbial community in a way that favors the spread of plant pathogens. Overall, the results suggest that the passage of fire can change the composition of the soil microbial communities, with significant effects on pathogenic and beneficial microorganisms. Our results contribute to the mechanistic understanding of the effects of wildfires within the forest plant communities and might contribute to planning the next generation of sustainable forest management strategies.

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Quick and simple method for *Plenodomus tracheiphilus* detection by real-time Loop-mediated isothermal amplification (LAMP) assay in laboratory conditions

E. Yahyaoui[1], S. Panno[1], A.G. Caruso[1], G. Agrò[1], P. Caruso[2], G. Licciardello[2], S. Di Silvestro[2], S. Davino[1]

[1]Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Viale delle Scienze, Ed. 5, 90128 Palermo, Italy; [2]Council for Agricultural Research and Economics (CREA), Research Centre for Olive, Fruit and Citrus Crops, Corso Savoia 190, 95024 Acireale, Italy. E-mail: andreagiovanni.caruso@unipa.it

Citrus mal secco disease, caused by the conidia-producing fungal plant pathogen *Plenodomus tracheiphilus*, has affected severely different cultivated citrus species, particularly lemon trees in the Mediterranean area. The disease diagnosis is still time-consuming and requires highly skilled individuals. Hence, it is crucial to perform early detection by laboratory tests to prevent the disease's spread. Therefore, this work aims to develop a quick and straightforward method to detect the fungus based on the real-time loop-mediated isothermal amplification (real-time LAMP) assay, comparing the canonical DNA extraction using a commercial kit with a rapid sample preparation method (membrane spot crude extract). A set of six LAMP primers was designed, targeting a 204-nucleotide sequence within the Internal Transcribed Spacer (ITS1-5.8S-ITS2) region of the nuclear rRNA genes. Sensitivity, specificity, accuracy, and membrane spot crude extraction were evaluated and compared to end-point and real-time PCR. The results showed that the developed real-time LAMP assay could detect the pathogen within only 15 minutes with a minimum target detection of 1.77×103 genome copies without cross-reactivity with other fungal DNAs or *Citrus* species. Moreover, the rapid sample preparation method gave a positive and reliable amplification within 60 minutes, with an acceptable reaction delay when compared to canonical DNA purification. In conclusion, the developed real-time LAMP assay coupled with rapid sample extraction and a portable real-time thermocycle can be a valuable tool for fast and predictive in-field diagnosis of citrus mal secco disease.

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Assessing past and future climate change from planetary to regional scales

D. Zardi

Center of Agriculture, Food, Environment, University of Trento, San Michele all'Adige, Italy. E-mail: dino.zardi@unitn.it

Plant pathogens - either fungi, bacteria, viruses, or other organisms - have specific environmental situations that can either favour or contrast disease development. Indeed, factors such as air temperature, humidity, precipitation and wind strength may control their spread and growth. Hence, the attitude of single geographic areas for optimal growth of plants, including the contrast of their pathogens, largely depends on their microclimates, i.e., the typical regimes of the above variables. However, these regimes are subjected to changes over time. Besides natural rhythms, such as the diurnal cycle, the turning of seasons, or other nonperiodic climate cycles (e.g., the El Niño Southern Oscillation, the North Atlantic Oscillation, etc.), recently, continuously growing mean temperatures have been associated with a globally changing climate. Such warming also affects the whole hydrological cycle and hence water availability for life, including plants and their pathogens. Earth observing systems, including direct and remote sensing, have documented changes that have occurred so far. Furthermore, numerical climate prediction models allow the evaluation of future climate at planetary scales based on expected scenarios of greenhouse gas emissions. However, the outcome of such models is available only at low spatial and time resolutions, which are too coarse compared to the typical time- and space-scales of weather factors affecting plants and pathogens. Suitable post-processing procedures must be applied to get those outcomes downscaled to the appropriate resolution. This goal is among the leading-edge developments of applied meteorology, pursued both through classical physically-based resampling techniques and, more recently, developing artificial intelligence algorithms.

Cyclic lipopeptide producers in the *Pseudomonas asplenii* and *corrugata* subgroups: mutualists or pathogens?

L. Zhou[1], B. Marahatta[1,2], J. De Rop[1], M. Spacapan[2], G. Dimaria[3], L. Miceli[3], N. Geudens[4], P. Spanoghe[1], J. Martins[4], V. Venturi[2], V. Catara[3], M. Höfte[1]

[1]Department of Plants and Crops, Ghent University, Belgium; [2]Bacteriology Group, International Centre for Genetic Engineering and Biotechnology (ICGEB), Italy; [3]Department of Agriculture, Food and Environment, University of Catania, Catania, Italy; [4]Department of Organic and Macromolecular Chemistry, Ghent University, Belgium. E-mail: monica.hofte@ugent.be

Lipopeptides (LPs) produced by plant-associated *Pseudomonas* spp. are specialized metabolites with diverse ecological roles in colonization, phytotoxicity, and defence against competitors and predators. Structurally, *Pseudomonas* LPs can be classified into at least 14 different families that differ in oligopeptide and macrocycle length. Most *P. fluorescens* lineage strains are LP mono-producers and secrete one or more LP variants from a single LP family. LP poly-producers typically produce two types of cyclic LPs, respectively from the Mycin and Peptin families, often in addition to a third cyclic or linear LP. Our research focuses on LP poly-producers from the *P. corrugata* and *P. asplenii* subgroup isolated from the rhizosphere or above-ground plant parts. Some of these LP poly-producers are plant pathogens on rice or tomato, while others are powerful biocontrol agents protecting the plant from fungal pathogens with LPs playing a dual role in pathogenicity and antagonism. Regulation of LP production in these organisms can be quorum-sensing (QS) dependent or independent. Notably, LP production is mostly QS-independent in plant pathogens attacking above-ground plant parts. In contrast, organisms that reside in the soil or rhizosphere harbour a well-conserved four-gene QS system crucial for LP production. Additionally, all these strains produce various non-QS LuxR regulatory proteins responding to environmental factors, further regulating LP production. We use a comprehensive approach, combining *in silico*, chemical, and molecular methods with plant bioassays to unravel how LP production shapes parasitic and mutualistic interactions with plants.

Identification of sensitive 'wild type' strains of *Puccinia hordei* and *Puccinia triticina* across Europe through air sampling

M. Zubair, S. Nadalini, C. Agius, B. Jaser, G. Manneveld

EpiLogic GmbH, Hohenbachernstrasse 19-21, 85354 Freising, Germany. E-mail: stefano.nadalini@epilogic.de

Plant growers are constantly facing new challenges in controlling fungal plant pathogen control, such as reducing fungicides available for use and constantly evolving plant pathogens. Strategies are in place to extend the effective use of existing fungicides, such as fungicide application plans and fungal pathogen resistance monitoring. Dwarf rust of barley (*Puccinia hordei*) and brown rust of wheat (*Puccinia triticina*) result in production losses worldwide and are an ongoing threat to cereal production. The Fungicide Resistance Action Committee has classified rust fungi as having a low risk for resistance development. However, Cyp51 mutations and differential expression levels were recently found in European wheat leaf rust samples, which may lead to demethylase-inhibitor fungicide resistance. Furthermore, the mutation I187F was reported in sub-unit SdhC (SdhC-187F) in populations from the UK and France, resulting in a 5 to 10-fold increase in adaptation to inpyrfluxam, a succinate dehydrogenase inhibitor fungicide. Thus, it is crucial to monitor the development of resistance. To deepen the understanding of mutation and resistance development of *P. hordei* and *P. triticina* across Europe, EpiLogic GmbH harvested rust isolates using a car-mounted air spore trap. The resultant isolates were screened for mutations, and bioassays were performed to monitor and compare ED_{50} values. Correlation analysis between mutation absence/presence and ED_{50} values identified sensitive 'wild type' *P. hordei* and *P. triticina* strains, to estimate the resistance development in populations, thus gaining a better understanding of adaptation to fungicides and mutation presence for dwarf rust of barley and brown rust of wheat.