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Italian Phytopathological Society

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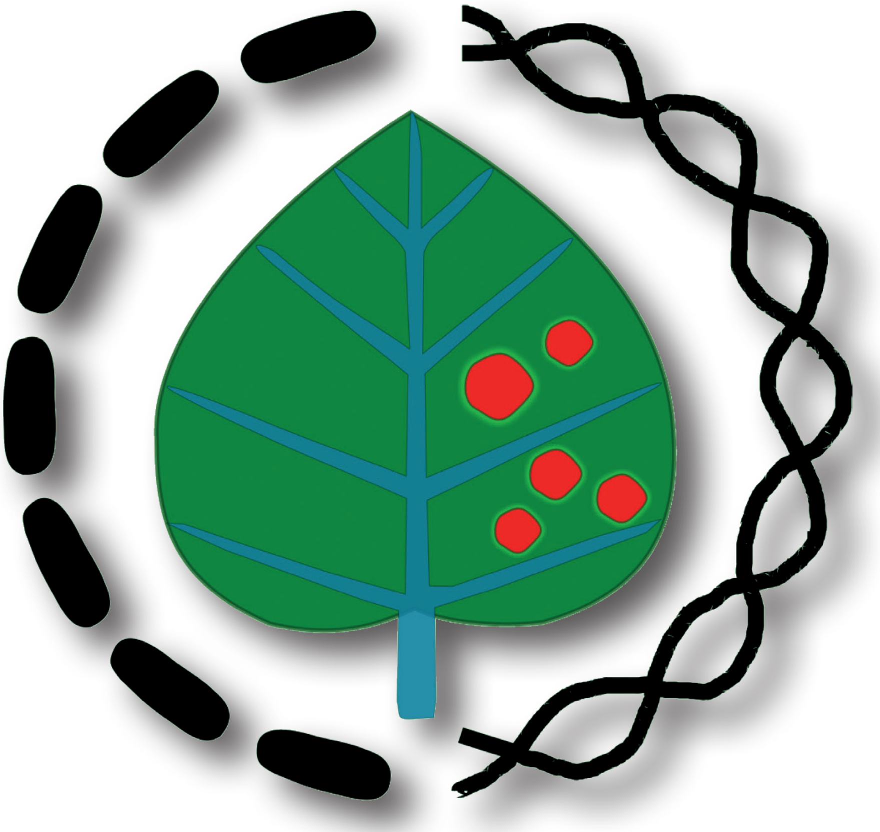
DELLE MARCHE

XXIV National Congress Italian Phytopathological Society (SIPaV)



BOOK OF ABSTRACTS

Ancona, 5-7 September, 2018

UNIVERSITÀ POLITECNICA DELLE MARCHE
Department of Agricultural Food and Environmental Sciences

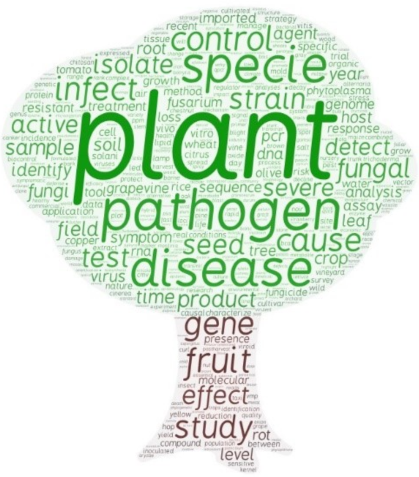
Edited by
Gianfranco Romanazzi, Lucia Landi, Sergio Murolo, Erica Feliziani, Valeria Mancini, Luisa Rubino

XXIV National Congress
Italian Phytopathological Society
(SIPaV)

Ancona, 5-7 September, 2018

Aula Azzurra

UNIVERSITÀ POLITECNICA DELLE MARCHE



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XXIV National Congress
Italian Phytopathological Society (SIPaV)
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CONFERENCE PROGRAMME

Wednesday 5th September

1.30-2.30 pm: Registration of participants and posters set up
2.30-3.00 pm: Opening of the SIPaV 2018 Congress and welcome address

3.00-4.30 pm: I Session – Phytoplasma diseases (*Chairs* Piero Attilio Bianco and Rita Musetti)

3.00-3.30 pm: Invited talk

Variability and function of Vmp adhesion related proteins give insight into the emergence of phytoplasma epidemics. S. Malembic-Maher, D. Desque, P. Salar, J.L. Danet, S. Duret, M.P. Dubrana, B. Batailler, J. Jović, S. Krnjajić, E. Angelini, L. Filippin, M. Monticone, D. Bosco, I. Ember, M. Kolber, M. Della Bartola, A. Materazzi, M. Maixner, L. Beven, J. Renaudin, N. Arricau-Bouvery, X. Foissac

3.30-4.30 pm

Detection of '*Candidatus* Phytoplasma solani' in roots from Bois noir symptomatic and recovered grapevines. L. Landi, S. Murolo, G. Romanazzi

Clues on the epidemiology of 16SrV-C phytoplasma on *Spartium junceum* in Sicily. S. Rizza, D. Di Pietro, V. D'Urso, C. Marzachì, M. Tessitori

Metagenome analyses reveal microbiota changes in *Citrus sinensis* affected by citrus decline disease in Iran. A. Passera, H. Alizadeh, M. Azadvar, F. Quaglino, A. Alizadeh, P. Casati, P.A. Bianco

Synthetic elicitors affect *Hyalesthes obsoletus* behavioral responses. P. Riolo, R. L. Minuz, V. Mancini, S. Ruschioni, G. Romanazzi, N. Isidoro

4.30-5.00 pm: Coffee break

5.00-5.30 pm: II Session – Forest pathology (*Chairs* Paolo Capretti and Angelo Mazzaglia)

Assessing the seasonal patterns of spore deposition of *Gnomoniopsis castaneae* in some chestnut orchards in northern Italy. L. Giordano, G. Lione, F. Sillo, P. Gonthier

Early warning methods for forest pathogens: a case study on pine canker. N. Luchi, P. Bartolini, F. Pecori, A.L. Pepori, A. Santini

5.30-6.00 pm: SIPaV "Giovanni Scaramuzzi 2018" Award (*Chairs* Maria Lodovica Gullino and Luisa Rubino)

6.00-7.00 pm: Factory of Ideas - Young Researcher Session (*Chairs* Alessandra Di Francesco and Valeria Mancini)

***Diaporthe* spp. associated to defected hazelnuts in Turkey.** R. Arciuolo, G. Chiusa, N. Spigolon, G. Castello, P. Battilani

***Pseudomonas corrugata* genome mining reveals a LuxR 'solo' with a role in virulence in tomato.** A. Anzalone, G. Licciardello, C. Strano, P. Bella, I. Bertani, V. Venturi, V. Catara

Sphingolipids in *Fusarium verticillioides – Zea mays* interaction. M. Beccaccioli, M. Salustri, A. Grottoli, M. Ludovici, V. Scala, M. Reverberi

Evaluation of the antagonistic activity of *Bacillus amyloliquefaciens* against fungal pathogens of wheat. R. Caracciolo, J. Cabrefiga, I. Mora, A. Fabi, E. Montesinos, L. Varvaro

Spread and stability of Tomato yellow leaf curl virus – IL23 in Sicily and partial displacement of its parental. A.G. Caruso, S. Panno, S. Davino

Study on bio-morphological and ultra-structural effects caused by onion yellow dwarf virus infection in "Rossa di Tropea" onion bulb. G. Micali, A. Tiberini, A. Lupini, A. Ciampa, I. Inzucchi, L. Tomassoli, M.T. Dell'Abate, G. Albanese

Morphological and molecular identification of seedborne fungi in squash (*Cucurbita maxima*). M. Moumni, M.B. Allagui, V. Mancini, S. Murolo, N. Tarchoun, G. Romanazzi

Metagenomic analysis of the aerial mycobiome of rice paddies. S. Franco Ortega, I. Ferrocino, S. Silvestri, I. Adams, D. Spadaro, M.L. Gullino, N. Boonham

Role of gluconic acid in the biocontrol activity of *Pseudomonas fluorescens* strain CHA0 against *Fusarium oxysporum*. D. Palmieri, G. Barone, F. De Curtis, G. Lima

Enzymes production by solid state fermentation of *Cryphonectria parasitica* on agro-industrial residue. S. Savino, E. Monti, E. Gobbi

Characterization of endophytic fungi isolated from grapevines by matrix-assisted laser desorption time-of-flight mass spectrometry (Maldi-Tof MS). A. Tóth, Cs. Kovács, E. Sándor

The expression of TAXI-I and TAXI-III xylanase inhibitors and of a *Fusarium graminearum* xylanase increases plant resistance to bacterial and fungal pathogens. S. Tundo, M.C. Paccanaro, L. Sella, I. Elmaghraby, I. Moscetti, R. D'Ovidio, F. Favaron

7.15-7.30 pm: **SIPaV High School Award, I Edition "Teach me as to take care for a plant (*Insegnami a curare una pianta*)"**

7.30 pm: Light welcome dinner and poster view

8.30-9.30 pm: "POD Talks" (*Chair* Gianfranco Romanazzi)
Ruggero Osler, Felice Scala

Thursday 6th September

9.00 am-11.00 am: Join Session SIPaV-APS about "Emerging and re-emerging plant pathogens" (*Chairs* Maria Lodovica Gullino and Lindsey J. du Toit)

Case studies of the complexity of seedborne and seed transmitted fungi affecting regional and global seed trade. L.J. du Toit, J.C. Correll

Seed transmission of viruses and viroids: implications for global seed movement. G. Munkvold, T. Pitman, and B. Falk

Emerging soilborne plant diseases caused by fungi and oomycetes in Italy. S.O. Cacciola

Grapevine Flavescence dorée: still a threat to European viticulture. S. Palmano, L. Galetto, D. Bosco, C. Marzachì

11.00-11.30 am: Coffee break

11.30-12.15 am: III Session – *Xylella fastidiosa* (*Chairs* Giovanni Paolo Martelli and Stefania Loreti)

Media monitoring to identify emerging plant health risks: the case of *Xylella fastidiosa.* F. Ferilli, G. Stancanelli, J. Linge, M.R. Mannino

***Xylella fastidiosa* subsp. *pauca* strain De Donno: a lipidomic point of view.** V. Scala, M. Reverberi, M. Salustri, N. Pucci, V. Modesti, S. Lucchesi, S. Loreti

Field experiments for the containment of the spread of *Xylella fastidiosa* in olive orchards. C. Dongiovanni, M. Di Carolo, G. Fumarola, D. Tauro, A. Ciniero, G. Altamura, F. Palmisano, V. Cavalieri

12.15-1.00 pm: IV Session – Mycotoxigenic fungi (*Chairs* Massimo Reverberi and Sabrina Sarrocco)

Occurrence of mycotoxigenic fungi on chestnut and hazelnut and management strategies. D. Spadaro, S. Prencipe, S. Valente, E. Piombo, A. Garibaldi, M.L. Gullino

***Fusarium graminearum* cerato-platanin proteins weaken cellulosic materials and enhance cellulase activity in an expansin-like manner.** A. Quarantin, C. Castiglioni, F. Favaron, L. Sella

Effect of soft wheat infection timing on the development of Fusarium head blight causal agents and on the accumulation of secondary metabolites in the kernels. G. Beccari, C. Arellano, L. Covarelli, F. Tini, M. Sulyok, C. Cowger

1.00-2.30 pm: Lunch and Poster Session

2.30-3.45 pm: V Session – Host-pathogen interactions (*Chairs* Matteo Lorito and Annalisa Polverari)

Abiotic factors effecting grapevine trunk disease incidence between vineyards in the Tokaj wine region, Hungary. C. Kovács[1,2], P. Balling[3], Z. Bihari[3], A. Nagy[4], E. Sándor

Fungal effector-omics in the genomics "*era*": *Verticillium* and *Fusarium* study case. J. Li, B.P.H.J. Thomma, L. Faino

Multifaceted chatting between *Trichoderma* and the plant. M. Ruocco, M.M. Monti, L. Gualtieri, M. De Palma, N. D'Agostino, M. Chiurazzi, L. Navazio, M. Tucci, M. Lorito

***N*-Acetyl cysteine-loaded chitosan nanoparticles to control oxidative stress in durum wheat.** V. Picchi, S. Gobbi, M. Fattizzo, F. Faoro

Hyperspectral reflectance, an innovative and useful tool for stress detection in plants. L. Cotrozzi, E. Pellegrini, J.J. Couture, C.D. Cruz, C. Nali, G. Lorenzini

3.45-4.30 pm: VI Session – Plant viruses (*Chairs* Salvatore Davino and Sergio Murolo)

Where virus taxonomy is heading to. L. Rubino, G.P. Martelli

Occurrence of viral pathogens and *Pseudoperonospora humuli* in hop plants in Italy. L. Ferretti, M. Luigi, F. Faggioli, A. Haegi

Two negative-stranded RNA viruses with unusual molecular features recently identified in citrus in Italy. B. Navarro, M. Minutolo, S. Zicca, M. Saponari, D. Alioto, F. Di Serio

Rapid and sensitive detection of onion yellow dwarf virus and iris yellow spot virus by reverse transcription loop-mediated isothermal amplification on 'Rossa di Tropea' onion. A. Tiberini, G. Micali, A. Manglli, G. Leo, G. Albanese, L. Tomassoli

4.45-5.15 pm: Coffee break

5.15-7:15 pm: General Assembly of SIPaV Members

7:30 pm: Bus leaving to Fortino Napoleonico, Portonovo (AN)

8.00 pm: Gala dinner at the Fortino Napoleonico (http://www.hotelfortino.it) (43°33'54.43"N, 13°35'33.43"E)

Friday 7th September

9.00-10.15 am: VII Session – Disease management (*Chairs* Franco Faretra and Slaven Zjalić)

9.00-9.30 am: Invited talk

Defining the host genetic control of the rhizosphere bacterial microbiota: a key to enhance plant adaptation to biotic and abiotic stresses. R. Alegria Terrazas, S. Robertson-Albertyn, L. Pietrangelo, R. Sharma, D. Bulgarelli

9.30-10.15 am

Resistance to the downy mildew agent in the Eurasian grapevine germplasm of Georgia (Caucasus). S.L. Toffolatti, G. De Lorenzis, G. Maddalena, A. Costa, P. Casati, O. Failla, D. Maghradze, F. Quaglino

Comparative effectiveness of phosphonate fungicides, calcium oxide and reduced doses of copper hydroxide for managing *Phytophthora* brown rot of citrus fruit. F. La Spada, S.O. Cacciola, A. Pane, F. Aloi, C. Stracquadanio, M.G. Li Destri Nicosia, G. Magnano di San Lio

Leafhoppers in the vineyard, preliminary data about their possible implication in grapevine leaf mottling and deformation epidemiology. V. Gualandri, F. Ghidoni, M. Benazzoli, C. Duso, V. Malagnini, F. Pedrazzoli, G. Angeli

10.15-10.45 am: Coffee break

10.45-11.45 am: VIII Session – Innovative tools in detection and molecular characterization (*Chairs* Giuseppe Lima and Giorgio Gambino)

New genetic resources for the causal agents of brown rot on stone fruits, *Monilinia fructicola*, *Monilinia laxa* and *Monilinia fructigena*. R.M. De Miccolis Angelini, D. Abate, S. Pollastro, C. Rotolo, D. Gerin, F. Faretra

Structure and dynamics of airborne fungal populations in organic and conventional vineyards. A. Abdelfattah, A. Ippolito, M. Wisniewski, S. Pangallo, S. Scibetta, S. Mosca, M.G. Li Destri Nicosia, G.E. Agosteo, G. Magnano di San Lio, S.O. Cacciola, L. Schena

A new real-time loop-mediated isothermal amplification (LAMP) assay to rapidly detect *Phytophthora ramorum* and *P. lateralis* invasive plant pathogens. C. Aglietti, A. Santini, P. Bartolini, P. Capretti, N. Luchi

Environmental genomics reveals the diversity of *Phytophthora* populations in phytocoenoses of "Complesso Speleologico Villasmundo-S. Alfio" Natural Reserve in Sicily. F. Aloi, A. Pane, F. La Spada, M. Evoli, R. Faedda, C. Stracquadanio, L. Schena, S.O. Cacciola

11.45-12.00 am: Close of the SIPaV 2018 Congress

LIST OF POSTERS

1. **The Apulia Region-funded Pilot Study: "Silver Bullet against *Xylella fastidiosa*" (SIX).** C. Altomare, S.L. Woo, E. Comite, G. Bleve, A. Gallo, M. Lorito
2. **Antimicrobial photodynamic treatment against plant pathogens: the power of anionic porphyrin against the grey mold agent (*Botrytis cinerea*).** V. Ambrosini, M. Issawi, V. Sol, C. Riou
3. **Change of activity of antioxidant enzymes under stress conditions of the environment.** A. Zh. Amirkulova, G. Romanazzi, G.V. Kurbanova, O.V Chebonenko
4. **Pathogenic endophytic fungi in transplanting poplar plants.** N. Anselmi, A. Giorcelli
5. ***Pseudomonas corrugata* genome mining reveals a LuxR 'solo' with a role in virulence in tomato.** A. Anzalone, G. Licciardello, C. Strano, P. Bella, I. Bertani, V. Venturi, V. Catara
6. ***Diaporthe* spp. associated with defected hazelnuts in Turkey.** R. Arciuolo, G. Chiusa, N. Spigolon, G. Castello, P. Battilani
7. **Development of a novel molecular tool to trace the invasive pathogen *Xylella fastidiosa* subsp. *pauca* in the olive European outbreak.** G.M. Balestra, M. Tatì, M.C. Taratufolo, Y. Jundi Rahi, V. Tagliavento, S. Ciarroni, A. Mazzaglia
8. **Selection and characterization of beneficial bacteria and seed bacterization of leguminoses as a biocontrol strategy against *Rhizoctonia solani* damping-off.** A. Barberio, D. Palmieri, G. Barone, C. Del Grosso, G. Lima, F. De Curtis
9. **Identification of candidate genes involved in resistance to Fusarium wilt race 0 in chickpea.** G. Barone, J.V. Die, F. De Curtis, J. Rubio, D. Palmieri, G. Lima, T. Millàn
10. **Innovative delivery of Cu(II) ions by a nano-structured hydroxyapatite to enhance the sustainable control of *Plasmopara viticola*.** E. Battiston, L. Antonielli, S. Di Marco, F. Fontaine, L. Mugnai

11. **Sphingolipids in *Fusarium verticillioides* – *Zea mays* interaction.** M. Beccaccioli, M. Salustri, A. Grottoli, M. Ludovici, V. Scala, M. Reverberi
12. **Effect of soft wheat infection timing on the development of Fusarium head blight causal agents and on the accumulation of secondary metabolites in the kernels.** G. Beccari, C. Arellano, L. Covarelli, F. Tini, M. Sulyok, C. Cowger
13. **Phytopathological problems associated with quinoa (*Chenopodium quinoa* Willd.) seed production in an area of central Italy.** G. Beccari, M. Quaglia, E. Pannacci, F. Damiani, L. Covarelli
14. **Ecophysiological response of pomegranate plants under long-term combined salt and ozone stress.** A. Calzone, A. Podda, G. Lorenzini, B. Maserti, C. Nali, E. Pellegrini, M. Tonelli, E. Carrari, Y. Hoshika, E. Paoletti
15. **Coexistence of *Caliciopsis pinea* and *Fusarium circinatum* on pine: interactions among fungal pathogens.** P. Capretti, D. Migliorini, J. Diez Casero, P. Martínez-Álvarez, N. Luchi
16. **Evaluation of the antagonistic activity of *Bacillus amyloliquefaciens* against fungal pathogens of wheat.** R. Caracciolo, J. Cabrefiga, I. Mora, A. Fabi, E. Montesinos, L. Varvaro
17. **Spread and stability of tomato yellow leaf curl virus – IL in Sicily and partial displacement of its parental.** A.G. Caruso, S. Panno, S. Davino
18. **Occurence of *Erwinia amylovora* foci in Sicily twenty years after eradication.** V. Catara, P. Bella, A. Caruso, G. Conti Nibali, N. Pucci, S. Loreti, G. Cavallaro, R. Fisicaro, G. Greco, V. Sinatra, D. Carta Cerrella, F. Conti
19. **Application of native *Trichoderma* spp. to confer suppressiveness to nursery potting mixes.** G. Chilosi, M.P. Aleandri, C. Morales-Rodriguez, A.M. Vettraino, E. Luccioli, A. Vannini
20. **Non-conventional compounds against powdery mildew: profiling grapevine phyllobiome and elicited defence responses.** W. Chitarra, C. Pagliarani, L. Nerva, M. Monchiero, S. Gonthier, M. Pugliese, M.L. Gullino, G. Gambino

21. **Epidemiological survey on *'Candidatus* Phytoplasma solani' and putative vectors in western Sicily.** G. Conigliaro, G. Lo Verde, P. Bella, S. Giambra, G. Romanazzi, V. D'Urso, H. Tsolakis, S. Burruano

22. **Evaluations on the effects of phenylpyrrole fungicides in *Botrytis cinerea* by RNA-Seq transcriptome analysis.** R.M. De Miccolis Angelini, C. Rotolo, S. Pollastro, F. Faretra

23. **New insights into biology, transcriptome analysis and control strategies of the cucurbit pathogen *Podosphaera xanthii.*** R.M. De Miccolis Angelini, S. Pollastro, P.R. Rotondo, C. Dongiovanni, C. Laguardia, D. Abate, C. Rotolo, F. Faretra

24. **Effectiveness of biocontrol rhizobacteria and a new natural organic fertilizer against soilborne pathogens on pot grown ornamental plants.** C. Del Grosso, C.G. Chavez Duran, D. Palmieri, D. Vitullo, C. Stracquadanio, F. De Curtis, S.O. Cacciola, G. Lima

25. **Effect of *Aureobasidium pullulans* strains against *Botrytis cinerea* on kiwifruit during storage and on fruit nutritional composition.** A. Di Francesco, M. Mari, E. Baraldi

26. **Effectiveness against strawberry postharvest decay of volatile organic compounds produced by biocontrol agents.** E. Feliziani, L. Oro, M. Ciani, G. Romanazzi, F. Comitini

27. **Effects of preharvest and postharvest fungicide applications for the control of gray mold on postharvest decay of strawberries and on fungicide residues on the fruit.** E. Feliziani, E. Monaci, L. Landi, S. Mattei, C. Vischetti, G. Romanazzi

28. **Metagenomic detection of viruses and viroids associated with isolates of citrus tristeza virus.** R. Ferraro, G. Scuderi, M. Russo, A. Catara, G. Licciardello

29. **Foliar fungal diseases in different cultivars of durum wheat in the Marche region.** G. Ferroni, S. Nardi, G. Mazzieri, C. Governatori, A. Perticarà, G. Romanazzi

30. **Impact on olive oil quality of olive leaf yellowing associated virus (OLYaV).** A. Fontana, A. Piscopo, A. De Bruno, A. Tiberini, I. Muzzalupo, G. Albanese

31. **Phenotypic and genotypic studies to evaluate Fusarium Head Blight Type I and Type II resistances in Italian wheat cultivars.** S. Francesconi, A. Mazzaglia, G.M. Balestra

32. **A new method for testing rice seeds, detection of *Magnaporthe oryzae* and *Fusarium fujikuroi* by loop-mediated isothermal amplification.** S. Franco Ortega, J. Tomlinson, J. Hodgetts, D. Spadaro, M.L. Gullino, N. Boonham

33. **Characterization of the secondary metabolite profile and phylogenetic analysis of *Alternaria* species isolated from basil.** S. Franco Ortega, I. Siciliano, G. Gilardi, P. Bosio, A. Garibaldi, M.L. Gullino

34. **Detection of *Fusarium oxysporum* f. sp. *lactucae* in soil, lettuce seeds and plants by LAMP assay.** S. Franco Ortega, J. Tomlinson, G. Gilardi, D. Spadaro, M.L. Gullino, A. Garibaldi, N. Boonham

35. **Metagenomic analysis of the aerial mycobiome of rice paddies.** S. Franco Ortega, I. Ferrocino, S. Silvestri, I. Adams, D. Spadaro, M.L. Gullino, N. Boonham

36. **Rapid detection of *Monilinia fructicola* and *Monilinia laxa* on nectarines through loop-mediated isothermal amplification.** S. Franco Ortega, J. Tomlinson, D. Spadaro, M.L. Gullino, N. Boonham

37. **Evaluation of the potential vector role for the alien planthopper *Ricania speculum* in Flavescence dorée phytoplasma epidemiology.** L. Galetto, M. Pegoraro, E. Rossi, A. Lucchi, S. Palmano, C. Marzachì, D. Bosco

38. **Genotyping by high-resolution melting of *Alternaria* species causing citrus brown spot.** F. Garganese, A. Ippolito, V. di Rienzo, C. Lotti, C. Montemurro, S.M. Sanzani

39. **Pathogenicity and possible biological control of fungi associated to Botryosphaeria dieback of grapevine in western Sicily.** S. Giambra, V. Mondello, G. Conigliaro, P. Bella, A. Alfonzo, S. Burruano

40. **Efficacy of different fungicides against *Allophoma tropica*, the causal agent of a new leaf spot on lettuce: results from *in vitro* and *in planta* tests.** G. Gilardi, M.L. Gullino, A. Garibaldi

41. **Preventative treatments in nursery with resistant inducers, compost and biocontrol agents for the management of the Fusarium wilt of lettuce**

under glasshouse and field conditions. G. Gilardi, M.L. Gullino, A. Garibaldi

42. **Land use and climate change trigger *Phytophthora* infestation in an amenity urban wood in Milan.** B. Ginetti, A. Benigno, S. Anderloni, P. Pirelli, T. Jung, S. Moricca

43. **Monitoring the spread of *Phytophthora* spp. in water bodies of green park areas of the Milan hinterland.** B. Ginetti, A. Benigno, S. Corsinovi, S. Anderloni, P. Pirelli, T. Jung, S. Moricca

44. **Recycling agro-industrial by-products using beneficial microorganisms.** L. Gioia, S. Lanzuise, A. Bottiglieri, E. Comite, A. Sicari, E. Monte, F. Vinale, M. Lorito, S.L. Woo

45. **Assessing the effects of different heavy metals on the development of poplar and willow clones: perspectives for dendroremediation.** L. Giordano, A. Giorcelli, P.M. Chiarabaglio, G. Lione, P. Gonthier, M.L. Gullino

46. **Monitoring the presence of mycotoxigenic fungi and mycotoxins on Italian rice during the growing season.** P. Giorni, T. Bertuzzi, M. Romani

47. **Assessing the variability of pathogenicity within a group of Italian isolates of *Fusarium verticillioides*, pathogen of *Zea mays*, differing in unique genes of virulence.** A. Grottoli, G. Giuliano, M. Beccaccioli, M. Blandino, D.W. Brown, V. Scala, M. Reverberi

48. **Preliminary detection of necrotrophic fungal species associated with local caraway and cumin seeds in the Cap Bon region of Tunisia.** S. Hassouna, M.B. Allagui, M. Moumni

49. **Preliminary investigations on '*Candidatus* Phytoplasma solani' genotypes associated with grapevine Bois noir in Iran.** E. Jamshidi, S. Murolo, S. Baghaee Ravari, G. Romanazzi

50. **The defense mechanism against *Meloidogyne javanica* is induced by beneficial microorganisms combined with chemicals in tomato *Solanum lycopersicum*.** M. Kavari, L. Landi, E. Mahdikhani Moghaddam, G. Romanazzi

51. **Identification of pathogenic fungi in rotten walnut fruits from different plantations in Hungary.** C. Kovács, F. Takács, A. Tóth, E. Sándor

52. **Whole genome sequence and annotation of the brown rot fungal pathogen *Monilinia fructigena*.** L. Landi, R.M. De Miccolis Angelini, S. Pollastro, D. Abate, F. Faretra, G. Romanazzi

53. **Survey of the presence of ochratoxigenic fungi in vineyards in northern Dalmatia.** J. Lončar, Z. Jakopovic, A. Lacic, K. Markov, M. Reverberi, S. Zjalić

54. **Effects of copper and biostimulants used for grapevine downy mildew control on grape amino-acid content.** V. Mancini, T. Garde-Cerdán, M. Carrasco-Quiroz, A. Servili, G. Gutiérrez-Gamboa, E.P. Pérez-Álvarez, G. Romanazzi

55. **First survey on *Moniliniae* affecting stone fruits in the Marche region.** V. Mancini, L. Landi, F. Chieti, S. Morini, G. Romanazzi

56. **Use of chitosan and other natural compounds to control grapevine downy mildew.** V. Mancini, A. Servili, E. Feliziani, F. Giammarino, F. Del Re, G. Del Gobbo, M. Mascetti, L. Pellegrini, M. Bastianelli, A. del Re, A. Marini, R. Cerusici, M. Gregori, S. Piancatelli, D. Marcolini, D. Coppa, S. Nardi, G. Romanazzi

57. **A bizarre endophytic *Fusarium* strain.** G. Manganiello, F. Vinale, R. Marra, A. Staropoli, S.L. Woo, M. Lorito, R. Nicoletti

58. ***WRKY* transcription factors, oxidative protection and signalling in *Salvia officinalis* plants exposed to ozone.** A. Marchica, R. Papini, M. Tonelli, F. Filippi, E. Pellegrini, G. Lorenzini, C. Nali, R. Bernardi

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72. **Oak decline in Marche region: monitoring of sanitary status in the Selva di Castelfidardo.** S. Murolo, J. Concas, F. Maiorano, L. Cingolani, A. Salerno, G. Romanazzi

73. **Chestnut blight in the Marche region (central-eastern Italy).** S. Murolo, J. Concas, A. Corvaro, L. Marchegiani, A. Moresi, G. Romanazzi

74. **Spatial analysis as a tool to monitor recovery in Bois noir infected vineyards of cv. ‘Chardonnay’.** S. Murolo, V. Mancini, J. Concas, G. Romanazzi

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76. **Jasmonates, salicylate and site-specific responses to phytoplasma infection in *Arabidopsis thaliana.*** L. Pagliari, C. Bernardini, V. De Rosa, M. Martini, M. Almeida-Trapp, A. Mithöfer, R. Musetti

77. **Influence of a biocontrol agent on *Penicillium expansum*-driven acidification and patulin biosynthesis.** D. Palmieri, C. Miccoli, G. Ianiri, G. Lima, F. De Curtis, R. Castoria

78. **Role of gluconic acid in the biocontrol activity of *Pseudomonas fluorescens* strain CHA0 against *Fusarium oxysporum.*** D. Palmieri, G. Barone, F. De Curtis, G. Lima

79. **Two years of tomato leaf curl New Delhi virus monitoring in Campania region.** G. Parrella, E. Troiano, R. Griffo

80. **Reduction of mycotoxigenic *Aspergillus flavus* infection and aflatoxin contamination in maize treated with the biological product AF-X: second year of field trials.** S. Pecchia, L. De Martino, A. Civello, G. Puntoni, A. Serra, E. Bulleri, G. Ragaglini, C. Tozzini, G. Vannacci

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83. **New insights into the infection process of *Fusarium fujikuroi* in rice using a GFP expressing isolate.** E. Piombo, L. Campos, D. Spadaro, M.L. Gullino, B. San Segundo de los Mozos, A. Infantino, M. Aragona

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85. **Use of MLVA as a promising tool for the identification and tracing of *Pseudomonas savastanoi* populations.** Y. Rahi, M.C. Taratufolo, M. Cerboneschi, S. Tegli, F. Valentini, A.M. D'Onghia, N.S. Iacobellis, G.M. Balestra, A. Mazzaglia

86. **Effectiveness of essential oils used under a hypobaric environment to control postharvest gray mold of strawberries.** R. Rajestary, L. Landi, E. Ortenzi, E. Feliziani, D. Sivakumar, G. Romanazzi

87. **Study of biovar-specific determinants in *Pseudomonas syringae* pv. *actinidiae* virulence.** A. Regaiolo, T. Colombo, C. Foresti, A. Polverari, E. Vandelle

88. **Influence of chitosan on the antioxidant status of tomato plants infected by cucumber mosaic virus.** N. Rendina, M. Nuzzaci, A. Scopa, A. Sofo, A. Cuypers, M. Manfra

89. **Vectors and candidate vectors of '*Candidatus* Phytoplasma solani' in vineyards.** R.L. Minuz, E. Ranieri, A. Serallegri, S. Murolo, G. Romanazzi, N. Isidoro, P. Riolo

90. **Eliciting, antimicrobial and film-forming properties of chitosan applied on fresh fruit and vegetables.** G. Romanazzi, E. Feliziani, D. Sivakumar

91. **There can be only one: two Flavescence dorée phytoplasma strains compete during plant infection.** M. Rossi, M. Pegoraro, M. Ripamonti, F. Veratti, L. Galetto, S. Palmano, C. Marzachì

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94. **A lipidomic approach to study the interaction of *Xylella fastidiosa* with *Olea europaea*.** M. Salustri, V. Scala, N. Pucci, A. L'Aurora, V. Modesti, M. Scortichini, M. Reverberi, S. Loreti

95. **Enzyme production by solid state fermentation of *Cryphonectria parasitica* on agro-industrial residues.** S. Savino, E. Monti, E. Gobbi

96. **Tramesan, an eco-friendly approach against Septoria disease complex in wheat.** V. Scala, C. Pietricola, L. Covarelli, M. Blandino, V. Farina, G.

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98. **Looking for novel control measures against the rice fungal pathogen *Pyricularia oryzae.*** L. Sella, A. Quarantin, C. Castiglioni, A. Bolzonello, M. De Zotti, F. Favaron, A.M. Picco, R. Govind, S. Tundo, S. Odorizzi, L.T. Đô, V.V. Van, N.M. Hung

99. **Phyogenetic diversity and toxigenic potential of *Fusarium tricinctum* Species Complex strains associated to Fusarium head blight on durum wheat.** M.T. Senatore, D. Spadaro, M.L. Gullino, G. Beccari, A. Prodi

100. **A fast and specific assay for the surveillance of the invasive forest pathogen *Heterobasidion irregulare* based on Loop-mediated isothermal AMPlification (LAMP).** F. Sillo, L. Giordano, P. Gonthier

101. **Biogenic extracellular synthesis of gold and silver nanoparticles by *Trichoderma harzianum* and *Trichoderma longibrachiatum* and their effectiveness against seed-borne fungal pathogens.** M. Spada, M.A. Mohamed, G. Puntoni, S. Pecchia

102. **Sensitivity of four Fusarium head blight causal agents to commonly used fungicides.** F. Tini, G. Beccari, E. Ciavatta, A. Onofri, D.M. Gardiner, L. Covarelli

103. **Characterization of endophytic fungi isolated from grapevines by matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS).** A. Tóth, C. Kovács, E. Sándor

104. **High incidence of cucumber mosaic virus in *Alstroemeria* hybrids in Campania region (southern Italy) and observation of symptoms recovery from initial infection.** E. Troiano, G. Parrella

105. **The expression of TAXI-I and TAXI-III xylanase inhibitors and of a *Fusarium graminearum* xylanase increases plant resistance to bacterial and fungal pathogens.** S. Tundo, M.C. Paccanaro, L. Sella, I. Elmaghraby, I. Moscetti, R. D’Ovidio, F. Favaron

106. **Innovative sustainable approaches for the management of potato pests and pathogens.** A. Tursunova, Y. Dyo, S. Purton

107. **New acquisition about the epidemiology of grapevine leaf mottling and deformation.** D. Valenzano, L. Simonetti, V. Malagnini, E. De Lillo, P. Saldarelli, R.A. Kubaa, P. L. Bianchedi, G. Angeli, V. Gualandri

108. **A putative trichothecene biosynthetic gene cluster in the biocontrol agent *Trichoderma gamsii*: preliminary investigations.** A. Vicente, R. Baroncelli, S. Sarrocco, R. Hermosa, E. Monte, G. Vannacci

109. **Insights on the cross-talk between *Trichoderma gamsii* and *Fusarium graminearum* at a distance: two opposite messages.** A. Zapparata, R. Baroncelli, L. Malfatti, M. Brandström Durling, C.P. Kubicek, M. Karlsson, G. Vannacci, S. Sarrocco

110. **Does timing of application influence the efficacy of *Trichoderma* in reducing wood fungal infections and in improving plant quality in grapevine nurseries?** S. Di Marco, A. Benigno, G. Carella, E. Metruccio, M. Nocentini, F. Osti, A. Pacetti, L. Mugnai

111. **Effects of natural compounds on postharvest gray mold infections on fresh strawberries fruit.** R. Rajestary, L. Landi, E. Feliziani, M. Magini, A. Servili, A.Zh. Amirkulova, A. Ramos Guerrero, D. Xu, G. Romanazzi

INVITED TALKS

Variability and function of Vmp adhesion related proteins give insight into the emergence of phytoplasma epidemics

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Variable membrane protein (Vmp) genes are powerful markers for tracing phytoplasma disease epidemics but their role in phytoplasma life cycle remains to be investigated. Genetic, ecological and cellular biology approaches have been undertaken to evaluate the impact of Vmps on phytoplasma biology. Since the first outbreaks, flavescence dorée epidemics had been associated to the introduction of the leafhopper vector *Scaphoideus titanus*, while Europe imported American phylloxera-resistant *Vitis* rootstocks. However, the geographical and ecological origin of the etiological agent, a phytoplasma, remained unclear despite evidences for a plant host-range not restricted to grapevine. The outcome of a European collaboration shows that this phytoplasma is endemic to European Alders and common in Clematis. Its emergence as an epidemic pathogen for grapevine is restricted to some genetic variants pre-existing in the wild plant host reservoir. The compatibility of this phytoplasma to the introduced *S. titanus* insect vector resulted from the preadaptation/compatibility of phytoplasma Vmps to other *Deltocephalinae* leafhoppers living on alders. Vmp organization is similar to adhesion related proteins (Arp) and the recombination ability of most phytoplasmas certainly favors the fast duplication of pre-adapted repeated domains. VmpA-coated fluorescent beads and recombinant *Spiroplasma citri* adhesion-defective mutant expressing VmpA, were used in *ex vivo* adhesion and *in vivo* ingestion assays. Results demonstrate that VmpA promotes adhesion to the epithelial cells of the leafhopper vector. This suggests a key role of Vmps in the life-style of phytoplasmas that rely on the adaptation to new insect vectors to expand their plant-host range.

Case studies of the complexity of seedborne and seed transmitted fungi affecting regional and global seed trade

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Numerous fungi and oomycetes have been detected on seed, including obligate plant pathogens, opportunistic plant pathogens, endophytes, saprophytes, and beneficial fungi. In this era of global seed trade, seedborne fungi and oomycetes are implicated increasingly in the emergence of fungal and oomycete diseases or strains/races of plant pathogens in new regions. *Phomopsis* (*Diaporthe*) and *Verticillium* associated with spinach (*Spinacia oleracea*) seed provide insightful case studies of seedborne fungi that illustrate: 1) complex and sometimes controversial circumstances that can develop when phytosanitary regulations are implemented to reduce the risk of moving seedborne fungi that are potential plant pathogens; 2) the need for taxonomic clarity as well as effective and efficient methods of differentiating species or races/strains of seedborne fungi; 3) the Herculean task of understanding all potential host plants of seedborne fungi, particularly necrotrophic fungi; 4) the difficulty of understanding the biological relevance of seedborne inoculum vs. alternative sources of inoculum in outbreaks of plant diseases; 5) the necessity but dearth of robust, internationally standardized, and biologically relevant seed health assays for seedborne pathogens that can be used with treated and non-treated seed lots; and 6) the demand for effective, non-phytotoxic, and economically-viable seed treatments for infested seed lots that reduce the risk of moving plant pathogens on seed. The case studies illustrate the many gaps in our understanding and ability to manage seedborne fungi effectively, and opportunities for the field of seed pathology to contribute significantly to protecting plant production and enhancing seed trade.

Seed transmission of viruses and viroids: implications for global seed movement

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Diseases caused by viruses and viroids continue to emerge in new geographies, and seeds are frequently implicated in the global redistribution of these pathogens. Although the actual risk associated with seeds as a pathway for pathogen introduction is frequently unclear, regulatory agencies employ cautious phytosanitary regulations and the seed industry must protect the quality of its products. The increasing complexity and volume of international seed movement, both during cultivar development and commercial trade, necessitates more intense vigilance toward preventing pathogen introductions. In recent years, significant attention has been focused on cucumber green mottle mosaic virus (CGMMV), maize chlorotic mottle virus (MCMV), and the Pospiviroid family, particularly potato spindle tuber viroid (PSTVd). CGMMV emerged in California and Canada in 2013, and Australia in 2014, associated with imported seeds. Efforts to eradicate the virus were abandoned in Australia, but continue in California. Meanwhile a collaboration between the seed industry and the USDA National Seed Health System was initiated to monitor imported cucurbit seeds for CGMMV contamination. Improved seed health testing methods also are being developed. Seed transmission of several pospiviroids has been demonstrated in tomato and other crops, but research results vary widely, and the risk of seed transmission of pospiviroids in commercial seed movement remains controversial. Nevertheless, there are widespread phytosanitary testing requirements, resulting in significant testing and sampling costs. In this presentation, phytosanitary measures that are available to manage the risks associated with seedborne viruses and viroids will be discussed, including seed testing methods, seed treatments, regulatory and eradication programs.

Emerging soilborne plant diseases caused by fungi and oomycetes in Italy

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Many emerging diseases in Italy are caused by soilborne fungi and oomycetes. These pathogens have peculiar epidemiological features. Typically, they affect roots and cause monocyclic diseases. However, soilborne pathogens, e.g. *Calonectria* species and their *Cylindrocladium*-like asexual morphs, may infect the canopy and cause polyciclic diseases. Defining an emerging infectious disease (EID) is not straightforward. There can be an emergence of a new disease, an emergence in an area not previously affected, the occurrence of a disease on a new host plant, the unforeseen increase of disease incidence as a consequence of favorable environmental factors, or there may simply be an increase in knowledge or awareness of a particular disease. The underlying cause of most EIDs is the anthropogenic introduction of alien pathogens. However, other concurrent factors may play a major role, e.g. climate change, such in the case of *P. cinnamomi* in Mediterranean oak forests in Sardinia and Apulia, severe weather events, such in the case of *Plenodomus tracheiphilus* in lemon orchards in Sicily, hybridization between related species, such as in the case of the invasive North American *Heterobasidion irregulare* and the endemic *H. annosum* in pine stands of Central Italy. Other cases of interspecific hybrids include *P.* x*cambivora* and *P.* x*pelgrandis*, in forests of *Fagaceae* and in ornamental nurseries, respectively. Conducive environmental conditions and host jump have been hypothesized to be responsible for the emergence of a disease of Iceland poppy caused by a new f. sp. of *Fusarium oxysporum*, probably originating from spontaneous species of *Papaveraceae*.

Grapevine Flavescence dorée: still a threat to European viticulture

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Flavescence dorée (FD) is one of the most important quarantine diseases of grapevine. It is associated with phytoplasmas and is transmitted by the grapevine-feeder *Scaphoideus titanus*. FD induces severe symptoms leading to fruit abortion up to plant death. The disease is a major problem of viticulture in several European countries, and Piedmont is the most affected Italian region. FD management is based on mandatory insecticide treatments and replacement of infected grapevines with certified heat-treated plants. A continuous research effort is directed to study host/pathogen interactions, mechanisms of phytoplasma infection, host response and plant defense mechanisms, to find alternative and sustainable control strategies. No genetic resistance to FD is known so far, and recent studies have shown different degrees of disease susceptibility among varieties of cultivated grapevines and rootstocks. The role of the spontaneous symptom remission (recovery) in FD epidemiology has been addressed, as well as the feasibility of its exploitation as a management strategy in heavily infected areas. On the other hand, genetic variability of FD phytoplasma isolates has been identified through an in depth analyses of several infection sites, and the epidemic outcomes of the possible competition between strains are under study. Recent monitoring has evidenced high vector populations within the wild compartments, pointing at the role of primary infections in the late year epidemics, despite compulsory insecticide treatments of the vineyards. The existence and possible application of virus infection of *S. titanus* to complement insecticide treatments is object of a recent H2020 funded project.

Defining the host genetic control of the rhizosphere bacterial microbiota: a key to enhance plant adaptation to biotic and abiotic stresses

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The rhizosphere, the thin layer of soil tightly adhering to roots, defines a distinct habitat for microbial proliferation. The microbial communities inhabiting this habitat are collectively referred to as the rhizosphere microbiota. Members the microbiota engage in associations with plant roots involving parasitism, commensalism and mutualism. Consequently, dissecting the molecular basis of these ‘Janus-faced’ plant-microbe interactions in the rhizosphere is considered a key to rational exploit the microbiota for sustainable crop production. My group uses barley (*Hordeum vulgare*) as a model to gain novel insights into the genetic basis of plant-microbiota interactions in the rhizosphere. Using a comparative metagenomics approach, we previously demonstrated that Elite varieties and wild barley ancestors host microbiotas of contrasting composition, possibly representing a footprint of plant domestication on the microbial communities inhabiting the rhizosphere. We recently extended this line of investigation by characterising the microbiota of a bi-parental population between an elite variety and a wild barley ancestor. By combining 16S rRNA gene sequencing profiles, as ‘quantitative traits’, with thousands of SNPs in the barley genome we compiled a map of the plant loci shaping the rhizosphere microbiota. Strikingly, we did not observe a linear relationship between number of loci and bacteria putatively controlled by them. Rather, our data suggest that microbiota composition in the rhizosphere is controlled, at least in part, by a few major alleles with a major effect. Here I will discuss the experiments that led us to these discoveries and their implications for basic science and translational applications.

SIPAV
"GIOVANNI SCARAMUZZI 2018"
AWARD

Developing an innovative tool to enhance the biological activity of active substances for the control of fungal diseases in *Vitis vinifera* L.

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Over the years, research in nanotechnology has led to the development of revolutionary smart materials in many fields. In agriculture, the nanotechnological approach is often applied to improve plant productivity, crop quality and disease management, through nano-sized materials. The present research investigates the application of the biomimetic inorganic nanostructured particles of hydroxyapatite as innovative delivery system for the protection of grapevines (*Vitis vinifera* L.). Through a multidisciplinary approach, the study aimed to evaluate the efficiency of hydroxyapatite in enhancing the biological activity of copper(II) compounds, for the control of relevant common diseases, like downy mildew, and complex fungal diseases, such as grapevine trunk diseases. The HA functionalized with copper(II) compounds was fully characterized and the biological stability was established by performing applications to leaf tissues of *V. vinifera* for plant protection purposes. The morphology, structure and composition of HA functionalized with different copper(II) compounds and sprayed on grapevine leaves was detected and finally, a different and specific delivery model was developed for each copper(II) salt. The models are based on the high pH difference between the copper(II) compounds. Based on the results achieved, the micrometric aggregation and shape of HA was described in relation to its stability when formulated with Cu(II) ions. A particular affinity was highlighted for the formulations based on copper sulfate pentahydrate (CuSPHy) and copper tribasic sulfate (CuTBS). Electron microscopy of the substances and *in planta* revealed a different delivery model for CuSPHy and CuTBS and the non-homologation of their aggregates to the nanostructures. Based on this first approach, the biological activity of HA functionalized with copper(II) compounds was further studied *in vitro* and *in vivo*, for the control of grapevine fungal pathogens. Results collected evaluating the disease severity and incidence on treated potted vines, inoculated with *P. viticola*, showed the HA effect with a trend significantly different for each copper(II) compound. Considering these promising results, further trials were performed to assess the stability of the treatments to rainfall washing-off effect, with positive results on the efficiency and persistence of the experimental treatments compared to commercial fungicides. The distribution and efficiency of HA functionalized with copper(II) compounds in the grapevine lignified tissues colonized by vascular pathogens was investigated through imaging methods in an experiment on propagating material. In this regard, the study revealed how these parameters can be highly variable, with consequences on pathogen colonization. Quantification of the copper revealed a different concentration, distribution and persistence

of copper on the treated plant material analyzed after the treatment and after harvest. Differences were related also to the copper compound applied, confirming the delivery models previously hypothesized. A parallel investigation based on transcriptomic analysis, allowed an appreciation of the potential role of innovative formulations based on HA and copper(II) compounds in stimulating plant defense responses.

In conclusion, combining the results of the physical, chemical, microscopical, biological and molecular approaches, the research investigated an interesting protective tool against two important grapevine fungal diseases. Overall, the experimental activities allowed: (i) to understand the interaction between delivery system, functional substance and grapevine tissues; (ii) to demonstrate the mechanism on which the higher efficacy of the functional substance is based; (iii) to collect new information on the mechanisms involved in symptoms expression by studying the plant defense reactions induced by the treatments.

FACTORY OF IDEAS
YOUNG RESEARCHER SESSION

Diaporthe spp. associated with defected hazelnuts in Turkey

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Hazelnuts are important products with high economic value. Kernel defects can occur and cause non-compliance with quality standards required by the market. *Diaphorte* spp. was recently suggested as the main causal agent of defected hazelnuts in the Caucasian region. The aim of this study was to investigate the fungi associated with defected hazelnuts in Turkey and confirm the role of *Diaporthe* spp. One hundred and eighty orchards, placed in seven hazelnut-growing regions of Turkey (Duzce, Sakarya, Samsun, Zonguldak, Trabzon, Ordu, Girensun) were sampled at early and full ripening. Collected kernels were observed for defects and separated in healthy and defected. One representative sample of healthy and defected hazelnuts (30 kernels each) was prepared per each region, and plated for isolation and identification of fungi. ITS1 (5′-CGGATCTCTTGGTTCTGGCA-3′) and ITS4 (5′-GACGCTCGAACAGGCATGCC-3′) primers were used for PCR analysis to confirm the identification of *Diaporthe* spp. in all the samples examined. The most frequently isolated fungi were *Penicillium* spp. and *Diaporthe* spp., with increasing incidence from early to full ripening only for the latter. *Diaporthe* spp. was significantly most frequently isolated from defected than healthy kernels. PCR gave a very high percentage of positivity in defected kernels (94% and 98% at early and full ripening, respectively) but healthy kernels were also frequently positive (more than 75%). *Diaporthe* spp. seems confirmed as the fungus associated with hazelnut defects in Turkey. The molecular approach followed needs improvement to be effective.

Pseudomonas corrugata genome mining reveals a LuxR 'solo' with a role in virulence in tomato

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Pseudomonas corrugata and *P. mediterranea* are the only species described as phytopathogens in the *P. fluorescens* group within the *P. fluorescens* lineage. They are included in the *P. corrugata* subgroup with *P. kilonensis*, *P. thivervalensis, P. brassicacearum* and a number of other *Pseudomonas* sp. strains mostly reported as biological control agents. Both species have a canonical LuxI/R N-acyl homoserine lactone-dependent quorum-sensing system (AHL-QS), PcoI/R in *P. corrugata* and PmeI/R in *P. mediterranea*. We analyzed the genomes of the other taxonomically related species and found that the AHL-QS system is common and highly conserved in all *P. corrugata* and *P. mediterranea* strains and in the BCA *Pseudomonas* sp. strain SHC52, but not in the other species of the subgroup. *P. corrugata* CFBP 5454 genome mining revealed the presence of 292 transcriptional regulators. Among them, an additional LuxR with the typical modular structure: an AHL-binding domain at its N-terminus and a HTH DNA-binding domain at the C-terminus. Since the inactivation of the only gene coding for a PcoI AHL-synthase completely abolished the AHL production in *P. corrugata*, it is possible that this additional regulator belongs to the LuxR "solos" family, LuxR which lacks a cognate LuxI. Homologs of this regulator, named SolR, were found also in other species of the *P. corrugata* subgroup. To investigate its role, a mutant strain was constructed. The SolR mutant maintains the ability to produce AHLs, to induce hypersentitive response in tobacco and pith necrosis in tomato. Nevertheless, it differs from the wild type strain being highly virulent.

Sphingolipids in *Fusarium verticillioides – Zea mays* interaction

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Sphingolipids are bioactive molecules involved in plant cell death (PCD) and associated with defence. They are considered signalling and structural molecules implicated in the regulation of membrane trafficking and formation of membrane domains during defence response. The *liaison* between sphingolipids and ear rot disease of maize caused by *Fusarium verticillioides* actually is the biosynthesis of fumonisins (FBs). *F. verticillioides* produces FBs able to inhibit the activity of ceramide synthase in plant host cell, leading to a severe sphingolipidome alteration. In this study, we characterized and quantified by LC-MS/MS the sphingolipids in maize ears artificially infected with *F. verticillioides*. Sphingoid base accumulation is one of the effects of exposing maize cells to FBs; this might activate the MPK6-salicylic acid pathway leading to PCD and activation of pathogen-related proteins. Moreover, complex sphingolipids are differentially present during the infection process; in fact, membrane rearrangements are linked with the formation of defence-related lipid rafts enriched in these sphingolipids. These findings suggest that *F. verticillioides* shapes the sphingolipid metabolism of maize, promoting its own growth and fitness. We hypothesize that the pathogen adopts this strategy to switch from an endophytic growth to a necrotrophic lifestyle.

Evaluation of the antagonistic activity of *Bacillus amyloliquefaciens* against fungal pathogens of wheat

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Wheat is one of the most important crop worldwide and its widespread distribution makes it constantly exposed to many fungal diseases. Nowadays the use of biocontrol agents (BCAs) is one of the most successful strategies for their control. This study deals with: (i) *in vitro* evaluation of the antifungal activity of three strains of *B. amyloliquefaciens* (Ba) against *Fusarium graminearum, Fusarium culmorum, Bipolaris sorokiniana* and *Septoria tritici*, (ii) molecular and chemical characterization of the molecules involved in this process, (iii) *in vivo* evaluation of the antifungal activity against *B. sorokiniana* and (iv) *in vivo* wheat growth promotion fostered by Ba strains. The inhibitory activity of bacterial growth broth filtrates against all the tested pathogens highlighted the nature of the molecules involved, that are released and are thermostable. Molecular identification of *ituA, fenD, surfA, bmyB* and *mycA* confirms the presence of the genes coding for the production of the most important cyclic lipopeptides (CLPs): iturins, fengycins and surfactins, that are reported as antifungal compounds. Their production was confirmed with chemical characterization of Ba filtrates, and the antagonistic assays performed with their fractions showed that fengycins are responsible for this antagonistic process. Ba cells and their filtrates controlled *B. sorokiniana* also *in vivo*, as it was the most *in vitro* inhibited pathogen. Moreover, Ba strains confirm to be potential biofertilizers for wheat, as they promoted seeds germination, seedling growth and root development. The results obtained confirm *B. amyloliquefaciens* as a valid BCA against the most important wheat pathogens, and its use can be recommended in the formulation of commercial bio-pesticides.

Spread and stability of tomato yellow leaf curl virus – IL23 in Sicily and partial displacement of its parental

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Tomato yellow leaf curl disease (TYLCD) is caused by viruses belonging to the genus *Begomovirus*. In Sicily, the disease is caused by tomato yellow leaf curl Sardinia virus (TYLCSV), tomato yellow leaf curl virus (TYLCV) and their recombinants. These viruses are subject to genetic recombination, thus increasing the number of new hybrid genomes. Since 2016, in the Ragusa province (Sicily), a recrudescence occurred of an aggressive syndrome caused by TYLCD to tomato plants. Fifty samples from plants showing begomovirus-like symptoms, were collected in the province of Ragusa from 10 different farms. Samples were geo-referenced and catalogued taking into account the cultivar, typology and commercial company. Twenty-eight of the 50 samples were cultivars with introgression of the *Ty-1* genes, which give tolerance against TYLCD. Total DNA was extracted from each sample for multiplex PCR analysis able to detect TYLCSV, TYLCV and their recombinants. Twenty-two samples were positive for TYLCSV/TYLCV and their recombinants, while 28 samples were positive only for TYLCV-IL23. Interestingly, all of the 28 positive samples for TYLCV-IL23 were cultivars with introgression of the *Ty-1* gene. To confirm the presence of TYLCV-IL23, the 28 samples were sequenced in both directions. The presence of TYLCV-IL23 appear to be strongly favored by the presence of tolerant hybrids that seem to represent, in this case, a bottleneck. Tolerant hybrids represent in Sicily about 90% of the cultivated tomato crops. This aspect is of great importance because it could pose a serious risk for future tomato cultivation in Sicily and in the Mediterranean basin.

Study on bio-morphological and ultrastructural effects caused by onion yellow dwarf virus infection in 'Rossa di Tropea' onion bulb

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'Rossa di Tropea' onion is a particular onion cultivated in Calabria (southern Italy), with a Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) trademarks. It is characterised by a high nutraceutical compounds content (i.e. phenolics, flavonoids, fructoligosaccharides, alk(en)yl cysteine sulphoxides) showing anti-inflammatory, anti-cholesterol, anticancer and antioxidant properties. 'Rossa di Tropea' onion is affected by onion yellow dwarf virus (OYDV, genus *Potyvirus,* family *Potyviridae*), which represents the most limiting biotic stress for this cultivar as it induces severe symptoms, as yellowing, dwarfing and stem twirling. To better investigate OYDV-'Rossa di Tropea' interaction pathway, the effects of viral infection on macroscopic features were analysed. In addition, using innovative tools like magnetic resonance microimaging (MRI), the OYDV effect on the overall tissue architecture of whole bulbs was investigated, including chemical-physical parameters such as water mobility deduced by variations of water protons at two times. This study was carried out as part of the planned activities of the project "SI.ORTO" funded by the Italian Ministry of Education, to evaluate OYDV infection effect on modulation and variation of onion secondary metabolites. Combination of traditional and innovative tools allowed to determine how OYDV alters the natural physiology by inducing water accumulation in bulb and leaf tissues as well as ultrastructural alterations of the cell wall, highlighted in bulbs by MRI imaging. All these effects resulted in an increased amount of free water in plant tissues, and consequently relevant water losses which affected bulb quality and shelf life in storage.

Morphological and molecular identification of seed-borne fungi in squash (*Cucurbita maxima*)

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Squash (*Cucurbita maxima*) is one of the most important vegetables grown in tropical and temperate regions. This crop can be affected by several diseases transmitted by the seeds, as an efficient vehicle to disperse pathogens over long distances. Even low percentages of seed infection can result in severe economic losses. Early detection of seed-borne fungi is the first step in the control of such diseases. Squash fruit were collected from two regions of Tunisia and were evaluated according to three levels of lesion extension: asymptomatic fruit; infected fruit showing lesions on the squash skin without colonization of the fruit cavity; and infected fruit showing lesions that colonized the fruit cavity. Following the blotter test, 16 fungal species were detected in the seeds. Seed-borne fungi were identified for all of the tested fruit samples, including the asymptomatic fruit. The most frequent seed-borne fungi were *Didymella bryoniae* (24.6%), *Alternaria alternata* (24.8%), *Fusarium moniliforme* (7.8%), *Fusarium solani* (6.9%), *Rhizopus* spp. (20.3%), *Myrothecium verrucaria* (3.3%), *Pleospora herbarum* (2.8%), and *Myrothecium roridum* (1.2%). Morphological identification was confirmed by molecular diagnosis using the available genus-specific and species-specific primers. Furthermore, specific primers were designed from DNA sequences in the internal transcribed spacer (ITS) regions of the nuclear rDNA to identify *Myrothecium* spp., *M. verrucaria*, *M. roridum*, and *Pleospora* spp. Correct identification of seed-borne fungi is a key factor for crop protection. Application of seed health testing methods, including conventional and molecular diagnostic tools, will help to improve seed quality and crop yields.

Metagenomic analysis of the aerial mycobiome of rice paddies

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Air-borne microbiome is a new topic that has been understudied in comparison with the microbiota present in other environments as soil, rhizosphere or water. Traditional methods as culture-based methods allow the study of only a small fraction of the organisms in the atmosphere. In this study, the aerial composition of fungi in a rice paddy has been examined during the crop production cycle (from June to September) using a DNA-based method (qPCR) to target two important rice pathogens, *Magnaporthe oryzae* and *Cochliobolus miyabeanus,* and using a high throughput sequencing (HTS) targeting the ITS region. The results demonstrated an increase in the alpha diversity analysis (Shannon-Wiener diversity index *H'*, the chao1 and total number of observed species) at the beginning of the trial (June), showing a higher level of complexity than at the end of the trial. The main taxa were identified by HTS where the relative abundance drove the cluster separation as a function of the time and temperature. The OTU core included *Cladosporium*, *Alternaria*, *Myrothecium*, *Epicoccum*, *Davidiella*, *Russulaceae*, *Leptosphaerulina*, *Magnaporthe*, *Aurciculibuller*, *Sporobolomyces*, *Lewia*, *Cochliobolus*, *Hyphodontia* and *Fusarium.* A parallel, oligotyping analysis on the main rice pathogens was performed to obtain a sub-OTU identification. The results revealed the presence of several characteristic oligotypes associated with monitoring time. In addition, changes on mycobiota composition were clearly detected in function of the air temperature. Indeed, temperature variations can drastically affect the mycobiota community structure with a possible impact on the development of rice diseases.

Role of gluconic acid in the biocontrol activity of *Pseudomonas fluorescens* strain CHA0 against *Fusarium oxysporum*

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The rhizobacterium *Pseudomonas fluorescens* protects different plant species against diseases caused by soil-borne fungal pathogens. Its main mode of action involves the production of antifungal compounds such as 2,4-diacetylphloroglucinol and pyoluteorin. Strains of this rhizobacterium have also evolved efficient mechanisms of pH modification, mainly based on acidifying the rhizosphere microenvironment trough oxidation of glucose in gluconic acid. In recent years, it has been shown that modifications of the environmental pH can dramatically affect fungal virulence and disease severity. The aim of this study was to assess the contribution of gluconic acid production by a selected strain (CHA0) of *P. fluorescens* in the control of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. Knockout bacterial mutants for glucose dehydrogenase (Δ*gcd*) or gluconate dehydrogenase (Δ*gad*) coding genes required for the conversion of glucose to gluconic acid and gluconic acid to 2-ketogluconate, respectively, were used. The results showed that the ability of strain CHA0 to control *F. oxysporum* f. sp. *lycopersici* is strongly influenced by gluconic acid production. Surprisingly the defective mutant Δ*gcd,* incapable of producing gluconic acid, proved much more effective in the biocontrol of the fungal pathogen both *in vitro* and *in vivo*, compared to the wild type and the Δ*gad* strains. The loss of GCD gene and the consequent incapacity of environmental acidification stimulates the production of chelating compounds (i.e. pyoverdine). Therefore, the Δ*gcd* bacterial strain acquired a greater advantage in the competition for iron and exerted a higher biocontrol activity against *F. oxysporum*.

Enzyme production by solid state fermentation of *Cryphonectria parasitica* on agro-industrial residues

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A variety of microorganisms are used to produce biofuels, enzymes, vitamins, antioxidants, animal feed, antibiotics, and other products through solid state fermentation (SSF) of agro-industrial wastes. In a SSF process, microorganisms grow in an environment with no or a very low content of free water. Although its historical importance for humans has been known for thousands of years, recently this process has gained greater importance in circular economy. The aim of the present study was to investigate the capability of *Cryphonectria parasitica* (Murr.) Barr to grow and produce enzyme(s) on waste. Esterase, amylase, cellulase and laccase production by four newly isolated *C. parasitica* strains was obtained by means of solid state fermentation using commercially available wheat bran. Various physico-chemical parameters were optimized, such as fermentation period, moisture content and growth temperature, to guarantee a good colonization of the substrate. After liquid-solid extraction from fermented wheat bran, optimum fermentation conditions for esterase and amylase production were the following: growth at 25 ºC, at a moisture content of 50% for 15 days. The results obtained suggest the feasibility of large-scale production of industrially relevant enzymes by *C. parasitica* in a SSF process of low-value materials. Furthermore, this SSF process offers notable economical and environmental advantages over submerged fermentation.

Characterization of endophytic fungi isolated from grapevines by matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS)

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PCR-based methods are now the most current and most reliable to identify fungi, although these procedures are time-consuming and expensive. During the last decades, matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS) has been regarded as an accurate and reproducible tool for microorganism identification. By measuring the size of the fungal proteins and analyzing the results using appropriate techniques, these analytical instruments identify species-specific spectra. Our study aimed at examining DNA-based identified filamentous fungi, isolated from grapevine by MALDI-TOF MS, to extend the database for a possibile use in the identification of microorganisms in the future. Fifteen isolates of *Alternaria*, *Epicoccum*, *Diplodia*, *Lasiodiplodia*, and *Fusarium* were subjected to molecular identification by ITS gene sequencing and MALDI-TOF analysis. Two extraction methods were tried in which formic acid or trifluoroacetic acid were used together with acetonitrile, and combined with sonication and MagNA Lyser cell disruption. Two different matrices (α-cyano-4-hydroxycinnamic acid and sinapinic acid) were also applied. The most effective disruption was obtained with formic acid and acetonitrile (1:1), whereas the most significant protein profiles were obtained using the α-cyano-4-hydroxycinnamic acid. Other treatments did not increase the efficiency of extraction. In the course of this study, we have found that the isolates showed individually distinctive species-specific spectra, consequently this method is appropriate for augmenting the database of filamentous fungi. We have also observed that there was a reduced set of peaks in the case of older mycelia, so it is advisable analysing younger fungal cultures. The result of this extensive work opens the possibility to generate a wide database, which offers a promising tool for an extremely rapid and reliable identification of plant pathogens and mutual endophytic fungi.

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The expression of TAXI-I and TAXI-III xylanase inhibitors and of a *Fusarium graminearum* xylanase increases plant resistance to bacterial and fungal pathogens

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During plant infection pathogens produce a wide range of cell wall-degrading enzymes (CWDEs) to break the host cell wall. Among CWDEs, xylanases are key enzymes in the degradation of xylan, the main component of the cell wall hemicellulose. In *Botrytis cinerea* the Xyn 11A xylanase was demonstrated to be a virulence factor. Wheat, whose cell wall is particularly rich of xylan, contains several xylanase inhibitors. In order to counteract the xylanase activity of *B. cinerea,* two *Triticum aestivum* xylanase inhibitors (TAXI), TAXI-I and TAXI-III, were transiently and separately expressed in tobacco leaves and Arabidopsis transgenic plants were also produced, constitutively expressing these *TAXI* genes. When challenged by *B. cinerea*, TAXIs agroinfiltrated tobacco plants showed a 20-25% reduction in symptoms and this effect was confirmed in the TAXI-III transgenic line. Instead, TAXI-I transgenic plants did not show any increased resistance to this pathogen. Since fungal xylanases can also induce necrosis and activate defense responses in plants independently from their enzymatic activity, a *Fusarium graminearum* xylanase (FGSG_03624) was tested for increasing plant resistance to bacterial or fungal pathogens. Transgenic Arabidopsis lines expressing an enzymatically inactive FGSG_03624 showed about 20% reduction of symptoms caused by *Pseudomonas syringae* pv. *maculicola*. The efficacy in reducing symptoms caused by bacterial infection was confirmed by the transient expression of FGSG_03624 in agroinfiltrated tobacco inoculated with *P. syringae* pv. *tabaci* (about 45% reduction). No symptoms reduction in tobacco and Arabidopsis was observed against *B. cinerea*.

ORAL PRESENTATIONS

Detection of '*Candidatus* Phytoplasma solani' in roots from Bois noir symptomatic and recovered grapevines

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Bois noir (BN) is a disease that is caused by '*Candidatus* Phytoplasma solani' in grapevine (*Vitis vinifera*). It is widespread in all European and Mediterranean viticultural areas, and it can induce severe damage to the quality and quantity of production. '*Ca.* P. solani' is usually detected in the leaves, where typical disease symptoms are seen. However, no information is available on the presence of this phytoplasma in grapevine roots. In this study, we investigated '*Ca.* P. solani' on roots collected from symptomatic, recovered, and asymptomatic grapevine plants of cv. 'Chardonnay' in a vineyard located in the Marche region (central-eastern Italy). A protocol using real-time quantitative PCR was developed according to the translation elongation factor *Tu* target gene. '*Ca.* P. solani' was detected in more than 50% of the roots from symptomatic plants, and more than 40% of the roots from recovered plants; no phytoplasma was detected in the roots from asymptomatic plants. These data demonstrate that phytoplasma can persist in the roots irrespective of the presence of disease symptoms on the plant.

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

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

Metagenome analyses reveal microbiota changes in *Citrus sinensis* affected by citrus decline disease in Iran

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Citrus decline disease was recently reported in Iran, affecting several citrus species grafted on Bakraee a local rootstock. Preliminary studies identified '*Candidatus* Phytoplasma aurantifoliae' and '*Candidatus* Liberibacter asiaticus' as putative etiological agents, but were not ultimately able to determine which one, or if an association of both pathogens, were causing the disease. The current study had the aim of characterizing the microbiota of citrus plants that were either asymptomatic, or showed either early or late symptoms, through the amplification of the V1-V3 region of the 16S rRNA gene, using an Illumina sequencer in order to: (i) clarify the etiology of the disease, (ii) describe the microbiota associated with different symptom stages. Our results suggest that liberibacter may be the main pathogen causing citrus decline disease, but cannot entirely rule out the possibility that a phytoplasma be involved as well. The characterization of microbiota described very different situations in the aerial part of the plants and in the roots, or between rootstock and scion: the leaves showed only two kinds of communities, either symptomatic or asymptomatic, while the roots showed a clear distinction between early and late symptoms. These results could lead to the identification of bacteria that are related to successful plant defense response and, therefore, to immunity to citrus decline disease.

Synthetic elicitors affect *Hyalesthes obsoletus* behavioral responses

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

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

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Gnomoniopsis castaneae Tamietti is an emerging fungal pathogen of sweet chestnut causing symptoms ranging from nut rot to leaves necrosis, up to bark cankers. To date, little is known about the epidemiology of this pathogen. The aims of this work were i) to detect and quantify the airborne inoculum of *G. castaneae*, ii) to assess the effect of seasonality on spore deposition, and iii) to study the correlation between spore deposition and climatic conditions. Samplings were performed between 2013 and 2015 in three orchards in the northwest of Italy using a novel and optimized spore trapping method combined with a real-time PCR approach. In each orchard, one permanent transect was established by installing 14 spore traps at approximately 15 m intervals. The airborne inoculum was sampled continuously every two weeks. The disease incidence was also assessed by performing fungal isolations from 40 ripe nuts collected from chestnut crowns in each orchard. Climatic data were obtained by installing thermo-pluviometric stations. Airborne inoculum of *G. castaneae* was detected in all sampling sites. The spore deposition showed different patterns depending on site and climatic conditions; however, similar patterns were recorded in the two years of samplings. On average, disease incidence ranged from 10% to 42.5% in 2013, from 5% to 57.5% in 2014, and from 0% to 40% in 2015. This study provides key information that may be used for the development of epidemiological models.

Early warning methods for forest pathogens: a case study on pine canker

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Introduction and invasion events in Europe have grown exponentially over the last years. The use of new methodologies is important to reduce and to prevent the entry and the establishment of diseases in Europe and to mitigate the impact of invasive and indigenous organisms in forestry, in terms of impacts on both ecosystems biodiversity and productivity. *Fusarium circinatum*, the causal agent of Pitch canker, is considered one of the most important invasive pathogens of conifers. At the early stage of colonization it causes bleeding cankers similar to *Caliciopsis pinea*, the causal agent of Caliciopsis canker, an increasing threat to pine stands in North America. Since *F. circinatum* is a quarantine organism, subjected to provisional emergency measures, its report immediately causes serious economic implications, while *C. pinea*, even if now emerging, is not regulated in Europe nor in the US. For this reason a reliable and accurate diagnostic tool able to distinguish between the two organisms was considered a priority. In this study we developed a duplex real time PCR assay for the simultaneous detection of *C. pinea* and *F. circinatum* in pine tissue. The test was able to detect DNA from a very large set of strains of both pathogens, even in the same host. Furthermore, this qPCR assay combined with spore trapping resulted in a suitable method for early detection of airborne fungal inoculum in pinewood. A continuous implementation of molecular detection tools is needed to improve the early warning systems of invasive plant pathogens.

Media monitoring to identify emerging plant health risks: the case of *Xylella fastidiosa*

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Following a request from the European Commission, EFSA has established a horizon scanning exercise for plant pests by automated monitoring of media and scientific sources. Currently about 560 plant pests are monitored (pest listed in the EU legislation and in the EPPO lists). In addition, the monitoring is active to retrieve information on new and unlisted plant pests. The sources are screened using the MEDISYS (Medical Information System) platform of the Joint Research Centre of the European Commission. The aim is to identify relevant information on pests that might be of quarantine concern for the EU. *Xylella fastidiosa* is a highly polyphagous and its hosts include citrus, almond, olive, and grapevine. It made its first confirmed European outbreak in Apulia, Italy, in 2013. Since its first occurrences, media and scientific articles have reported its findings in Corsica and PACA region, in France, in the Balearic Islands, Valencian Community and Madrid Community, Spain. Reports indicate that *X. fastidiosa* continues to spread and to damage severely olive orchards and other crops in Europe. The media monitoring shows that *X. fastidiosa* is a highly discussed topic, with many articles referring daily to detection, prevention and control of the pest in different European countries. The EFSA media monitoring results are regularly presented and discussed at the Plant Health section of the Committee on Plants, Animals, Food and Feed. This case illustrates how media monitoring can support the management of control measures, the understanding of their impacts and societal response to plant health threats.

Xylella fastidiosa subsp. *pauca* strain De Donno: a lipidomic point of view

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The bacterial cells organize and modulate their lipid membrane composition in order to survive under different environmental conditions. Lipids provide different biological functions related to membrane formation, energy storage and stress responses. Regarding this latter, the bacteria employ the lipid entities to modulate motility, biofilm formation and virulence. This well conserved “molecules” represent also a class of signals exchanged during the perception, signal transduction and regulation of defence mechanism in the host-pathogen interaction. LC-TOF and LC-MS/MS were adopted to assess the lipid composition of *X. fastidiosa* subsp. *pauca* strain De Donno, the causal agent of CoDiRO. Different lipids class were characterized and quantified (namely, polar and non-polar lipids, free fatty acids and oxylipins) in bacterial cells and cultural filtrate, highlighting a different lipid profile during the bacterial growth. The lipid profile of *X. fastidiosa* subsp. *pauca* strain De Donno during the interaction with the model plant *Nicotiana tabacum* Petit Havana SR1 was also assessed. In particular, specific class of lipids (e.g. ornitholipids and oxylipins) accumulate differently in infected plant tissues (petiole and laminar leaf) compared to uninfected ones.

Field experiments for the containment of the spread of *Xylella fastidiosa* in olive orchards

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The control of plant diseases caused by insect-vectored pathogens relies primarily on effective strategies for the reduction of the vector populations. The emergence of the insect-vectored bacterium *Xylella fastidiosa* in southern Italy, and more recently in different EU Countries, called for research on the characterization of the xylem-feeder populations and identification of candidate European vectors. Surveys identified the spittlebug *Philaenus spumarius* as one of the most abundant species, in different countries and crops, as well as the predominant vector spreading the infections on olive trees in southern Italy. This finding called for the development of control strategies to reduce the population of this highly polyphagous and widespread insect species. Besides, supporting actions for the mechanical control of the ground vegetation to effectively reduce the juvenile populations, there was a need to develop tools for the control of the adults. From 2015 to 2017, several formulations based on different active substances (chemical and organic) with different mechanism of action were tested for the control of nymphs and adults of *P. spumarius*. Indeed, in the last two years, experiments were carried out, for evaluating the reduction of the bacterial transmission rates upon applications of kaolin on olive canopies. Applications of insecticides showed that among the synthetic products, neonicotinoids and pyrethroids yielded the highest mortality rates and persistence, both for the control of the juveniles and adults, whereas lower efficacy was recorded for the natural and inert compounds. Reduction of the transmission rates have been so far detected upon applications with kaolin.

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Occurrence of mycotoxigenic fungi on chestnut and hazelnut and management strategies

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Chestnuts and hazelnuts are affected after harvesting by species *Penicillium* and *Aspergillus*. Several strains of *Aspergillus* spp. and *Penicillium* spp. were isolated from chestnut. *A. flavus* resulted the dominant *Aspergillus* species, followed by *A. oryzae* var *effusus*, *A. tamarii*, *A. parasiticus* and *A. toxicarius*. Forty percent of the *Aspergillus* spp. strains produced aflatoxins *in vivo,* while most strains were pathogenic on chestnut. Twenty species of *Penicillium* spp. were identified. *P. bialowiezense* was dominant in the fresh chestnuts, while *P. crustosum* was more frequent in dried chestnuts, chestnut granulates, chestnut flour and indoor chestnut mills. Around 70% of *Penicillium* spp. isolated were pathogenic on chestnut, while 59% of the strains could produce at least one mycotoxin on chestnuts. *P. expansum* was able to produce patulin, chaetoglobosin A and roquefortine, while *P. bialowiezense* produced C. Mycophenolic acid. Cyclopenins and viridicatins were produced by *P. crustosum, P. polonicum, P. solitum* and *P. discolor*. The genomes of eight species of *Penicillium* spp. were sequenced to search for secondary metabolite clusters. A new HPLC-MS/MS method was developed to detect twenty metabolites produced by *Penicillium* spp. on nuts. A LAMP assay and a Taqman PCR were developed for *Aspergillus flavus*. Drying conditions of chestnuts and hazelnuts are critical to reduce *Aspergillus* spp. and aflatoxin contamination. Monitoring, prevention and control are the strategies to manage *Aspergillus* spp. and *Penicillium* spp. on nuts. Besides these strategies, traditional static hot air roasting and infra-red rays roasting, together with cold atmospheric pressure plasma could be used for nut detoxification from aflatoxins.

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

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

Effect of soft wheat infection timing on the development of Fusarium head blight causal agents and on the accumulation of secondary metabolites in the kernels

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Fusarium head blight (FHB) results in production loss and damaging contamination of kernels and can be caused by several *Fusarium* species. The objective of this research was to assess, in a greenhouse trial on soft wheat, how FHB was influenced by infection timing (0, 3, 6 or 9 days after anthesis, daa) by the aggressive species *F. graminearum* compared to the relatively weak species *F. avenaceum*, *F. poae* and *F. acuminatum*. Assessment of FHB development were: symptoms in heads (visually measured), fungal biomass (quantified by q-PCR) and accumulation of fungal secondary metabolites (quantified by LC-MS/MS) in kernels. With regard to symptoms, *F. graminearum* was unaffected by infection timing, while the weaker pathogens caused greater severity at later timings. In contrast, the accumulation of *F. graminearum* biomass was strongly affected by inoculation timing (3 daa ≥ 6 daa ≥ 0 daa = 9 daa), while colonization by the weaker pathogens was less influenced. Similarly, *F. graminearum* secondary metabolite contamination was affected by infection timing (3 daa ≥ 6 daa ≥ 0 daa = 9 daa), while that of the weaker species was less influenced. However, secondary metabolites produced by these weaker species tended to be higher from intermediate-late inoculations (6 daa). Overall, infection timing appeared to play a role particularly in *F. graminearum* development and mycotoxin accumulation. Secondary metabolites of weaker *Fusarium* species may be relatively more abundant when environmental conditions promote spore liberation later in anthesis, while metabolites produced by *F. graminearum* are relatively favored by earlier conducive conditions.

Abiotic factors effecting grapevine trunk disease incidence between vineyards in the Tokaj wine region, Hungary

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The Grapevine Trunk Diseases (GTD) are one of the most serious diseases worldwide for the grapevine (*Vitis vinifera* L.). It is a complex disease, and infection can be latent without visible disease symptoms for years, but the factors affecting the disease appearance are still only suspected. Grapes are the largest fruit crop in Hungary, moreover there is also great cultural importance in the historic Tokaj Wine Region, as a producer of the world's botritized wine called "aszú". Five vineyards were observed with all together 22,794 grapevines between 2013 and 2015 in the Tokaj Wine Region. The records of external GTD symptoms (foliar symptoms with wood necrosis or dieback) were carried out yearly, three to five times, between May and August. Vineyards were planted on different soils (two on cambisols, while three on slope sediment from luvisols) at different time (1992, 1994, 1999, and two in 2003). Disease incidence (DI) was determined every year for each vineyard. DI varied from 0.2 to 42.1%. The old vineyards showed higher DI than the young ones, while lower DI was observed in vineyards planted on a slope than those planted on a terrace. Finally, vines planted on luvisols were less affected than the ones planted on cambisols.

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Fungal effector-omics in the genomics "*era*": *Verticillium* and *Fusarium* study case

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Plant pathogens are specialized organisms that can manipulate and/or suppress plant immunity. Effectors are small-secreted proteins which are key factors used by the pathogen to manipulate host immunity and establish successful infection. *Verticillium dahliae* is a soil-borne fungus that causes Verticillium wilt disease in a more than 200 plants. *V. dahliae* strains are assigned to "pathotypes" based on their virulence on a particular host. For instance, *V. dahliae* strains that are highly virulent and cause rapid and severe defoliation on cotton (*Gossypium hirsutum*) and olive (*Olea europaea*) are referred to as strains of the defoliating (D) pathotype; the strains that are less virulent and only induce wilting symptoms without defoliation on the same host are referred as non-defoliating (ND) pathotype. In this study, we used comparative genomics and transcriptomics to identify the effector protein behind the manifestation of defoliation symptoms. The analysis showed that two D pathotype-specific candidate genes are highly expressed during cotton colonization. Surprisingly, the two-candidate effector genes appeared to be 100% identical suggesting a recent duplication. We showed that both genes contribute to the defoliation in cotton and, therefore, both are pathogenicity factors on cotton. Furthermore, cotton plants grown in a solution of D protein and water showed the characteristic defoliation phenotype suggesting that the effector protein is directly responsible for the symptoms. Further genomics analysis using the genome of about 3,000 fungal genomes showed that the D protein is conserved among other *Verticillium* spp. and *Fusarium* spp suggesting a common origin of the D gene.

Multifaceted chatting between *Trichoderma* and the plant

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In a natural environment plant roots and soil microbes are in constant association. The result is a fine interplay based on signals and chemical messages continuously shared by the players. Microbes reveal their presence to plants by releasing molecules referred to as Microbe Associated Molecular Patterns. Among those, *Trichoderma* spp. are common components of the soil and rhizosphere microbiome strongly influencing plant growth, development and resistance to biotic and abiotic stresses. Our studies are aimed at understanding which are the players involved in the multifaceted chatting between *Trichoderma* and the plant. Here we present the results of a whole-transcriptome study of the interplay between tomato roots and the beneficial strain *T. harzianum* T22. In this study, we detected large transcriptomic perturbations in both *T. harzianum*-treated tomato roots and in *Trichoderma* mycelia interacting with tomato. Moreover, we have verified that root colonization by a selected *Trichoderma* beneficial strain modifies the outcome of the complex, multiplayer interaction among plants, aphids and their natural enemies, by modulating Volatile Organic Compounds composition. We have also further characterized one of the *Trichoderma* MAMPs, the HYTLO1 hydrophobin, demonstrating that when exogenously added to a *Lotus japonicus* cell culture, it can trigger a signal transduction pathway leading to the activation of defense genes in a Ca^{2+}-dependent manner. Moreover, we showed that perception of HYTLO1 by the plant occurs at the cell surface, and that the fungal secreted hydrophobin is selectively located in the apoplastic space, where it forms a web-like network with patches close to the plasma membrane.

N-Acetyl cysteine-loaded chitosan nanoparticles to control oxidative stress in durum wheat

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Modern durum wheat cultivars are more prone to ozone and UV oxidative stresses because of their high photosynthetic efficiency and leaf gas exchanges, which cause a greater pollutant uptake. This in turn generates an increased ROS production that can be hardly kept under control by the antioxidant system of the plant and affects the final yield with reduction up to 25%. Most of these cultivars show typical leaf symptoms, characterized by small regular chlorotic spots that usually appear at mid-end of April and may evolve in necrotic lesions, often mistaken for pathogen attack. With the aim of mitigating oxidative stress in wheat, we used chitosan nanoparticles (CHT-NP) for the delivery of the antioxidant compound *N*-Acetyl cysteine (NAC) to plants, growing either in greenhouse or in open field. NAC loaded NPs were prepared adding 0.5 mg/ml NAC to the CHT solution before ionotropic gelation with Tripolyphosphate (TTP). Greenhouse experiments evidenced that CHT-NPs-NAC were actually able to increase the level of leaf antioxidant pool (AsA+DHA and GSH+GSSG). However, the results of the field trial, while confirming the rising of AsA level, at least in the first phenological stages, were less conclusive. The presence of NAC did not seem to have a significant effect on the leaf antioxidant pool, though the grain yield was tendentially higher only in NAC treated parcels. Furthermore, both NAC loaded and unloaded CHT-NPs partially reduced the symptom severity and increased the weight of 1000 seeds, thus showing a moderate mitigation of ozone injury.

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Hyperspectral reflectance, an innovative and useful tool for stress detection in plants

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The need for rapid, automatic and non-destructive technologies for early detection of biotic and abiotic stress is an important issue for plant pathology and related disciplines. One such emerging technology is hyperspectral reflectance (HR, i.e., high-resolution measurement of reflected light, both at the remote and proximal levels). Due to improvements in the sensitivity of instruments, as well as in computation power and chemometric modeling methods, it enables the estimation of a variety of plant traits and physiological processes based on the optical properties of living foliage. The ability to capture even minimal changes in vegetation spectra caused by biotic and abiotic stress can assist in better understanding plant response under unfavorable conditions. Also, HR can be used for reliable, precise and accurate estimation of disease severity, the degree of sensitivity to abiotic stress, assessment of germplasm for disease resistance, yield loss prediction, monitoring and forecasting of epidemics, as well as to understand fundamental biological processes. Firstly, this work presents basic concepts of vegetation spectroscopy, concerning plant-light interactions, instrumental set-up, and spectral data analysis. Furthermore, recent research is reviewed to show the potential of HR to early detect plant reaction to pathogens (e.g., fungi, viruses) and abiotic factors (e.g., drought, air pollution). Finally, original case studies using spectral data collected on PVY-infected potato plants and wheat inoculated with an aggressive strain of *Magnaporthe oryzae* (*Lolium* pathotype) are presented, showing the ability of HR to early detect the temporal and spatial signature of these agents responsible of important shifts in plant physiology.

Where virus taxonomy is heading to

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Virus taxonomy is a dynamic discipline, which is subjected to continuous changes for allocating and managing new information about viruses. Traditionally, viruses used to be classified based on a variety of characters that include biological and molecular features. However, with the increasing importance of next generation sequencing (metagenomics), genetic characteristics like DNA, RNA and protein sequences, are becoming predominant over biological traits for the identification of novel viruses. Nevertheless, whether the metagenomic approach will ultimately be retained as sufficient by itself for assigning the rank of species to newly reported sequences is still unclear. Currently, virus species are grouped into genera, families and orders. The increasing divergence between taxa has prompted the International Committee on the Taxonomy of Viruses (ICTV) to expand the standing four-rank taxonomy structure (species-genus-family-order) to a virus classification including 15 ranks, up to the "realm" rank. Consequently, virus diversity will be addressed with a stepwise approach, first defining the species as the basal (i.e. lowest) rank then, when feasible, establishing higher ranks, by taking into consideration characters like protein structure, type and polarity of nucleic acid, etc. In addition, a standardized Linnean-style, binomial species naming format is under study, which would follow the format used in the taxonomy of other biological entities.

Occurrence of viral pathogens and *Pseudoperonospora humuli* in hop plants in Italy

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Hop (*Humulus lupulus* L.) is known to be one of the essential ingredients of brewing industry. In Italy, the number of craft breweries has strongly increased over the last few years and several farmers have begun cultivating hops. None or only a little knowledge is available on the occurrence of pathogens in Italian hop crops. In 2017 a field survey was carried out in hop yards located in different Italian regions (Tuscany, Emilia-Romagna, Latium and Basilicata) to investigate on the occurrence of the main viral (viruses and viroids) and fungal pathogens. A total of 50 leaf samples from hop plants belonging to several commercial cultivars were collected and analyzed. In addition, 11 spontaneous hop plants from areas surrounding commercial hop yards (2) and areas far from cultivated hops (9) were sampled. Molecular tests based on RT-PCR technique were performed for the detection of hop stunt viroid (HSVd), hop latent viroid (HLVd), citrus bark cracking viroid (CBCVd), apple mosaic virus (ApMV), arabis mosaic virus (ArMV) and of viruses belonging to the genus *Carlavirus*. Morphological observations of mycelium and/or reproductive structures were carried out for the identification of *Pseudoperonospora humuli* in symptomatic leaves. HLVd were detected in all samples collected from commercial varieties and in the two wild hops collected from areas surrounding commercial hop yards. ApMV and carlaviruses were also found respectively in 36.1% and 54.1% of the tested samples, in single or mixed infection. *P. humuli* were found in all investigated geographical areas, with an average infection rate of 16%.

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Two negative-stranded RNA viruses with unusual molecular features recently identified in citrus in Italy

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In an attempt of identifying the causal agent of a severe citrus disease first described in the early 1930s and named citrus concave gum, a new negative stranded RNA (nsRNA) virus was identified by high-throughput sequencing (HTS). Being associated with the disease, this virus was tentatively named citrus concave-gum-associated virus (CCGaV). With a bipartite genome composed of an RNA1 encoding the RNA-dependent RNA polymerase (RdRp) in the negative strand and an ambisense bicistronic RNA2 coding for the nucleoprotein (NP) and the putative movement protein (MP), CCGaV showed structural and phylogenetic features supporting the proposal of classifying it as a representative member of a new species to be allocated in the new genus *Coguvirus* in the Order *Bunyavirales* (https://talk.ictvonline.org/files/proposals/taxonomy_proposals_plant1/m/plant01/7405). A second nsRNA virus, resembling CCGaV has been recently identified by HTS in citrus and provisionally denoted citrus bunya-like virus (CBLV). The molecular and structural features of CBLV genomic RNAs, the signatures of the proteins it encodes and phylogenetic analyses support the classification of CBLV and CCGaV in the same genus and suggest a possible modular genome evolution for these viruses. These findings, supporting the hypothesis that plant-infecting nsRNA viruses most likely have evolved from an invertebrate-infecting ancestor, several times as independent events, will be discussed. Specific methods for detecting both CCGaV and CBLV and results of a field survey on the distribution of both viruses in Southern Italy will also be presented and discussed.

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Rapid and sensitive detection of onion yellow dwarf virus and iris yellow spot virus by reverse transcription loop-mediated isothermal amplification on ‘Rossa di Tropea’ onion

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Onion yellow dwarf virus (OYDV) and iris yellow spot orthotospovirus (IYSV) are two important viral pathogens of seed and bulb onion crops. In particular, on ‘Rossa di Tropea’ onion, granted with a Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) trademarks, these pathogens represent the most limiting biotic stress. Despite OYDV is the most important virus in terms of spread, severity of symptoms and damage, the detection of both pathogens is necessary to maintain high quality standard and avoid production losses. Two reverse transcription loop mediated isothermal amplification (RT-LAMP) assays were developed. The specificity, sensitivity, repeatability and reproducibility of assays were validated according to EPPO standard PM7/98. In addition, the RT-LAMP assays were tested on both leaf and bulb tissues, comparing several nucleic acids extraction methods aimed to simplify sample preparation phases. The results showed the reliability of the method for both OYDV and IYSV detection, with a limit of detection (LOD) comparable to real time reverse transcription polymerase chain reaction (RT-qPCR). Furthermore, the easiness of sample preparation and the more than acceptable LOD, indicated these RT-LAMP assays as rapid and simple.

Resistance to the downy mildew agent in the Eurasian grapevine germplasm of Georgia (Caucasus)

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The oomycete *Plasmopara viticola* is the causal agent of downy mildew, one of the most important diseases affecting grapevine cultivation worldwide. *P. viticola* is an obligate parasite and biotrophic pathogen of members of the *Vitaceae*, introduced in Europe from North America in 1878. While it causes limited damages to American and Asian grapevine species, severe epidemics leading to yield losses are frequent in the Eurasian grapevine species, *Vitis vinifera*. The possibility that *V. vinifera* could be resistant to *P. viticola* has been largely unexplored in the past, due to absence of co-evolution with the pathogen and weak genetic diversity of the main cultivars. The recent discovery of the great genetic variability of the Caucasian grapevine, Georgian in particular, opened new perspectives on resistant traits in *V. vinifera*. A large collection of 600 *V. vinifera* accessions of Caucasian origin, cultivated and wild, has been phenotypically characterized for resistance to *P. viticola* through experimental inoculation in laboratory and disease assessment in open field. This led to the discovery of 33 accessions with a resistance level from medium to high, all from Georgia. The Georgian accession, Mgaloblishvili, in particular, showed unique resistance traits based on comparative transcriptomic analysis and confocal microscopy analyses. In conclusion, the cradle of viticulture, located in Georgia (Caucasus), represents an important source of resistance to *P. viticola* that could be exploited for genetic improvement of grapevine, one of the most specific, environmentally safe and innovative tools for plant disease management.

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Comparative effectiveness of phosphonate fungicides, calcium oxide and reduced doses of copper hydroxide for managing *Phytophthora* brown rot of citrus fruit

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Copper (Cu) fungicides are traditionally used against citrus fruit brown rot (FBR) caused by *Phytophthora citrophthora* in the field. However, there is concern about the environmental impact of Cu due to its toxicity and persistence in the soil. The objective of this study was to evaluate natural substances and reduced doses of Cu to control FBR in order to minimize dependency on Cu. In a first series of experiments, carried out in two 20-year-old orange orchards in Sicily from 2010 to 2015, treatments included three doses of Cu (1, 0.75 and 0.5 kg/ha) as Cu hydroxide, Fosetyl Al 300 g/hl (commercial product, a.i. 80%) as a reference product, and control treated with water. Another set of experiments was carried out in other two 20-yr-old orchards from 2014 to 2016. Treatments included potassium (K) phosphite 250 ml/hl (commercial product, P_2O_5 30% and K_2O 27.5%), Ca oxide 20 l/ha (commercial product, a.i. 33.6%), Cu oxychloride at 1 kg/ha, and control treated with water. In both set of experiments, products were applied in autumn as a single normal volume foliar spray. Phosphonate fungicides were the most effective products in both series of experiments. All three doses of Cu were effective. However, the dose of 0.5 kg/ha showed a lower persistence with a significant reduction of efficacy from 40 to 60 days after the treatment. The effectiveness of Ca oxide against FBR, albeit significant, was not satisfactory. Results indicate that the intake of Cu in citrus orchards can be reduced using appropriate strategies.

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

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

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New genetic resources for the causal agents of brown rot on stone fruits, *Monilinia fructicola*, *Monilinia laxa* and *Monilinia fructigena*

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Monilinia fructicola (MFRC), *Monilinia laxa* (MLAX) and *Monilinia fructigena* (MFRG) are the most important pathogens causing the brown rot disease of stone and pome fruits. The sources of genetic variation as well as genomic and transcriptomic data remain largely unexplored for the three fungal species. The molecular characterization of the *MAT1* loci showed that MFRC, MLAX and MFRG have a bipolar heterothallic mating system, typical of other self-sterile Ascomycetes. Single isolates carry only one of two alternative idiomorphs, each containing a specific couple of genes (*MAT1-1-1* and *MAT1-1-5* in *MAT1-1*; *MAT1-2-1* and *MAT1-2-10* in *MAT1-2*). The populations of the three *Monilinia* species show a balanced distribution of mating types indicating a potential outcrossing in all the geographic areas in which the pathogens are present. ISSR markers suggested that asexual reproduction is prevalent, but that sexual recombination occurs in MFRC populations in Italy. The complete transcriptomes of MFRC, MLAX and MFRG was *de novo* assembled from Illumina sequence reads (about 70 million per species) and deposited in public database (https://www.ncbi.nlm.nih.gov/Traces/wgs; accessions GGAK00000000, GGAL00000000 and GGAM00000000). Comparative analyses among orthologous transcripts revealed transcripts over-expressed (FC≥8 and FDR≤0.05) or unique in MFRC (65), MLAX (30) or MFRG (31) that are involved in important biological and physiological processes, such as morphogenesis and development, pathogenesis, biosynthesis of secondary metabolites and detoxification systems. The obtained results will be useful to clarify aspects of their biology and physiology, including adaptability to different environmental conditions and plant-pathogen interactions, which can be of help for improving the disease management.

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

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

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

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

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

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The "Complesso Speleologico Villasmundo-S. Alfio" (Melilli, Siracusa) was established as a Natural Integral Reserve in 1998. It sits on a karstic calcareous subsoil and includes two deep river valleys delimiting a plateau. Because of its naturalistic and environmental features it has been recognized as a *Site of Community Importance - SCI* - (92/43/EEC). As part of a broader study aimed at investigating the causes of the decline of native vegetation in Natural Reserves in Sicily, the diversity of *Phytophthora* populations in six different phytocoenoses in this Natural Integral Reserve has been studied, including the riparian wood *Platano-Salicetum pedicellate*, the termophylic evergreen wood *Pistacio-Quercetum ilicis*, the Mediterranean maquis *Myrto communis-Pistacietum lentisci*, the garrigue with *Sarcopterium spinosum*, the termophylic wood *Oleo-Quercetum virgilianae* and the riparian bush community with *Nerium oleander*. *Phytophthora* isolates were recovered from rhizospere soil and river water using leaf baits and selective isolation medium. They were identified on the basis of morphological characteristics and ITS-rDNA sequence analysis. Overall, eight *Phytohpthora* species belonging to four phylogenetic clades (I, II, VI and VIII) were recovered from five out of six phytocoenoses, including *Phytophthora asparagi*, *P. bilorbang, P. cryptogea*, *P. lacustris*, *P. multivora*, *P. nicotianae*, *P. plurivora* and *P. syringae*. With the exception of *P. lacustris*, all these species are aggressive plant pathogens. Leaf bait combined with molecular identification of isolates proved to be a valuable tool to study the diversity and ecology of *Phytophthora* in natural ecosystems.

POSTERS

The Apulia Region-funded Pilot Study: "Silver Bullet against *Xylella fastidiosa*" (SIX)

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Since 2013 *Xylella fastidiosa*, the bacterial causal agent of the Olive Quick Decline Syndrome (OQDS-CoDiRO), has threatened the existence of oliviculture in the Salento area, southern Apulia, and has sounded the alarm for possible epidemic spread of the disease. The bacterium, once introduced into the xylem by the insect vector *Philaenus spumarius*, colonizes the vessels and provokes occlusions that impair the upward transport of water/sap, leading to scorch/dieback of leaves, shoots and branches and, ultimately, plant death. OQDS threatens not only olive production, but also natural ecosystems, landscapes and tourism, causing enormous economic and social impact to the Region. *X. fastidiosa* is a quarantine pathogen, to which control is mandatory by law. However, the pathogen is polyphagous, and numerous cultivated and wild plant species are potential hosts of *Xylella*, thus contribute to rapid distribution of the disease. Effective control measures appear difficult to obtain, and to date, no successful, practical management practices are available to limit spread. Attempts have also been made to recover already afflicted olive trees. In this Apulia Region-funded pilot study, initiated in 2017, two field trials were conducted to test the curative efficacy of treatments with a bio-stimulant (a bioactive natural compound) and an anti-septic formulation (containing elementary silver particles) to control the advancement of disease in "infected" olive trees. The active compounds were administered by a combination of endotherapy and foliar sprays, and the level of bacterial infection was monitored by quantitative RT-PCR. We report on the first year of experimentation.

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Antimicrobial photodynamic treatment against plant pathogens: the power of anionic porphyrin against the grey mold agent (*Botrytis cinerea*)

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Recently, an antimicrobial photodynamic treatment (APDT) relying on the use of photosensitizers that generate reactive oxygen species (ROS) when photoactivated, proved effective against plant pathogens, including the fungus *Botrytis cinerea*. In previous studies, we worked on the plant side of the APDT strategy using porphyrins as photosensitizers. While tomato and Arabidopsis plantlet growth was clearly altered by cationic porphyrins, their growth and development were not affected in the presence of anionic porphyrins even when tested at 50 µM. Thus, our idea was to study how a plant pathogen (*Botrytis cinerea*) responds to porphyrins, especially the anionic ones. In a first attempt, we studied how *B. cinerea* mycelium responded to porphyrins and determined their minimum inhibition concentration (MIC). Both anionic and cationic porphyrins were able to kill the fungus but MIC of anionic and cationic porphyrins was 1.5 and 3.5 µM, respectively. To gain insight into the fungal anti-oxidative enzymes involved in response to photoactivated porphyrin, the reaction of a 4-day-old mycelium was assayed. In parallel, as *Botrytis* has grapevine fruits as targets, the plant response to anionic porphyrin was monitored at the vegetative level for grapevine, and strawberry for fruits by means of phenotype and anti-oxidative components such as tocopherol and enzymes. Taken together, the preliminary results strongly suggested the possibility that an APDT approach can be developed using anionic porphyrin to control plant pathogens without disturbing plant and fruit development.

Change of activity of antioxidant enzymes under stress conditions of the environment

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The earliest response of a plant organism to stress factors is the generation of reactive oxygen species (ROS) that damage various cell components. In this regard, the goal of the study was to study the effect of fungicides and pathogenic fungus on changes in the activity of wheat antioxidants jointly and separately. For the experiment, the Arai spring wheat seeds were planted in containers with a wet substrate and processed in several variants: a suspension culture of the fungus *Fusarium graminearum*, normal fungicides (tenazol 12% of s.c., tebuconazole, 120 g/l), Bastion 34% w.s.c., carboxin 170 g/l + tiram 170 g/l), fungicides together with the fungus, the control was treated with water. When treated with pathogenic fungus, the activity of CAT and PO slightly exceeded the control both in shoots and in the roots of wheat seedlings. Exposure to the fungicide tenazol led to the induction of CAT in the shoots and plant roots 5-fold compared to the control. With the joint action of the fungicide with the pathogen, the level of CAT activity in the shoots did not exceed the control, and in the roots it was 4 times higher than the control variant. After treatment with tenazol, PO activity in shoots and roots varied within control. The bastion opposite induces PO almost 1.5 times in both parts of wheat germs. Thus, it is established that the pathogenic fungus induces a plant protective response to stress, expressed in the activation of antioxidants.

Pathogenic endophytic fungi in transplanting poplar plants

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In new poplar plantations transplanted plants often die because of attacks by *Discosporium populeum* (Sacc.) B. Sutton, *Cytospora* spp. and *Phomopsis* spp. The ability of these fungi to live asymptomatically inside healthy tissues is well known. The purpose of this research was to verify if this phenomenon might be due to infections that occurred undetected in the nursery. To this end, in 2007 two nurseries were established with the poplar clone 'I-214' (*Populus* × *canadensis*) in Casale Monferrato (Northern Italy) and Viterbo (Central Italy) and were subjected to different fertilization and irrigation regimes. In the second year, just before transplant, the incidence of fungal endophytes was surveyed and all plants resulted healthy. *D. populeum* was found in Casale Monferrato, *Cytospora* sp. in Viterbo, *Phomopsis* sp. in both, always in plants suffering from water deficiency. A group of dehydrated plants were rehydrated in water, planted in Casale Monferrato with another group of dehydrated ones to detect the incidence of possible attacks of cortical pathogens. At the end of spring, severe attacks were detected, in this new plantation, on the dehydrated plants and no attacks in the rehydrated ones. This proved that, from a practical point of view, it is possible to reduce the risks of transplant crisis by minimizing water stress in the nursery, avoiding dehydration during the transplant phase and promoting good hydration before planting.

Development of a novel molecular tool to trace the invasive pathogen *Xylella fastidiosa* subsp. *pauca* in the olive European outbreak

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Xylella fastidiosa subspecies *pauca* is the causal agent of the Olive Quick Decline Syndrome (OQDS), one of the most severe phytopathological alert today. In the Apulia region, olive groves have been harshly affected since the first report in 2013 but this pathogen could theoretically spread all over the entire Mediterranean olive growing area, where suitable environmental conditions for the pathogen exist. The aim of this research is the development of a molecular diagnostic tool that can provide information about the origin of the pathogen, its population dynamics and diffusion pathways. The methodology chosen is the VNTR (Variable Number of Tandem Repeats) analysis, which is a very sensitive method for bacterial genotyping but it is also fast, low-cost, and repeatable. It has already been successfully applied on other subspecies of *Xylella fastidiosa* on different hosts. The TRs formerly published were tested *in silico* on the genome of *X. fastidiosa* subsp. *pauca* strain De Donno (accession No. CP020870). Furthermore, 20 new TR loci were identified and related primers were designed. About 40 TR loci were amplified on the DNA of few isolates of *X. fastidiosa* subsp. *pauca* from the Italian outbreak. The genotyping results obtained are encouraging. The prospective effectiveness of the assay in tracing OQDS strains is discussed.

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Selection and characterization of beneficial bacteria and seed bacterization of leguminoses as a biocontrol strategy against *Rhizoctonia solani* damping-off

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The set up and the implementation of eco-friendly tools in agriculture minimize the use of chemical pesticides and the consequent negative impact on environment and human health. The selection and use of beneficial microorganisms is the most interesting strategy to control soilborne pathogens in the rhizosphere. In a such context, our research is addressed to identify and select bacterial isolates with high and optimized activity against the main soilborne pathogens. Over 300 bacterial isolates from different ecosystems were characterized for both their potential antagonistic activity against soilborne fungal pathogens and their potential beneficial effect on the plant growth. Some of the selected bacteria not only showed a remarkable antagonism in dual culture tests toward *Rhizoctonia solani* but in *in vivo* assays of seed bacterization either increased germination of *Pisum sativum* seeds and reduced the incidence of *R. solani* damping off symptoms. Researches are in progress for a deep molecular characterization of the selected bacterial strains, to better elucidate the mechanisms of biocontrol agent-host plant-fungal pathogen interaction and to optimize the use of the most effective bacteria through the seed bacterization method.

Identification of candidate genes involved in resistance to Fusarium wilt race 0 in chickpea

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Fusarium oxysporum f. sp. *ciceris* (Foc) is one of the main biotic agents limiting the chickpea production in the world. In the absence of effective control tools for field disease management, the constitution and use of resistant crop cultivars through genetic studies, represent the most appropriate approach to prevent pathogen attacks and production loss. In order to include new markers associated with resistant reaction, mapping populations derived from crosses with the genotype JG62, resistant to Foc race 0, was studied. JG62 is an Indian accession of chickpea that carries the resistance gene ($Foc0_1/foc0_1$). In previous studies, this gene was already mapped on linkage group 5 of chickpea genetic map. In this study three recombinant inbred populations (RIP) of chickpea (RIP1, RIP7 and RIP10) have been used. Our aim was to refine the position of the resistance gene including new markers in the three populations. Seven microsatellites (SSR, simple sequence repeat) markers were selected in the genomic area near by the microsatellite targeting $Foc0_1/foc0_1$. Four of them resulted polymorphic: i) two in RIP1 (CaGM20918 and CaGM20716); ii) three in RIP7 (CaGM20918, CaGM20942 and CaGM20920); iii) all of them were monomorphic in the RIP10. To get useful information about molecular functions, the genomic region delimited by the marker most associated with the resistance gene was annotated with Blast to go. Downstream of the results obtained a list of candidate genes was proposed and a detailed information for each gene was reported.

Innovative delivery of Cu(II) ions by a nano-structured hydroxyapatite to enhance the sustainable control of *Plasmopara viticola*

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In organic viticulture, protection against *Plasmopara viticola* is essentially ensured by the use of cupric fungicides, which is expected to be restricted by European Countries because of its ecotoxicological profile. Research on innovative forms of copper as well as the optimization of its distribution and persistence appear to be the most promising approaches to enhance the control of *P. viticola*. The present research investigates the delivery properties of biomimetic synthetic hydroxyapatite (HA) to enhance the biological activity of Cu(II) ions. To this aim, four Cu(II) compounds were formulated with HA and applied in a preliminary *in vitro* antifungal assay against *Botrytis cinerea* as test reference pathogen, and in consecutive *in-planta* assays against *P. viticola* under greenhouse conditions. The *in vitro* results highlighted a different degree of inhibition by each Cu(II) compound according to their dosage and indicating also the delivery role potentially played by HA, especially on the insoluble copper salts. Under greenhouse conditions, further findings were gained especially on the efficacy of variable percentages of HA into the formulations, on the influence of dose variation of the formulation and the treatment efficacy and persistence under rain-washing effect. The study revealed promising findings on the formulation based on the HA particles and the most soluble copper salt, copper sulfate pentahydrate, which proved to be highly efficient in reducing both disease severity and incidence in all the experimental conditions, likewise suggesting a deeper investigation of functional models and co-formulation process on the insoluble Cu(II) compounds.

Phytopathological problems associated with quinoa (*Chenopodium quinoa* Willd.) seed production in an area of central Italy

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Quinoa is a widely cultivated crop for human consumption in the Andean Regions (South America), which is recently expanding into areas far from those of origin. In 2017, in a quinoa field trial near Perugia (Umbria, central Italy), emergence failures of the varieties ‘Titicaca’, ‘Riobamba’ and ‘Real’ were observed. Seed multiplication had been performed during the previous year in the same area. Conversely, seeds belonging to ‘Regalona’ variety, coming from Chile, showed an optimal emergence. For these reasons, phytopathological investigations on seed lots were carried out. Visual observations showed a higher incidence of brown/necrotic symptomatic seeds of the ‘Titicaca’, ‘Riobamba’ and ‘Real’ varieties (93, 77 and 70%, respectively) in comparison to ‘Regalona’ (17%). *In vitro* germination was completely absent in the symptomatic seeds of the first three varieties, while 73% of symptomatic ‘Regalona’ seeds were able to germinate. Germination differences of the asymptomatic seeds of the four varieties (33, 40, 70 and 100%, respectively) were also recorded. *In vitro* fungal isolations and identifications (by partial *ITS* and *tef1α* region sequencing) showed a high incidence of a member of the *Fusarium incarnatum-equiseti* species complex (FIESC) in the symptomatic seeds of ‘Titicaca’, ‘Riobamba’ and ‘Real’ (83, 70 and 90%, respectively). This pathogen was also detected (30, 27 and 37%, respectively) in their asymptomatic seeds. No FIESC members were detected in ‘Regalona’. Isolates belonging to the genera *Alternaria* and *Aspergillus* were also obtained. This study highlights that particular attention should be given to phytopathological problems during quinoa seed production and storage.

Ecophysiological response of pomegranate plants under long-term combined salt and ozone stress

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The progressive salinization of soils irrigated with salty water and salt-water intrusion of groundwater bodies can limit crop production in many areas, especially in the Mediterranean basin. The current background tropospheric ozone (O_3) levels are high enough to negatively affect plant physiological and productive performances. In this work, one-year-old saplings of pomegranate (*Punica granatum* L., cv. 'Dente di cavallo') were exposed to two levels of O_3 [AOT40 values were 21.51 in ambient air (AA), and 58.74 ppm h, in 2AA] and two levels of salinity denoted as No Salt (NS) and Salt (S, the electrical conductivity and pH of the irrigation water were 5.5 mS cm^{-1} and 7.6 with 50 mM of NaCl) for four consecutive months in an O_3 FACE open air facility. Under O_3 (alone or in combination with salt), plants developed visible stipples of browning tissue localized in the interveinal adaxial leaf surface. At ecophysiological level, salt stress further affected the photosynthetic performance (-17% compared to AA_NS). By contrast, salinity did not induce oxidative damage [as confirmed by unchanged malondialdehyde (MDA) levels]. Under 2AA conditions, O_3 alone reduced the stress tolerance, as confirmed by the production of reactive oxygen species (+10 and +225% of anion superoxide and hydrogen peroxide, respectively), the increase of superoxide dismutase activity (+9%) and the concomitant membrane denaturation (+198% of MDA content). According to Bansal calculation, the combination of both stressors had a synergistic effect in terms of oxidative damage and increased activity of catalase.

Coexistence of *Caliciopsis pinea* and *Fusarium circinatum* on pine: interactions among fungal pathogens

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Coexistence of pathogenic fungi on the same host has been often reported in plant pathology. As for pine trees there are several examples on needles and shoots that, in temperate regions, could be contemporary affected by different pathogens. Less frequently, different fungal species, responsible for cankers on branches and stems, have been found on the same host. Recently in Spain, in a plantation near San Sebastian de Garabandal (coord-*ETRS89 383997, 4784740 UTM 30N*), during a survey, a sample was collected from a canker on a *Pinus radiata* affected by *Fusarium circinatum* (Nirenberg & O'Donnel). The pathogen, included in the A/2 Eppo list, is present in Spain and Portugal on pine species causing severe mortality. A careful observation of the canker showed that the pine was infected also by *Caliciopsis pinea* Peck ascomycetous fungus responsible for resinous cankers on pine, reported both in Europe and North America. The *P. radiata* bark showed a number of perithecia of *C. pinea* actively producing ascospores. The pathogen was isolated, DNA extracted from the mycelium and its taxonomical position confirmed after sequencing (amplification of ribosomal DNA ITS region and Elongation Factor gene). Dual cultures of *C. pinea* and *F. circinatum* tested in petri dishes on PDA showed weak reactions, confirming their possible coexistence. According to the literature, the environmental conditions of both fungi are very similar. Since both ascomycetes produce the same symptoms on the same species of pine, care should be taken during monitoring in the forest to avoid the risk of misidentification.

Occurence of *Erwinia amylovora* foci in Sicily twenty years after eradication

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The monitoring of fire blight of rosaceous plants by the Regional Plant Protection Service of Sicily, continues to be very intensive since 1991 when *Erwinia amylovora* was first reported in a single field located near Milazzo (Messina) and successfully eradicated. In June 2014, during the survey campaign symptoms of fire blight were observed in two pear orchards on plants of the cv. 'Abate', in Enna province, and cv. 'Carmen' on the slopes of Mt. Etna. Both fields, according to the rules, were eradicated. Demarcated areas were established at the end of 2016 after the diagnosis of approximately 100 foci against the near 350 inspections carried out in 2015-2016. The "Protected Zone" status of this area located in Eastern Sicily was compromised and a mandatory integrated disease control protocol was established. Diagnosis was performed according to the EPPO protocol for diagnosis of fire blight in symptomatic plants (EPPO, 2013). While processing numerous assays a LAMP protocol with ICGENE system was standardized to be used for a rapid presumptive diagnosis or for colonies identification. Both LAMP and real-time PCR were able to detect *E. amylovora* also when the pathogen was not isolable. A collection of more of 100 *E. amylovora* strains was set up for further studies. The analysis of the pEA29 repeat region sequences of representative strains suggests that more strains were responsible of the outbreaks. The 2017 survey carried out with near 200 inspections showed a considerable reduction of positive sites (approximately 20 infected sites in the demarcated areas).

Application of native *Trichoderma* spp. to confer suppressiveness to nursery potting mixes

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Populations of *Trichoderma* species were characterized and selected from the rhizosphere of holm oak (*T. hamatum* T19, *T. asperellum* T20, and *T. virens* T21), olive (*T. asperellum* T2, *T. hamatum* T3, and *T. harzianum* T6) and lavender (T. *asperellum* T12 and *T. harzianum* T14) grown in nursery, and from spent coffee grounds (SCG) (*T. atroviride* Ta3, Ta4) to select effective antagonistic and use them to amend nursery growth substrates. The representative *Trichoderma* isolates were tested for the *in vitro* antagonism against a panel of pathogens including *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Fusarium circinatum*, *Phytophthora nicotianae*. The mycelial growth of pathogens was differently and directly affected by each *Trichoderma* isolate and its volatile and non-volatile metabolites, suggesting multiple mechanisms in the antagonistic activity. The application of a T-complex of *Trichoderma* isolates (T3 T6, T20 and T21) to a peat-based substrate decreased the root rot of lavender caused by *R. solani* and *S. sclerotiorum*. The highest efficacy against the oomycete *P. nicotianae* was shown by *T. harzianum* T6 alone. The application of the T-complex in soil significantly increased the germination rate of *Pinus radiata* infected seeds with *F. circinatum* and decreased seedlings mortality. Peat-SCG substrate significantly reduced damping off of cress by *P. nicotianae* but failed to suppress that by *S. sclerotiorum*. When the peat-SCG substrate was inoculated with *T. atroviride* a consistent decrease of damping off was recorded. The application of a combination of local antagonists through the amendment of potting soil may have potential in the sustainable production of nursery plants.

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Non-conventional compounds against powdery mildew: profiling grapevine phyllobiome and elicited defence responses

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The reduction of antimicrobial treatments and mainly the application of environmentally friendly compounds, such as resistance elicitors, is an impelling challenge for grapegrowers in the optic of a more sustainable viticulture. We addressed this research to study how non-conventional compounds reduce leaf fungal attack and influence the phyllobiome. Pathogenicity tests were conducted on potted *Vitis vinifera* 'Nebbiolo' and 'Moscato' infected with the powdery mildew agent (*Erysiphe necatrix*) and maintained in greenhouse. The efficacy of diverse resistance inducers was assessed on infected plants treated with either conventional (acibenzolar-S-methyl) or alternative (laminarin, potassium phosphonate) fungicides. In both the cultivars, the most effective products in terms of significant inhibition of the pathogen were laminarin and acibenzolar-S-methyl. The effect of the antifungal treatments in shaping the foliar microbial community of powdery-mildew infected grapevines was evaluated by: i) community-level physiological profiling (CLPP) by using Biolog EcoplatesTM; ii) high throughput sequencing of ITS and 16S regions; iii) depict of virome, viroids and phytoplasma; iiii) analysis of cuticular wax content and secondary metabolites. Cuticular waxes on leaf epidermis was overall higher in 'Nebbiolo' than in 'Moscato', pointing to distinct defense mechanisms between the two genotypes, likely induced, besides the pathogen presence, by the applied alternative compounds. This trend was confirmed by CLPP results obtained by performing sole-carbon source-utilization tests on leaf extracts. Further experiments are ongoing to integrate microorganism profiling with metabolomic data in order to gain a comprehensive picture of the alternative fungicide effects on phyllobiome and defense-related responses.

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Epidemiological survey on '*Candidatus* Phytoplasma solani' and putative vectors in western Sicily

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Bois noir (BN), associated with '*Candidatus* Phytoplasma solani' is the most important grapevine yellows, widespread in Europe and Mediterranean regions. During a two-year survey (2014-15) carried out in a 10-year-old vineyard located in San Giuseppe Jato (PA), BN symptoms were observed on cvs 'Chardonnay', 'Pinot Noir' and 'Nero d'Avola'. Incidence of the disease increased over the two years, reaching values of about 34% on cv. 'Chardonnay'. '*Ca.* P. solani' was detected on symptomatic leaf samples from all cultivars with the highest percentage on cv. 'Chardonnay', followed by 'Pinot noir' and 'Nero d'Avola'. Further surveys were performed in order to highlight the epidemiology of the disease by monitoring '*Ca.* P. solani' presence in wild herbaceous species and potential vectors and by molecular characterization of related strains. Weed samples were collected within and at the border of the vineyard, whereas plant- and leafhoppers individuals were monitored from June to August of each year, using yellow sticky traps replaced at weekly intervals. Eleven weed species and about 2000 insect specimens were collected and identified at species or genus level by morphological feature. No *Hyalesthes obsoletus* was found. '*Ca.* P. solani' was detected in 5 weed species and 9 out of 22 taxa of the different insect specimens: *Anaceratagallia laevis*, *Empoasca decipiens*, *E. vitis, Empoasca/Zigina* sp., *Hauptipia provincialis*, *Neoaliturus fenestratus*, *Selachina apicalis*, *Zygina rhamni* and *Z. serpentine*. RLFP analysis of the *tuf* gene of '*Ca.* P. solani' recovered from symptomatic grapes, weeds and insect specimens revealed the presence of only *tuf* type-b.

Evaluations on the effects of phenylpyrrole fungicides in *Botrytis cinerea* by RNA-Seq transcriptome analysis

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The use of synthetic fungicides is still very common for the management of grey mould caused by *Botrytis cinerea*. However, mode of action and resistance mechanism in the target pathogen for several fungicides, including the phenylpyrrole fludioxonil, are not completely understood. Whole transcriptional changes caused by the exposure to different concentrations of fludioxonil (EC_{50} and EC_{75}) in two strains of the pathogen, one sensitive and one resistant to fludioxonil, were investigated using Illumina sequencing and RNA-Seq analysis to identify affected genes and metabolic processes. Overall, about 930 differentially expressed transcripts (DETs; Fold change $\geq |6|$ and RPKM ≥ 5) were identified in each strain comparing fludioxonil-exposed versus the unexposed colonies. Comprehensive functional enrichment analysis showed that redox processes were associated to up- and down-regulated DETs, while biosynthesis of secondary metabolites and cell wall and surface proteins were strictly related with the up-regulated ones. Membrane-lipid metabolic processes were up-regulated in the sensitive strain and down-regulated in the resistant strain. Fungal growth as well as regulation and signalling, transmembrane transport, response to stress and protein metabolism were more frequently related to down-regulated DETs. Among transcripts associated with transport and regulation, the *mrr1* gene, encoding the transcription factor responsible for Multi-Drug Resistance type 1 (MDR1)-related *atrB* overexpression, was up-regulated in the fludioxonil-resistant strain. The results provide new knowledge that might be useful to plan novel sustainable disease management strategies, increasingly oriented to preserve effectiveness of chemicals and minimize environmental impact.

New insights into biology, transcriptome analysis and control strategies of the cucurbit pathogen *Podosphaera xanthii*

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Podosphaera xanthii is the main causal agent of cucurbit powdery mildew (CPM) in southern Italy, where the disease management is difficult and requires numerous fungicide sprays per season. The effectiveness of several biological control agents (BCAs), botanicals (BOTs) and fungicides was evaluated in a field trial on *Cucumis melo* local ecotype ‘Barattiere’. Orange oil, sulphur, potassium bicarbonate and laminarine showed a good effectiveness and reduced the CPM severity on leaves as compared to the untreated control. Sexual reproduction increases genetic variation and can promote the differentiation of new more adapted genotypes. *P. xanthii* is a heterothallic fungus; both idiomorphs *MAT1-1* and *MAT1-2* were detected in more than half of 23 monitored sites and, according to the whole data, the *MAT1-1* and *MAT1-2* are in 1:1 ratio. The capability of six *MAT1-1* and ten *MAT1-2* isolates in yielding fertile crosses and differentiate chasmothecia was proved under laboratory conditions. Chasmothecia were also detected, although sporadically, in the field. RNA-Seq experiments (Illumina technology) were carried out using two isolates of opposite mating types and their cross to obtain a detailed *de novo* Trinity-based assembly of the transcriptome of *P. xanthii*. The 23,065 transcripts obtained were employed in: i) the analysis of differential expressed transcripts; ii) the identification of putative effectors, transposable elements (TEs) and mycoviruses; iii) the molecular characterization of the *MAT1* locus in *P. xanthii*. The availability of new genomic and transcriptomic data will promote new insights on the population biology of the pathogen and CPM sustainable management.

Effectiveness of biocontrol rhizobacteria and a new natural organic fertilizer against soilborne pathogens on pot grown ornamental plants

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Control strategies to protect forestry and ornamental plants from diseases caused by soilborne pathogens in nursery are based on the adoption of preventive tools such as plant genetic resistance, healthy propagation material, nursery sanitation, and particularly on use of synthetic agrochemicals. However, chemical control is increasingly limited because of environmental and toxicological risks and consequent legislative restrictions. Under this scenario, integrated and biological strategy based on antagonistic microorganisms and/or organic bioactive substrates could be promising and eco-friendly alternative control tools. By the present investigation we evaluated *in vitro* and *in vivo*, on pot grown milkwort and oak plants, the inhibitory activity of four selected strains of beneficial rhizobacteria (*Bacillus amyloliquefaciens* BO7, *Ranhella aquatilis* 36, *Pseudomonas fluorescens* 57 and *Serratia marcescens* 59) and a new organic plant-growth bioactive amender against the soilborne pathogens *Fusarium oxysporum*, *Fusarium solani* and *Phytophthora cinnammomi*. Results of *in vitro* experiments evidenced different degrees of inhibitory activity against *Fusarium* spp. and *P. cinnammomi* of either the selected biocontrol bacteria and the organic bio-active amendment applied at different concentrations. The *in vivo* experiments evidenced that all tested products were compatible with milkwort and oak plants or even had beneficial effects on their growth. Moreover, on artificially inoculated plants the tested products evidenced different degree of disease symptom reduction with a better activity exerted by the biocontrol bacteria *B. amyloliquefaciens* strain BO7. Results are discussed also with regard to the possible implementation of these new eco-friendly tools for the control of *F. solani* and *P. cinnammomi*, and possibly against other soilborne pathogens on nursery grown plants.

Effect of *Aureobasidium pullulans* strains against *Botrytis cinerea* on kiwifruit during storage and on fruit nutritional composition

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Kiwifruit, wounded at the equator or by pedicel removal, to simulate the stem end wound, were treated with *Aureobasidium pullulans* (strains L1 and L8) and subsequently inoculated with conidia of *Botrytis cinerea* (10^4 conidia ml^{-1}). Fruits were stored at -1°C in normal refrigeration (NR) or in controlled atmosphere (CA) (2% O_2; 4.5% CO_2). After four months, both antagonists significantly reduced the disease in all experiments, L1 better than L8. In NR, their efficacy was higher than 80%. In CA, the disease reduction was lower: between 30% (L1) and 60% (L8). The ability of both strains to compete with the pathogen for nutrients was tested in kiwifruit juice (0.5%) with *in vitro* experiments. Both strains significantly reduced germination of pathogen conidia in water and in juice. HPLC analysis was performed to define the amino acid composition of kiwifruit juice upon L1 and L8 treatment. Both strains greatly increased the concentration of both glutamic and aspartic acids and stimulated the production of new amino acids such as serine, glycine, threonine, arginine, alanine, valine, leucine, glutamine (for L1) and isoleucine (for L8), although at low concentrations. Each amino acid displayed an antifungal effect against mycelium growth of *B. cinerea.* In conclusion, L1 and L8, cold tolerant and active in CA, can effectively be applied to control the stem end rot of kiwifruit in long storage.

Effectiveness against strawberry postharvest decay of volatile organic compounds produced by biocontrol agents

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Strawberries are popular fruit due to their pleasant taste and nutritional value. However, strawberry storage is challenging, as postharvest pathogens can develop very easily, because the fruit are very succulent. Gray mold caused by *Botrytis cinerea* is the main pathogen, although many other fungi can cause severe production losses. In conventional agriculture, fungicides are commonly applied in field to prevent the development of gray mold and other decay pathogens. However, low-impact pest management such as biocontrol agents are promising alternatives to this fungicide use. The present study investigated the effectiveness of the volatile organic compounds (VOCs) produced by biocontrol agents for the control of strawberry postharvest decay. In particular, the VOCs of *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* were tested *in vitro* against *B. cinerea*, *Monilinia fructicola*, *Alternaria alternata*, *Aspergillus carbonarius*, *Penicillium digitatum*, *Cladosporium* spp. and *Colletotrichum* spp., and *in vivo* on strawberry fruit. In both experiments, the VOCs controlled the growth of postharvest pathogens. Solid-phase microextraction gas-chromatography analyses revealed that the main components of these VOCs was ethyl acetate, which was also effective for reduction of the *in-vitro* growth of strawberry pathogens. Similarly, ethyl acetate was effective against gray mold on strawberry fruit. Although ethyl acetate showed promise as an alternative to fungicides in the control of postharvest fruit decay, further studies are necessary before its practical application.

Effects of preharvest and postharvest fungicide applications for the control of gray mold on postharvest decay of strawberries and on fungicide residues on the fruit

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After harvest, fruit in general, and succulent fruit such as strawberry in particular, easily undergo fungal spoilage. *Botrytis cinerea* is the causal agent of gray mold, which represents the main postharvest strawberry disease, and even a limited infection can spoil entire fruit lots. In conventional agriculture, fungicide applications are repeated in the field from the strawberry flowering until the harvest, to control postharvest gray mold. Although in more recent years consumer concerns of the presence of fungicide residues on fruit have increased, fungicides remain the most effective means for controlling postharvest fruit decay. The aims of the present study were to compare the effectiveness of the active principles pyrimethanil, boscalid, fludioxonil, and cyprodinil applied either preharvest or postharvest for the control of postharvest strawberry decay, and to measure the consequent fungicide residues on the strawberry fruit. Regardless of the time of application, these fungicides reduced postharvest decay of strawberry fruit that were cold-stored for one week and then exposed to shelf life. In particular, fludioxonil and cyprodinil almost prevented postharvest strawberry decay, while pyrimethanil and boscalid reduced the disease by almost half, as compared to the untreated control. Moreover, these data showed that at 0, 4, 8, and 12 days after the treatments, the fungicide residues on strawberry fruit were always below the maximum residue levels.

Metagenomic detection of viruses and viroids associated with isolates of citrus tristeza virus

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Tristeza diseases are caused by six different strains (VT, T30, RB, T68, T36, T3) of citrus tristeza virus (CTV). Symptoms occur and vary based on a) the citrus species and cultivar infected, b) the genotype or mixture of genotypes present, and c) the environment and other stresses. To study the role of the population of CTV genotypes together with other viruses and/or viroids concurring to the final phenotype, eight CTV isolates, characterized by conventional tests, were sequenced by NGS of the small RNAs. Sub-reads were aligned with a set of 47 genomes of categorized citrus viruses and viroids. Approximately 50% of reads aligned with one CTV reference genome with a coverage of 100%: six with VT, one with T30 and one with T36. A variable percentage of reads also mapped with T3 and T68 genotypes. A field sample from China (HU-PSTS) showed a consistent number of reads of other citrus viruses and viroids. Some of these were confirmed: citrus yellow vein clearing virus (CYVCV), citrus tatter leaf virus (CTLV), hop stunt viroid (HSVd) and citrus dwarfing viroid (CDVd). Field samples from Sicily (P3R1 and P3R3) showed multiple viroid reads: CDVd, HSVd, citrus exocortis viroid (CEVd) and citrus viroid IV (CVd-IV), confirmed by RT-PCR. *Citrus viroid V* (CVd-V) and citrus bent leaf viroid (CBLVd) showed less than 10,000 reads. The results confirm that metagenomic analysis is reliable and cost-effective to diagnose multiple infections although to date the role that each component of this population plays on the final phenotype still needs to be verified.

Foliar fungal diseases in different cultivars of durum wheat in the Marche region

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Durum wheat is one of the most important crops in Italy. The Marche region is listed after Sicilia and Puglia in the group of the main producers of this cereal. Many different foliar fungal diseases can affect durum wheat and reduce yields. Those that are very common in central Italy are powdery mildew, brown leaf rust, wheat yellow rust, *Septoria tritici* blotch, and *Stagonospora nodorum* blotch. The health of the last three leaves of the plants has an important role in the quantity and quality of the crop production. To investigate foliar disease incidence, trials were carried out through 2016 and 2017 in two cropping areas in the Marche region that showed different soils and climates. Eleven different durum wheat cultivars that are commonly grown in the Marche were selected, with symptom evaluation carried out for each one. The incidence of each listed fungal disease on the leaf blade of the last three leaves on 10 plants per plot was recorded in two surveys. No treatments were performed to control pests or diseases. The infection index varied across the two growing areas, with lower development of these diseases in the hilly field, compared to the valley field. Overall, the least susceptible cultivars to foliar diseases were 'Monastir' (1.9%), 'Saragolla' (2.1%), 'Iride' (2.4%), 'Ettore' (2.4%), and 'Claudio' (2.6%). For both years, brown leaf rust was the most frequent foliar disease. Those data are useful for cultivar selection according to growing area, to obtain high quality durum wheat.

Impact on olive oil quality of olive leaf yellowing associated virus (OLYaV)

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Among the fifteen virus species found in olive trees, olive leaf yellowing associated virus (OLYaV, genus *Closterovirus*) showed to be the most frequent especially in southern Italy where it was found with a very high incidence. Virus interference on olive production has been rarely studied, so a study of OYLaV impact on oil quality was undertaken. Six trees of ‘Leccino’ (*L*) and six of ‘Ottobratica’ (*O*) were selected for their sanitary status [three healthy/virus-free (H) and three OYLaV-infected (I) plants] in an olive orchard located in Calabria region (southern Italy). From H and I plants, olive fruits were harvested from *L* and *O* trees when mature status was achieved. Further, free acidity, peroxide value, spectrophotometric absorptions K_{232} and K_{270}, were determined according to methods reported in EEC regulation n. 2568/91. In both cultivars (*L/O*) and in both sanitary status (H/I) almost oil tested parameters were not statistically different with some exceptions. Oils derived from ‘Ottobratica’ I plants had free acidity significantly lower than oil from H plants (0.25% I *vs* 0.35% H). K_{232} of oil from H ‘Leccino’ was significantly lower than oil from I trees (1.29 H *vs* 1.35 I). Even if some quality parameter differences between H and I oils were found, it is important to highlight that all oils can be considered in extra virgin olive oil category, within the UE range accepted maximum limit. All these preliminary results suggest that, except for K_{232} values, OYLaV does not interfere negatively in oil quality, while surprisingly does it positively in free acidity.

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Phenotypic and genotypic studies to evaluate Fusarium Head Blight Type I and Type II resistances in Italian wheat cultivars

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Wheat is one of the most cultivated crop in the world. Fusarium Head Blight (FHB) disease is manly caused by *F. graminearum*, which proliferates and spreads intracellularly followed by development of FHB symptoms, i.e. necrosis and bleaching of the heads resulting in shrivelled kernels. The infection of plant tissue with fungal pathogens is closely linked with changes in metabolic pathways, such as photosynthesis, stomatal conductance and volatile organic compounds (VOCs) biosynthesis, suggesting that certain physiological aspects could be associated with disease resistance. The aim of this work was to identify and characterize the main genes involved in wheat VOCs biosynthesis and stomatal conductance and to evaluate the FHB Type I and Type II resistance of three Italian wheat cultivars: ‘Palesio’, ‘Marco Aurelio’ and ‘Claudio’. Current results underlined that the three cultivars could have a FHB Type I tolerance but no FHB Type II resistance (100% of infected spikelets). In particular, cv. ‘Marco Aurelio’ showed the most interesting infection progress: FHB Type I resistance was clearly evident, since the level of infected spikelets reached a maximum of 30%. To better understand these types of resistance, a RT-qPCR will be carried out and the weight of a thousand seeds will be determined, in order to quantify the mycelial biomass inside the spikes and to better understand the plant ability to reduce pathogen diffusion. Fifteen candidate genes were isolated from ‘Palesio’, ‘Claudio’ and ‘Marco Aurelio’ genome and the next step will be to evaluate their role during the plant-pathogen interactions.

A new method for testing rice seeds, detection of *Magnaporthe oryzae* and *Fusarium fujikuroi* by loop-mediated isothermal amplification

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Magnaporthe oryzae and *Fusarium fujikuroi,* the causal agents of rice blast and bakanae disease, respectively, are two of the most important rice pathogens. Both are seed-transmitted and can produce up to 30% of estimated losses of total production on a worldwide basis. Traditionally the identification of seed-borne pathogens, following the ISTA rules, consists in a 400-seed plating method with microscopic identification of 10-days post-plating rice seeds. The certification of pathogen-free seeds is essential for the control of the pathogens that can be easily spread by this propagation material. However, morphology-based methods may result in mis-identifications of the pathogen because of the high number of shared characteristics between phylogenetically related species. In this study, we developed two LAMP assays for *F. fujikuroi* and *M. oryzae*, designed on the elongation factor 1-alpha and calmodulin genes, respectively. Both assays were validated according to the international EPPO standard (PM7/98) in terms of specificity, sensitivity, repeatibility and reproducibility. The assays were validated using DNA from rice seeds, showing a limit of detection of 100-999 fg of DNA for *F. fujikuroi* and 10-99 pg of DNA for *M. oryzae.* Two different DNA extraction methods combined with the LAMP assays were used to extract the DNA from infected rice seed lots and were compared with the traditional culturing method. The results demonstrated that LAMP assays are fast, specific and reliable for detection of *F. fujikuroi* and *M. oryzae* in seed-testing laboratories, overcoming the drawbacks of traditional identifications.

Characterization of the secondary metabolite profile and phylogenetic analysis of *Alternaria* species isolated from basil

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Alternaria spp. are the causal agents of Alternaria leaf-spot of basil. This disease has recently been reported in Italy. The correct identification of the different species has traditionally been based on morphological features and molecular data. Nevertheless, this fungus underwent multiple reclassifications. In this study, a better characterization of *Alternaria* isolates from basil was sought using morphological characteristics, phylogenetic analysis of seven genomic regions, and secondary metabolite profile. The best-resolute regions allowing the identification of the majority of the isolates as *A. alternata* were OPA 1-3 and OPA 10-2. Morphological characteristics and sporulation groups helped to discriminate *A. tenuissima* from *A. alternata* isolates. All isolates in the *A.* sect. *Alternaria* were demonstrated to be mycotoxigenic and pathogenic to basil with an enhanced production of mycotoxins on this host, as compared with the *in vitro* conditions used in this work. The combined results and, especially, the mycotoxin production allowed differentiating some species within this species group as *A. arborescens* and *A. tenuissima.*

Detection of *Fusarium oxysporum* f. sp. *lactucae* in soil, lettuce seeds and plants by LAMP assay

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Fusarium oxysporum f. sp. *lactucae* (FOL) is a worldwide-distributed soil- and seed-borne pathogen that causes Fusarium wilt of lettuce. Four races have been identified of this *forma specialis*, which is currently spreading to new areas within Europe, with new records of race 1 and race 4 in North Europe. Several molecular tools have been used to identify this pathogen in different propagation material. Due to its harmfulness, it can cause a total crop loss, so the distribution of infected seeds can threat the worldwide production of lettuce. In this study we developed, optimized and validated a LAMP assay for the detection of FOL in lettuce seeds, soil and plants. The LAMP assay was designed on the sequence-characterized amplified region (SCAR) obtained in a previous rapid amplification of polymorphic DNA assay and was validated according to the international EPPO standard (PM7/98) in terms of specificity, sensitivity, repeatability and reproducibility. The sensitivity of the assay using DNA and artificially inoculated lettuce seeds, in individual lettuce seed testing and batches at different infection rate, reached detection limits as low as 0.004% infected seeds. Thus LAMP was experimentally proved as a reliable laboratory tool for the detection of FOL in lettuce seeds lots, which can assist in avoiding the dissemination of the pathogen to new areas.

Rapid detection of *Monilinia fructicola* and *Monilinia laxa* on nectarines through loop-mediated isothermal amplification

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Monilinia laxa and *Monilinia fructicola* are two of the causal agents of brown rot, one of the most important diseases of *Prunus*, *Malus* and *Pyrus* species. Losses caused by these fungi can reach up 90% of the total production, as the flowers can be infected during blooming, as well as the twigs and fruits, especially during postharvest. Both fungal species have a worldwide distribution occurring in north and south America, Australia and Japan. After its introduction in Europe in 2001, *M. fructicola* became widespread affecting pears, apples, plums, sweet cherries, apricots, peaches, blackberries and nectarines. Due to the high yield losses caused to peaches, apricots and nectarines *M. fructicola* was included in 2005 in the A2 EPPO List of quarantine organisms. Traditionally, *M. fructicola* identification has been carried out using different DNA-based assays. With this study, however, LAMP, a quick method able to identify these pathogens during postharvest was designed and validated, taking into account its specificity, sensitivity, repeatability and reproducibility in accordance with the international EPPO standard (PM7/98). The sensitivity of the technique was checked by monitoring the fruits of two nectarine cultivars stored at two different temperatures. LAMP assays were compared at different time points against the quantification of the inoculum assessed by previous qPCR assays, demonstrating that they can detect a low number of cells. These LAMP methods could be a useful tool for the monitoring of brown rot causal agents in the field and during storage.

Evaluation of the potential vector role for the alien planthopper *Ricania speculum* in Flavescence dorée phytoplasma epidemiology

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Ricania speculum (Walker) is an exotic planthopper, accidentally introduced in Europe and officially reported in Italy (Genoa, Liguria) in 2014. It is now also spread in Tuscany (provinces of Massa Carrara, Lucca, Pisa). *Ricania speculum* is highly polyphagous on herbaceous and woody plants, either wild or cultivated, and can cause damages by sup suction, honeydew emission and egg-laying. This phloem sap-sucking insect often colonizes grapevines and *Clematis vitalba* plants, which are known hosts of Flavescence dorée phytoplasma (FDp), a quarantine plant pathogen causing severe damages to European viticulture. The purposes of this work were to determine if *R. speculum* could be a vector of FDp and assess its eventual impact on epidemiology of this grapevine disease. Nymphs of *R. speculum* were isolated on FDp-infected broad beans to evaluate acquisition efficiency and then transferred onto grapevines, broad beans and *C. vitalba* plants to determine transmission capability. *Ricania speculum* was able to acquire FDp, but not to transmit it to any of the inoculated plant species. The pathogen multiplication rate measured within *R. speculum* was significantly lower than that observed within the vector *Euscelidius variegatus*. Consistently with absence of transmission, the phytoplasma was almost never detected in salivary glands dissected from *R. speculum* individuals exposed to FDp. Under our conditions, the role of *R. speculum* in spreading FDp is unlikely to be of any concern. Nevertheless, population and spatial distribution of this alien planthopper are rapidly increasing, and therefore attention threshold in monitoring this pest across Europe should be maintained very high.

Genotyping by high-resolution melting of *Alternaria* species causing citrus brown spot

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Alternaria brown spot is one of the most important diseases of tangerines and their hybrids worldwide. To set up effective control strategy, the accurate detection and identification of the species responsible of the diseases is crucial. However, the characterization based on morphology and/or multilocus genetic approaches is time consuming, requires great expertise, and sometimes is not conclusive. Therefore, the setup of a rapid and efficient DNA-based assay might be of paramount importance. The High-Resolution Melting (HRM) analysis represents an interesting tool for the uncovering of nucleotide variations as small as one base difference, and as such, relevant to species characterization. In the present investigation, a HRM assay based on the *Alternaria* barcoding region OPA1-3 was set up. Specimen strains of the main citrus-associated *Alternaria* species and morphotypes generated distinct and normalized profiles, allowing their differentiation when HRM-tested. Moreover, when the assay was used to screen an *Alternaria* collection from citrus fruit and leaves, it distributed the 180 isolates in three independent clusters, readily and consistently resolved. Isolates were identified as belonging to the species *Alternaria alternata* and the species complex *A. arborescens*. Within *A. alternata,* the morphotypes *alternata* (77% of the collection) and *limoniasperae* (17% of the collection) were present. Although further validation experiments will be performed to optimize the assay for a diagnostic use, this HRM approach might represent a rapid, sensitive, and specific method for the detection and identification of *Alternaria* spp. responsible for citrus brown spot disease.

Pathogenicity and possible biological control of fungi associated to Botryosphaeria dieback of grapevine in western Sicily

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Botryosphaeria dieback is a widespread grapevine trunk disease caused by species of fungi within the family Botryosphaeriaceae. Symptoms of the disease include bud necrosis, leaf chlorosis, vascular discoloration of the wood, cankers and dieback. In 2013 typical symptoms of Botryosphaeria dieback was observed in western Sicily and four species associated with the syndrome were isolated and identified as *Diplodia seriata*, *Lasiodiplodia mediterranea*, *Neofusicoccum parvum* and *Neofusicoccum vitifusiforme*. In order to fulfill Koch's postulates, pathogenicity tests of eighteen isolates were performed on two-year grapevine cuttings of cv. 'Inzolia'. Six months after inoculation all the Botryosphaeriaceae isolates induced vascular discoloration of the wood with differences between the species and within the isolates. *D. seriata* B22 was the most virulent isolate showing the longest mean lesion length, significantly different from those caused by any other Botryosphaeriaceae isolates. The control of Botryosphaeria dieback is difficult and integrate pest management is necessary to reduce disease incidence. In recent years, the use of biocontrol agents has been proved a promising and valuable strategy for the control of plant diseases. *Bacillus amyloliquefaciens* AG1, isolated from grapevine wood tissue, has been reported to be effective against some fungi associated to grapevine trunk disease. In this study, the antimicrobial activity of *B. amyloliquefaciens* AG1 was evaluated by well diffusion assay against Botryosphaeriaceae fungi associated to Botryosphaeria dieback in western Sicily. Cell-free supernatant of *B. amyloliquefaciens* AG1 showed significant antifungal activity, inhibiting fungal growth of all tested isolates.

Efficacy of different fungicides against *Allophoma tropica*, the causal agent of a new leaf spot on lettuce: results from *in vitro* and *in planta* tests

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Phoma leaf spot, caused by *Allophoma tropica* (syn. *Phoma tropica*), was first observed on lettuce grown under plastic tunnel in northern Italy since 2011, causing significant yield loss. This study was carried out to test a number of fungicides in order to establish their ability to reduce the mycelial growth of *A. tropica* in an *in vitro* assay, and to control the pathogen in *in vivo* trials, on plants grown in pots under glasshouse conditions. The most effective fungicide *in vitro* was penconazole (ED_{50} of 1 to 3 mg/l and an MIC value of 30 mg/l). Fludioxonil, iprodione, boscalid and pyraclostrobin were found to be the most effective fungicide labelled on lettuce, in inhibiting the mycelial growth of *A. tropica*, even if differences have been observed among the four isolates tested *in vitro* using the last two products. In the presence of an average disease severity in the untreated control, the best disease severity reduction (up to 90%) was provided nine days after the application of fludioxonil, mancozeb, pyraclostrobin + boscalid, propiconazole and iprodione. Fludioxonil and iprodione showed a persistent effect (up to 90%) still being evident 12 days after the application. In the presence of a high disease severity in the untreated control, nine days after the application of fludioxonil, mancozeb, iprodione and pyraclostrobin + boscalid reduced disease severity up to 80%. Moreover, copper provided a disease severity reduction from 84 to 89%, applied as preventative treatment, resulting of special interest, particularly for crops grown under organic farming rules.

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Preventative treatments in nursery with resistant inducers, compost and biocontrol agents for the management of the Fusarium wilt of lettuce under glasshouse and field conditions

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Potassium phosphite, acibenzolar-S-methyl, green compost and biocontrol agents (*Bacillus subtilis* Qst713, *Trichoderma asperellum* + *T. gamsii* and *Pseudomonas* strains) were applied in a preventative way, in nursery, in experimental and commercial situations, to soils artificially or naturally infested with *F. oxysporum* f. sp. *lactucae* race 1, and to lettuce cultivars moderately susceptible to the pathogen. In general, the results of the different treatments in the naturally infested soil were similar to those observed in the glasshouse trials in the presence of artificial inoculation with the pathogen on ‘Volare’ and ‘Novelski’ lettuce cultivars. Potassium phosphite provided a consistent disease reduction (48 to 62% in the artificially infested soil and 60 to 75% in the naturally infested soil). The effects of adding 10% compost to a peat growing medium in the nursery, followed by a soil mixing application at lettuce transplanting, significantly reduced the Fusarium wilt (50-59% efficacy) and improved the fresh biomass production. When tested under field conditions, the commercially available *Trichoderma* spp. and *Bacillus subtilis,* together with experimental strains of *Pseudomonas*, applied at the nursery level, provided a disease reduction of 30 to 78%. The early application of the different control measures in the nursery conditions and at lettuce transplant can be considered interesting because carried out at a more localized level, with reduced amounts of products. However, their use in practice should be probably integrated with other control strategies.

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Land use and climate change trigger *Phytophthora* infestation in an amenity urban wood in Milan

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A plant-health survey was carried out to discover the cause of a widespread decline of trees in a park in the southwestern periphery of Milan. The park area had previously been used to grow Gramineae (including rice) but was forested starting in the seventies. Many single trees, especially maple (*Acer pseudoplatanus*) showed signs of stress, mostly attributed to drought. To counteract this problem, during the summer, groups of trees were watered, using the water that flows abundantly through the park. Declining trees exhibited extensive dieback of the branches, and sticky exudates oozing from the base of the trunks. When the bark was stripped off, large and conspicuous necrotic areas were observed. A serological test (Pocket Diagnostics) was positive for *Phytophthora* spp. Samples of positive tissue yielded in purity a hitherto unknown species of *Phytophthora*. This new taxon was named *P. acerina*, after the host-tree it came from. An examination of the roots of declining trees showed that the fine roots had been destroyed and their absorbent capacity impaired by the pathogen. Consequently, these trees were unable to absorb and exploit the water in the soil around them, even though it was available in abundance. It is considered that the conversion of the soil from agricultural to forest land, and also changes in climate, which have put the trees under stress and made them more susceptible to parasites, as well as the decision made to water large parts of the wood, were all factors in predisposing trees to *P. acerina* attack.

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Monitoring the spread of *Phytophthora* spp. in water bodies of green park areas of the Milan hinterland

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The recent discovery of a number of harmful *Phytophthorae* in parts of the Milan hinterland has prompted a study to determine the extent to which the water in this area has been contaminated by them. The water bodies of various areas in the outskirts of Milan were studied: the Parco Boscoincittà, the Parco dei Fontanili, the water purification plant 'Nord Milano', the Termovalorizzatore, the Giretta wood, and the Parco delle Cave. These areas were examined with the baiting technique and detected 85 *Phytophthora* isolates, identified by both conventional and molecular methods. Based on macro- and micro-morphological characteristics, the most representative isolates were selected and characterized by PCR-amplification of the ITS region of ribosomal DNA and of a fragment of the *coxI* gene of mitochondrial DNA. The amplicons were subjected to RFLP. The following species were identified: *P. acerina*, *P. megasperma*, *P. lacustris*, *P. inundata*, *P.* taxon *PgChlamydo* and *P. gonapodyides*. The discovery of these micro-organisms is all the more worrying as some of them are known to cause serious damage to plants. Some of these pathogens were found for the first time in the areas examined, and this seems to indicate that climate change is now favouring their dispersal. In view of the reproductive biology of the *Phytophthorae*, and the many ways in which these organisms are able to spread, it seems imperative constantly to monitor the areas found to be at risk, and also to take steps to limit the spread of these oomycetes as much as possible.

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Recycling agro-industrial by-products using beneficial microorganisms

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Olive pomace (OP) is a by-product from olive oil extraction that is difficult to dispose of, causes environmental and health concerns. Recycling of OP can reduce storage and disposal costs, plus provide the opportunity to generate additional income for farmers. The aim of this work was to test the ability of filamentous fungi to grow on OP, to reduce the phenolic and phytotoxic compounds, and develop applications for the recycled wastes. A preliminary screening of the fungal candidates was carried out *in vitro* to evaluate mycelial growth on OP at different concentrations (from 1 to 100% w/v). The most tolerant genera belonged to *Aspergillus*, *Clonostachys*, *Talaromyces*, *Beauveria* and *Trichoderma*. Generally, microbial growth was inhibited only at the highest OP concentrations; *Trichoderma* spp. and *A. niger* showed the greatest ability to grow on the OP substrates. Since *Trichoderma* is a potential biocontrol agent it was selected for further study. A shaker flask experiment, with 50% w/v OP (OP50), was performed to evaluate conidia production by *Trichoderma*, and the metabolic profiles of phenolic compounds in the *Trichoderma*-treated OP were analyzed by LC-MS qTOF. The effects of the fungal-treated OP were tested on the germination and growth of tomato both in *in vitro* and *in vivo* conditions. Preliminary results showed that OP50 treated with some *Trichoderma* strains (used at 10% w/v) improved seed germination over untreated OP50. Ongoing analysis of the biocontrol ability of the isolated fungi is being conducted with common plant pathogens. These findings may open new opportunities to re-use OP residues for agricultural purposes.

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Assessing the effects of different heavy metals on the development of poplar and willow clones: perspectives for dendroremediation

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

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

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Rice is an important crop for Italian agriculture, covering 230,000 ha and represents about 50% of the European production. Climate change is creating several problems in rice cultivation areas because of conditions prone to mycotoxin producing fungi. This could lead rice to have mycotoxin contamination higher than fixed levels by European Commission (Regulaments CE 1881/2006, UE 165/2010, UE 488/2014 and UE 1006/2015). For this reason, a study was planned taking into account nine different rice varieties cultivated in three different areas with three different sowing densities. Rice samples were collected four times along the growing season from milk stage to harvest. An additional sampling time was considered taking into account rice overstaying in field for other 15 days after harvest time. Rice samples were analysed for fungal incidence, in particular for *Fusarium* spp. and *Aspergillus* spp., and for AFB_1, DON and sterigmatocystin (STC), a mycotoxin recently signalled in rice, using, respectively, HPLC-FLD, GC-MS and LC-MS/MS. *Fusarium* spp. were the most represented fungal species found in rice (mean incidence from 0 to 19%) and were found mainly at harvest. *Aspergillus* spp. were found with low incidence, in particular *A.* section *Flavi* (found mainly at milk stage), *A.* section *Nigri* (found mainly at harvest) and *A. versicolor* (found from milk stage to harvest and even able to increase in samples left in field after harvest time). Mycotoxins tested resulted always below legal limits, however STC resulted >0.1 µg/kg in some samples. No differences were among the three sowing densities considered.

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

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The *Fusarium fujikuroi* species complex (FFSC) is one of the largest *Fusarium* complexes. Phylogenetic and molecular analyses show a close relationship within the FFSC species; they may have distinct phenotypic traits, like mycotoxin production, host-specificity and supernumerary chromosomes (SCs) in addition to species-specific core chromosomes that may even differ among isolates in presence/absence, length and gene-abundance. SCs are important in the biology of pathogenic fungi. In a previous study, adopting a bioinformatic approach, we ascertained the presence of "extra" genomic regions (EGRs), a putative SC, in the genome of an Italian *F. verticillioides* isolate ITEM10027, which lacked in the *Fv*. reference genome *Fv*7600. For assessing the putative peculiarity of this EGRs within the Italian fungal strains, we collected *Fv* samples from maize kernels sampled in the whole Po valley, Northern Italy. To select a subset of strains, we analyzed the EGRs presence by a PCR approach (presence/absence). The genomes of 24 strains were sequenced by Illumina MiSeq. A bioinformatic pipeline able of highlighting *inter* and *intra* specific differences within the EGRs among the 24 samples was designed. An interesting set of genes with a Gene Ontology differing among the 24 *Fv* strains were found. *In vivo* pathogenicity assays on *Zea mays* kernels indicated significant difference among the 24 samples. We can argue that some of the unique and specific genes found in the 24 strains may provide a sort of "gain of function" in the pathogenicity toolkit that can possibly explain the difference emerged in the pathogenicity assays *in planta.*

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

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

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

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Bois noir (BN) is induced by '*Candidatus* Phytoplasma solani' ('*Ca*. P. solani'), and represents an important grapevine yellows disease that causes severe damage to vineyards in a number of countries. In Iran, BN was recorded recently, but there is no information available on the molecular types and their epidemiology. Investigations were carried out from 2015 to 2017 from July to September in the main grapevine-growing regions of Iran. Red and white berry cultivars were observed during the surveys, and leaf samples were collected from vines showing reddening or yellowing of the leaves, leaf rolling, flower drying, shriveled bunches, and/or small black pustules along the canes. After total DNA extraction, amplification of 16SrDNA was carried out. Most of the symptomatic samples were positive using the universal primer R16F2n/R2. Infection from '*Ca*. P. solani' was confirmed using the specific primers for stolbur detection, fStol/rStol. Amplification of the *tuf* and *vmp*1 genes was carried out, followed by restriction fragment length polymorphism analysis, to gain more detailed insight into the molecular diversity of these '*Ca*. P. solani' strains associated with grapevines. All of the tested samples belonged to *tuf*-type b. The restriction fragment length polymorphism analysis of *vmp*1 allowed five different *vmp*1-types to be defined, with V10 as the most prevalent. These molecular data will contribute to better understanding of the epidemiology of grapevine BN in Iran.

The defense mechanism against *Meloidogyne javanica* is induced by beneficial microorganisms combined with chemicals in *Solanum lycopersicum*

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Root-knot nematodes (RKNs) *Meloidogyne* spp. are considered to be the most damaging pests of a broad range of cultivated crops. Control of RKN using conventional methods is difficult because of its wide host range and the low inherent resistance of hosts against this nematode. Instead of the use of nematicides, host resistance to this nematode can be induced by application of abiotic and/or biotic agents, which results in broad-spectrum responses. In this study, the defense response was investigated using quantitative real-time PCR (RT-qPCR) in the Early Urbana tomato (*Solanum lycopersicum*) cultivar that is susceptible to *Meloidogyne javanica*, following treatments with three beneficial microorganisms (root drenching) combined with chemical treatments (foliar application) that were previously identified as effective against *M. javanica*. Tomato root samples were collected and analyzed at 0, 24, 48, 72 h post-inoculation (hpi) with the following treatments: *Trichoderma harzianum* combined with hexanoic acid (TH+HX); *Pseudomonas fluorescens* combined with 10 mM HX (PF+HX); TH combined with 20 mM riboflavin (TH+RF); TH combined with 20 mM thiamine (TH+TI); nematode inoculation (positive control); and water inoculation (negative control). The expression of pathogen-related protein 1, phenylalanine ammonia-lyase, proteinase inhibitor 1, salicylic acid-binding protein 2, and the peroxidase genes increased over time. The elicitors tested showed gene transcript accumulation that was significantly higher than that induced by the pathogen in the compatible interaction (positive control). These data demonstrate that the plant responses to compatible interactions and elicited by treatments are qualitatively similar, but quantitatively different.

Identification of pathogenic fungi in rotten walnut fruits from different plantations in Hungary

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The walnut production value is approximately 5.1% of the fruit production in Hungary. The production area and the yield have increased considerably and steadily over the past five years. Therefore, walnut is one of the most dynamically developing segments of the domestic fruit industry. New pathogens and pests that threaten walnut production have emerged in domestic plantations as a result of climate change in Eastern-Europe, and of imported planting material. The aims of this study were: (i) to isolate and identify pathogenic fungi from rotten walnuts collected in Hungarian orchards; (ii) to examine their fungicide sensitivity. Pathogenic fungi were isolated from different international (cvs 'Fernor', 'Pedro') and national (cvs 'Milotai 10', 'Milotai bőtermő', 'Milotai kései', 'Alsószentiváni 117', 'Tiszacsécsi 83') walnut cultivars. *Diplodia* sp. and *Diaporthe* sp. were isolated and identified in the highest proportion from rotten walnuts during 2015, 2016 and 2017. *Diaporthe* sp., *Fusarium* sp. and *Diplodia seriata* were identified based on morphology and molecular markers. The preliminary results of the fungicide sensitivity tests showed that Folicur Solo and the Manzato 75 DF, containing tebuconazole and mancozeb inhibited most effectively mycelial growth. However, further studies are necessary to develop an efficient plant protection technology against the detected pathogen populations.

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Whole genome sequence and annotation of the brown rot fungal pathogen *Monilinia fructigena*

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Monilinia fructigena, *M. laxa* and *M. fructicola* are examples of the several apothecial ascomycetes that can cause brown rot and blossom blight in pome-fruit and stone-fruit trees. Worldwide, these result in serious economic damage and losses for crops of the family *Rosaceae*. Here, we describe the sequencing and assembly of the *M. fructigena* strain Mfrg269. A hybrid assembly strategy was applied using a combination of 2× 92-bp paired-end reads (Illumina Sequencing Technology; HiScanSQ platform; SELGE Network Sequencing Service, Bari, Italy) and long 20-kb reads (Pacific Biosciences Sequencing Technology; PacBio RSII platform; Macrogen Inc., NGS Service, Geumcheon-gu, Seoul, South Korea). A final assembly of 131 scaffolds that corresponded to a total size of 43.125 Mb, and ~210× sequencing coverage. In more detail, this defined 42.05% GC content, N50 scaffold length 767.732 kb, scaffold L50 of 20, and maximum scaffold size of 1,863,841 bp. About 83% of the RNA-Seq reads mapped on the draft version of the final genome. Functional annotation data were obtained using the Augustus software implemented in the BLAST2GO PRO package (v.4.1.9), using *Botrytis cinerea* as the model species and the RNA-Seq reads as the guide. This predicted 10,511 genes, with 10,811 transcripts that coded for 9,970 predicted proteins that were functionally annotated. This genome sequencing of *M. fructigena*, as well as for other *Monilinia* spp. involved in brown rot diseases, will serve as a valuable resource for studies on populations biology and plant–pathogen interactions.

Survey of the presence of ochratoxigenic fungi in vineyards in northern Dalmatia

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The long tradition of wine production in Dalmatia started during the Roman Empire. Today in northern part of Dalmatia the production of high quality wine is becoming ever more important activity. Due to the geographic position and climatic conditions, one of the possible treats for wine industry in the region is contamination with ochratoxin A (OTA). To evaluate the risk and plan the appropriate prevention, in June and early September of 2016 and 2017 were taken samples of soil as well as of leafs and buries of red grapes (variety 'Merlot') in four vineyards, different for position, cultivation methods, microclimatic and pedological conditions, in Zadar county. From the samples, the present mycoflora was isolated and morphologically determined. All potential OTA producers, black and yellow *Aspergilli*, were tested for the production of the toxin. The ochratoxigenic strains were isolated in all four vineyards, but their occurrence was low. From 4256 isolated fungal species, about 10% were determined as potential OTA producers and only 0.7% of total strains resulted ochratoxigenic. There were no significant differences in results among the vineyards. All confirmed producers were determined as *Aspergillus carbonarius* (Bainier) Thom. Even if the presence of ochratoxigenic strains in vineyards was confirmed, the analysis of most and wine produced in those vineyards in those years showed no traces of OTA. The results indicate that, to maintain the OTA free wine, the grape growers and wine producers in the region should pay attention and apply all the appropriate measures to prevent the phenomenon.

Effects of copper and biostimulants used for grapevine downy mildew control on grape amino-acid content

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The long-term use of copper-based fungicides against grapevine downy mildew (GDM) has led to an accumulation of this heavy metal in vineyard topsoils across many European countries. This promotes negative consequences for the agroecosystem, including decreased earthworm populations, microbiological and enzymatic alterations, lowered soil pH, and reduced grapevine growth. As a consequence, in organic agriculture, the use of copper fungicides is currently restricted by European Regulation 473/2002. Several strategies have been developed to reduce GDM symptoms, including the use of biostimulants or resistance inducers, which include chitosan and laminarin applied alone or in combination, or alternating with copper-based formulations. However, there is little information available regarding the effects of their applications on the amino-acid composition of grape must. The aim of this study was to evaluate the effects on grape amino-acid composition of weekly foliar applications to cv. 'Montepulciano' grapevines of laminarin with *Saccharomyces* spp. extracts (LamE) and chitosan, applied individually or in combination with copper formulations, compared with two copper products and an untreated control. The results showed that all of the treatments affected the concentrations of several amino acids. Copper-based formulations significantly decreased the concentrations of several free amino acids, especially when applied individually. The least effect on the amino-acid composition of the must was after application of LamE, individually or alternating with copper hydroxide. These results provide further information on the negative effects of copper on grape quality, which can be reduced when copper is applied in a strategy that includes the use of LamE or chitosan.

First survey on *Moniliniae* affecting stone fruits in the Marche region

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Brown rot is the most important disease of stone fruit in warm and humid climates. These conditions can induce blossom blight and brown rot, with the latter developing both in the field and even more so during storage, transport and shelf life. The casual agents are four fungal species of the genus *Monilinia*: *Monilinia fructicola*, *Monilinia laxa*, *Monilinia fructigena*, and *Monilinia polystroma*. Other species such as *Monilinia mumecola* and *Monilinia yunnanensis* have also been reported as pathogens recently. In this study, a survey was carried out during spring of 2018 across several stone-fruit orchards in the Marche region, and in particular in Valdaso and Valle del Foglia, to identify the species responsible for brown rot. Samples were taken from infected fruit and twigs, and total DNA was extracted using the cetyltrimethylammonium bromide method. Molecular identification was performed using the common reverse primer MO368-5 and three forward primers: MO368-8R (specific for *M. fructigena* and *M. polystroma*), MO368-10R (specific for *M. fructicola*), and Laxa-R2 (specific for *M. laxa*). The amplified products were electrophoresed through 1.5% agarose gels in 1× TBE buffer, stained with GelRed dye, and visualized under ultraviolet light. The most common species recorded in this first survey on fruit and twigs were *M. laxa* and, to a lower extent, *M. fructicola*. Considering the different virulences of these species, knowledge about the presence of *Moniliniae* is essential for planning appropriate disease management strategies. Further investigations are required to explore the frequency of occurrence of each species and the resistance to the most commonly used fungicides.

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Use of chitosan and other natural compounds to control grapevine downy mildew

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In recent decades, the most widely used technique to control grapevine downy mildew (GDM) has been the application of copper compounds. As these have often been applied at up to 8 kg/ha/year, especially in organic vineyards, this has led to the accumulation of this heavy metal in the topsoil, with negative consequences on the agroecosystem. Consequently, the use of copper fungicides in organic agriculture has been restricted to 6 kg/ha/year by Regulation EU 473/2002. Some studies have identified natural compounds that can be used to reduce or eliminate copper-based formulations that have shown interesting qualities in terms of GDM control, such as chitosan and laminarin. In a first set of trials, applications of chitosan and of a mixture of laminarin and *Saccharomyces* spp. extracts significantly reduced GDM symptoms. In following trials, these compounds were applied throughout the season alone or under three different strategies with copper hydroxide, which halved copper use (3 kg/ha/year) compared to the EU threshold. Over the years of these investigations, good control of GDM was obtained especially with chitosan, when applied both alone and in a strategy with copper hydroxide. In the experimental trials, chitosan alone was as effective as the copper formulations, which indicates the possibility for the control of GDM even without copper application. Chitosan hydrochloride has been approved as a basic substance for use as a plant protection product, by Regulation EU 563/2014. Now grapevine growers have new tools to manage GDM. However, these data obtained in experimental trials need to be confirmed with larger scale applications.

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A bizarre endophytic *Fusarium* strain

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The *Fusarium* strain A1021B is an endophyte isolated from *Euonymus europaeus* (collected at the Astroni Natural Reserve in Naples, Italy). It has been assigned to the *Fusarium incarnatum-equiseti* species complex (FIESC) after a preliminary assessment by the identification service of the Fungal Biodiversity Centre (Utrecht, the Netherlands). The production of secondary metabolites (SMs) in Czapek-Dox broth (CDB) and Potato Dextrose Broth (PDB) by strain A1021B was investigated using a metabolomic approach. LC-MS qTOF analysis revealed that fusarinol, a derivative of the mycotoxin fusaric acid (FA), was the main SM produced in CDB, whereas FA was the most abundant compound found in the PDB filtrate. This result was in contrast with the literature reporting that FIESC strains are known producers of trichothecenes, while FA has been detected only in few cases. In order to evaluate if A1021B was able to produce trichothecenes, the presence of six genes involved in trichotecene biosynthesis (TRI-1, TRI-4, TRI-5, TRI-8, TRI-11 and Gene F) was confirmed by PCR, and the production of these compounds in a substrate of maize kernels was also analyzed. Interestingly, the cultivation of the fungus in the presence of plant material, in the dark, enhanced the production of the trichothecene toxin T-2. The phylogenetic analyses of A1021B in the FIESC complex are ongoing. These data indicate that this endophyte represents an unusual case of a *Fusarium* strain that produces both FA and trichothecenes, and whose biosynthesis is affected by growth conditions.

WRKY transcription factors, oxidative protection and signalling in *Salvia officinalis* plants exposed to ozone

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Tropospheric ozone (O_3) is a global air pollutant that causes negative effects on plant life. The objective of this work is to give a characterization of the signalling pathway activated in response to O_3 and to elucidate the potential role(s) of *WRKY* transcription factors in regulating oxidative protection and signalling in *Salvia officinalis* plants exposed to O_3 (200 ppb, 5 h) in controlled environmental conditions. At the end of the treatment, leaves appeared symptomless. During the treatment and throughout the recovery period an alteration of membrane integrity was observed as confirmed by the marked increase of the content of thiobarbituric acid. An accumulation of hydrogen peroxide and a concomitant reduction of anion superoxide was detected, confirming that an oxidative burst had occurred. O_3 triggered increases in phytohormones content already after 1 h of exposure. This seems to demonstrate that such signaling molecules (i.e. salycilic and jasmonic acids) could be considered a fast response to O_3-perception. To identify *WRKY* transcriptional factors involved in the oxidative burst, BLASTX analysis against the current assembly of the *S. miltiorrhiza* sequence genome was performed using *Arabidopsis* WRKY protein sequences as queries. A total of six gene sequences were predicted for sage *WRKYs*. The relative gene expression analyses of *WRKY* were assessed by qRT-PCR. *WRKYs* were induced by O_3 at different time-points. No significant expression level changes were observed during the recovery period. This suggests that *WRKYs* should be considered important players in the complex signalling processes during plant O_3 responses.

Monitoring the presence of *Cryphonectria parasitica* in Azerbaijan chestnuts

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The ascomycete *Cryphonectria parasitica* (Murrill.) M.E. Barr. is the causal agent of chestnut blight which resulted in death of American and European chestnuts. The transmissible hypovirulence, due to the infection of mycovirus of the family of Hypoviridae, has been reported to reduce the parasitic growth and sporulation capacity of *C. parasitica*, acting as a natural biocontrol agent. *C. parasitica* was first reported in Azerbaijan in 2003. Here we present the first results of a field campaign performed in four districts in the north part of Azerbaijanaimed at identifying the possible presence of mycovirus. Twenty fungal samples were collected in each Gabala, Sheki, Zaqatala and six in Quba districts. After visual characterization, about 45 isolates were identified as *C. parasitica*, and the identification was confirmed by PCR barcode analyses. Total RNA was extracted and PCR analysis was performed. Data showed the presence of dsRNA in several isolates from all the four sampled areas. The sequence of Azerbaijan dsRNA is highly homologous to that of European one, suggesting that the mycovirus could be spread from Europe to Asia. Interestingly, native gel electrophoresis followed by in-gel staining, showed a negative correlation between the activity of laccase, an enzyme involved in the growth and virulence of fungi and dsRNA presence, opening to the use of this enzyme as virulence biomarker. Overall, our results open a possible way for the biocontrol of chestnut blight in Azerbaijan based on spread the hypovirulent strains of the fungus as it was done in many European countries.

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Genetic diversity of mycoflora occurring on olive trees in Salento areas endemically affected by *Xylella fastidiosa* sub-species *pauca*

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Olive (*Olea europea* L. var. *sativa*) is a typical and traditional plant of Mediterranean area. In southern Italy, olive represents an economically important tree crop and, at the same time, a natural landscapes, since millennial trees fill the countryside view. In some cases, agronomic techniques as irrigation, high nitrogen fertilization, intensive cultivation, and no-tillage could induce micro-climatic and environmental conditions suitable for the development of fungal pathogens on olive trees. Moreover, in the last years the quarantine pathogen *Xylella fastidiosa* is causing the Quick Decline Syndrome of olive trees in wide areas of Salento, with a rapid dieback of twigs and branches followed by death of the entire tree. The co-occurrence of fungal pathogens together with *X. fastidiosa* has been reported as con-cause of the olive decline. We have been isolating two hundred ninety-five fungal strains from trunks, branches, twigs, bark and root of both symptomatic and asymptomatic olive trees, grown in Salento. Based on similar morphological traits, we grouped the fungal strains and 75 representative ones were molecularly characterized by sequencing a partial region of the two informative *ß-tubulin* and *actin* genes. Blast analysis assigned the fungal strains to 15 genera, most of which include species associated to olive tree dieback: *Alternaria*, *Arthrinium*, *Chaetomium*, *Cladosporium*, *Colletotricum*, *Diplodia*, *Epicoccum*, *Fusarium*, *Neofusicoccum*, *Paraconiothyrium*, *Phaeoacremonium*, *Phoma*, *Phomopsis*, *Pleurostomophora*, *Trichoderma*. Further analyses, including the sequencing of ITS region, are currently in progress to identify all strains at species level and assess phylogenetic analyses according with a multi-locus gene sequence approach.

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Young students and plant health: bringing to the light the environmental protection issues

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Young students can act, even if with modest impact, as multipliers for the environmental education activities, transmitting the knowledge acquired about environmental issues to their grandparents, parents and peers. In order to achieve its objectives, the research must open its doors to both children and teachers, giving them the opportunity to directly observe the activities developed by the researchers in up-to-date facilities. A work-related learning national programme helps research centres and departments to involve secondary schools and improve their awareness regarding plant health. More students needed to approach scientific disciplines, often seen as difficult and not entertaining. Particularly, initiatives for attracting more women in science, with special reference to plant pathology, must be mentioned. Plant health is a key factor to reduce hunger and rural poverty. Over the last years, about 3,000 children were educated and 200 young students from secondary schools spent their work-related learning programme in the field of plant pathology. At Agroinnova the experience gained with them convinced us that it is possible to communicate, with the adequate language and activities, the research done in an agricultural research centre to a young audience. The research community at large, including plant pathology, is facing more challenges with limited human resources and funding available. Young people are, in general, capable of understanding the objectives and motivations of the research. They help to bring to the light some misconceptions about agriculture that could otherwise pass unnoticed.

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Influence of CO_2 and temperature on the *Blumeria graminis* f. sp. *tritici/*wheat pathosystem

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The combined effect of different temperatures and CO_2 levels was estimated on the *Blumeria graminis* f. sp. *tritici* (*Bgt*)/wheat pathosystem. *Bgt*-inoculated and healthy plants were exposed under phytotron conditions to six CO_2 and temperature combinations: (1) 450 ppm CO_2 (ambient CO_2) + 18-22 °C (low temperature), (2) 850 ppm CO_2 (elevated CO_2) + 18-22 °C (low temperature), (3) 450 ppm CO_2 (ambient CO_2) + 22-26 °C (medium temperature), (4) 850 ppm CO_2 (elevated CO_2) + 22-26 °C (medium temperature), (5) 450 ppm CO_2 (ambient CO_2) + 26-30 °C (high temperature), and (6) 850 ppm CO_2 (elevated CO_2) + 26-30 °C (high temperature). *Bgt*/wheat pathosystem was evaluated by measurement of different fungal and plant parameters such as disease index, fungal DNA quantity, pathogenesis-related (PR) gene expression, plant death incidence, chlorophyll and carbohydrate content. Powdery mildew progress was influenced significantly by both CO_2 and temperature, and their interaction. The most favorable conditions for the powdery mildew development on wheat were low temperature and ambient CO_2. Although high CO_2 did not favor disease development, it affected the plant vitality. Pathogen growth was strongly inhibited by elevated temperatures with both CO_2 conditions, and typical disease symptoms could not be observed. The PR transcripts showed different levels of expression between six phytotron conditions. Real-time PCR quantification permitted a sensitive pathogen detection at the early stages of the disease. This study suggested that Mediterranean area with average warming increase might result in lower incidence of wheat powdery mildew, unless the pathogen would adapt to elevated temperatures.

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Pythium spp. in vegetable crops: genetic diversity and sensitivity to mefenoxam and azoxystrobin

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Recently, severe yield losses caused by *Pythium* spp. were observed in Italy on lettuce, lamb's lettuce, spinach, and bean crops. *Pythium* diseases are mainly controlled by two fungicides classes, phenlyamides and QoIs, with mefenoxam and azoxystrobin as representatives, respectively. The present study aimed to estimate the sensitivity profiles of six *Pythium* species to mefenoxam and azoxystrobin, and their genetic diversity by ITS, RNA polymerase I and cytochrome b gene sequences. For mefenoxam, the inter-species sensitivity was quite variable and many resistant isolates were observed in all *Pythium* species, but without RNA polymerase I polymorphisms. For azoxystrobin, inter- and intra-species sensitivity was quite stable, with exception of one *Pythium paroecandrum* isolate, which showed reduced sensitivity and few cytochrome b polymorphisms. Based on ITS and cytochrome b phylogenetic analyses, *Pythium* species were divided in globose- and filamentous-sporangia clusters. This study evidenced the importance of well-structured chemical control strategies adapted to each *Pythium* species. For efficient use of mefenoxam, previous identification and sensitivity assays of *Pythium* species are necessary due to its quite variable intrinsic activity and possible presence of resistant isolates. Use of products with alternative modes of action and mixture of products may additionally support a delay in resistance development. The rather stable intrinsic activity of azoxystrobin among species and absence of resistant isolates may allow its application without species differentiation as long as it is applied preventatively to control fast-growing aggressive isolates and at sufficient rates to control less sensitive isolates.

The research that has led to these results has received funding from the European Union Horizon 2020 research and innovation program under grant agreement number 634179 "Effective Management of Pests and Harmful Alien Species-Integrated Solutions" (EMPHASIS).

Preharvest treatments modified surface molds population and the shelf life of tart cherry (*Prunus cerasus* L.)

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The economic loss of fruit is estimated to be around 20-25%, mainly due to postharvest diseases. In the case of cherry (*Prunus avium*) and tart cherry (*Prunus cerasus*), half of the lost was estimated to be caused by molds. Preharvest fungicide application was shown to be effective against fungal decay of different stone fruits during storage. Fluopiram was applied two weeks before harvest, and its efficiency was examined on three Hungarian cultivars ('Érdi bőtermő', 'Újfehértói fürtös' and 'Petri'). The CFU and the morphology-based identification of molds were done after harvest. Molds were isolated from rotten fruits during shelf life and identified based on morphological and molecular characters. Disease incidence (DI) was also determined at 20 °C. These observations were repeated after six weeks storage at 0-2 °C, in normal and modified (MAP) atmospheres. Surface mold contamination of fruits was higher at harvest than after six-week cold storage. Similar mold species were isolated from different cultivars, and storage conditions did not affect this finding. Fluopiram applications reduced both the number of molds and rotten fruits. However, its positive effect on the shelf life was not so clear after the storage. Modified atmosphere packaging could decrease rotting in some cases.

Postharvest fungal diseases of pomegranate fruit in southern Italy

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Pomegranate (*Punica granatum* L.) is an increasingly important crop in Apulia, where most of the Italian production occurs. The main yield problem is related to postharvest losses caused by fungi. The present research was conducted using fruits of various cultivars (Acco, Mollar de Elche, Wonderful, and Wonderful One), from local markets, orchards, and packinghouses located in southern Italy. The main fungi observed on stored fruit were *Botrytis cinerea*, *Pilidiella granati*, *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp. (*sensu lato*), *Colletotrichum acutatum s.s.,* and *Cytospora punicae*. The first three pathogens infect the fruits through the calyx area during blossom, then spread to the entire fruit. Moreover, *B. cinerea* was responsible for harmful latent infections, developing during cold storage, which was a critical stage also for *C. acutatum*. Two different species of *Alternaria*, causing "black heart" or "black spot", were identified: *A. alternata* and *A. arborescens*. *Aspergillus* species belonged to section *nigri,* which includes putative toxicogenic species. The main *Penicillium sensu stricto* species observed were *P. glabrum*, *P. adametzioides,* and *P. brevicompactum*. Few isolates of *Talaromyces albobiverticillius* were also present. These two genera, sharing very similar macroscopic features, were mainly present on decayed stamens and wounds. In the present study, *P. granati* and *C. punicae*, mostly reported as etiological agents of pomegranate trunk canker, were isolated from fruits. The macro- and micro-morphological aspects and the molecular characterization of the pathogens allowed species identification. Pathogen identification might contribute to control postharvest rots and reduce the related economic losses.

Incidence and genotype prevalence of citrus tristeza virus in Campania region, southern Italy

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A large-scale survey to monitor virus incidence of citrus tristeza virus (CTV) was carried out in 2016-2018 in Campania, southern Italy. The direct tissue blot immunoassay (DTBIA) indicated that 13.8% of samples collected from citrus fields (orchards and private gardens) and 3.2% from garden centers were infected with CTV. No infected trees were detected in the collection of lemon mother trees located at CRAA-Improsta farm of Campania Region. Since CTV isolates may differ in their pathogenicity based on genotype composition, fourteen CTV isolates recovered in Campania were characterized by molecular approaches including real time RTqPCR, Multiple Molecular Markers analysis (MMM) and sequence analysis of 5'end of genomic region (RdRp). Results showed that the CTV isolates spreading in Campania contain genotypes categorized as T30, VT and T3. The finding of the latter two genotypes further confirms the occurrence in different Italian citrus-growing areas of isolates harboring potentially severe strains of the virus, as previously reported in Calabria and Sicily. Conversely, genotypes T68, T36 and those overcoming the resistance of *Poncirus trifoliata* resistance-breaking isolates (RB) were not detected.

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In vitro efficacy tests against *Fusarium* spp. causing garlic dry rot

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Garlic is cultivated worldwide and, according to FAOSTAT, in 2016 1.5 million ha were given over to this crop. Starting from 2002, *Fusarium proliferatum* was identified as the main causal agent of garlic dry rot during storage, but with infections starting during the growing season. Seed sanitation seems crucial to prevent pathogen spread. Thus, this study was designed in view of preparing guidelines for farmers to prevent/control dry rot in the field and during storage. *Fusarium proliferatum* (MPVPG29) recovered from garlic during the 2017 growing season was used in *in vitro* trials. Six fungicides were considered, all including triazoles alone or in mixture, and four competitor/antagonist biocontrol agents (BCA). i.e. *Trichoderma gamsii*, *Fusarium oxysporum* IF23, *Bacillus subtilis*, *Streptomyces griseoviridis* K61. Media spiked for chemicals (four concentrations, 1-1000 ppm) and dual cultures for BCAs were used. Inoculated dishes were incubated at three temperatures (10-15-25 °C) and the percentage of growth inhibition was recorded after 7-14-21 days. All chemical products were able to stop fungal growth at 100 ppm concentrations. Procloraz+propiconazole showed a 94% reduction followed by tebuconazole (57% reduction) even at 1 ppm. Regarding BCAs, *T. gamsii* was the most effective in reducing *F. proliferatum* growth (mean reduction = 84%), followed by *S. griseoviridis* (mean reduction = 60%). Results are very promising and a field trial is ongoing to verify the performances of these BCAs in garlic crops.

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Trichoderma **spp. differ in volatilome profile: are these differences detectable also *in vivo*?**

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Volatile organic compounds (VOCs) are a small portion of the total metabolites produced by the organisms, and their unique properties enable them to mediate important ecological interactions, including those between fungi and plants. In particular, fungal VOCs may be beneficial to plants by activating defense responses and priming them against pathogen attack, as well as by providing growth promotion. The potential utility of fungal VOCs in agriculture is therefore evident, with a growing interest in identifying the specific compounds within the blend of fungal VOCs. The aim of this work is to detect volatile molecular markers of different *Trichoderma* species, in order to define diagnostic VOC profile patterns to identify *Trichoderma* spp. directly *in vivo*, in the soil. A challenge is to investigate soil fungal communities by studying their VOC profile, avoiding extraction steps. For this purpose, we used the PTR-Qi-TOF-MS technique to first analyze VOCs headspace of four *Trichoderma* isolates (*T. harzianum* T22, *T. asperellum* B6, *T. longibrachiatum* MK1 and *T. atroviride* P1) and successively to perform analyses on soil inoculated with the four studied strains. PTR-TOF is based on an innovative, rapid and highly sensitive VOCs detection method that combines a high resolution time-of-flight mass spectrometer (TOF-MS) with a PTR ion source. Preliminary results showed quali-quantitative differences in volatomes of *Trichoderma* spp. analyzed: in particular, the most abundant volatile compounds were ethyl acetate in *T. longibrachiatum* MK1, and 6-PP in *T. asperellum* B6 and *T. atroviride* P1; two volatile compounds styrene and sesquiterpenes were instead present in all the analyzed isolates.

Molecular identification of '*Candidatus* Phytoplasma prunorum' and '*Candidatus* Phytoplasma solani' associated to stone fruit yellows in Marche region

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In the Marche Region, stone fruit production is particularly important in Valdaso valley, where mainly peaches, apricots and plums are grown. Over the last years, these crops have been affected by several phytosanitary problems, and a program aiming to reduce of pesticide applications (AAA Valdaso) involved most of growers of the valley. European stone fruit yellows, induced by '*Candidatus* Phytoplasma prunorum', has frequently been detected in northern Italy and sporadically in central and southern Italy. The aim of our study was the molecular identification of phytoplasma associated with plum, apricot and peach yellows from plants with severe yellows symptoms in Valdaso and surrounding areas. Forty symptomatic leaf samples were collected from Japanese plum (25), apricot (6) and peach (9) plants and their total DNA was extracted. Seven peach samples were infected by '*Candidatus* Phytoplasma solani', while 7 apricot and 17 plum (cv 'October Sun') samples were infected by '*Ca*. P. prunorum'. From The BLASTn analysis carried out on selected peach, apricot and plum samples identified a high sequence homology with '*Ca*. P. solani' AM933139 (99.0%) and '*Ca*. P. prunorum' AM933142 (99.6%), respectively, as available in the GenBank database. The infection of plum and apricot with '*Ca*. P. prunorum' is common in all areas where the stone fruits are grown. Whereas, the presence of '*Ca*. P. solani' in peach can be ascribed to the presence of the phytoplasma in the weeds of the area, that usually is transmitted to grapevine inducing Bois noir, and occasionally can be transmitted to peaches.

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Oak decline in Marche region: monitoring of sanitary status in the Selva di Castelfidardo

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Over the past few decades, oak decline has been associated to climate change, which has been characterized by reduced rainfall and increased temperatures. In 2015, the sanitary status of Selva di Castelfidardo (SIC IT5320008) was assessed during the summer. Here, 561 plants of genus *Quercus* were monitored, with their geographical positions recorded using GPS tools, and reported in a map (Google Earth). Some plants (7%) showed emission of black exudates, with necrotic tissue found under the bark. About 50% of the plants showed excessive proliferation of shoots along the trunk. For 32% of these *Quercus*, charcoal cankers were recorded, as caused by *Biscogniauxia mediterranea*. For both the healthy and symptomatic plants, the charcoal canker diameters were recorded and the plants were divided into 12 diameter classes. Most of the plants with charcoal canker symptoms showed diameters from 10 to 30 cm (mean diameter, 15 cm), while the healthy plants showed diameters from 20 to 30 cm (mean diameter, 26 cm). The high density of plants of the same age was due to absence of any silvicultural operations since 1960. This caused great competition for space, light, and nutrient uptake, and hence conditions of stress for the plants. These effects were amplified by the environmental stress conditions, which increased susceptibility to *B. mediterranea*, a pathogen that is considered to be a secondary invader or an opportunistic fungal pathogen. These preliminary data are essential for identification of inoculum sources and for application of management plans to mitigate these stress factors.

Chestnut blight in the Marche region (central-eastern Italy)

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Chestnut blight is caused by the fungus *Cryphonectria parasitica*, and represents one of the most ecologically important diseases of *Castanea* spp., as it can rapidly kill the trees. In Europe, mitigation of the disease severity occurred spontaneously through the CHV-1 mycoviruses, which reduced the virulence of the fungus. From 2013 to 2016, surveys were carried out in the provinces of Ascoli Piceno and Fermo, where the production of chestnut was drastically reduced and the incidence of chestnut blight ranged from 20% to 60%. The severity of the symptoms appears to be related to the age of the plants, and mostly to the silvicultural practices adopted. In some situations, the severity of the decline of chestnut is related to infestation by the chestnut gall wasp. *C. parasitica* was isolated and identified in about 90% of the bark samples collected during the surveys. The virulent/ hypovirulent *C. parasitica* phenotypes were determined through analysis of the morphological properties and pathogenicity tests. A number of hypovirulent strains were isolated that belonged to four main vegetative incompatibility genotypes that correspond to the vegetative compatibility groups EU12, EU17, EU5, and EU2. DsRNA of CHV-1 was extracted from representative hypovirulent isolates as mainly responsible for the hypovirulence, and then sequenced using next-generation sequencing methods with the Illumina platform. These records of the hypovirulent *C. parasitica* isolates, the results of the vegetative compatibility tests, and the genome sequence of CHV-1 are essential for the future management of this chestnut disease in the Marche region using biological control methods.

Spatial analysis as a tool to monitor recovery in Bois noir infected vineyards of cv. 'Chardonnay'

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In Europe, one of the most recurrent grapevine yellows is Bois noir (BN), which has becoming a seriously limiting factor for production targets. Infected vines can show spontaneous disappearance of disease symptoms, which is known as 'recovery'. Knowledge of the recovery rate is basic for decisions to be made about future management of a vineyard, as if the number of symptomatic plants is high, the grower can evaluate the need to uproot the vineyard. In a commercial vineyard of cv. 'Chardonnay' grafted on Kober 5BB (*Vitis berlandieri* × *V. riparia*) rootstock established in 2004, surveys have been carried out over last 7 years (2009 to 2015) to evaluate the spread of BN symptomatic plants over time. For each survey, bidimensional maps were constructed using the SYSTAT program, with the plotting of symptomatic and symptomless/ healthy vines, to spatially monitor the epidemiology of the BN. In 2009, 30% of the plants (491/1629) showed BN symptoms. During the following years, the number of symptomatic plants initially increased (44% in 2010; 50% in 2011), but then decreased (26% in 2012; 25% in 2013; 13% in 2014; 9% in 2015). Therefore, the recovery rate ranged from 20% in 2010, and increased to 81% in 2014, and settled at 70% in 2015. The distribution of the vines with symptoms showed a clustered pattern. Monitoring of the spatial distribution of BN allows evaluation of the disease progress over time, along with the recovery rates. When combined with the molecular epidemiology of the BN strains, these data can provide growers with useful information to support decisions in the management of vineyards.

Impact of strategies with reduction in fungicide use on grapevine diseases

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Grapevine is one of the two crops, with apple, requiring the highest input of pesticides for disease control. This is true for both organic cultivation (risk of copper accumulation in the soil) and integrated management with the use of synthetic fungicides. In the framework of the project "LIFE GREEN GRAPES, New approaches for protection in a modern sustainable viticulture: from nursery to harvesting" we compared different strategies, in the same vineyard, for grapevine protection including both organic and integrated protection. The traditional farm strategy is compared with a progressive reduction in fungicide input thanks to the support given to the vine by defence inducers, biocontrol agents and natural substances acting as plant strengthener. All treatments were introduced on the base of the risk indication given by a Decision Support System acting on all diseases affecting vines in the area. The impact of the different defence protocols was evaluated in Tuscany, in three portions of the same area, comparing the role of different exposure and soil on the diseases development, in Apulia, on integrated managed table grape, and in Cyprus, on organically managed table grape. Surveys were carried out following the EPPO guidelines at different growth stages on incidence and severity of downy and powdery mildew, on black rot (which is often present expecially in Tuscan vineyards), and on early development of trunk disease symptoms. The trials will be repeated for another three years to get a full evaluation of applicability of fungicide reduction in farm conditions.

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Jasmonates, salicylate and site-specific responses to phytoplasma infection in *Arabidopsis thaliana*

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Phytohormones are major actors in the sophisticated survival strategies developed in response to biotic and abiotic stresses. Several hormones take part to a complex signalling network, but jasmonate (JA) and salicylic acid (SA) play a crucial role. JA- and SA-mediated signalling pathways are mutually antagonistic. SA signalling is deemed to activate defences against biotrophic and hemibiotrophic pathogens, while JA is mainly though to induce resistance against necrotrophic pathogens. Phytoplasma are obligate biotrophic parasites associated with hundreds of diseases affecting many important crops. Considering the crude methods used to control phytoplasma diseases, a deeper knowledge on the defence mechanisms recruited by the plant to face phytoplasma invasion is required. In this work, phytohormone response was studied in *Arabidopsis thaliana* plants infected with '*Candidatus* Phytoplasma asteris' (strain Chrysantemum yellows, 16Sr-I). Phytohormone titre was measured in leaves of healthy and infected plants, in two tissues, the main-vein and the lamina. Differently from what was expected for biotrophic pathogens, phytohormone measurements on midrib tissue revealed an increase of JA and a decrease of SA levels. Since the veins are the sites where the direct interaction between phytoplasma and host occurs, this response could be interpreted as the "true" plant reaction to the infection. However, because separation of the midrib from the lamina might cause a wounding response, further experiments were performed to confirm a possible role of JA and/or SA upon phytoplasma infection and their ability to multiply. Thus, phytoplasma titre was measured in wild-type and Arabidopsis mutant lines, *dde2-2* and *sid2-2,* which are defective in JA and SA production, respectively.

Influence of a biocontrol agent on *Penicillium expansum*-driven acidification and patulin biosynthesis

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Penicillium expansum attacks stored fruit and produces patulin. Fruit tissue acidification by the pathogen plays a significant role in the infection process and pH is known to affect mycotoxin biosynthesis. The optimal pH range for patulin biosynthesis is between 4 and 5. Biocontrol is a promising alternative/integration for preventing postharvest diseases and patulin contamination. Recently, we reported that biocontrol yeasts increase the specific rate of patulin biosynthesis by *P. expansum* (µg/ng fungal DNA) in apples, although they lower the overall level of contamination. In this work, we examined the influence of the biocontrol yeast *Cryptococcus laurentii* LS28 on *P. expansum*-driven acidification and on patulin production. *Penicillium expansum* strain 7015 was grown in buffered medium at pH values from 3 to 8. Patulin production (µg/g mycelium) was the highest at pH 5, while only traces were detected at pH 3. In unbuffered medium, *P. expansum* quickly lowered medium pH to values below 3, whereas pH decreased more slowly, reaching values ≥ 4, when the pathogen was co-cultured with LS28. A similar pattern of pH was observed in a substrate mimicking the apple tissue, in which LS28 and *P. expansum* were co-cultured: the yeast delayed the pathogen-driven acidification, thus counteracting a key strategy of the fungal virulence, but kept the pH values in the optimal range for patulin biosynthesis. In a biocontrol assay, sub-optimal concentration of LS28 cells increased the rate of patulin biosynthesis (µg/g decayed tissue) in the apple tissue. These results suggest the possible ambivalent role of pH modulation by LS28 in i) counteracting the infection process, ii) increasing the specific rate of patulin biosynthesis by *P. expansum*.

Two years of tomato leaf curl New Delhi virus monitoring in Campania region

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Since 2016, Campania Region has joined the national surveillance program on the basis of Regulation 652/2014 where tomato leaf curl leaf curl New Delhi virus (ToLCNDV) is comprised in the "group three" which, although contemplating non-quarantine organisms, considers them potentially dangerous for the EU. Between the Campania Region Phytosanitary Service and the Institute for the Sustainable Plant Protection of the CNR, is in force an agreement for the establishment of a "Regional Unit of Phytosanitary Coordination". According on this collaboration, the monitoring and laboratory diagnosis of the ToLCNDV were assigned to the IPSP-CNR, which has developed a protocol for the specific molecular diagnosis of ToLCNDV. Two years (2016-2017) of field observations, samplings and laboratory analysis, carried out mainly in zucchini crops, have provided various indications, especially epidemiological: crops realized by early showing (March-April) and those realized in the hinterland hills, avoiding the *Bemisia tabaci* infestations, were not affected by ToLCNDV; the highest disease incidence occurred in field crops from June until late October (corresponding to the maximum presence of the vector); low density of vector populations (1-2 individuals per plant) were sufficient to generate severe epiphytotics of ToLCNDV; after an initial phase of the disease characterized by severe symptoms, as a result of early seedlings infection, most of the infected plants showed later a partial recovery of symptoms, to which corresponds a certain level of production, although limited and not of good quality; very low level of genetic variability was found among ToLCNDV field isolates.

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Reduction of mycotoxigenic *Aspergillus flavus* infection and aflatoxin contamination in maize treated with the biological product AF-X1: second year of field trials

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Aflatoxin contamination of maize, used for both human and animal consumption, is a serious constraint for final users health. Biocontrol is one of the measures that proved to be effective in aflatoxin reduction. This strategy is based on the application of non toxigenic *Aspergillus flavus* strains in maize fields to displace aflatoxin-producers during crop development. Field trials, for the second year running, in two different Tuscany locations using maize hybrids of maturity class FAO 400 and 600 were conducted. Geo-referenced plots (1 ha) were treated or not treated with the biological product AF-X1 (25 kg ha^{-1}) and kernel samples from plants at five sites on the two diagonals of each plot were collected. 100 maize kernels from each plot were surface sterilized and plated on PDA amended with streptomycin. Seeds mycoflora was assessed and all the *Aspergillus* spp. colonies were transferred on AFPA, CZ, CAM and YES media in order to determine toxigenic and non toxigenic *A. flavus* isolates. Moreover, maize flour samples were analyzed for aflatoxins by HPLC-FLD. The percentage range of non toxigenic *A. flavus* was higher in treated plots compared to untreated plots (31.5-91.3% *vs* 1.7-41%) and the average ratio of non toxigenic/toxigenic isolates was 79 and 6 in seeds from treated and non treated plots, respectively. The overall average aflatoxin B_1 concentration in maize from treated plots was 1.8 µg kg^{-1} and in untreated plots was 9.5 µg kg^{-1}. The percent reduction in aflatoxin B_1 concentration ranged from 67.7% to 99.7%.

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Hyperbaric atmospheres in the induction of resistance to *Botrytis cinerea* in tomatoes

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Tomato is an essential item worldwide for human diet. Its use is beneficial in several nutritional and medicinal aspects, contributing to the prevention of various diseases, including cancer. The fungus *Botrytis cinerea* is one of the main agents causing post-harvest losses in tomato. Its control is carried out mainly by the use of chemical treatments, which is increasingly undesireble by consumers. The use of hyperbaric treatment is an innovative technology that can induce synthesis of tomato defense compounds. The objective of this work was to study the effect of hyperbaric treatments on the induction of resistance to *B. cinerea* fungus in Italian type tomatoes cv. 'Paipai' stored at 22 °C. The tomatoes were submitted to pressure levels of 100 (Control), 200, 400, 600 and 800 kPa for a period of 48 h. After application of the treatments, tomatoes were inoculated with 10 µl of *B. cinerea* suspension (2×10^5 conidia ml^{-1}). The fruits remained at 22 °C and 75% RH for 6 days and were assessed every 2 days. The incidence and severity of the disease, accumulated loss of fresh mass, firmness, soluble solids content, titratable acidity and ascorbic acid were evaluated. The pressures of 600 and 800 kPa reduced the incidence of the disease by 25% in relation to the control, highlighting the treatments of 600 and 800 kPa that also presented the lowest severity of the disease. There was a tendency to increase ascorbic acid content with increasing applied pressure, signaling the induction of defense compounds in tomatoes.

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Genome and transcriptome of two strains of *Metschnikowia fructicola* provide new insight on the biocontrol mechanisms against postharvest diseases

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The yeast *Metschnikowia fructicola*, the base of a commercial biofungicide, is an efficient biological control agent of postharvest diseases of fruits and vegetables. Several mechanisms of action are exploited by *M. fructicola* to inhibit postharvest pathogens, including the release of iron-binding compounds, the induction of defense signaling genes, the production of cell wall degrading enzymes and high amounts of superoxide anions. The whole genome sequence of two strains of *M. fructicola* was sequenced by PacBio and Illumina sequencing, enabling to obtain a high-quality draft genome of 93 contigs, consisting of approximately 26 Mb. 564,302 homologous single nucleotide polymorphisms (SNPs) were detected by comparing the genomes by SNP calling, suggesting a high intraspecific variability. The genome is exceptionally large when compared with those of closely related organisms, and the most probable cause of this size is a recent whole genome duplication event, especially considering the high rate of homology found among the genes predicted in *M. fructicola* (5,132 out of 8,629 genes have a homolog). The genome was also searched for the CAZyme genes, and 1,145 such putative genes, an exceptionally high number, were identified. A transcriptomic analysis of *M. fructicola* placed in contact with either grapefruit peel tissue or *Penicillium digitatum* revealed a high level of CAZyme genes expression when the yeast was placed in wounded fruit tissue. This assembly represents an extraordinary resource for researchers studying *M. fructicola*, making them able to pursue comparative genomics studies aimed at explaining the biological control activity and the extreme variability of this yeast.

New insights into the infection process of *Fusarium fujikuroi* in rice using a GFP expressing isolate

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Fusarium fujikuroi (teleomorph: *Gibberella fujikuroi*) is the main seed-borne pathogen of rice, causal agent of Bakanae, a disease that in the last years has become of increasing economical concern in many Italian rice-growing areas. A virulent *F. fujikuroi* isolate was tagged with the green fluorescent protein (GFP) gene, using *Agrobacterium tumefaciens*-mediated transformation, and the virulence of the GFP isolate has been confirmed. By using the GFP isolate, fungal development during the *F. fujikuroi*/rice interaction was analysed by LASER scanning confocal microscopy (LSCM). The infection of rice roots was investigated from 24 h to 12 days post-inoculation both in resistant and susceptible cultivars. Roots of resistant genotype seem to trigger a hypersensitive response at the infection site and LSCM analysis of root sections allowed the visualisation of fungal growth within host tissues. Gene expression analysis of genes involved in pathogenesis and hypersensitive response is currently under way, by qPCR on the *F. fujikuroi*-infected rice roots. Analysed genes include chitinases, peroxidases and genes involved in gibberellin synthesis. The knowledge of plant infection and colonization mechanisms, together with the host response, will provide useful information for developing better control strategies of the pathogen and for improving breeding programmes for bakanae resistance.

Molecular identification and characterization of phytoplasmas infecting tomato in Oltrepò pavese (northern Italy)

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Tomato is affected by economically important phytoplasma diseases, such as stolbur, tomato big bud, tomato yellows, tomato witches'-broom, tomato little leaf, and hojia de perejil. Such diseases, characterized by different geographic distribution patterns, have been associated with eight distinct '*Candidatus* Phytoplasma' species in at least 30 countries throughout the world. In Italy, phytoplasma diseases of tomato were associated with infections by '*Ca*. Phytoplasma solani', '*Ca*. Phytoplasma asteris' and phytoplasmas of taxonomic groups 16SrIII and 16SrV. The present work aimed to investigate the aetiology of a disease affecting tomato in Oltrepò pavese (Lombardy, North Italy). During field surveys, carried out in a tomato plantation in August 2015, symptoms typically associated with phytoplasma infection (leaf purpling, witches'-broom, flower and fruit alterations, and stunting) were observed on tomato plants. Based on *16S rRNA* gene amplification and nucleotide sequence analyses, '*Ca.* P. solani' was detected in 82% of symptomatic tomato plants and in bindweed plants. Molecular characterization based on *tufB* and *stamp* gene nucleotide sequence analyses revealed the presence of two '*Ca*. P. solani' strain types: the type *tufB*-b/St5, identified respectively in 83% and 100% of tomato and bindweed infected plants, and the type *tufB*-a/St18 identified in 17% of tomato infected plants. These results suggest that '*Ca*. P. solani' ecology in the examined agro-ecosystem is associated mainly with the bindweed-related host system. Based on such evidences, it should be interesting to evaluate the incidence dynamics of the disease and to investigate its epidemiology by tracing the movements of '*Ca*. P. solani' throughout neighbouring fields.

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

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

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

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

Study of biovar-specific determinants in *Pseudomonas syringae* pv. *actinidiae* virulence

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Kiwifruit bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) is a quarantine plant disease that is threatening global kiwifruit industry, where Italy plays a leading role worldwide. Currently, control measures are mainly based on the use of copper, for which the European Union has concerns due to its accumulation in the soil and its possible negative environmental effects. Moreover, the possible development of Psa resistance to copper is an event already emerging in several Countries. Such a scenario would rapidly leave kiwifruit growers with no chance to efficiently control the pathogen. We are currently attempting to decipher molecular signals involved in Psa virulence induction and promotion. This will allow the identification of targets for designing new control strategies based on the inhibition of the infection process, instead of killing bacteria, thus averting the appearance of new antibiotic resistances, in accordance with the new trend of bacterial disease control also developed in medical field. To that purpose, we produced a Psa-specific microarray chip carrying whole genomes of Psa strains of the best-studied biovars (1, 2 and 3). Microarray analyses were performed with wild-type strains of the three mentioned biovars for a deep investigation of biovar-specificities to provide insights into molecular mechanisms possibly controlling Psa aggressiveness.

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

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

Vectors and candidate vectors of ‘*Candidatus* Phytoplasma solani’ in vineyards

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Bois noir (BN) is the most widespread grapevine disease in Europe, which can cause severe production losses. ‘*Candidatus* Phytoplasma solani’, associated with BN, is transmitted from host plants to grapevine by two cixiid species, *Hyalesthes obsoletus* and *Reptalus panzeri*. However, other polyphagous planthoppers and/or leafhoppers are probably involved in the epidemiological cycle of BN because these two vectors are absent or their population density are not correlated with BN incidence in some European areas. Dispersal of the phloem-feeding species and their relationship with weed abundances were studied in a vineyard, where a high incidence of BN has been recorded. Adults were sampled (from May to October 2016) using yellow sticky traps placed in a regular geo-referenced grid. Moreover, an aspirator was used to collect insects on the vineyard borders. Spatial analysis by distance indices was performed on total insect captures and wild plant species abundances too. The most abundant species collected inside the vineyard were *Neoaliturus fenestratus*, *Thamnotettix zelleri*, *Psammotettix alienus*, *Euscelis lineolatus*, *Laodelphax striatellus* and *Exitianus capicola*. Only three specimens of *H. obsoletus* were captured on the traps. The most abundant species collected on the vineyard borders were *P. alienus*, *E. lineolatus*, *Anaceratagallia laevis*, *N. fenestratus*, *Doratura homophila*, *Recilia schmidtgeni*, *L. striatellus*, *Toya propinqua*, *E. capicola* and *T. zelleri*. Preliminary results of molecular investigations allowed to identify ‘*Ca*. P. solani’ in *A. laevis, E. capicola*, *E. lineolatus, M. crocea, N. fenestratus*, *H. obsoletus, H. scotti* and *P. alienus.* Spatial distribution maps and association/dissociation with weeds are reported.

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

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Chitosan is a natural biopolymer obtained from arthropod exoskeletons or fungi by incomplete deacetylation of chitin. It is known for its biocompatibility and bioactivity and it is used in slimming diets or as stabilizer in tablets. When applied on fruit and vegetables, chitosan produces a coating on the treated surface, with chemical and physical properties depending on the acid used to dissolve it. Since '90 of last century, a wide literature is available on the effectiveness of chitosan in the control of postharvest decay of fruit and vegetables, obtained following postharvest dipping or preharvest spraying. The biological activity of chitosan depends on its eliciting, antimicrobial and film-forming properties, which based on literature and our experience, can account for 30-40%, 35-45%, and 20-30%, respectively, depending on the rate of application, coated fruit or vegetable and infecting pathogen. A standard application rate for the control of postharvest decay is at 1%, except for the control of *Penicillia*, where higher concentrations may be needed to provide a good effectiveness. The biopolymer was approved by Reg. EU 2014/563 as basic substance for plant protection purposes, and a long list of commercial products is available. Based on its multiple biological activities, it is expected an increases of its popularity in the control of plant diseases in sustainable strategies aiming to reduce the application of other fungicides in both IPM and organic agriculture.

There can be only one: two Flavescence dorée phytoplasma strains compete during plant infection

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Flavescence dorée phytoplasmas (FDp, 16SrV) are plant pathogenic non-cultivable bacteria associated with a severe and epidemic disease of grapevine. Despite the high genome sequence identity of the two reference strains FD-C and FD-D, their frequencies in cultivated grapevine are unbalanced. In particular, FD-C overcomes FD-D during plant infection. To investigate this phenomenon, *Catharanthus roseus* plants were graft-infected with the two strains. A first group of plants was infected with the two strains simultaneously, whereas a second group was infected with either of the two strains sequentially. Since the apical grafting seemed to foster FDp infection, different combination of lateral and apical grafting were applied to avoid artificial benefit due to the graft position. The infection was monitored over time and strain-specific diagnosis of symptomatic leaves was periodically performed up to 120 days. At the end of the experiments, roots were also collected and FD-C and –D loads were measured. Since the first sampling date, FD-C was more effective in plant colonization, and this was confirmed until the end of the experiment, when more than 70% of plants were positive only for FD-C. Moreover, more than the 90% of the analyzed roots were infected with FD-C only. These results strongly support a competition between FD-C and FD-D strains during plant infection/colonization and suggest the hypothesis that FD-C is more effective in plant infection. Moreover, these data are coherent with the observed different frequencies of FD-C/D strains under field conditions, and may contribute to explain the biology behind this phenomenon.

Associations and causal relations in the decline of kiwi in Calabria

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In Calabria (southern Italy) kiwi (*Actinidia chinensis* Planch.) covers 2.665 hectares and yields a total of 75,000 tonnes of fruit. Over the past five years, the decline of young vines throughout the growing area, has led to substantial economic losses. A survey carried out in 2017 in three different locations showed that the roots of symptomatic vines have been affected by black, sunken and necrotic lesions, sometimes associated with hyphae of *Armillaria* sp. The base of the trunk showed necrotic areas and xylem plugged with black inclusions, more severe in water-gaining sites and in those with impeded subsoil drainage. When portions of the discolored wood of crown roots and the trunks of rot vines exhibiting typical symptoms were transferred onto potato dextrose agar (PDA), a variable number of colonies were obtained. Of these, *Phytophthora* sp. and *Fusarium* sp. were isolated both from the roots and soil in the three locations. DNA extraction from the mycelia of Fusarium-like colonies, amplification and sequencing of the internal transcribed spacer (ITS) region of rDNA, performed with primers ITS1/ITS4 led to the identification of *Fusarium solani*. Multiplex PCR from the symptomatic wood of vines growing in one of the three locations also revealed single or multiple infections of *Cylindrocarpon macrodidymum* and *C. pauciseptatum,* which were not detected by conventional isolation on PDA. Since these three species identified are soil borne pathogens affecting many fruit trees, the results suggest that, in Calabria, they may play a role in the complex etiology of the destructive decline affecting kiwi.

Apricot yellows disease associated with '*Candidatus* Phytoplasma phoenicium' in Iran

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Almond witches' broom (AlmWB) associated with '*Candidatus* Phytoplasma phoenicium' is one of the economically important diseases of almond in Iran and Lebanon. In 2012-2015 surveys of AlmWB, apricot yellows (AprY) disease was observed in Fars province of Iran. The characteristic symptoms of the disease were leaf yellowing, inward leaf curl, scorch of leaf margins, shortened internodes, production of rosettes at the tip of branches, decline, stunting, and death. Healthy bitter almond and apricot seedlings, grafted with shoots of AprY-affected trees, exhibited phytoplasma-type symptoms. The 16S rDNA fragment F2n/R2 was amplified by nested-PCR from both AprY-affected trees and grafted seedlings. Nucleotide sequence identity, presence of species-specific signature sequences, and phylogenetic analysis of 16S rDNA allowed the assignation of detected phytoplasma strains to the species '*Ca*. P. phoenicium'. *In vitro* and *in silico* RFLP analysis of the fragment F2n/R2 allowed the affiliation of AprY phytoplasma to a variant of subgroup 16SrIX-B. Within '*Ca*. P. phoenicium' (16SrIX-B) population strains, identified in this and previous studies, 16 genetic lineages were determined by the combination of 19 single nucleotide polymorphisms (SNPs) identified within 16S rDNA nucleotide sequences. Interestingly, AprY phytoplasma strains belong to a unique genetic lineage distinguished by the presence of three lineage-specific SNPs. This first report of '*Ca*. P. phoenicium' in association with AprY disease in Iran opens new perspectives on the epidemiology of AlmWB phytoplasma, suggesting its possible adaptation to other fruit trees.

A lipidomic approach to study the interaction of *Xylella fastidiosa* with *Olea europaea*

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In the last years, *Xylella fastidiosa* became a severe threat for the European Community. In particular, *X. fastidiosa* subsp. *pauca* is associated with the Olive Quick Decline Syndrome, a disease causing severe losses in the Apulia region. So far, prevention is the most pursued strategy to restrain disease spread. Metabolomic studies, and specifically, in our case, the lipidomic ones, could provide useful information, either for prevention, detection (potential marker of infection) or for a possible remediation, i.e. by revealing pathogen pathways to be possibly blocked. In this study, naturally infected and healthy olive samples (cv. 'Ogliarola salentina') were collected in an affected olive orchard (Lecce and Taranto provinces, Apulia region) to investigate their lipidomic profile. In particular, HPLC-ToF untargeted analysis methods were used to acquire lipidome profiles and HPLC-MS/MS targeted methods to characterize and quantify different lipids. The untargeted analysis highlighted oxylipins, free fatty acids, glycerolipids and phosphoglycerolipids within the different lipid classes that were analysed. Based on these results, quantitative and semi-quantitative analysis of specific entities within each of these class of lipids both in healthy and infected samples was provided. A scenario has emerged, in which the Xf-infected olive samples presented at least one bacterium-specific lipid, i.e. 10-hydroperoxy-octadecenoic acid, and other host-pathogen shared lipids that are significantly higher in amount in comparison with healthy samples. To corroborate lipidomic profiles, the molecular quantification of bacterial charge through real time PCR is under way. This explorative approach will provide a robust basis for further targeted biological assays.

Tramesan, an eco-friendly approach against Septoria disease complex in wheat

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Wheat is the most cultivated cereal representing the main source of plant proteins in human food. However, up to 50% of worldwide wheat production may be lost because fungal diseases. The Septoria disease complex (STB) is critical, being *Parastagonospora nodorum* and *Zymoseptoria tritici* among the principal foliar pathogens on wheat. Currently, chemical fungicides (e.g. strobilurins, SDHI, triazoles and imidazoles) are widely used to challenge crop diseases. The chemicals may cause the insurgence of resistant fungal strains and, not less important, polluting the environment with possible toxic effects on humans and animals. The European Regulation 128/2009 aimed to control the use of pesticides; *de facto,* this regulation eliminates several of them from the market. All this prompts the research to find eco-friendly novel alternatives to chemical pesticides; in particular, agents based on living microorganism or natural products from them are studied to control plant diseases. One of the mechanisms of action of this novel bio compounds generation lays on the stimulation of plant defences; this phenomenon is called "defence priming". It exploits natural ability of the plant immune system to act through inducible responses. Here we use purified and semi-purified Tramesan, a polysaccharide from the secretome of the basidiomycete *Trametes versicolor,* to assay its ability to prime wheat defences against septoriosis in greenhouse and field trials, under artificial and natural infection in three different crop seasons (2016-2017-2018). The results indicate that Tramesan is a promising solution to contrast the Septoria disease complex.

First report of grapevine Syrah virus-1 infecting grapevine (*Vitis vinifera*) in asteroid mosaic-associated virus infected grapevine in Sardinia

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Grapevine (*Vitis* spp.) is affected by several graft transmissible agents causing crop losses and reduced plant vigor. During 2016 spring 24 berry samples from cv. ‘Cannonau’ plants, characterized by star-shaped chlorotic spots, low grapy grown and with suffering look have been collected in a north Sardinian vineyard. Total RNA was extracted with the RNeasy Plant mini kit (Qiagen) and retrotranscription together with libraries preparation were achieved by using the TrueSeq RNA kit. The obtained reads were mapped to the virus genome database (NCBI) and alignments were observed, for three samples, on large genome portions of grapevine Syrah virus-1, a member of the family *Tymoviridae*, identified for the first time in Sardinia, liable responsible, in other areas of the world, of the “decline” symptoms of Syrah grapevines and observed for the first time as an emerging disease in France. On the same samples of cv. ‘Cannonau’, it was detected the presence of grapevine asteroid mosaic associated virus (GAMaV), a poorly known maculavirus belonging to the Fleck complex, characterized by star-shaped chlorotic spots on the leaves, stunted vines producing little or no fruit. These multiple infections include novel viruses in Sardinian vineyards, even if at the moment no specific symptoms could be attributed to either virus and further work is required to determine the prevalence, pathological properties, vectors and the important of the economic impact of these two viruses.

Looking for novel control measures against the rice fungal pathogen *Pyricularia oryzae*

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The filamentous fungus *Pyricularia oryzae* is the main causal agent of the rice blast disease, which accounts for 10-30% yield losses per year globally. The objective of this project, which is part of the Scientific and Technological Cooperation Agreement between the Italian Ministry of Foreign Affairs and International Cooperation and the Department of International Cooperation of the Ministry of Science and Technology of Vietnam, is to identify new pathogen targets and new molecules to control rice blast disease. *P. oryzae* uses a large number of degrading enzymes active on cell wall polysaccharides and lignin to penetrate and invade the rice plant tissues. Since these enzymes could be potential targets for plant inhibitors, candidate *P. oryzae* genes encoding enzymes particularly expressed during the infection process have been deleted from the fungal genome and the characterization of the obtained mutants is in progress to identify enzymes essential for fungal virulence on rice. In addition, new ecofriendly antimicrobial peptides, analogs of the natural *Trichoderma longibrachiatum* peptaibol, have been synthesized and tested *in vitro* against several *P. oryzae* strains from different geographic origin. The screening has allowed to identify some peptides very effective in inhibiting spore germination and fungal growth that could be used *in vivo* to confirm their efficacy in protecting rice from the blast disease.

Phylogenetic diversity and toxigenic potential of *Fusarium tricinctum* Species Complex strains associated to Fusarium head blight on durum wheat

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Several *Fusarium* species are agents of Fusarium head blight (FHB), a serious worldwide cereal disease causing qualitative and quantitative yield losses. A lot of information is available for the main fungal species, such as *F. graminearum* and *F. culmorum*, involved in FHB. However, the “less aggressive” species, like *F. acuminatum* and *F. tricinctum*, belonging to the *Fusarium tricinctum* Species Complex (FTSC), have poorly been studied until now. A population of FTSC strains isolated from Italian durum wheat grains was molecularly characterized by partial sequencing of the translation elongation factor 1-alpha region (*TEF1α*) and RNA polymerase second largest subunit region (*RPB2*). Furthermore, greenhouse and field experiments were carried out in order to define their pathogenetic role. The strains capacity to biosynthesize, *in vitro* and *in vivo*, secondary metabolites (enniatins A, A1, B, B1 and beauvericin) was also investigated by HPLC-MS/MS. The molecular characterization allowed the identification of two phylogenetically distinct populations of *F. tricinctum* without evident biological differences. Specific symptoms caused by *F. tricinctum sensu stricto* strains were observed. Inoculated durum wheat heads showed "peacock eye"-shaped necrosis and necrotic spots at the glume level. Secondary metabolites production was independent from the phylogenetic group. *In vitro*, enniatins were produced in significant amounts but no beauvericin was detected. *In vivo*, the enniatin levels were higher than the beauvericin ones. These results allowed to clarify the role of *F. tricinctum sensu stricto* in the disease while further studies are necessary to better understand the involvement of each member of FTSC and their relationship within the FHB complex.

A fast and specific assay for the surveillance of the invasive forest pathogen *Heterobasidion irregulare* based on Loop-mediated isothermal AMPlification (LAMP)

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The North American species *H. irregulare* Garbel. & Otrosina was accidentally introduced in central Italy during the World War II. The pathogen has become invasive and is currently distributed in pine and oak stands over about 105 km of coast around Rome, often in association with significant mortality of Italian stone pine (*Pinus pinea* L.) trees. Based on its observed and potential impact, *H. irregulare* is recommended for regulation in Europe by the European and Mediterranean Plant Protection Organisation (EPPO). In the frame of the EU Project EMPHASIS (grant agreement 634179), a fast and specific molecular diagnostic tool based on Loop-mediated isothermal AMPlification (LAMP) coupled with two different DNA extraction methods was developed and optimized for the detection of *H. irregulare*. The LAMP assay was successfully validated both in controlled conditions and in the field on different samples, including fruiting bodies, infected plants, wood colonized by the pathogen, and airspora collected by using woody spore traps. The detection of the pathogen in airspora was optimized both in terms of methods and in terms of length of incubation period of traps. The limit of detection of this assay is approximately 20 picograms of target DNA with a detection time of about 40 minutes. This molecular diagnostic tool may be used for the surveillance in the ports of entry of wood imported from North America and for the monitoring of pine forests, especially those located in a buffer zone surrounding the infested area.

Biogenic extracellular synthesis of gold and silver nanoparticles by *Trichoderma harzianum* and *Trichoderma longibrachiatum* and their effectiveness against seed-borne fungal pathogens

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Nanoparticles present a multifunctional platform for a diverse range of applications in the modern agricultural concept of precision farming due to their small size, high surface to volume ratio and unique optical properties. Antimicrobial nano-materials, pathogen detection by the use of nano-sensors, spray-induced gene silencing (SIGS) are only some of the potentialities of the nanotechnology in crop protection. The biosynthesis of gold and silver nanoparticles (AuNPs and AgNPs) by *Trichoderma harzianum* and *T. longibrachiatum* was evaluated. Fungi were isolated from deep-sterilized tissues of *Cupressus sempervirens* and *Gladiolus* cv. Red Balance, respectively, and were molecularly identified using ITS and *tef-1* sequences analyzed by *Trich*OKEY and *Tricho*BLAST. Cell-free extracts of the fungi were challenged with 1 mM silver nitrate and 0.5 mM tetracholoroauric acid solutions. The formed nanoparticles were characterized by means of spectroscopic and microscopic analyses including UV-VIS spectroscopy, TEM and XRD, EDAX and FT-IR analyses. Antifungal activity using a microdilution assay in 96-well microtiter plates against *Colletotrichum lupini*, *Fusarium oxysporum* f. sp. *basilici* and *Botrytis cinerea* was assessed. The results were transformed to percentage of controls and the IC_{50} and IC_{90} values were graphically obtained from the dose-response curves. The AgNPs of *T. harzianum* and *T. longibrachiatum* strongly reduced the growth rate of all tested pathogens (85-100%) and only the AuNPs of *T. longibrachiatum* gave rise to a reduction of growth between 18 and 56% depending on the pathogen tested. The promising results obtained open up opportunities for further research on effective and ecofriendly solutions for the control of seed-borne diseases.

Sensitivity of four Fusarium head blight causal agents to commonly used fungicides

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Fusarium head blight (FHB) is one of the most serious diseases of wheat. It is caused by fungi belonging to the genus *Fusarium.* Recently, a variation in the composition of the FHB community was observed in some wheat cultivation areas of Italy. In detail, *F. avenaceum* and *F. poae* increased their frequencies while a lower *F. graminearum* presence and a negligible *F. culmorum* incidence were simultaneously observed. These shifts within the FHB complex may have been caused by fungicides used to control the disease in the field. Therefore, the present study was carried out to evaluate, both *in vitro* and in field trials, the activity of the commonly used fungicides: Ares 5 SC (tebuconazole), Caramba (metconazole), Proline (prothioconazole) and Sportak 45 EW (prochloraz) towards four FHB causal agents. Fungicides efficacy was assessed by *in vitro* experiments evaluating fungal growth in liquid cultures (PDB) and by q-PCR quantification of fungal biomass in grain samples harvested in the field. *In vitro* results revealed that low rates of all fungicides caused the incomplete reduction of fungal development, especially of *F. poae* and *F. avenaceum*. These two species showed, at the same time, a lower sensitivity to Caramba in comparison to the other fungicides. In field trials, all fungicides showed to possess an activity against the four *Fusarium* species. However, *F. avenacum* showed a lower sensitivity to Caramba. Finally, an additional *in vitro* experiment, carried out with different *F. graminearum* and *F. avenaceum* strains, confirmed the significantly lower sensitivity of *F. avenaceum* to Caramba.

High incidence of cucumber mosaic virus in *Alstroemeria* hybrids in Campania region (southern Italy) and observation of symptoms recovery from initial infection

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Alstroemeria is a perennial flowering plant native to South America very appreciated for its lily-like blooms with a wide range of colors and commonly used as cut flowers. It is vegetatively propagated by division of rhizomes making it prone to viral infections which have detrimental effects on crop production and quality. During autumn 2015, *Alstroemeria* hybrids exhibiting virus-like symptoms were observed in some protected cultivations of Boscotrecase municipality (Campania region). Symptoms, consisting in bright yellow mosaic and streaks along the leaf, slight leaf area reduction and stunting, were observed in almost 90% of the plants. Serological and molecular analysis were performed on the leaves of 20 symptomatic plants in order to detect the presence of arabis mosaic virus and cucumber mosaic virus (CMV) by using ELISA commercial kits; tobacco rattle virus, potyviruses, carlaviruses, tospoviruses and ophioviruses by using RT-PCR with genus-specific and specie-specific primer pairs. Only CMV was found associated to the symptomatic plants and by *in silico* restriction analysis of the partial CP gene the Alstroemeria isolate was classified as belonging to subgroup II. Interesting, after an initial severe symptoms development, plants visibly recovered from viral infection and symptoms progressively decreased, leading to reduced symptoms or symptomless leaves at the apices. This behavior was studied in particular in plants kept under observation in greenhouse for three consecutive years. Preliminary results obtained by semi-quantitative RT-PCR revealed a comparable level of CMV RNA accumulation between upper symptomless and median symptomatic leaves after one month from the beginning of the recovery stage.

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Innovative sustainable approaches for the management of potato pests and pathogens

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Kazakhstan is an agro-industrial country, where the agro-industrial complex is an important part of the economy. The most destructive pests of potato industry, one of most important of the Country, are late blight, caused by *Phytophtora infestans*, and Colorado potato beetle (*Leptinotarsa decemlineata*). Late blight and Colorado potato beetle are usually managed through the application of pesticides in both organic agriculture and IPM. However, more sustainable strategies are needed to manage both those pests. Colorado potato beetle larvae and adults feed on leaves of solanaceae (potato, sweet pepper, tomato). As the beetles accumulate in their bodies the toxic alkaloids contained in the shoots and leaves of the Solanaceae, they are inedible for most birds and animals, and therefore have few natural enemies. In the absence of control measures, crops can be completely destroyed. We applied a novel technology based on production of a powder from transformed microalgae that will act as a biopesticide against major crop pests. Feeding insects with double-stranded RNA (dsRNA) molecules can result in mortality through a cellular process called RNA interference. The design of dsRNA ensures that it targets only the specific species and does not give rise to resistance within the pest population. We have designed a new expression vector (pYMD1) for high-level expression of dsRNA in the chloroplast of the alga *Chlamydomonas reinhardtii* and created transgenic lines producing dsRNA targeting the essential actin gene of *L. decemlineata*. The efficacy of the powdered alga as a biopesticide when dusted onto potato plants will be tested.

New acquisition about the epidemiology of grapevine leaf mottling and deformation

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Grapevine Pinot gris virus (GPGV) was discovered in Italy in 2012 and successively in several grape-growing regions worldwide infecting different varieties. Studies associating GPGV with symptoms of leaf mottling and deformation (GLMD) showed that different strains of the virus responsible for eliciting or not the symptoms exist and that *Colomerus vitis* (Pagenstecher) collected from infected grapes were able to transmit GPGV to healthy grapevines. GPGV represents a potential threat for grapevine production in Europe and elsewhere. Acquisition and transmission by an arthropod vector is central to the infection cycle of the majority of plant pathogenic viruses. Filling the gap of information of epidemiological aspects of GPGV strains/*C. vitis* interactions would help in implementing efficient strategies of control of the associated GLMD disease. The current study was aimed at identifying the main drivers of GPGV spread and define the epidemiology of GLMD disease in North-eastern Italy vineyards.

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A putative trichothecene biosynthetic gene cluster in the biocontrol agent *Trichoderma gamsii*: preliminary investigations

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Trichoderma gamsii T6085 is a promising biocontrol agent against *Fusarium graminearum* and *F. culmorum*, two of the major causal agents of Fusarium head blight (FHB) of wheat. This beneficial isolate can reduce disease incidence in the field, controling pathogen growth and preventing mycotoxin, such as the trichothecene deoxynivalenol, accumulation. Trichothecenes are sesquiterpenoids described as mycotoxins, phytotoxic molecules or plant signal inducers. Analysis performed on the genes involved in the biosynthesis of these molecules in 14 fungal species showed that gain/loss, functional changes and rearrangement on these genes have taken place during evolution. A putative trichothecene biosynthetic gene cluster has recently been found in the sequenced genome of *T. gamsii* T6085. It contains a *TRI5* gene (trichodiene synthase), whose encoded protein shows a high similarity with other TRI5 proteins described in the genus. The other proteins show a low sequence similarity and different conserved domains when compared with other TRI proteins described in *Trichoderma*. This could lead to the hypothesis that T6085 might produce novel metabolites belonging to the trichothecene family. In order to better characterize this cluster and to study the syntenic organization in our isolate we looked for orthologous genes using a bioinformatic approach. In addition, with the aim to determine whether T6085 can produce trichothecenes, 12-day-old supernatants were analysed by HPLC and RMN. Our results could lead to the identification of new genes and secondary metabolites that may be involved in biocontrol strategies of FHB.

Insights on the cross-talk between *Trichoderma gamsii* and *Fusarium graminearum* at a distance: two opposite messages

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We investigated the cross-talk at a distance between the promising biocontrol agent *Trichoderma gamsii* T6085 and the mycotoxigenic pathogen *Fusarium graminearum* ITEM 124, the causal agent of Fusarium Head Blight, both at the physiological and the molecular levels. To this end, a genome-wide transcriptome analysis focused on the sensing phase (before contact) of the interaction between the two fungi was carried out. The analysis of differentially expressed genes (DEGs) revealed two opposite behaviours. While an overall up-regulation occurred in *F. graminearum* ITEM 124 ('buzzing mode'), the gene patterns of *T. gamsii* T6085 were mainly down-regulated ('stealth mode'). The functional analysis of DEGs revealed that *T. gamsii* T6085 won the competition for iron by up-regulating a ferric reductase (one of the top-ranked up-regulated genes) involved in iron uptake, whereas *F. graminearum* ITEM 124 down-regulated the expression of seven stress-related enzymes having iron as cofactor. Furthermore, while *F. graminearum* ITEM 124 up-regulated the entire repertoire of four killer toxin-like chitinases, *T. gamsii* T6085 decreased the level of interaction at a distance by down-regulating four out of six genes coding for secreted chitinases ('stealth mode'). Finally, *F. graminearum* ITEM 124 grew faster in presence of *T. gamsii* T6085, supporting the high metabolic activity ('buzzing mode'). This study shows how the communication between *Trichoderma* and *Fusarium* is definitely regulated before contact. The study of gene expression profiles unravels a strongly different behaviour of the two fungi when they face each other. This opens new fields of investigation towards the successful development of *T. gamsii* T6085 as biocontrol agent.

Does timing of application influence the efficacy of *Trichoderma* in reducing wood fungal infections and in improving plant quality in grapevine nurseries?

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Wood pathogens of grapevine are recognized to be already present in propagation material, even if once in the field infections through pruning wounds are another source of infection. In these last years, the availability of biological control products increased. Application timing of a product based on *Trichoderma atroviride* SC1 (Vintec®) was tested in a commercial nursery to establish the influence of application timing on the quality of the grafted vine and level of infection in the nursery. The formulation was applied at three stages: rehydration, callusing, basal callus formation followed by soil drenching. In addition, the effect of the combined applications was also tested. All treatments were compared with controls (another *Trichoderma*-based product, plants treated with synthetic fungicide and untreated plants). Surveys included assessment of plant quality and viability after callusing and after a growth season in the nursery soil, root development and quality by video image analysis. The final grafted vines, ready for sale, were used for isolating the mycoflora, thus showing a direct influence of application at different stages of the grafted cuttings production and the fungi and in particular fungal colonization by wood pathogens. The rehydration stage showed to be the better performing application in reducing fungal infections, whereas the applications at the basal callus followed by soil drenching produced plants with a better root quality, and a different increase of the certifiable plants depending on the rootstock tested. The use of biological control products requires a lot of care in detecting the most useful application timing.

Effects of natural compounds on postharvest gray mold infections on fresh strawberries fruit

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The strawberry (*Fragaria* × *ananassa*) is a climacteric fruit and recognized for sensory features, However, it has a short postharvest life, due to the intrinsic physiology and the high presence of spoilage microorganisms. Gray mold, caused by *Botrytis cinerea*, is the most important postharvest disease, followed by Rhizopus rot, caused by *Rhizopus stolonifer*, and other minor rots (Mucor rot, blue mold, and anthracnose, induced by *Mucor* spp., *Penicillium expansum* and *Colletotrichum gloeosporioides*, respectively).The approach of control treatment using natural compounds as a safe products is considered in this study. The antifungal effects of chitosan edible coating, with a list of commercial products (Chito Plant powder, Chito Plant solution, Kaitosol, OII-YS) and other commercial natural compounds (3 logy, DF-100 Forte, Prev-AM plus, Humic acid, Methyl jasmonate) was tested on naturally inoculated fresh strawberries stored at 5 and 20 °C. Among the tested compounds, the chitosan formulation Chito Plant powder performed best in the control of gray mold, which was the most frequent postharvest decay. Further studies are needed to better understand the mechanisms of action and to test the product in large scale trials.

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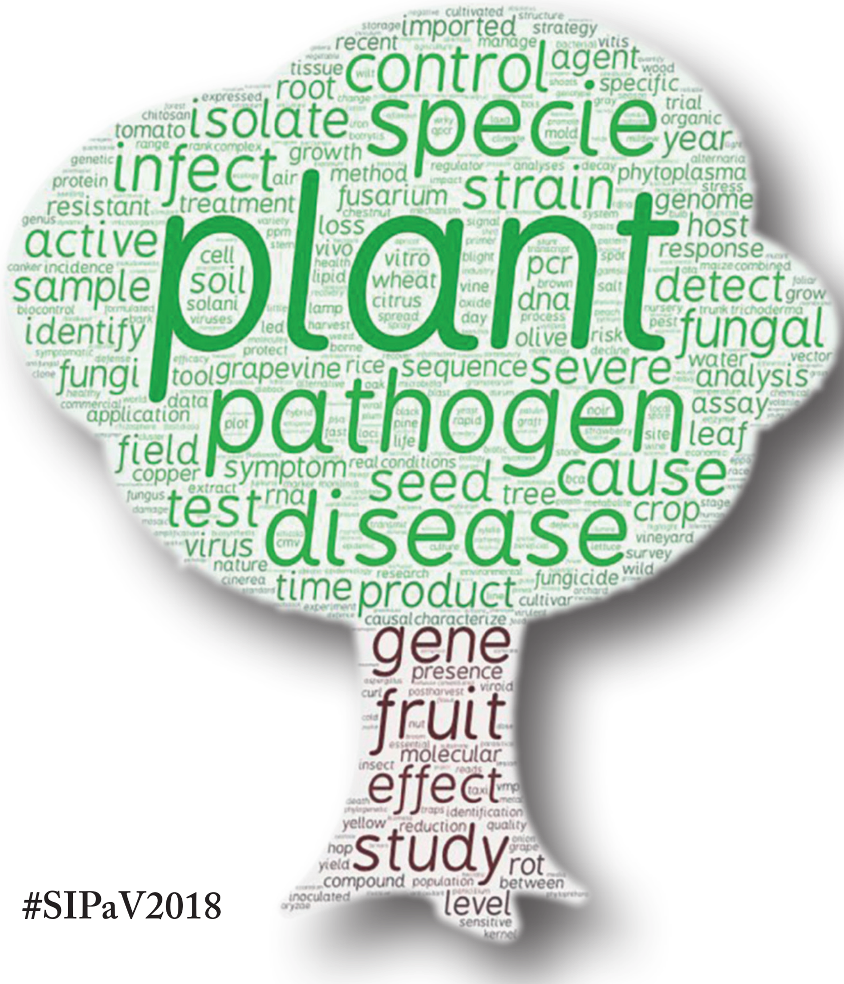
Notes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