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ABSTRACT

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

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

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

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

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

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

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

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

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