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ECOLOGY AND EPIDEMIOLOGY OF THE EMERGING PLANT PATHOGEN
GNOMONIOPSIS CASTANEAE

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Gnomoniopsis castaneae is an emerging fungal pathogen causing nut rot on Castanea sativa, or living as an endophyte in the green tissues of the tree. The disease has been reported since the mid-2000s in European countries such as Italy, France and Switzerland. To date, the ecology and the epidemiology of G. castaneae have been mostly unexplored. The main goals of this work were: I) testing the association between the climate and the incidence of G. castaneae; II) assessing the influence of the plantation density on the spatial pattern of the nut rot infection through the development of a new geostatistical test; III) investigating some possible ecological interactions between G. castaneae and the exotic pest Dryocosmus kuriphilus (Asian chestnut gall wasp); and IV) comparing a large set of Castanea spp. cultivars and the wild-type of C. sativa to assess their levels of susceptibility to G. castaneae.

A combined approach involving isolations, molecular analyses and the application of non-conventional statistical techniques was used. The methods were adapted depending on the specific aims of each experiment.

In 2011, based on the inspection of 12 sites, the incidence of G. castaneae in north-western Italy was comprised between 20% and 93%. Climatic analyses showed that warmer temperatures were associated with higher levels of G. castaneae incidence, while no significant effect of the rainfall was detected. A series of models (GnoMods) was fitted, resulting in equations predicting the incidence of the disease at site level as a function of the temperatures of the months before harvesting. The GnoMods were significant (P<0.05) and provided good predictive performances, as shown by the strong correlation between observed and predicted values resulting from the external validation (Spearman ρ>0.72; P<0.05).

The comparison of areas with the same number of chestnut trees significantly clustered (P<0.05) or randomized (P>0.05) indicated that the spatial distribution of the trees carrying nuts infected by the pathogen was randomized (P>0.05), regardless of the plantation density. The incidence of trees infected was not significantly different among areas (52.9%-71.4%; P>0.05). The results suggest that the plantation density was not related to the spatial distribution of G. castaneae. To perform these analyses, new geostatistical tests (Mean Distance Tests - MDT) were developed, validated and embedded in a user-friendly software. MDT displayed reliable outcomes (power>80%; type I error<0.05) and can be applied to test the spatial pattern of any plant disease when the variable of phytopathological interest is categorical.

DNA analyses showed that 40% of the adults of D. kuriphilus emerging from galls colonized by G. castaneae carried inoculum of the pathogen, yet the inoculum was not viable, since no fungal colonies could be isolated. Consequently, the pest is an unlikely vector of G. castaneae. On average, the emerging adults were significantly more abundant in galls colonized than in galls not colonized by the fungus (3.76 vs. 2.54, P<0.05), indicating a possible fungus/pest synergy. The combined results of the Monte Carlo simulations, validated by isolation trials on chestnut buds inspected for D. kuriphilus eggs, showed that G. castaneae colonized 33.8% of the buds before oviposition, and that fungal colonization and oviposition were not associated (odds ratio 0.98, 0.71-1.33 95% CI). Consequently, the higher number of insects in galls
colonized by *G. castaneae* might have been the effect of an interaction occurring after oviposition, probably during the insect development.

The incidence of *G. castaneae* was compared between 85 chestnut cultivars and the wild-type of *C. sativa* growing in homogeneous environmental conditions. The statistical analyses showed that only cultivars significantly more susceptible (P<0.05) than the wild-type (22% of the total number of cultivars in 2013 and 55% in 2014) could be identified. *G. castaneae* was isolated not only from *C. sativa* nuts, but also from nuts of Asian chestnut species and hybrids. The validation analysis revealed no significant association between the most susceptible cultivars detected in 2013 and 2014 (odds ratio 2.85; 0.18-176.61 95% CI). The results suggest that the levels of susceptibility of the chestnut cultivars and the wild-type were substantially homogeneous.

In conclusion, the temperatures are strongly associated with the incidence of *G. castaneae*, while the plantation density of the orchards, the interaction with *D. kuriphilus* and the chestnut cultivars seem to play a negligible, or minor, role in the epidemiology of this plant pathogen.