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# ECOLOGY AND EPIDEMIOLOGY OF THE EMERGING PLANT PATHOGEN *GNOMONIOPSIS CASTANEAEE*

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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  $\rho > 0.72$ ;  $P < 0.05$ ).

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.