# Some Observations On the "Hypovirulence" of Chestnut Blight in Italy

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ABSTRACT.— Abnormal cankers caused by *Endothia parasitica* occur in chestnut areas of Italy. Hypovirulent isolates of the fungus have been obtained from abnormal cankers and four types of *E. parasitica* differentiated. The natural establishment of hypovirulent strains has resulted in the reinitiation of chestnut cultivation.

The damage caused by chestnut blight resulted in the abandonment of chestnut cultivation in the mountains of Italy. However, in recent years two new events have modified the present situation: the price of chestnuts and the loss of virulence in *Endothia parasitica* (Murr.) P. J. & H. W. And. Presently, chestnut blight is not the problem it was 20 or 30 years ago when the disease destroyed all the chestnut growing areas. Now, recultivation is possible because of "hypovirulence" of the blight organism.

## EVOLUTION AND SYMPTOMATOLOGY

Chestnut blight was officially discovered in Italy, near Genoa, in 1938, and in a few years all of the Italian chestnut areas were affected with the disease, causing total destruction in many regions (Biraghi, 1946). *Endothia parasitica* attacks the sprouts, branches and trunks of *Castanea sativa*

(Mill.). During the first stages of attack the inner tissues of the bark develop yellow-rust red areas with irregular, slightly raised edges. The tissues of the bark turn dark and are destroyed by the fungus. In the older branches the infection is irregular, slightly raised and violet-red in color. The fungus grows through the bark tissues, eventually surrounding the branch and killing the upper portion of the stem. The canker is then characterized by the pronounced splitting of the bark. Infected stems react by sprouting many small branches below the canker. The outer parts of the bark contain red- to orange-colored pycnidia, that with suitable conditions of temperature and humidity, produce conidia.

In 1950, it was observed that some chestnut areas of Liguria had many sprouts and branches with cankers that showed abnormal growth. This same phenomenon took place in many other Italian chestnut areas. Although the first stages of the disease appeared like a normal canker, abnormal cankers developed that were characterized by a more or less pronounced swelling of the bark. The canker fully surrounded the shoots or the branches, but did not kill them. Many abnormal cankers are often found on living sprouts, but bark lesions are few, pycnidia production is low and perithecia have not yet been observed. The buds below the canker do not vegetate, and we have not observed the development of the small branches. Under the bark, the tissues are living because of the external growth of the fungus. The cambium is alive and produces a reaction tissue that results in the elimination of the fungus.

## THE "HYPOVIRULENT" ISOLATES

In order to explain the appearance of abnormal cankers, two hypotheses were suggested; increased resistance of the chestnut trees to the blight organism, and/or the loss of virulence in the fungus. Biraghi (1968) admitted the existence of some resistant chestnut trees, but not the sudden resistance of a whole population, independent of varieties or ecological conditions. The second hypothesis was implicated when Bonifacio and Turchetti (1972) sampled cankers from many chestnut areas of Italy, particularly in Tuscany. Bark samples from cankers were collected, especially where the regression of disease was observed. Isolations of *E. parasitica* from these samples produced strains of different morphological type than isolations from normal cankers.

Grente and Sauret (1969b) and Bonifacio and Turchetti (1972) by single conidial isolation have described four types of cultures; a) virulent strains typical of the species *E. parasitica,* b) white isolates which seldom produce pycnidia, c) pigmented strains which bear large numbers of very small pycnidia, d) strains with intermediate features. The differential criteria of the *E. parasitica* strains were based on morphological characters.

Bonifacio and Turchetti (1972) considered the physiological behavior of the fungus to verify the reason for the loss of virulence in the parasite towards *C. sativa* that Grente (1965) had previously described for some isolates of the fungus coming from the Lombard Prealps. After inoculating chestnut trees with the different isolates a remarkable difference appeared. The results could not be explained by differences in host reaction because the phenomenon was verified by inoculation tests on sprouts from the same stump. The sprouts developed reactive tissues and were living even though fully surrounded by the canker. Only a few sprouts have died in the nine years since inoculation.

When many sprouts of the same stump were inoculated with normal, white, pigmented and intermediate strains of *E. parasitica* obtained by single conidial isolation, we observed a different behavior pattern from the first inoculations. The normal isolate was injurious while the white, the pigmented, and the intermediate isolates were not. The different patterns of growth in the bark were confirmed by inoculation tests on chestnut shoots in Erlenmeyer flasks, using the procedure reported by Puhalla and Anagnostakis (1971).

The different strains also had different phytotoxic activity. The isolates were incubated in Erlenmeyer flasks with Knop medium with an addition of yeast-extract and dextrose for 30 days at room temperature. The filtered medium was then mixed with charcoal and immersed in methanol for 12 hours at 15 C and the filtrate evaporated in a "Buchi" roto-evaporator. Young tomato plants were immersed in 1 ml of a solution made by adding 100 ml of water to the dried extract. The filtrates of the normal fungus were more injurious than the pigmented, white and intermediate filtrates.

Cultures of the fungus in potato dextrose broth with added pyrogallol showed inhibition of normal isolates at the 0.04 percent concentration. The growth of white and pigmented isolates was inhibited at the 0.035 percent and 0.03 percent concentrations respectively. All isolates died when they were incubated in a 0.04 percent pyrogallic solution for a month. These results confirm that various physiological differences exist in the isolates of *E. parasitica*.

We distinguish four types of *E. parasitica* isolates; a) the normal type with pycnidia of medium size forming 10 to 15 days after the inoculation, b) the white type with fewer pycnidia but larger than normal type, forming 30 days after the inoculation, c) the red-orange pigmented type with many abnormal pycnidia forming 10 to 15 days after the inoculation, and d) the intermediate type with pycnidia uniformly distributed on all the colony. These isolates correspond to "N", "B", "JR" and "V" identified by Grente and Sauret (1969a).

Grente and Sauret (1969a), obtained from the

normal isolate only normal types. When white isolates were single spored they formed normal, pigmented, white and intermediate types. Some pigmented strains after the second to third inoculation on agar media, formed normal, pigmented and white isolates.

Bonifacio and Turchetti (1972) also obtained by successive isolation normal types from normal isolates and pigmented types from pigmented isolates. But, Bonifacio and Turchetti (1972) obtained pigmented isolates, from abnormal cankers, while the pigmented isolates of Grente and Sauret (1969b) were from single spore isolation of white strains.

## CONCLUSIONS

Hypovirulent isolates are abundant in the Italian forests. They are able to reduce the virulence of normal isolates through hyphal anastomosis. This is a reasonable explanation of the natural reestablishment of chestnut. For this reason there has been a decrease in the development of the disease and renewed interest has developed in chestnut cultivation.

In France, Grente realized the potential for biological control and has initiated a program of artificial dissemination of hypovirulent isolates by inoculating hypovirulent strains along the margins of the normal cankers. Hypovirulent strains transmit hypovirulence to normal isolates, slowing canker growth so that healing occurs. The selection of the appropriate hypovirulent isolates is important because they must be compatible with virulent strains or anastomosis of hyphae may not occur.

Chestnut blight is still a problem with grafted stock. Since *E. parasitica* is a wound parasite, the tissues of the graft favor attack by the parasite. Many grafts have failed in the Tuscany area as a result of infection. All types of grafts are susceptible. From dead graft samples, we have obtained normal and hypovirulent strains. Even though the fungus has lost virulence, protection of the grafts is necessary. Some experiments with Benomyl mixed with Vinavil have not been successful, while copper salts have effectively protected the grafts. Because grafting is an important means of propagating valuable chestnut varieties, the Silvicultural Institute and the Plant Pathology Institute of the University of Florence have begun further experiments that we hope will hasten the recultivation of chestnut, a very important tree to Italy.

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