ENDOTHIA PARASITICA ON NUTS OF CASTANEA DENTATA

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ABSTRACT.--The *chestnut blight fungus was reported on nuts of Castanea sativa in Pennsylvania in 1915. Visual examination of germinating American chestnuts in the spring of 1981 indicated that 38 percent, 88 of 234, were infected with Endothia parasitica. These were collected from trees at Lockwood Experimental Farm, Hamden, in an area where cytoplasmic hypovirulent strains have been introduced. Our initial identification was ed on the presence of orange mycelial fans just below the epidermis of the* nut *shell and on fruiting pustules that erupted through the shell. Apparent E. parasitica was isolated free of contaminants from 26 of 41 infected nuts tested. Fourteen of these isolates displayed varying degrees of abnormal morphology in culture. Two of the isolates are hypovirulent based on a pathogenicity test on American chestnut. Several isolates are being tested for double-stranded RNA. Infection of the nut shell does not appear to affect germination or health and vigor of seedlings through the first growing season. The means by which these nuts become infected and possible significance for spread of hypovirulent strains is being examined.*

The chestnut blight fungus, *Endothia parasitica,* is normally spread by the transport of ascospores and conidia and perhaps by mycelial fragments. Long distance spread may result from the physical movement of infected stems and subsequent dispersal of spores and mycelium. Infection arising from nuts has generally been assumed to be by surface contamination which could be eliminated by surface disinfection (Lanza 1950). However, more than a half century ago, one investigator (Collins 1913; 1915) reported finding infected chestnuts lying on the ground in Pennsylvania and Delaware. The tree species, identified only in the second paper, was *Castanea sativa,* the European chestnut. Infections of *E. parasitica* on American chestnuts, *C. dentata,* have not been reported.

Methods and Results

1980 Harvest

In the fall of 1980, we collected American chestnuts from field-planted 14-year-old trees at the Lockwood Farm, Hamden, Connecticut, in an area where cytoplasmic hypovirulent strains have been introduced. The nuts were stratified in peat moss and kept at 4 C during the winter, and in February were sown in flats of moist peat moss in the greenhouse.

Visual examination of the germinating nuts five and nine weeks after sowing indicated that a total of 38 percent (88 of 234) were infected with *E. parasitica.* This determination was based on the presence of orange mycelial fans just below the epidermis of the nuts and on fruiting pustules that had erupted through the shell (Figure 1).

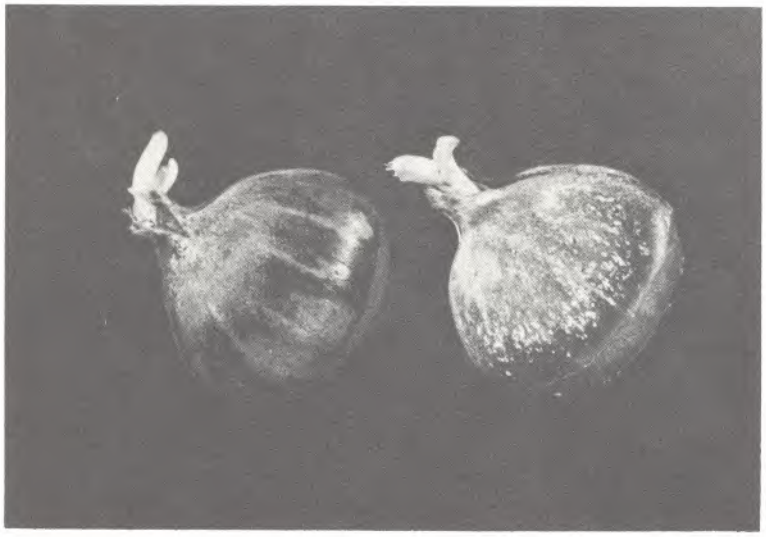

Figure 1. Nuts of *C. dentata* infected with *E. parasitica*: left mycelial fan, right pycnidia erupted through shell.

Twenty-six nuts with fruiting bodies were sampled and *E. parasitica-like* isolates were obtained from all of them. No *E. parasitica* was recovered from the 15 nuts with only mycelial fans, but this may have been due to contamination. Two of the nuts with mycelial fans later produced fruiting bodies and *E. parasitica-like* isolates were recovered from these also.

Twenty-six isolates were examined for morphology and pathogenicity. Twelve were classed as morphologically normal while the other 14 ranged from almost normal to highly abnormal in appearance. Two dsRNA extractions were run on 7 of the most abnormal isolates; no dsRNA was detected.

The 26 isolates were inoculated into American chestnut trees in May 1981. Measurements taken in September indicated that three of the isolates were much less pathogenic than normal and two other isolates had intermediate pathogenicity.

Seedlings which were produced from the infected nuts were field planted during summer 1981. Observations in late fall indicated that tree vigor and survival was not affected by the presence of *E. parasitica* on the nuts and none of the tree stems appeared to be infected from the nut.

1981 Harvest

In September 1981, we harvested unopened burs from six American chestnut trees at Lockwood Farm. The burs were placed in a moist, cool root cellar

for a week prior to removing the nuts from the opening burs. None of the nuts were observed to be infected at that time.

Nuts of each tree were divided into three equal groups of 50 to 100 nuts and placed in plastic bags with damp peat moss. One group was kept in the root cellar (about 13 C), another placed in a refrigerated room (about 4 C), and the third kept at room temperature (about 21 C).

The nuts were examined after 11 weeks for the presence of *E. parasitica.* Some nuts from each of the six trees had apparent *E. parasitica* infections (Table 1). Nuts that were stored at room temperature showed the most infection (i.e., mycelial fans or pustules). None of the nuts stored at the cooler temperatures produced fruiting bodies that erupted through the shell. More nuts from the 1981 harvest will be examined and *E. parasitica-like* isolates characterized.

Table 1. Number of nuts apparently infected by *E. parasitica* over total stored at three different temperatures, 1981.

	Approximate storage temperature - 11 weeks		
Tree	21 C	13 C	4 C
1	40/100	0/100	0/100
2	57/100	4/100	0/100
3	23/100	0/100	3/100
4	77/100	7/100	12/100
5	63/75	3/75	4/83
6	44/50	9/50	1/42
Total	304(57.9%)[a/]	23(4.4%)	20(3.8%)

[a/] Fruiting bodies erupted through the shell of 54 of the 304 (18 percent) infected nuts.

Discussion

The reports by Collins and our original observations of infected nuts do not preclude the possibility that infection occurs on the ground after release from the bur. Our more recent results suggest, however, that a high proportion of the nuts become infected while still on the tree and enclosed within the bur. A period of incubation is necessary before such infections produce the typical orange mycelial fan. There was no consistency as to where the infection appeared on the nuts, occurring at the stylar end, hilum, or in between. Some infected nuts were weeviled but many unweeviled were also infected. Therefore, oviposition does not seem to be responsible for the infections. Infection by germination of *Endothia* spores on the style and subsequent growth into the shell is a possibility.

Conclusions

Whether nuts infected with *E. parasitica* have any role in the dissemination of hypovirulent strains was not conclusively determined. It is clear, however, that nuts apparently free of *E. parasitica* at harvest may have latent infections in the shell which would not be killed by a surface sterilant. It is important that infected nuts not be exported to blight-free areas such as the western United States, Argentina, New Zealand, or Australia where blight susceptible chestnut trees are now being grown.

Literature Cited

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