Testing *Endothia parasitica* Strains for Vegetative Incompatibility

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ABSTRACT.—Vegetative incompatibility can be detected in the laboratory by pairing *Endothia parasitica* strains on agar media. Preliminary field results indicate an involvement of vegetative incompatibility in hypovirulence transfer in the host.

Vegetative incompatibility has been reported for many Ascomycetes and a few Basidiomycetes (Anagnostakis, 1977). It is usually controlled by several nuclear genes, and the results are: 1) lack of hyphal anastomoses, 2) death of cells after anastomosis, or 3) production of heterokaryons or heteroplasmons that cannot compete with the homokaryons present.

This system in *Endothia parasitica* (Murr.) P. J. & H. W. And. results in a large number of compatibility groups. On agar media in the laboratory, strains in the same group will merge with each other where the colonies meet, and the hyphae will anastomose (Fig. 1). Strains in different compatibility groups form a barrage on certain agar media; the colonies will not merge, and lines of pycnidia usually form along the barrage. On segments of chestnut stems, a similar barrage line forms between unlike strains (Fig. 2).

I am concerned about vegetative incompatibility, because transfer of hypovirulence between strains requires hyphal anastomosis. Therefore, vegetative incompatibility could be responsible for the failure of some hypovirulent strains to cure treated virulent cankers.

Caten (1973) and Handley and Caten (1975), who have studied vegetative incompatibility in *Aspergillus*, report that cytoplasmic genes are transferred from strain to strain with the highest frequency when the strains are in the same compatibility group. There is, however, some transfer when strains are in different groups depending on how many, or which, compatibility genes are different among them. We have field data on canker cure with hypovirulence suggesting that *E. parasitica* is similar. If the hypovirulent is in the same vegetative compatibility (v-c) group as the virulent strain causing the canker, restriction is fairly rapid. If the strains are in different v-c groups, control may be rapid, slow, or nonexistent.

Therefore, to cure a specific canker, or group of cankers, the most efficient way is to treat with a mixture of hypovirulent strains representing several v-c groups. If this fails, we can determine the v-c groups of the virulent strains causing the cankers, and treat with hypovirulent strains known to control those v-c groups.

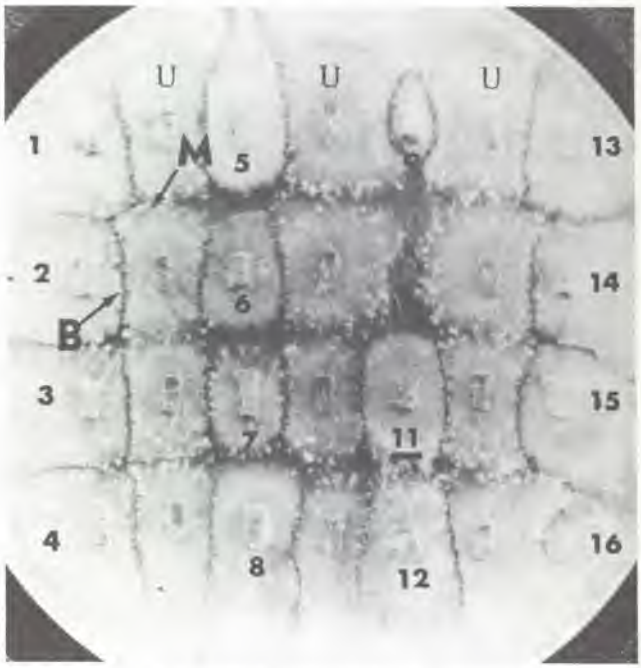

Figure 1. *Endothia parasitica* mass mycelial isolate (labeled U for unknown) paired with v-c group type strains 1-16. Merging (M) and barrage (B) can be seen between strains at arrows. An older culture was used for v-c type 10, and has not grown well enough to give a clear compatibility result (subsequent tests showed the unknown to be incompatible with this tester strain). The morphology of the strain used here as the v-c 11 tester is "difficult," as discussed in the text. It is densely branched, highly pigmented, and often does not form distinct lines of pycnidia along barrage areas.

Strains can be typed in the lab by pairing small pieces of agar containing mycelium on Difco potato dextrose agar (PDA) at 25 C in the dark. Other media have not been as suitable as Difco PDA. The cultures used should not be more than seven days old. The pieces should be uniform and placed about 1 cm apart. Other temperatures do not work as well, and light causes so much sporulation that the results are hard to score. I usually examine test plates with both front and back lighting. If one of the cultures used is older than the other, the dif-

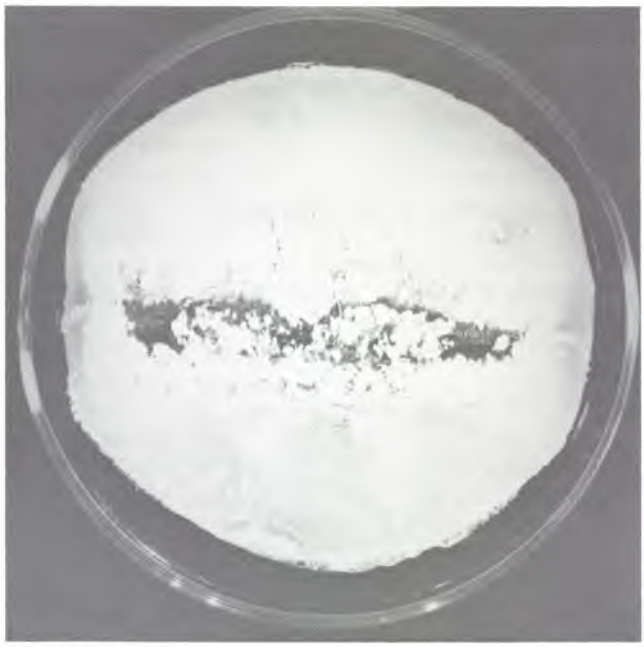

Figure 2. Autoclaved segment of *Castanea dentata* stem supported by 4 percent water agar in a glass petri dish, and inoculated with (top) EP-42, v-c group 5, and (bottom) EP-2, v-c group 10. The two strains have grown over the stem and formed a barrage upon meeting (center).

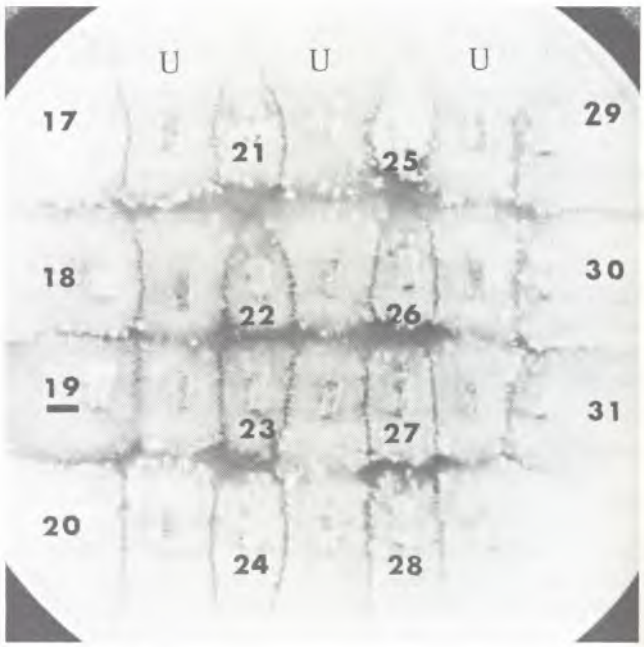

Figure 3. *Endothia parasitica* mass mycelial isolate (U) paired with v-c group type strains 17-31. An unusual interaction with tester 19 has affected the morphology of the tester but not of the unknown. A weak barrage reaction can be seen between the colonies.

ference in initial growth rate (Fig. 1) will be a problem. Occasionally stocks maintained in the lab for a long time by mass transfers will develop "difficult" morphology, i.e. dense branching habit, highly pigmented, early sporulation (Fig. 1). These characteristics interfere with v-c tests and seem to be under the control of cytoplasmic genes (perhaps mitochondrial?). Single-sporing (plating spores on *Endothia* complete agar medium [Puhalla and Anagnostakis, 1971] and selecting single colonies for transfer) often yields some segregants with more typical morphology.

Another problem I have encountered in v-c testing is an interaction which produces an altered appearance of one or both in contact without a typical barrage (Fig. 3). Usually only one of the pair is affected, mycelium is thin, there is little aerial mycelium and the colony may be a reddish buff color instead of white (which would be normal in the dark). I class this as an incompatible reaction.

I am now pairing strains for sexual crosses to obtain genetic information about the determinants of vegetative incompatibility. Based on the number of groups that we have so far (46), I expect at least six genes if they have two alleles each (this would yield 64 v-c groups).

I am also pairing hypovirulent strains from various v-c groups with our 46 v-c group testers (all virulent) in chestnut stems, to find out which combinations will lead to canker control.

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