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Recovery of White and Gray Isolates of Ceratocystis fagacearum from Red Oaks

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Recovery of White and Gray Isolates of *Ceratocystis fagacearum* from Red Oaks[1]

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Abstract. Boles of four *Erythrobalanus* oaks were inoculated with white and gray isolates of *Ceratocystis fagacearum* at diametrically opposed loci. Bole and branches were sampled in an orderly manner when a tree exhibited moderately severe foliar wilt symptoms. Isolations were made on PDA plates. Of the four red oaks, two yielded only the white isolate; the remaining two yielded both isolates. In no instance were both isolates recovered from the same branch, but all sections from the main trunk yielded both, and in several instances both isolates were observed growing from the same chip. This pilot experiment and the findings of other workers indicate that any factor effecting survival of one isolate of *C. fagacearum* over others is manifested at the time of germination of inocula, and that mycelia of different isolates have little significant inhibitory effect on each other.

The presence of a factor which effects the survival of one strain of *Ceratocystis fagacearum* (Bretz) Hunt over other strains has been demonstrated experimentally using mixed culture inoculations (Barnett and Jewell, 1954; Barnett and Staley, 1953). Apparently this factor is in effect in nature since rarely has there been more than one compatibility-type isolated from naturally infected trees (Boyce and Garren, 1953; Yount, 1954). Results of artificial inoculations and investigations of naturally infected stands indicate the selection, if such exists, of one strain over others is random (Barnett and Jewell, 1954; Yount, 1954). These results also indicate survival of one strain over others is determined during or shortly after germination of spores in the penetration court. An experiment was undertaken to determine whether or not establishment of different strains of *C. fagacearum* in the same tree would occur if the inocula were introduced at different loci.

MATERIALS AND METHODS

Two strains of *C. fagacearum* were utilized: Isolate 300, exhibiting the usual grayish color in culture, and Isolate 2126B, an albino

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isolate. Both are B compatibility-type. By using this combination one can determine the isolates recovered macroscopically, without resorting to spermatization tests.

Four oaks of the subgenus *Erythrobalanus* between four and seven inches DBH were inoculated with each strain at diametrically opposed loci four feet above the ground. Three-week-old cultures of the oak wilt fungus growing on PDA slants were smeared on the upper surfaces of 1½ inch chisel cuts into the boles. The chisel cuts were closed, the excess agar acting temporarily as a seal.

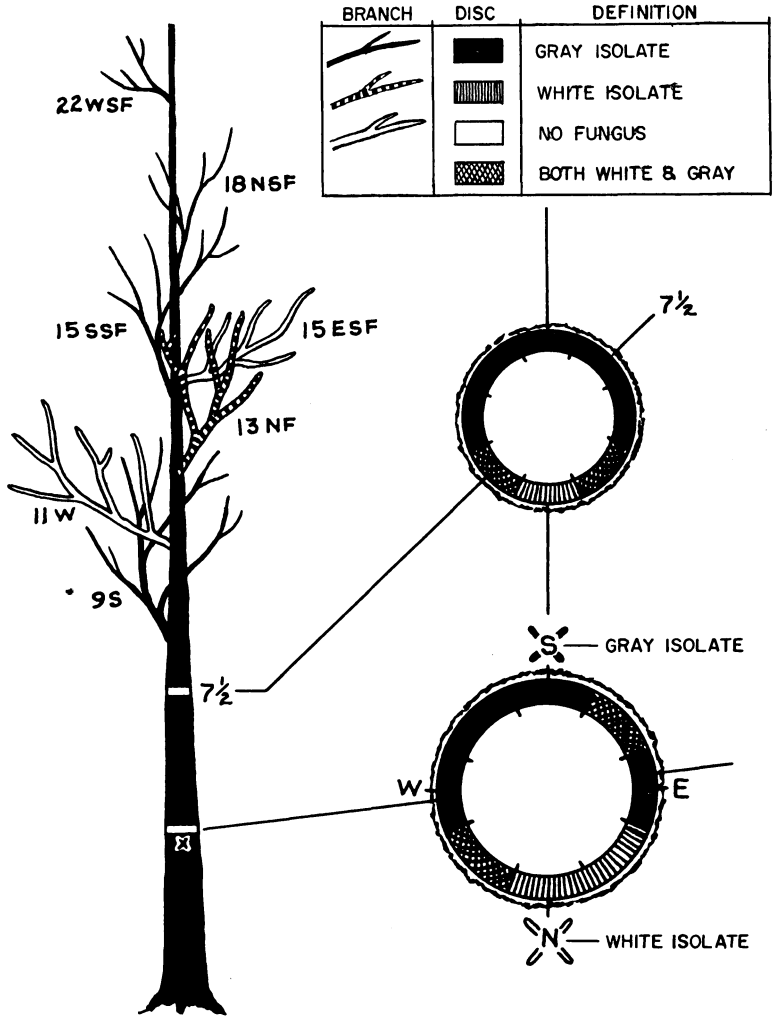

Figure 1. Diagram of Tree E-2, felled thirty-one days after inoculation (Courtesy R. Albertson).

The inoculated trees were felled when they exhibited moderate to severe foliar wilt symptoms. Discs were cut from the bole at eight-foot intervals starting immediately above the inoculation sites. Samples were taken from ten to twelve branches representing most aspects of the tree. The number of samples from one branch was somewhat dependent on the size and deliquescence of that branch.

Four to eight isolations were made along the periphery of each bole using a special heavy-shanked gouge. Isolations were made from each branch sample according to standard procedures (Barnett, 1953). Two percent PDA was used throughout the experiment. Culture plates were incubated 12-14 days at 23°-25°C.

Results

Two of the four inoculated red oaks yielded only the albino strain of the oak wilt fungus. The remaining two trees yielded both strains. Tree E-2 (Figure 1) was sampled differently than the others. Only two discs were removed from the bole, one immediately above and one 7½ feet above the inoculation site. At least one isolate was recovered in every isolation attempted from bole discs. In four instances, both isolates were recovered from the same isolation locus. Twice both isolates were observed growing from the same chip.

Apparently the gray isolate was more widely distributed than the albino isolate in Tree E-2. The gray isolate was recovered from six loci on the lower disc; the albino isolate was recovered from four. On the upper disc the gray isolate was recovered from seven loci, the white isolate from three.

Seven branches from Tree E-2 were sampled and cultured. Of these, four yielded the gray isolate, one the albino isolate, and two neither. Of the latter, one branch exhibited no foliar symptoms at the time of felling. None of the branches sampled yielded both isolates of *C. fagacearum*. The fungus was recovered at several locations on each of the branches inhabited by the gray isolate, but the albino isolate was recovered only once from the branch yielding it.

Both isolates of *C. fagacearum* were recovered from Tree E-3 (Figure 2). Thirty-two isolations were made from five bole discs—eight isolations each from the lowest two discs, six each from the next two discs, and four from the highest disc. Of these, nine yielded only the gray isolate, 11 only the white isolate, two yielded both isolates, and 10 yielded neither isolate. Twice both isolates were observed growing from a single chip.

Eleven branches were sampled from Tree E-3. Of these, five yielded the gray isolate, two the albino isolate, and four yielded neither. Again, no branch yielded both isolates.

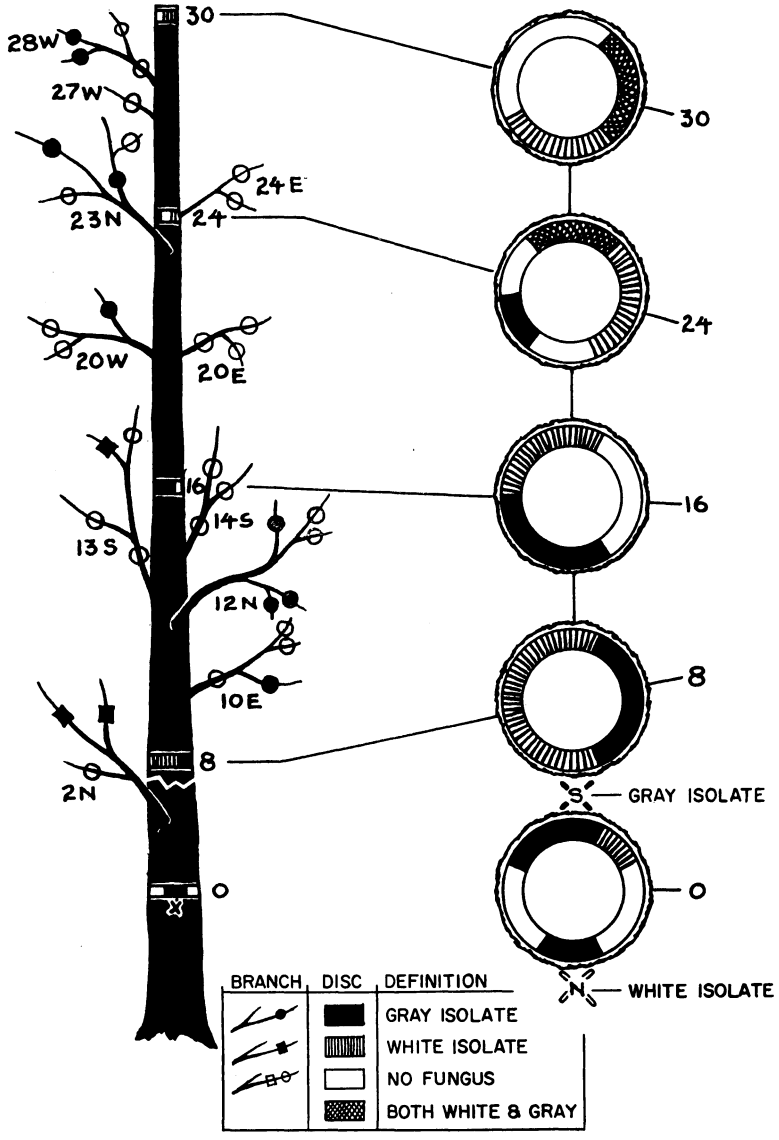

Figure 2. Diagram of Tree E-3, felled forty-one days after inoculation (Courtesy R. Albertson).

Discussion

Results from this experiment, combined with findings reported by other workers (Barnett and Jewell, 1954; Barnett and Staley, 1953; Boyce and Garren, 1953), show that if such a survival factor as has been proposed is in existence in nature, its effects are manifested at

the time of or shortly after germination of inoculum. When conidia or ascospores from two or more strains of *C. fagacearum* are introduced at the same locus, usually only one strain becomes established. However, when two strains were introduced at different loci, both strains became established in the host. In several instances both isolates were recovered together from the boles of the hosts. This indicates the mycelia of the two strains have little inhibitory effect on each other after spores germinate and mycelial colonies become established.

Both isolates were never recovered from the same branch during this experiment. Assuming the translocation of spores by the transpiration stream, perhaps the branches sampled had become colonized by conidia of only one strain. If conidia of both strains were transported more or less simultaneously, the survival factor or factors may have effected the establishment of one strain to the exclusion of the other. If conidia of one strain were transported to a branch and had germinated before infestation by conidia of the other strain, perhaps some factor(s) produced by the established strain rendered the localized area of the branch unfavorable for germination of conidia of the other strain. It will require further research to explain why only one isolate was recovered from each branch sampled in this experiment.

The possibility also exists that both isolates might have been recovered from one branch had more branches been sampled.

The selection phenomenon is difficult to reconcile on the basis of interaction between strains if observations of artificial cultures are considered. The only apparent inhibitory effects of one strain of *C. fagacearum* on another is spatial in nature.

After this apparent selection phenomenon is more clearly understood, several easily recognized strains of the oak wilt fungus may be used to great advantage in host colonization and transmission studies.

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