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Infection of *Quercus Macrocarpa* by White and Gray Isolates of *Ceratocystis Fagacearum*[1]

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Abstract. Cultural studies were made of 348 bur oak branches inoculated with white and gray isolates of *Ceratocystis fagacearum* at diametrically opposed loci. Approximately 15 per cent of the branches developed foliar symptoms of oak wilt, yielded *C. fagacearum,* or both. More than one-third of the visibly diseased branches did not yield this fungus. Part of the failures were attributed to competition from other fungi and bacteria, isolated commonly from wood of both inoculated and uninoculated branches. No branches yielded both isolates of the pathogen. Distribution of the two isolates among trees indicated preferential susceptibility of the host many contribute to the apparent competition *in vivo* among isolates of *C. fagacearum.*

Past investigations using both naturally and artifically infected oaks have rarely shown more than one strain of *Ceratocystis fagacearum* (Bretz) Hunt present within a diseased tree (Barnett and Jewell, 1954; Barnett and Staley, 1953; Boyce and Garren, 1953; Hepting *et al.,* 1951; Yount, 1954). Artificial inoculation with two macroscopically distinct isolates at diametrically opposed loci on the main stem of the red oaks resulted in the reisolation of both isolates from trunk sections, but only one isolate from individual branches (Marchetti, 1960). An experiment, utilizing material for a histological study, was undertaken to obtain further information on this competition phenomenon among isolates of *C. fagacearum.*

MATERIALS AND METHODS

Two strains of *C. fagacearum* were utilized: Isolate 50B, exhibiting typical gray-brown coloration in culture, and Isolate 2126B, an albino isolate. Both were B compatibility-type.

From 40 bur oaks (*Quercus macrocarpa* Michx.) located in Pilot Knob State Park, Hancock County, Iowa, 348 branches, varying in diameter outside bark from 9 to 21 mm and in length from 45 to 180 cm, were inoculated with each strain at diametrically opposed loci. One or two drops of a conidial suspension containing approximately 10^5 spores per ml were injected into holes drilled into the xylem at the base of each

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branch. The holes were drilled with a finger-twist brace and 0.0250 inch bit in 1960 and a portable battery-powered drill in 1961. Disposable polyethylene syringes and aluminum needles (1960) or glass syringes and steel needles (1961) were used as injectors.

Beginning 9 June 1960, six series of inoculations were made at two-week intervals. In each series, 48 branches were inoculated. Samples of six inoculated branches were collected at weekly intervals after injection. Six inoculated branches of each series were left for collection in 1961. Starting 1 April 1961, four series of inoculations were made at three-week intervals. Three inoculated branches were collected every two weeks for eight weeks after inoculation. Three branches were collected 12 weeks after inoculation.

Branches were taken at random within each series. They were sawed flush with the main stem, and a linear reference was inscribed on the bark corresponding to the radius of the inoculation site of the white isolate. Branch samples were 10-15 cm in length. One to four additional samples were taken depending upon length and deliquescence of a branch. All samples from a given branch were numbered, wrapped with a sketch of the branch in aluminum foil, appropriately marked and stored in a portable cooler for transport to Ames.

A medium consisting of 0.5 percent potato dextrose -2 per cent agar was used for culturing. Enough bark was removed from the basal end of the sample to expose approximately five cm of wood. The reference line on the bark was reinscribed on the wood with a felt-tipped pen. A Masonite [R] surface was swabbed with alcohol; a pair of anvil-type cutters and the exposed portion of the branch were dipped in alcohol. All were set aflame. Five discs were cut from the sample, the first being discarded. Petri plates had been marked previously on the bottom with a reference line. Discs were placed in the plate serially, top-side-up, in clockwise order, with reference lines on the discs toward the reference line on the plate (Figure 1). Culture plates were incubated at 22-24°C for 12-14 days.

Results

The incidence of successful inoculations appeared to be very low; 293 out of 348 inoculated branches neither developed foliar symptoms of oak wilt nor yielded *C. fagacearum* in culture. The success obtained in attempts to reisolate the pathogen from diseased branches was poorer than expected. The inciting organism was isolated from 28 of the 50 inoculated branches that developed foliar symptoms. Seven of the remaiinng 22 branches were dead at the time of collection and, therefore, were not

likely to have yielded *C. fagacearum*, regardless of the technique used. The inoculations made on 23 June 1960 were the most successful for that year. (Table 1).

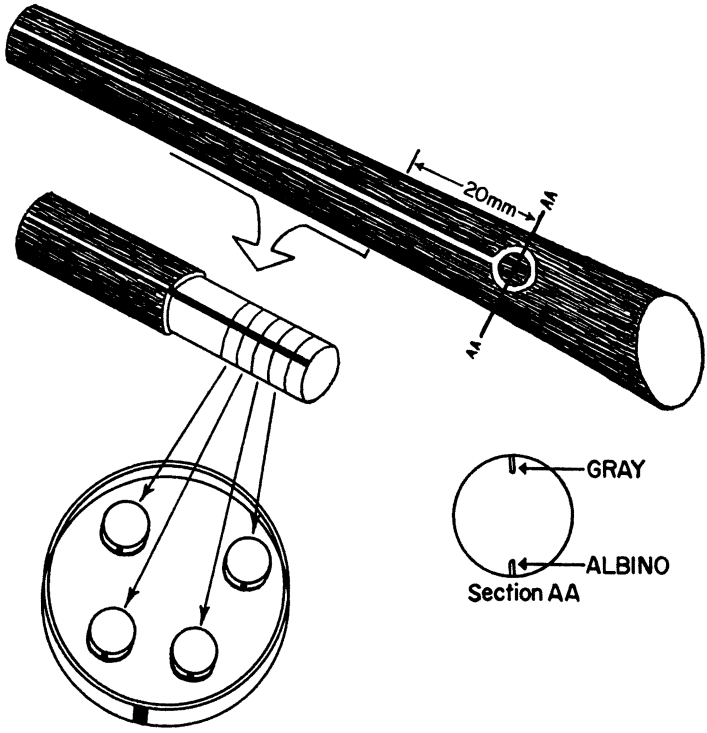

Figure 1. Inoculation and sampling for culture of bur oak branches and the ultimate disposition of sample discs in the culture plate (Courtesy R. Albertson)

Table 1. Distribution of diseased bur oak branches inoculated on 23 June 1960 among trees, and recurrence of symptoms on the trees in 1961, Pilot Knob State Park, Hancock County, Iowa.

Tree	No. branches inoculated	No. diseased	Symptoms on trees in 1961
8	10	7	dead
9	6	0	none
10	10	3[a]	none
11	8	3[b]	staghead; no new foliar symptoms
14	9	1[c]	none
15	5	3[b]	staghead; no new foliar symptoms

[a] One branch, collected in summer of 1961, neither yielded *C. fagacearum* in culture nor developed recurrent foliar systems.

[b] One branch, collected in summer of 1961, yielded *C. fagacearum* in culture, but developed no recurrent foliar symptoms.

[c] Yielded *C. fagacearum* in culture, but developed no foliar symptoms.

Other fungi and bacteria were isolated consistently from bur oak branches, regardless of treatment. Less than three per cent

of approximately 1100 plates contained wood discs with no emergent microflora. Except on rare occasions, the oak wilt organism and any other microorganisms appeared mutually exclusive in any given branch disc.

The form-genera most commonly encountered among the fungal contaminants were *Trichoderma, Phomopsis, Phoma, Coniothyrium* and *Alternaria.* These fungi grew more rapidly than *C. fagacearum* and all except *Alternaria* usually covered the wood discs within the first week of incubation.

The frequencies of symptom expression and recovery of the pathogen increased as the period between inoculation and collection increased. In 40 of the 55 branches that were considered successfuly inoculated, symptoms or recovery of the fungus were first noted between four and eight weeks after inoculation. Two branches that were inoculated on 21 July 1960 developed no foliar smyptoms until the following July. The albino isolate was isolated from both branches.

Certain trees appeared to have been preferentially susceptible to one or the other isolate (Table 2). *C. fagacearum* was isolated from two or more branches of eight trees. Generally, all the branches from the same tree yielded the same isolate. No branch yielded both isolates.

Table 2. Distribution of isolates of *C. fagacearum* among inoculated branches of 14 bur oaks in Pilot Knob State Park, Hancock County, Iowa

Tree	Inoculation date	No. branches yielding *C. fagacearum*	Gray	White
2	6-9-60	1	0	1
6	6-9-60	1	1	0
13	6-9-60	1	0	1
8	6-23-60	4	4	0
11	6-23-60	3	0	3
14	6-23-60	1	0	1
15	6-23-60	3	3	0
22	7-7-60	2	0	2
24	7-7-60	1	0	1
29	7-21-60	2	0	2
50	4-22-61	1	1	0
54	5-13-61	5	4	1
55	5-13-61	2	0	2
60	6-1-61	6	2	4
14		33	15	18

On all trees, there were several to many uninoculated branches interspersed among those selected for treatments. With one exception, inoculated branches developed symptoms one to three weeks before uninoculated branches on the same tree. In this exception, foliar symptoms on uninoculated branches on the

upper crown and on two inoculated branches were first noted on the same day.

Discussion

Approximately 15 per cent of the branches inoculated by the techniques described had become diseased by the time of collection. Undoubtedly, part of the apparent failure to infect can be attributed to the sampling schedule. About 75 per cent of the diseased branches developed foliar symptoms four or more weeks after inoculation. By the end of this fourth week, half of the initial population of inoculated branches had been eliminated by periodic sampling. Presumably other branches would have developed oak wilt symptoms had they not been eliminated beforehand.

Many other fungi and bacteria were isolated from bur oak branches. The presence of a diverse microflora in xylem of white oaks has been reported (Wood and Peterson, 1959). It is probable that such contaminants masked in culture the presence of *C. fagacearum* in infected bur oak branches. Possibly the activities of certain microorganisms in the xylem rendered branches unfavorable for the establishment of the oak wilt organism. The activities of the internal microflora of bur oaks are unknown, and little is known about competition *in vivo* between *C. fagacearum* and other microorganisms.

Although the oak wilt organism did not appear to compete well with other organisms on an agar medium, it may be more competitive in the environment of the branch. In testing this, two branches, which yielded only contaminants by culturing discs, were cultured by placing small chips from prominently discolored wood on the medium. *C. fagacearum* was recovered from these chips. Thus, the growth of the oak wilt pathogen could have been inhibited by other microorganisms in the disc method used.

The distribution of isolates of *C. fagacearum* in infected branches of the same tree indicated preferential susceptibility of certain trees to one or the other isolate. The chances of the distribution of isolates occurring independently of the trees involved were very slight (Cochran, 1954; pp. 425-427), assuming that each infection was an independent event and that each isolate had an equal opportunity to become established in each branch. It is unlikely that any inconsistencies in inoculating techniques should favor consistently one or the other isolate in all branches of a given tree. On nearly all trees inoculated, branches developed foliar symptoms before other branches. If the infections of all the inoculated branches had resulted

from only one or two successful inoculations, then, presumably, uninoculated branches interspersed among the inoculated branches should have had as much chance of becoming infected by *C. fagacearum* as branches in which inoculations were unsuccessful. Therefore, it was indicated that each infection of inoculated branches had been an independent event in most, if not all, instances and that certain trees were preferentially susceptible to one or the other isolate of *C. fagacearum.*

The results obtained from certain inoculation periods indicated certain trees were resistent to infection by either isolate. None of the inoculations in some trees was successful, whereas a high proportion of inoculations was successful in others, all being inoculated on the same date. Again assuming that each branch infection was an independent event, the chance occurrence of such a distribution of infected branches among trees appeared slight.

The effects of the oak wilt disease were in evidence throughout the areas of the park in which experimental trees were selected. Probably a high proportion of susceptible bur oaks has been eliminated from the population. Consequently, it is probable that the frequency of resistant trees among those selected for this experiment was greater than it would have been a decade or two ago.

Literature Cited

Barnett, H. L. and F. F. Jewell. 1954. Recovery of isolates of *Endoconidiaphora fagacearum* from oak trees following mixed culture inoculations. Plant Dis. Rptr. 38:359-361.

——————— and J. M. Staley. 1953. Isolation of compatability types of *Endoconidiophora fagacearum* from oak trees naturally and artifically inoculated. Phytopathology *43*: 341-343.

Boyce, J. S., Jr., and K. H. Garren. 1953. Compatability types of the oak wilt fungus in 23 Appalachian trees. Phytopathology *43*:644-645.

Cochran, W. G. 1954. Some methods for strengthening the common[2] tests. Biometrics *10*:417-451.

Hepting, G. H., E. R. Toole and J. S. Boyce, Jr. 1951. Perithecia produced in an unpaired isolate of *Chalara quercina* and its possible significance in oak wilt control. Plant Dis. Rtpr. 35:555.

Marchetti, M. A. 1960. Recovery of white and gray isolate of *Ceratocystis fagacearum* from red oaks. Iowa Acad. Sci. Proc. 67:109-113.

Wood, F. A. and J. E. Peterson. 1959. Fungi isolated from oak-wilted-infected and apparently healthy oak trees. (Abstract) Phytopathology *49*:555.

Yount, W. L. 1954. Identification of oak wilt isolates as related to kind of inoculum and pattern of disease spread. Plant Dis. Rptr. 38:293-296.