Longevity of Ceratocystis fagacearum in Ammate Treated and Nontreated Root Systems

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ABSTRACT

Two roots were removed from each of 292 wilt-killed oaks during 1965 and isolations were made using Barnett's medium. During 1966 and 1967, two roots from each of 49 Ammate-treated stumps were treated similarly. *Ceratocystis fagacearum* was isolated from 23 of 45, 21 of 101, 1 of 50, 2 of 64, and 0 of 30 of the nontreated trees dead 1 to 5 years, respectively. The fungus was obtained from 17% (4 of 24) of the trees treated with Ammate 1 yr before sampling and from none of those trees treated 2 yr before sampling (0 of 25). Roots remained solid for 2 yr after tree death, but then rapidly deteriorated. These data indicate that the control methods used in Pennsylvania reduce the amount of inoculum available for spread by root grafts or by root inhabiting insects.

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Oak wilt in in Pennsylvania is usually characterized by wilt of a single tree within a stand. If left untreated, such infection centers often enlarge. After treatment, which consists of cutting all species in the same oak group (subgenus – Erythrobalanus or Leucobalanus) within a 14.25m (50-ft) radius of the wilted tree and applying Ammate (ammonium sulfamate) at the rate of 0.45 kg (1 lb) of the 95% formulation/0.31m (ft) diam of the stump surface. Even so, trees on edges of treated areas sometimes develop symptoms (breakovers).

Breakovers have been observed to occur 1 to 4 yr after control. In Pennsylvania, there were 32 breakovers treated out of 271, and 49 out of 294 treated centers in 1965 and 1966, respectively (Jeffery 1965, 1966. *Unpublished* reports. Penna. Dept. Agric.). The number of breakovers, the proximity of the newly infected trees to the old treated centers, and the distance between infection centers, indicated that introduction of inoculum from distant sources was improbable.

Trees found dead at the time of treatment are left untreated due to the hazards involved during felling. The number of trees found dead and left standing without treatment during 1965 and 1966 were 693 and 815, respectively (Jeffery 1965, 1966. *Unpublished* reports. Penna. Dept. Agric.). The fungus within the root system of these trees could also function as inoculum for spread from the infection center.

Craighead and Nelson (4) reported that Ammate was not effective in killing roots unless applied before 50% wilt had occurred. They also questioned the fungitoxicity of Ammate to *C. fagacearum*.

In West Virginia, Amos (1) and Amos and True (2) succeeded in culturing the fungus from roots of 25% of trees treated 1 yr and from a small unreported percentage of trees dead 2 yr before sampling. Chemicals are not used in the West Virginia oak wilt control program; where a deep girdle to the heartwood is made at stump height and the bark is removed to the ground. Amos (1) concluded that the roots of infected trees were unimportant as a major source of inoculum except for root graft spread.

Studies by Skelly (8) concerning root-inhabiting insects as vectors of *Ceratocystis fagacearum* (Bretz) Hunt demonstrated a need to determine the longevity of the fungus in the root systems of infected trees. The presence of the oak wilt fungus in roots of wilt-killed trees has been established. Yount (10), in a limited study, examined the roots of Ammate-treated stumps in Pennsylvania, and was able to isolate the fungus from roots of one of three trees treated 3 yr prior to sampling, from five of eight trees treated 2 yr prior to sampling, and from five of six trees treated 1 yr before sampling.

This study was initiated to determine the longevity of the oak wilt fungus in the roots of Ammate-treated and nontreated trees. Associated insect activity in the various root systems was also recorded and these observations were reported elsewhere (9).

MATERIALS AND METHODS.—A pilot study was initiated during 1966 to determine the sample size needed to indicate the presence or absence of *C. fagacearum* in the root system of an infected tree. Two roots from three trees that had died, 1 (two trees) and 2 (one tree) yr before from infection by *C. fagacearum* were excavated, excised, and taken to the laboratory for isolation. Since the majority of trees to be sampled later were those that had died either from root or main stem inoculation, or from "natural" infection, one tree was selected in each of these categories for this initial study. The bark was removed and the wood surface was flame-sterilized with 95% ethanol. Chips were removed with a small wood chisel and placed onto Barnett's oak wilt agar (3). When *C. fagacearum* was observed growing from these chips, the root system of the tree was excavated to an average distance of 1.3 m and taken to the laboratory for further isolations. Four chips were removed from sample points every 15 cm along the upper and lower surfaces of each root and at depths of approximately 1.0 and 2.5 cm. Root length and the apparent amount of decay were noted.

Two large roots from each of 292 trees to which no control measures had been applied, were sampled during 1965, 1966, and 1967. These were trees that could be accurately dated as to year of death, and which had been killed by *C. fagacearum*. The trees had been either

TABLE 1. Occurrence of *C. fagacearum* in root systems of nontreated and Ammate-treated oak trees

Years dead	Nontreated			Ammate-treated		
	No. sampled	No. positive	Percent positive	No. sampled	No. positive	Percent positive
1	45	23	51	24	4	16
2	101	21	21	25	0	...
3	50	1	2	...	...	...
4	64	2	3	...	...	...
5	32	0	0	...	...	...
Total	292	47	16.1	49	4	8

inoculated during previous studies, or had died from natural infections.

Root sections were stored at 2 C until isolations were made. Barnett's medium was used for all isolations and streptomycin sulfate was added at 250 μg/ml to suppress the development of bacteria. Forty chips were removed from each root (five chips/plate, eight plates/root); an attempt was made to isolate from all areas of the root sections. Plates were incubated at 21 C for 10 to 14 days and then examined for the presence of *C. fagacearum*. In this manner, two roots were sampled from each of 45, 101, 50, 64, and 32 trees that had died from 1 to 5 yr prior to sampling, respectively.

During 1966, sections of two roots were removed at the ground line from each of 24 and 25 stumps treated with Ammate in 1965 and 1964, respectively.

The locations of infection centers to which control practices had been applied were obtained from the Pennsylvania Department of Agriculture, Harrisburg, Pa. Treated stumps of trees infected at the time of control and designated by a nail driven part way into the cut surface were sampled.

Roots of several dead trees that were standing and left untreated in the plots were also sampled. The majority of these trees (14 of 17) had died in 1963, 3 yr prior to this investigation. All other procedures were identical to those used for the noncontrol root systems.

RESULTS.—To determine a reliable root sample size for the presence or absence of *C. fagacearum* in any particular root system, 2,064 chips were removed from a total of 516 individual isolation points on 28 roots from three trees showing typical disease symptoms. *Ceratocystis fagacearum* was consistently isolated only from buried portions. Only 2 of 28 roots sampled did not yield the fungus; both of these were from the same tree. One was entirely exposed and one was completely buried. By using binomial probability analysis, the isolation success rate for a one-root sample was $P = 0.928$ and for a two-root sample was $P = 0.995$. The two-root sample size was used in all subsequent studies. A sample of two roots was selected for each tree or stump to be studied.

The data for isolations made from nontreated and treated root systems are presented in Table 1. These data are based upon a total of 23,360 chips and 3,925 chips removed from roots of nontreated and Ammate-treated trees, respectively.

Ceratocystis fagacearum was present in the roots of 51% of the nontreated trees sampled that had died 1 yr before sampling. The fungus was present in the roots of 21% of the trees dead 2 yr and then the percent recovery dropped sharply to 2, 3, and 0% for trees dead 3, 4, and 5 yr, respectively.

The fungus was isolated from 16% (4 of 24) of the root systems tested which were treated 1 yr before with Ammate, but from none of the trees dead 2 yr. Trees treated 3 yr before were not tested.

The amount of decay found in the roots increased with length of time after death. In general, roots remained in good condition for 2 yr; extensive decay was observed in the roots of trees dead 3 yr. Decay was first evident at the distal ends of roots and in roots that were severely attacked by sapwood decay fungi.

Roots of the additional 17 trees, that were dead and standing within the treated infection centers were tested for the presence of fungus. Although it was not definitely known that these trees were killed by *C. fagacearum*, their proximity to the infection center and the presence of mat scars on some of them indicated that they may have died from infection during previous years. *Ceratocystis fagacearum* was not detected in any of these root systems, but most of the trees (14 of 17) had been dead for 3 yr. This agrees with the results for nontreated trees presented in Table 1.

DISCUSSION.—The results of these studies of nontreated and treated root systems indicate that a source of inoculum of *C. fagacearum* exists, particularly in the roots of nontreated trees. The number of nontreated trees found dead within infection centers during 1965 and 1966 alone was 1,508 (Jeffery 1965, 1966. *Unpublished* reports. Penna. Dept. Agric.). The avg number of wilting trees per infection center in 1965 and 1966 was 1.96 and 2.80, respectively. The average number of nontreated trees per infection center during each year was 2.56 and 2.76. The large number of these trees together with the ability of *C. fagacearum* to survive in roots for up to 3-4 yr suggests that they may harbor a significant source of inoculum.

The longevity of *C. fagacearum* in root systems of nontreated trees is important from another aspect. Aerial surveys are only 25%-50% efficient in Pennsylvania (6). Gillespie (5) reported only 14% to 25% efficiencies in other states. If aerial surveys are at least 50% effective and all detected centers are controlled, then 50% of those trees dead 1 yr and left untreated still harbor the fungus, 20% do so for 2 yr and so on. Hence, the root systems of one out of four infected trees in Pennsylvania harbor the fungus for 1 yr after death.

It is apparent that treating stump surfaces with Ammate reduced the ability of *C. fagacearum* to survive in the root systems. The fungus was not obtained from any of 25 trees treated 2 yr prior to sampling and from

only 17% of trees sampled 1 yr after treatment.

Different control practices may in part explain the different rates of spread of oak wilt in West Virginia and Pennsylvania (7). Large differences were noted in the breakover numbers of treated infection centers in the two states with West Virginia having 650 in 1965 and Pennsylvania having less than 30. The addition of numerous nontreated trees each year and the lack of use of a chemical treatment may account for the rapid increase in the numbers of infection centers and the increase in the incidence of breakovers in West Virginia as compared to Pennsylvania.

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