

Ceratocystis fagacearum Not Transmitted by Ambrosia Beetles

H. W. Wertz, J. M. Skelly, and W. Merrill

Former Graduate Assistant, former Research Assistant, and Associate Professor, respectively, Department of Plant Pathology, The Pennsylvania State University, University Park 16802.

Contribution No. 607 from the Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station. Authorized for publication as Journal Series Paper No. 3935.

Supported in part by funds received from the Pennsylvania Fair Fund administered by the Pennsylvania Department of Agriculture.

Accepted for publication 5 May 1971.

ABSTRACT

Monarthrum fasciatum, *Xyleborus saxeseni*, and *X. xylographus* did not attack or transmit *Ceratocystis fagacearum* to healthy red oak seedlings, saplings, or mature trees. *Xyloterinus politus* attacked healthy red oaks of all ages and tunneled into the

xylem, but transmission of the pathogen did not occur. Although high percentages of the insects acquired the fungus in vitro, few insects retained the fungus longer than 24 hr. *Phytopathology* 61:1185-1187.

Oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, is a problem in oak forests from central Pennsylvania to western North Carolina and from central Minnesota to northeastern Texas. Although in the past two decades much of the disease cycle has been elucidated, the means of establishment of new infection centers is still unknown. The pathogen is adapted for insect dissemination (15), and new infection centers continue to develop annually in spite of control programs (5, 8) and in areas where sporulating fungus mats do not develop (10).

Results of several studies indicated that ambrosia beetles (Coleoptera: Scolytidae) were potential vectors because (i) the pathogen survives up to 4 years in the buttress roots of wilted trees (13); (ii) in Pennsylvania, such roots are nearly always heavily infested with one or more of four species of ambrosia beetles (13, 16); (iii) young adult insects emerge from these roots carrying the pathogen in or on their bodies (1, 3, 9, 13, 14); (iv) such adults fly about in oak stands (9) and subsequently may feed on healthy, weakened or dead trees (1, 2, 3, 11, 12); and (v) one species common in oak already had been implicated as a possible vector of *C. ulmi* (4). However, transmission studies had never been attempted with ambrosia beetles.

The following studies were done to determine whether any of the four species of ambrosia beetles common in wilted oaks in Pennsylvania could transmit *C. fagacearum* to healthy red oaks.

MATERIALS AND METHODS.—Preliminary studies were made in 1966 and 1967 in the greenhouse and in screened insectaries using potted red oak (*Quercus rubra* L.) seedlings or bench-grown saplings. *Xyleborus saxeseni* (Ratzeburg), *X. xylographus* (Say), and *Xyloterinus politus* (Say) were collected from infested roots and stumps; we have been unable to rear these insects in the lab. The wood was split with axes and knives, or shaved with a drawknife; individual insects were removed with forceps, placed in glass vials, and stored at 2 C for up to 3 weeks until needed.

The beetles were maintained 36 to 40 hr on 10-day-old colonies of *C. fagacearum* grown in petri dishes on Difco oak wilt agar, then caged immediately onto the plants. The cages consisted of small cylinders of

35-mesh nylon screening placed over the seedlings or wrapped around the bole of the saplings; insects were placed into the cage and the top was closed and sealed.

Ambrosia beetles normally attack thick-barked portions of the tree rather than thin-barked branches or twigs, as represented by the saplings and seedlings used in the greenhouse and insectary studies. Therefore in 1968, field transmission studies were done with the above three species and with *Monarthrum fasciatum* (Say). The insects were collected and handled as before. Experimental plots were located in Perry County, Pa., and were known to have been free of oak wilt for the previous 5 years; thus, the chance of a naturally occurring infection confounding the results was minimal. The trees ranged from 5- to 40-cm-diam breast height. Some had thin smooth bark; others had thick rough bark. Cages consisted of 4-cm² pieces of nylon screening stapled to the bark over fissures wherever possible, because we had observed that the insects initiated tunnels in such sites. Three or sometimes four beetles were used/cage because not all beetles become infested while feeding. Also, in most species only the female tunnels, and it is difficult to determine the sex of insects without injuring them. All caging was done on 1 June. The average delay between the time the beetles were removed from the cultures and the time they were caged was 1 hr.

Based on the results of the above studies, a larger transmission study was made in 1969 using only *Xyloterinus politus*. Trees containing *X. politus* were located during the fall and winter of 1968; most of the trees had died of oak wilt during the summer of 1967. Sections of the bole and roots containing the galleries in addition to numerous individual beetles collected in vials were taken to University Park and stored at 2 C until needed. Insects were collected, fed on cultures of *C. fagacearum* as before, and caged on the trees on 28 May. The average delay between the time the beetles were removed from the cultures and the time they were caged was 1 hr.

Because some beetles escaped from the cages used in 1968, another type of cage was used on half the trees in 1969. A ring of Silicone Bathtub Seal (G.E. 2561-71D) 2 cm in diam was applied to the tree, generally

over a bark fissure or other irregularity, 3 weeks prior to caging so that all traces of solvent would be gone when the beetles were placed in the cage (Fig. 1). Screening was stapled over the ring to form a chamber about 8 mm deep and 2 cm in diam. Because the effect of sealer on the beetles was unknown, the cages were stapled directly to the bark of the remaining trees. Five (or sometimes four) insects were placed in each cage to increase the chances of getting at least one tunnel/tree.

In 1968 and 1969, insects which had not fed on *C. fagacearum* were caged on similar trees to serve as controls. In all studies, the pathogenicity of the isolates used was determined by inoculating four trees with about 5 ml of a conidial suspension containing ca. one million conidia/ml.

In 1969, the spermatization technique (6) was used to determine the percentage of beetles carrying viable inoculum. Fifty beetles were picked at random from those feeding on the fungus. Each was crushed in 5 ml of sterile distilled water. The resulting suspension was brushed over a 10-day-old colony of the opposite mating type of *C. fagacearum*. As a check on the receptivity of the two isolates, a few ml of spore suspension of the isolate used for feeding were brushed over colonies of the opposite mating type.

Results of the 1969 field studies raised questions concerning the length of time the pathogen remained viable in or on the insects. Therefore 35 *X. politus* were fed for 36 hr on 10-day-old cultures of *C. fagacearum*, then placed on small sections of oak bark in deep petri dishes to simulate cage conditions. The presence of the pathogen on these insects was determined by the spermatization technique at various times after removal; eight or nine insects were used per time period. In a second study, 50 *X. politus* were fed,

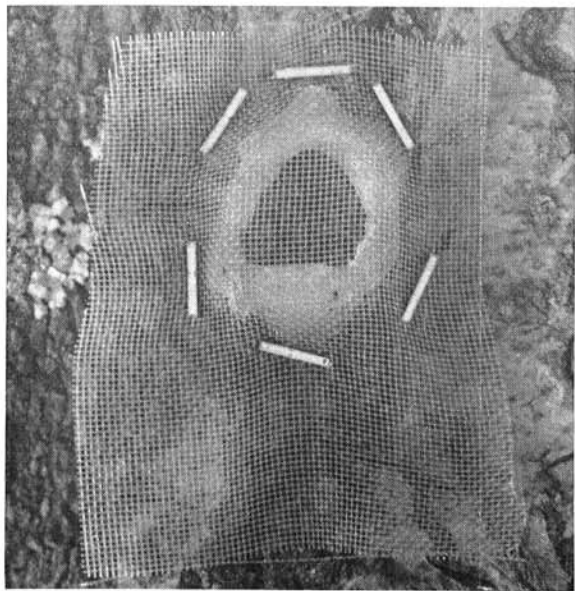


Fig. 1. Insect cage of nylon screening stapled over ring of silicone rubber sealer ($\times\frac{3}{4}$).

stored, and tested in the same manner; ten insects were used/time period.

RESULTS.—In 1966, a total of 66 *Xyloterinus politus* were caged in 12 cages on five red oak saplings about 3 cm in diam in the greenhouse. Eleven tunnels into the xylem were formed, but no transmission occurred. All inoculated check trees wilted and died.

In 1967 a total of 75 *Xyloterinus politus* caged on 40 seedlings formed 18 tunnels, but no transmission occurred. Twenty-six *Xyleborus saxeseni* and 18 *X. xylographus* were caged on 22 seedlings. No feeding by either species was observed. All inoculated check trees wilted and died.

In 1968, 31 *Monarthrum fasciatum*, 48 *Xyleborus saxeseni*, 49 *X. xylographus*, and 47 *Xyloterinus politus* were used. Only *Xyloterinus politus* attacked healthy red oaks; 5 out of 47 (10.6%) of the beetles bored into the xylem. Depth of the tunnels varied; maximum penetration was about 8 mm into the xylem. Sap oozed from the insect tunnel in one of the five trees. However, no wilt symptoms developed in any of the trees. Check trees inoculated with a conidial suspension wilted and died by 26 August 1968.

In the 1969 transmission study, 949 *X. politus* were caged on 191 trees. When the cages were removed 2 weeks after initial caging, there were 246 visible entrance holes in the trees. The nature of the bark may have obscured holes in some instances. Seventeen trees had no visible tunnels; on three of these, the cages had been destroyed by some unknown agent and all the beetles had escaped. The remaining trees had from one to four tunnels/tree. As in the 1968 studies, sap oozed from the tunnels on 110 trees. In several instances, the sap attracted Nitidulids and, indeed, three cages contained Nitidulid larvae. None of the 174 trees with tunnels showed oak wilt symptoms as of 15 August 1970. Trees inoculated with a conidial suspension to check pathogenicity had wilted and died by 1 August 1969. The spermatization studies showed that 84% (42/50) of the beetles carried the pathogen when they were removed from cultures of the fungus and caged on the trees. Check matings of the two isolates yielded fertile perithecia in all cases, indicating good receptivity.

Figure 2 shows the percentages of insects carrying viable inoculum at various times after removal from cultures of *C. fagacearum* and maintenance on oak bark. Check matings of the isolates used in both studies showed good receptivity.

DISCUSSION.—The silicone ring and mesh cages had no apparent effect upon the longevity or tunneling activity of the beetles. Because such cages allow virtually no escape, they are a better type of cage for such studies than screening alone.

Monarthrum fasciatum, *Xyleborus saxeseni*, and *X. xylographus* did not attack healthy red oaks of any size or age. However, in rearing studies in the laboratory these insects tunneled readily into sawdust media and into small oak bolts placed in screened insectaries. If these species would not attack living trees when forced (i.e., under cage conditions), it is highly im-

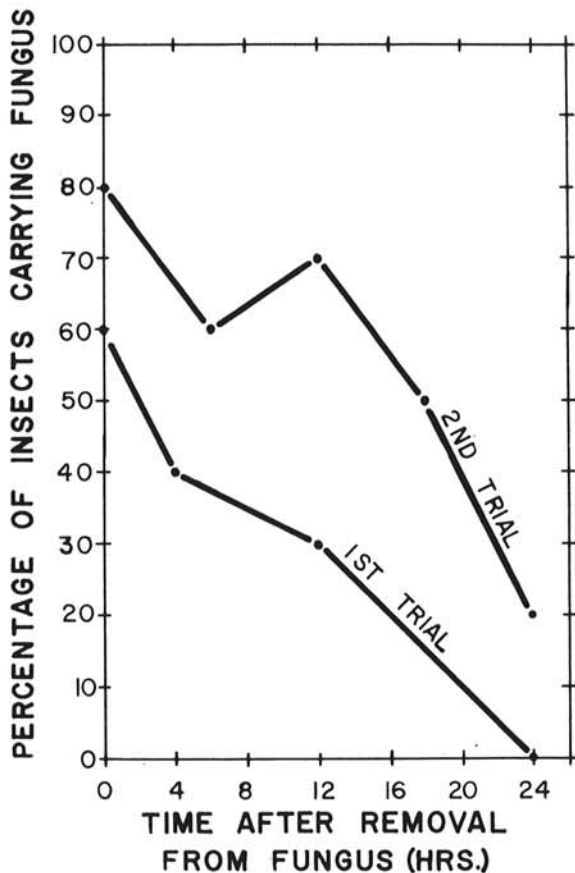


Fig. 2. Percentages of *Xyloterinus politus* carrying fungus propagules at various times after removal from cultures of *Ceratocystis fagacearum*.

probable that they would attack living trees in nature. Hence, it appears that these three species are not involved in establishment of new oak wilt infection centers.

Xyloterinus politus attacked healthy red oak seedlings, saplings, and mature trees of all sizes, but did not transmit the pathogen. The time required for penetration to the xylem of mature trees is unknown, but on saplings, penetration was complete within 24 hr. The pathogen would have remained viable for this period of time (7).

In preliminary studies, we found that if individual beetles, maintained for 24 hr on colonies of *C. fagacearum*, were macerated in a few ml of distilled water and the resulting suspension was placed into fresh wounds on red oaks, the trees wilted. Thus, the insects initially carried sufficient inoculum to inoculate the trees; yet transmission did not occur. This raised questions concerning the longevity of the fungus in the insects.

Initially, 60-80% of the beetles carried the fungus (Fig. 2), but only a small percentage of the beetles placed on bark carried the fungus 24 hr, and probably none carried it longer than 36 hr. Thus, it would seem

that in the time required for the insect to tunnel into the xylem in some manner, the inoculum it had been carrying was lost.

Studies elsewhere indicate that the pathogen is carried internally in Scolytid beetles (C. O. Rexrode, *personal communication*). Such inoculum may be voided in the fecal pellets before the insect penetrates to the xylem; this may occur rapidly if the insect is engaged in heavy work such as tunneling. Thus, infection would not occur.

In summary, 5 years of field and greenhouse studies have demonstrated that the four species of ambrosia beetles commonly associated with wilted oaks in Pennsylvania are unable to transmit *C. fagacearum* to healthy red oaks, and hence are not the vectors involved in the establishment of new oak wilt infection centers.

LITERATURE CITED

- BATRA, L. R. 1963. Ecology of ambrosia fungi and their dissemination by beetles. *Kansas Acad. Sci. Trans.* 66:213-236.
- BLETCHLY, J. D. 1961. A review of factors affecting ambrosia beetle attack in trees and felled logs. *Empire Forest. Rev.* 40:13-18.
- CRAIGHEAD, F. C., C. L. MORRIS, & J. C. NELSON. 1954. Pennsylvania studies of insect vectors of the oak wilt fungus—a summary. Pa. Dep. Forests Waters, Harrisburg. 9 p.
- FINNEGAN, R. J., & C. GAGNON. 1964. *Xyloterinus politus* (Say), another possible vector of the Dutch elm disease. *Can. Dep. Forest. Bi-mon. Prog. Rep.* 20(6):2-3.
- HIMELICK, E. B., & H. W. FOX. 1961. Experimental studies on control of oak wilt disease. *Univ. Ill. Agr. Exp. Sta. Bull.* 680. 48 p.
- JEWELL, F. F. 1954. Viability of the conidia of *Endoconidiophora fagacearum* Bretz, in the fecal material of certain Nitidulidae. *Plant Dis. Repr.* 38:53-54.
- MEREK, E. L., & C. L. FERGUS. 1954. The effect of temperature and relative humidity on the longevity of spores of the oak wilt fungus. *Phytopathology* 44:61-64.
- MERRILL, W. 1967. The oak wilt epidemics in Pennsylvania and West Virginia: an analysis. *Phytopathology* 57:1206-1210.
- MERRILL, W. 1968. A summary of recent oak wilt research at The Pennsylvania State University. The 1968 oak wilt program in Pennsylvania. Pa. Dep. Agr. Bur. Plant Ind., Harrisburg. p. 1-12.
- NORTHEAST FOREST EXPERIMENT STATION. 1967. Report, 1966. USDA Forest Serv. 98 p.
- REXRODE, C. O. 1968. Tree-wounding insects as vectors of the oak wilt fungus. *Forest Sci.* 14:181-189.
- SHIGO, A. L. 1966. Defects in birch associated with injuries made by *Xyloterinus politus* (Say). *U.S. Forest Serv. Res. Note NE-49.* 7 p.
- SKELLY, J. M. 1968. Root inhabiting insects as possible vectors of *Ceratocystis fagacearum* (Bretz) Hunt. Ph.D. Thesis, Pa. State Univ. 131 p.
- STAMBAUGH, W. J., C. L. FERGUS, F. C. CRAIGHEAD, & H. E. THOMPSON. 1955. Viable spores of *Endoconidiophora fagacearum* from bark and wood-boring beetles. *Plant Dis. Repr.* 39:867-871.
- TRUE, R. P., H. L. BARNETT, C. K. DORSEY, & J. G. LEACH. 1960. Oak wilt in West Virginia. *W. Va. Agr. Exp. Sta. Bull.* 448 T. 119 p.
- WERTZ, H. W. II. 1970. Four species of ambrosia beetles as vectors of *Ceratocystis fagacearum* (Bretz) Hunt. M.S. Thesis, Pa. State Univ. 13 p.