# Nonsurvival of Ceratocystis fagacearum in Frass of Oak Bark Beetles and Ambrosia Beetles

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ABSTRACT

Viable propagules of *Ceratocystis fagacearum* were not detected in the frass of oak bark beetles and ambrosia beetles formed the spring after initial infection and death of red oaks in Pennsylvania. This indicates that wind dissemination of frass-borne propagules is not involved in long-range dissemination of the pathogen, at least in Pennsylvania.

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*Additional key words:* Vascular wilt, oak wilt, *Pseudopityophthorus* spp., Scolytidae.

Oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, was first described in 1942 in Wisconsin, and is now distributed from Texas to Minnesota and from South Carolina to Pennsylvania (22, 27). Although in the past 30 years much has been learned about this disease, the means of the long-range overland spread of the fungal pathogen is still not known.

Numerous studies showed that sap-feeding beetles (Coleoptera: Nitidulidae) are usually associated with sporulating mycelial mats, and are able to transmit the pathogen from mats to wounds on healthy trees (13, 16, 24). Sources of inoculum other than mats and insects not associated with mycelial mats must be responsible for much of the overland spread of the pathogen; however, because (i) mats are rarely formed in the southwestern portion of the oak wilt range yet the number of new infection centers continues to increase (25); (ii) in areas where mat production has been low because of drought conditions, the number of new infection centers formed annually has not changed (15); and (iii) the rate of spread of the disease is not related to the quantity of mats produced (9).

The only source of inoculum other than mycelial mats consists of mycelium and occasional spores in the xylem or cambial areas of infected trees. This inoculum is accessible primarily to wood or bark-boring insects. Thus, because of their feeding and breeding habits, certain species of wood and bark-boring beetles (Coleoptera: Buprestidae, Cerambycidae, Scolytidae) are potential vectors (8, 18).

Of the Scolytids, both ambrosia beetles and bark beetles commonly infest wilted and dead oaks. Although the roots and lower boles of wilted oaks often are heavily infested with the ambrosia beetles (*Xyleborus saxeseni* Ratz., *X. xylographus* Say, and *Xyloterinus politus* Say), and despite the fact that these insects can acquire the pathogen from the wood (23), they are unable to transmit the pathogen to healthy oaks (26).

Recent research by Iton (10) in the West Indies, however, has shown that *Ceratocystis fimbriata* Ellis & Halstead, cause of a vascular wilt disease of cacao (*Theobroma cacao* L.), has wind-disseminated chlamydospores. These spores form in frass of ambrosia beetles of the genus *Xyleborus* Eichhoff, and are liberated as frass is forced out the insects' galleries. Since much frass is liberated by bark and ambrosia beetles tunneling in wilted and dead oaks, spores of *C. fagacearum* could likewise be liberated into the air and be wind-disseminated to wounds on healthy oaks.

Scolytid bark beetles, *Pseudopityophthorus* spp., are usually abundant in the smaller branches of diseased trees (4, 21) and are known to make breeding attacks on healthy oaks from spring throughout the summer (19). Up to 22% of the oak bark beetles acquire the fungus from

trees during the same season that symptoms have developed (2, 4, 19, 21), and can inoculate healthy oaks during their feeding activities (5, 6). This figure is misleading because it is doubtful that insects which emerge during the summer are important vectors; most inoculation of oaks appears to occur from late May to early July (7, 11, 17). Insects active at this time have overwintered in trees which wilted and died the previous summer. Indeed, only 0.4% of the oak bark beetles carry the pathogen as they emerge in the spring from trees which died the previous season (20).

Ascospores and conidia of *C. fagacearum* remain viable for only 3 days at temperatures above 31 C (14); mycelium probably survives only a few days longer at the same temperatures (3). Hence the fungus does not even survive through the summer in the smaller branches of the crown which are the breeding sites of *Pseudopityophthorus* spp. Overwintering of the fungus in frass in bark beetle galleries could explain, however, how bark beetles emerging from branches in the spring carry the fungus, even though the fungus cannot be isolated directly from the xylem of those branches at that time. Indeed, the results of studies at the Northeastern Forest Experiment Station suggested the pathogen survived in such frass (C. O. Rexrode, *personal communication).*

The following studies were done to determine if viable propagules of *C. fagacearum* occurred in frass of ambrosia beetles and oak bark beetles formed the spring after the trees had died of oak wilt.

MATERIALS AND METHODS.—Ten red oaks which died of oak wilt during the summer of 1971 were felled in mid-May 1972. Six of the oaks had been inoculated; four died from natural infection. At the time of felling, five of the trees had mats on them, clearly indicating the presence of the fungus. The fungus was isolated from the boles of the other five trees.

The bark was peeled from bark beetle-infested branches to locate the galleries. The diameter of the branches varied from 2 to 10 cm. A dissecting needle with a flattened end slightly narrower than the width of the tunnels was used to trace the tunnels and collect any frass present within them. The frass was transferred to a sterile 15-ml glass vial which contained 1.0 ml of sterile distilled water. Between collections of successive samples, the transfer needle was washed with sterile distilled water to avoid possible carry over of propagules. Approximately 100 samples were collected per week from 17 May to 4 August in 1972.

All trees felled in the bark beetle study were cut into bolts 2 m long. The ends were coated with a fiberglass-resin mixture to retard desiccation. The bolts then were placed in screened insect rearing cages. The bark surfaces of these bolts were periodically inspected for frass deposits which usually extruded 10 to 30 mm from the tunnels. This frass was scraped with a sterile scalpel into sterile 15-ml glass vials containing 1 ml of sterile distilled water. Approximately 125 samples were collected per week from 31 May to 25 July 1972.

The conidiation technique was used to test each sample for the presence of the fungus (12). Each suspension of frass and water was divided in half and brushed onto colonies of "A" or "B" mating types of the fungus using sterile pipe cleaners. Colonies used were between 12 and 17 days old (1). For each 100 samples, five controls were used to insure that both "A" and "B" mating types were receptive at the time of conidiation. These controls consisted of conidiating "A" or "B" mating types with the opposite strain of the fungus. Conidiation experiments usually were performed within 24 hours of the time of collection of the frass samples. When it was necessary to keep samples for longer than 24 h, they were stored in a refrigerator at 10 C. At no time were samples kept longer than 48 h before conidiation.

Plates were examined for perithecia under a dissecting scope at 10 to 14 days, and again at 3 weeks after conidiation. All perithecia found were examined under a microscope to determine if they were fertile.

RESULTS.—None of the 915 oak bark beetle frass samples, and none of the 1,015 ambrosia beetle frass samples yielded fertile perithecia. In all cases, check matings of both "A" and "B" cultures yielded abundant fertile perithecia, indicating that the isolates were receptive.

A subsample of 75 "A" and 75 "B" plates conidiated with ambrosia beetle and bark beetle frass revealed that 36% developed sterile perithecia. Sterile perithecia arise spontaneously (1).

A species of *Pyrenochaeta* developed commonly in plates conidiated with bark beetle frass, and somewhat less frequently in those conidiated with ambrosia beetle frass. Of 250 samples of oak bark beetle frass collected on 31 May 1972, 154 (62%) yielded this fungus. When oozing conidia, the pycnidia of the *Pyrenochaeta* sp. superficially resemble the perithecia of *C. fagacearum* oozing ascospores. This fungus was encountered with decreasing frequency from May to August.

A *Graphium* sp. also was present in the frass of ambrosia and bark beetles at about equal frequency. Of 300 samples of bark beetle and ambrosia beetle frass, 2.7% yielded the *Graphium* sp. This species also could be confused with *C. fagacearum* unless examined under the microscope.

DISCUSSION.—Viable propagules of *C. fagacearum* were not present in the frass of oak bark beetles or ambrosia beetles in Pennsylvania oaks the spring and summer following wilt and death of the trees.

If the oak wilt fungus does not survive in insect frass or in the wood of the upper branches of wilted trees, oak bark beetles must acquire the fungus during the same season that symptoms develop and somehow retain the pathogen in or on their bodies until the following spring. The fungus appears to be carried internally in *Pseudopityophthorus* spp. (C. O. Rexrode, *personal communication).* It is not known whether the fungus is retained in the digestive tract or in special structures such as mycangia.

Because oak bark beetles attack not only red oaks but also the more resistant white oaks, a dilution factor is involved. Thus although 0.4% of the beetles may carry the fungus after emerging from infected trees (23), the actual percentage of effective vectors, i.e., those feeding on red oaks, is undoubtedly much lower, and therefore results in fewer successful transmissions. This may explain the slow rate of spread of oak wilt.

In conclusion, wind dissemination of frass-borne propagules following beetle tunneling activity the spring

after symptom development does not occur, at least in Pennsylvania. These studies also show that relationship of the oak bark beetle to oak wilt differs from that of the well-known elm bark beetle-Dutch elm disease complex.

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