Infection of Trembling Aspen by *Hypoxylon mammatum* Through Cicada Oviposition Wounds

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ABSTRACT

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An outbreak of the periodical cicada (*Magicicada septendecim*) in 1976 caused extensive oviposition injury to 1- and 2-yr-old branch wood of trembling aspen (*Populus tremuloides*) in a plantation established from progeny of controlled crosses. The incidence of Hypoxylon canker significantly increased, beginning in 1978. By 1982, 78% of the new cankers were associated with cicada wounds. Branch infections developed into lethal cankers on the main stems of many trees. Susceptibility to infection appeared to differ among the progeny. Cicada wounds also were associated with cankers on surrounding wild aspen. Periodic high rates of infection in localized areas may be attributed to wounding by this insect.

Additional key words: forest management, *Saperda inornata*.

Hypoxylon canker, which is caused by *Hypoxylon mammatum* (Wahl.) Mill, is the most lethal disease of trembling aspen, *Populus tremuloides* Michx. It has been estimated that *H. mammatum* infects 12% (19) and kills from 1 to 2% of the aspen annually (5). Although it has been the subject of research for many years, the etiology of the disease is not completely known.

Aspen bark has been demonstrated to be toxic to mycelium (17) and ascospores (12) of *H. mammatum*. Hypoxylon cankers have been associated with branch stubs and dead branches (7,18). French and Oshima (12) suggested that infection takes place at branch axils where the toxic green layer of bark is absent.

Evidence indicates that inoculum of *H. mammatum* is present on both healthy (1) and wounded bark (16). Bark moisture and competing microorganisms may influence the likelihood of infection and growth of *H. mammatum* (8,9); however, most investigators agree that a wound is necessary for infection.

Wounds on aspen made by insects have been implicated by many investigators as infection sites for *H. mammatum*. Infections have been reported at wounds made by species of *Oberea*, a wood-boring beetle (7); the poplar borer, *Saperda calcarata* Say (14,16); gall-forming *Saperda* spp. (15), and galls of *S. concolor* LeC. (= *S. inornata*) (18). Anderson et al (4) found that 95% of the Hypoxylon cankers on plantation-grown aspen originated in galls produced by *S. inornata* Say. Hypoxylonlike cankers also were associated with galleries of *S. inornata* and *O. schaumii* LeC. by Nord and Knight (20).

Our objective in this investigation was to determine the importance of cicada oviposition wounds in the infection of aspen by *H. mammatum*.

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MATERIALS AND METHODS

In 1971, a 1-ha plantation consisting of 290 trees of *P. tremuloides* was established at the Oconto Seed Orchard on the Nicolet National Forest near Langlade, WI. Seed for planting stock was obtained from controlled crosses using floral budwood collected from trees throughout the geographic range of aspen in Minnesota. Trees were planted at a 3 × 3-m spacing. Weeds were controlled by mowing.

In the summer of 1976 a severe outbreak of the periodical cicada (*Magicicada septendecim* (L.)) occurred; these have a 13- or 17-yr life cycle compared to the 2- to 5-yr life cycle of dog day cicadas. Some adults emerge each year somewhere within their range because of overlapping broods. Plantation and surrounding wild trees received heavy oviposition injury (Fig. 1). Plantation trees were examined several times each year from 1976 through 1982 for infection by *H. mammatum* at these cicada wounds. Similar wounds on surrounding wild aspen were randomly sampled in 1978 and 1979. Branch age, branch diameter, extent of wound closure, canker size, and the presence of fruiting structures of *H. mammatum* were recorded. Isolations were made on 2% malt extract agar from wounds with and without canker symptoms.

In 1980, 25 pole-sized (12–20 cm [5–10 inches] in diameter) wild aspen adjacent to the seed orchard were selected along a transect and felled. All cicada wounds associated with Hypoxylon canker symptoms were recorded. Also, along the edge of this stand, 100 standing aspen were randomly selected and examined for Hypoxylon cankers associated with cicada oviposition wounds. Ten additional aspen ranging from 4.1 to 9.4 cm in diameter at breast height (DBH) were felled, cut into sections and the entire trees, including branches, were brought into the laboratory for closer examination. Number of wounds, number of cankers associated with wounds, and presence of fruiting structures of *H. mammatum* were recorded. Isolations were made from all wounds by plating tissue fragments on 2% malt extract agar.

RESULTS

Plantation trees. One- and 2-year-old branch wood of plantation and wild aspen was extensively injured by oviposition during the 1976 cicada outbreak. Injury to all branches within crowns was common. Many branches were completely girdled and killed as a result of the oviposition wounds.

The incidence of Hypoxylon canker increased 2 yr after the cicada outbreak and fluctuated thereafter (Table 1). Oviposition wounds made by cicadas were the most frequent sites of infection. Cankers on branches and small main stems were usually less than 8 cm long (Fig. 2). Fruiting structures were present only on a few of these cankers in 1978. However, hyphal pegs were present on these cankers when examined in 1979.

Inspection of plantation trees from 1980 through 1982 revealed new cankers at the cicada wounds made in 1976. A few cankers developed at oviposition wounds made by dog day cicadas after 1976. Saperda galls were also infection sites (Table 1). Trees frequently had multiple cankers; some had as many as 10 branch and main stem Hypoxylon cankers. Perithecia were present in 1980 on many of the cankers recorded in 1978. Generally, hyphal pegs formed from 1 to 2 yr after visible symptoms developed, and perithecia formed 1–2 yr after hyphal pegs. Fruiting was most often observed directly on or adjacent to the wound.

As of November 1982, 76 of the 494 cankers on plantation trees were first noted on the main stem, the remainder on branches. Of the 76 main stem cankers, 25 originated at cicada wounds, 12 at saperda galls, and 39 either had no identifiable wound or were centered around a dead branch or branch stub. An additional 148 main stem cankers have resulted from branch infections; 130 originated at cicada wounds, nine at saperda galls, and nine from wounds of unknown origin. Growth of the fungus from a branch wound into the main stem occurred over distances up to 30.5 cm on branches ranging in diameter from 2.5 to 5.1 cm. Sixteen trees were killed by Hypoxylon main stem cankers: three died at 2 yr, nine died at 4 yr, two died at 5 yr, and two died at 6 yr after disease symptoms were first detected.

Canker incidence on the various parent and progeny trees differed greatly. Disease index (ratio of the number of cankers to the number of trees of that cross planted) ranged from 0 to 4.9. For example, 18% of the trees in the plantation had the same pistillate parent (49), but 35% of all cankers were on these trees. The above pistillate parent and a pollen parent (38) each had a disease index of 1.5; however, their progeny had a disease index of 4.9.

TABLE 1. Number and origin of new cankers of *Hypoxylon mammatum* on trembling aspen in a Wisconsin plantation.

	Newly infected trees		Type of wound associated with cankers (no.)		
Year	Number	Percent[a]	Cicada	Saperda	Unknown
1976	6	2	4	1	1
1977	6	2	3	3	0
1978	70	24	120	11	5
1979	17	6	24	2	1
1980	39	13	105	7	21
1981	29	10	109	14	11
1982	13	4	21	15	16
Total	180	62	386	53	55

[a] Based on observation of 290 trees.

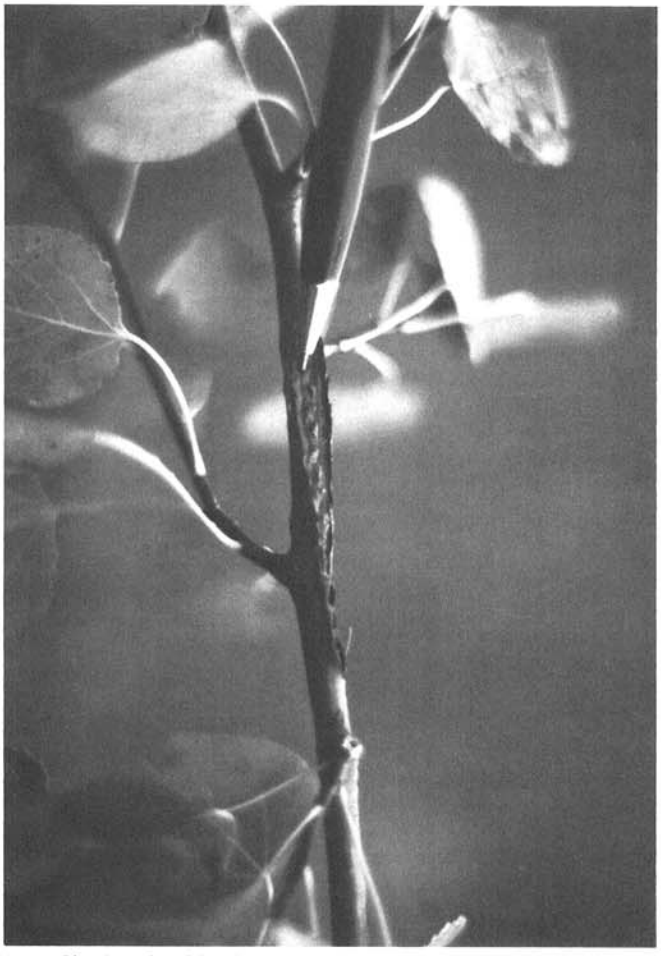

Fig. 1. Cicada oviposition injury to aspen.

Fig. 2. Aspen infected by *Hypoxylon mammatum* at cicada oviposition wound.

Wild aspen. In 1978, a random sample of 365 cicada wounds made in 1976 on wild aspen revealed that 47 (13%) were associated with Hypoxylon cankers. Hyphal pegs were present on two of these cankers. *H. mammatum* was isolated from one nonsymptomatic wound. One feature common to all was that *H. mammatum* was recovered from only incompletely closed wounds. Cankers averaged 69 mm in length. Many did not visibly extend beyond the wound (Figs. 3 and 4). In 1979, 204 cicada wounds made from 1976 to 1978 on wild aspen were examined. Fourteen (7%) were associated with Hypoxylon cankers. The average canker length was 53 mm. No fruiting structures of *H. mammatum* were present on these cankers at this time. Hypoxylon cankers were only associated with open wounds. Complete wound closure was evident on 25% of all wounds examined in 1978 and 33% in 1979.

A total of 29 Hypoxylon cankers associated with cicada wounds were found in the crowns of 18 of the 25 trees felled in 1980. Hyphal pegs and perithecia were present on many of them. Of the 100 standing trees adjacent to the plantation, 35 had a total of 49 cankers associated with cicada wounds and five associated with wounds of unknown origin. On the 10 trees dissected in the laboratory, there was a total of 505 cicada wounds and 26 (5%) were infection sites (Table 2). Only two of the above trees were free of cankers. *H. mammatum* was recovered in pure culture from five nonsymptomatic cicada wounds. One of two saperda galls, and six of 11 wounds of unknown origin also yielded *H. mammatum*.

TABLE 2. Branch wounding by cicada and prevalence of *Hypoxylon* canker on wild aspen

Tree number	Cicada wounds	Cankers associated with cicada wounds (no.)
1	65	5
2	24	2
3	8	0
4	71	6
5	63	2
6	57	6
7	49	1
8	61	0
9	47	2
10	60	2
Total	505	26 (5%)

DISCUSSION

Much of the previous literature on the prevalence of Hypoxylon canker of aspen pertains to main stem cankers. In this paper, we report on branch infections as well as main stem infections. Main stem cankers observed during this study often resulted when the fungus grew from an infected branch into the main stem. As of November 1982, 35% of the cankers first detected on branches resulted in main stem cankers. Thus, a high prevalence of infected branches increases the chance that lethal main stem cankers will develop. In a previous study to determine the importance of branches as initial infection sites, pruned portions of aspen stems had fewer Hypoxylon cankers than unpruned aspen (21).

Investigators have reported that Hypoxylon infection rates are not static (2,23). In our study canker incidence significantly increased 2 yr after trees were heavily wounded by cicada and fluctuated yearly thereafter. Branch diameter, tree vigor, wind breakage, competing microorganisms such as species of *Cytospora*, and environmental factors influence infection and the likelihood of

Fig. 3. Cicada wound with no visible symptoms beyond oviposition injury. Note white mycelium in incompletely closed wound.

Fig. 4. Bark removed from wound in Fig. 3. Note extent of fungus colonization beyond injury.

branch cankers entering the main stem.

Oviposition wounds made by cicadas provide an ideal entry for *H. mammatum* as evidenced by the fact that 78% of all cankers on plantation trees were associated with these wounds. Results were similar in surrounding wild aspen stands, indicating that periodic outbreaks of cicada may account for an occasional high canker incidence in affected stands.

Periodical cicadas emerge in large numbers in late spring and early summer and move into many species of trees and woody vegetation. Females use their sawlike ovipositor to make deep slits several centimeters long into small stems and branches where they deposit many eggs in two parallel rows. Eggs hatch in several weeks, the nymphs drop to the ground and burrow into the roots of plants where they remain until they complete their development.

Adult cicadas cause no visible feeding injury. We doubt that they come into contact with inoculum and act as vectors of *H. mammatum*, because they spend many years developing underground.

Although the type of inoculum responsible for infection of aspen through cicada wounds is not known, we speculate that ascospores are involved. Ascospores adhering to branch surfaces could be washed into the wounds by rain, or become incorporated into the wounded tissues during the egg-laying process. Incubation of *H. mammatum* in wounds for 2 yr or more before visible symptoms develop is not unexpected. Aspen stems artifically inoculated with mycelium of *H. mammatum* placed in wounds have taken 19 mo to become symptomatic (M. E. Ostry, *unpublished*). Several cicada wounds had no canker symptoms, yet when dissected, revealed small colonies of *H. mammatum* growing deep within the wound and often partially surrounded by callous tissue. Assuming ascospores are responsible for infection, many months can elapse for spore germination, colony growth, toxin production (22), and visible symptom development. In Minnesota, it has taken an average of 22 mo for symptoms to develop after artificial inoculation of saperda galls with ascospores (N. A. Anderson and M. E. Ostry, *unpublished*).

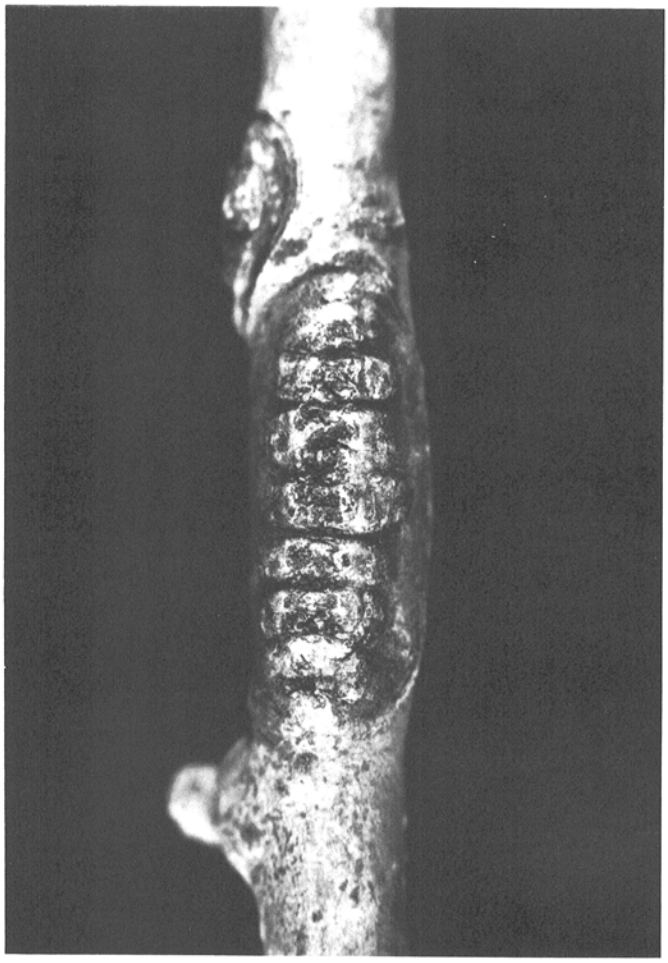

Fig. 5. Typical completely closed cicada wound on *Populus grandidentata*.

Progeny of the various crosses exhibited a range of susceptibility to *H. mammatum* as indicated by the disease index. Mechanisms that may be responsible for this apparent variation in resistance are not yet known; however, the degree of wound closure on trees is important. Our studies indicate that host response to wounding is a critical factor for infection. Wounds that were completely calloused and closed tight were, with one exception, not infection sites. In a previous study, it was found that pruning wounds were seldom sites of infection by the fungus (21). A prerequisite for infection of aspen by *H. mammatum* appears to be a wound into the xylem that remains open and retains the moisture needed for spore germination and mycelial growth. Wounds made on *P. tremuloides* by cicada and *S. inornata* meet these requirements. Complete closure of cicada wounds was common on bigtooth aspen (*P. grandidentata* Michx.) in the surrounding stand and no cankers were found on this species (Fig. 5).

Aspen bark is subject to wounding by many biotic and abiotic agents but conditions for successful infection by *H. mammatum* and canker development are met in only a few of them as indicated by the 5 to 13% of cicada wounds resulting in canker development in this study. Similarly, only 1% of all galls of *S. inornata* were associated with cankers in a previous study (4). Many chemical, biological, and physical factors (6,10) as well as the genetic variability of the host (11) and of the pathogen (3,13) may influence infection. Knowing the kinds of wounds associated with infection will add to our understanding of the disease cycle and will enable forest managers to identify aspen stands with a high risk of infection by *H. mammatum* so they can begin management strategies to minimize losses.

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