Insect Wounds as Infection Sites for *Hypoxylon mammatum* on Trembling Aspen

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ABSTRACT

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Two plantations of aspen (*Populus tremuloides*) from controlled crosses were established to study the process of infection by *Hypoxylon mammatum* and host resistance to this canker-causing fungus. The Rosemount, MN, plantation was in an agricultural area, the Langlade, WI, plantation in an aspen forest. Of the 169 cankers on trees at the Rosemount plantation, 95% originated in galls induced by *Saperda inornata* (Coleoptera: Cerambycidae). Less than 1% of the total galls were infected. At the Langlade plantation, seven hypoxylon cankers originated in oviposition wounds formed by *Cicada* sp., and four cankers in *S. inornata* galls. Infection of aspen by *H. mammatum* through *S. inornata* galls has been observed in wild aspen.

The most important canker disease of aspen, *Populus tremuloides* Michx., is caused by *Hypoxylon mammatum* (Wahl.) Miller. Annual mortality due to the disease has been estimated at 1–2% of the aspen grown on 7.1 million hectares in Minnesota, Wisconsin, and Michigan. The wood fiber loss each year due to the disease exceeds one million cords (2).

A half century of research has provided many important facts about the disease. The "edge effect" indicates that Hypoxylon cankers are more frequent on the edges of than within stands (2). Canker incidence is inversely related to stand density (1,5), and more cankers occur on young than on older trees (1). New cankers usually occur on the upper bole or in the crown of older aspen trees (6). A "typical Hypoxylon canker" often has a dead branch stub near the center of the canker. Branch or stem cankers with a central branch that is not broken by wind retain their leaves into the winter, and these "flags" often indicate recent Hypoxylon infection (4). The disease is not distributed uniformly over the range of aspen in the Lake States. The highest incidence of infected trees is in northern Wisconsin and Upper Michigan and the lowest is in northern Minnesota (1).

Living aspen bark contains pyrocatechol, two glycosides, and an undetermined phenol, which are toxic to ascospores (7) and hyphae (10). The fungus is a wound parasite of sapwood tissue (4,9,16) and produces phytotoxins that cause necrosis of host tissue (9).

The purpose of this study was to obtain information on the infection process, and the long-term goal was to determine the genetic control of host resistance.

MATERIALS AND METHODS

Two plantations of aspen from controlled crosses were established at Rosemount, MN, and Langlade, WI. The parent trees were selected from the geographic range of aspen in Minnesota. Included were trees from clones with high, intermediate, and low levels of infection. In addition, several trees were included that had formed callous tissue to restrict canker growth.

Floral budwood was collected in March of each year, beginning in 1965. The budwood was placed in water in the greenhouse to induce flowering. Selected crosses were made by placing branches with pistillate flowers and mature staminate catkins in a polyethylene bag and shaking them gently. The polinated, pistillate branches were kept in cold water in the greenhouse until seed was mature. Seed from the various crosses was sown in autoclaved soil. Individual seedlings were transferred to 11.4-cm pots after 4–6 wk and held in the greenhouse until planting. Vegetatively propagated root cuttings from 16 parent trees were included in the plantings.

The trees were planted in the field at 3.05 × 3.05 m spacing and observed each year throughout the growing season. Occurrence of Hypoxylon cankers, canker origin, location on the tree, size, and stage of spore development were noted. Hypoxylon cankers were identified by the presence of hyphal pegs (pillarlike structures that push up the host periderm to release conidia), perithecia, or the mottled black and yellowish white diseased bark. Insect wounds on branches and stems of trees were identified and the time of year and type and extent of wound were recorded.

The Rosemount plantation consisted of 575 trees from 46 crosses planted in 13 yr. The trees ranged in height from 0.3 to 8.3 m. This plantation was in an agricultural area and the nearest wild aspen stands were 0.8 and 3.7 km distant, and only the latter stand had trees infected by *H. mammatum*. To determine the prevalence of *H. mammatum* ascospores in the plantation, four roto-rod spore samplers were run during July and August 1977. The Langlade plantation, established in 1971 in a commercial aspen area on the Nicolet National Forest, had 290 trees from 38 crosses. The trees were 2–6 m tall and cankered wild aspen were less than 0.4 km away. A Malathion-Black Leaf 40 spray was applied three times to the trees in the Langlade plantation in 1973 and 1974 to control grasshoppers. No insecticides or fungicides were used on the trees at Rosemount.

RESULTS

As of July 1978, 169 Hypoxylon cankers were noted on aspen at the Rosemount plantation; 160 (95%) of the cankers were associated with galls made by the poplar-gall saperda (*Saperda inornata* Say), and 114 of the infected trees had a single canker. Fourteen trees had two cankers, four trees had three, two trees had four, and one tree had seven. Twenty-nine cankers originated in *S. inornata* galls on the main stem; 27 cankers began in galls on living branches. The fungus grew from the site of the gall down the branch and into the main stem of the tree. The remaining 104 cankers were

Fig. 1. A, Egg niche of *Saperda inornata*.**B,** A 1-yr-old gall with *S. inornata* larva in gallery. **C,** Young *Hypoxylon mammatum* canker beginning in an *S. inornata* gall. **D,** A 1-yr-old *H. mammatum* canker with hyphal pegs on an *S. inornata* gall. **E,** Young *H. mammatum* canker on *S. inornata* gall, 19 April 1977. **F,** Canker pictured in **E**, July 1978. Fungus has migrated 15 cm down the branch and into the main stem. **G,** Appearance of old *S. inornata* galls on open-grown aspen. Stem and gall at right infected with *H. mammatum*. **H,** Nectrialike canker in which the fungus migrated from an insect gall into the main stem.

associated with galls on live branches.

Insect galls induced by *S. inornata* were first noted on trees in the Rosemount plantation in August 1973. Exit holes were associated with the galls. The oviposition wounds were thought to have been made in 1972. In July 1976, 3,031 *S. inornata* galls were counted and 13 Hypoxylon cankers producing hyphal pegs were found associated with these galls. Only three ascospores were obtained on the spore traps during July and August 1977.

On 25 November 1977, 89 small cankers were first noted and 86 of them were associated with *S. inornata* galls. By 6 July 1978, hyphal pegs had formed on 81 cankers.

At the Langlade plantation six Hypoxylon cankers were noted in 1976 and six in 1977. In 1976 four cankers were associated with injury caused by *Cicada* sp. (Homoptera: Cicadidae), one was associated with an *S. inornata* gall, and the infection court of the other canker could not be determined. In 1977 three cankers were associated with *Cicada* wounds and three originated in *S. inornata* galls.

At the Rosemount plantation, progenies involving parent trees 28A and 51 appeared to be susceptible to infection. When 28A was used as a pistillate parent in seven different crosses involving 87 trees, 43 cankers were found. Of the total number of cankers found to date, 25% were on 15% of the total number of trees.

Floral budwood was collected from five parent trees near Duluth, MN, within 150 m of each other. When these trees numbered 51, 51A, and 51B were used as pistillate parents, and 51C and 51D as pollen parents in 18 different crosses involving 203 trees, 77 cankers (46% of the total) developed.

A disease index (DI) (number of cankers)/(number of trees) was computed. The DI was 0.12 for parent trees, 0.28 for all the progenies in the Rosemount test, 0.38 for progenies with at least one of the five 51-series parents, and 0.49 for progenies of crosses with 28A as the pistillate parent.

DISCUSSION

The type of wound made by *S. inornata* appears to be ideal for infection of aspen by *H. mammatum*. The adult female oviposits on small shoots and branches usually less than 15 mm in diameter (14). A single egg is deposited in a U-shaped niche gnawed by the female (Fig. 1A) (14). Several egg niches are usually made at the same level on a branch. The eggs hatch in approximately 14 days, the larvae bore a horizontal tunnel in the outer xylem, and a globase to spindle-shaped gall develops in response to the insect feeding. To overwinter, the larvae move into the central portion of the gall where they bore a tunnel parallel to the pith (Fig. 1B). The larvae maintain a frass ejection-port, and to emerge, the adult makes an exit hole approximately 2 mm in diameter (14).

The relationship between the insect and the fungus that was so clearly demonstrated at the Rosemount site may help explain the infection process, the stand density-canker incidence phenomenon, the pattern of infection on individual trees, the nonrandom distribution of the disease in the Lake States, and host resistance to this disease.

The insect tunnel is below the highly toxic bark tissue (7) and the overwintering tunnel is formed in the xylem where the fungus can grow and produce its phytotoxin.

The fact that the insect lays its eggs in branches and stems usually less than 15 mm in diameter may explain why usually more cankers occur in young than in older trees and why new cankers are higher in older trees.

The "typical canker" long noted by plant biologists consisted of a dead branch stub in the center of the canker. This might be explained as follows: The canker fungus infected the aspen at the site of the Saperda gall on a branch (Figs. 1C, D, and E), grew down the living branch, and entered the main stem (Fig. 1F). The branch was weakened by the gall and broken off by the wind. This sequence of events was noted on 27 cankers at Rosemount, MN.

S. inornata is a sunloving insect and prefers open grown aspen and branches of aspen growing along the edge of a stand (13) (Fig. 1G). The greater incidence of canker in stands of low density and along stand edges probably is correlated with insect activity.

Canker incidence is highest in northern Wisconsin and the Upper Peninsula of Michigan and lowest in extreme northern Minnesota. A 1-yr life cycle has been reported for *S. inornata* in the Lower Peninsula and a 1-2 yr life cycle in the Upper Peninsula of Michigan (13). A 2-yr life cycle was reported in Manitoba and Saskatchewan and possibly a 3-yr life cycle in northern populations of these provinces (12). A prolonged life cycle could reduce the number of galls formed and reduce the probability of the fungus entering this type of wound.

Other insects also have been reported to cause wounds in which the Hypoxylon canker fungus can become established. Oviposition wounds of *Cicada* sp. on aspen at the Langlade plantation were infected and in other areas infection has occurred through *Oberea* sp. (Coleoptera: Cerambycidae) wounds (4,14). Nord and Knight (14) found seven Hypoxylonlike cankers associated with *S. inornata* galls and seven cankers with *Oberea schaumii* galleries in a survey of 12 stands of large trees. In six sucker stands they found one Hypoxylonlike canker associated with an *S. inornata* gall and one with an *O. schaumii* wound. Manion (11) noted 10 Hypoxylon cankers associated with *S. concolor* (= *S. inornata*) galls. Manion (11) also noted that many Hypoxylon cankers appeared to originate at the base of 1 or 2 yr old dead branches. Graham and Harrison (8) found Hypoxylon cankers associated with *Saperda calcarata* wounds on *Populus grandidentata* Michx.: they also stated that lepidopterous wood borers lay eggs in the callous tissue around branch stubs or unhealed wounds and that these oviposition wounds are infection courts for the canker fungus. In our study, other canker fungi also infected aspen through galls formed by *S. inornata*. At the Rosemount plantation, many cankers initiated by *Cytospora* sp. started when the branch was killed or weakened by the *S. inornata* gall. Three *Nectria*- or *Ceratocystis*-type cankers had infected gall tissue and grew down the branch and into the main stem of the tree (Fig. 1H).

The insect gall made by *S. inornata* can be the infection site of aspen by *H. mammatum*. How and when infection occurs is not known. The following observations made at the Rosemount plantation may have a bearing on this problem. We found few Hypoxylon ascospores; as of July 1978 only three cankers had produced mature ascospores. During the limited spore trapping period, only three ascospores were obtained. As mentioned, this plantation is in an agricultural area with the nearest infected wild aspen 3.7 km (2.5 miles) away. Hyphal pegs, on which conidia form, were found in all but 8 of the 169 cankers. With apparently so few ascospores available for infection, perhaps an insect transmits infected tissue into the Saperda gall to cause infection. Rogers and Berbee (15) reported that isolated conidia of *H. mammatum* germinate but do not produce colonies on nutrient agar. They suggested that conidia of this fungus are spermatia. Bagga and Smalley (3) found that infection but not "running cankers" developed when conidial suspensions were injected into leaf axils of aspen plants derived from rooted cuttings. The role of ascospores and conidia in relation to insect wounds needs further study.

Hypoxylon infection through *S. inornata* galls has been difficult to detect in wild stands in the past, but it was very evident on aspen in the Rosemount plantation. A combination of events probably led to this. Small trees were observed at least twice monthly during the growing season. The wide spacing of the trees, addition of seedlings each year, and isolation of the plantation from wild aspen insured a high population of insects. Also, some of the progeny trees in the study apparently were more susceptible than the parent trees. Finally, no insecticides were used in this plantation.

Our opinion is that information on the mode of infection might provide valuable insights into resistance mechanisms. With this in mind, the experiment was designed to include a number of susceptible parent trees. Inclusion of these parents and their progenies in the breeding program provided plant material that appears to be susceptible and enabled us to study the insect-fungus-aspen relationship. It is too early to comment on resistance mechanisms and their inheritance, but we noted that certain crosses have progenies with fewer *S. inornata* galls than do other crosses.

Infection through insect wounds is not unique to aspen in the

Rosemount plantation. Since these studies were initiated, we found stands in Minnesota, Wisconsin, and Michigan with cankers associated with insect injuries. Insect wounds are not the only means by which infection can occur, but they do appear more important than previously thought. In tree breeding programs, attempts to obtain resistance to *H. mammatum* may involve artificial inoculation. An important component of resistance could be overlooked using this method, namely the response of aspen to insect wounds.

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