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Sixty-five Years of Research on Hypoxylon Canker of Aspen

The 1924 paper by Povah (37) was the first of a long line on the subject of Hypoxylon canker of aspen (Fig. 1). A diverse and distinguished array of plant pathologists began their careers researching this disease, and many eminent contributors trained in other disciplines have also been drawn to this disease system. A rapid scan of the literature on Hypoxylon canker identifies more than 80 authors. This article provides an opportunity for many to reminisce on ideas that never fully developed, for others to see how current participants are interpreting or misinterpreting earlier contributions, and for those who have never been involved to ponder why.

Bier (9) was the first to submit a thesis on Hypoxylon canker, and many of the concepts and statements recurring with regularity since its publication in 1940 are based on it. The often repeated statements that the economic importance of aspen will increase and that Hypoxylon canker is the most destructive disease of this future resource originate from this paper. We will address the current validity and appropriateness of these statements and discuss seven points:

1. This is a widely distributed, genetically variable disease system;
2. Important gaps still exist in our understanding of the disease cycle;
3. Chemical defenses and responses of the host have been characterized and evaluated, but we still cannot explain when and where host infection takes place;
4. Current research suggests a more complex involvement of toxic pathogen metabolites in host-parasite interactions than was initially thought;
5. Interacting environmental factors are still too confusing to be effectively used in management recommendations;
6. Our understanding of the genetics of disease resistance is slowly emerging and suggests possible routes toward management of the disease in intensive fiber production plantations; and
7. Hypoxylon canker is only one of many disease and insect problems that need to be considered in aspen management.

Hosts and Geographic Range

The substrate range of *Hypoxylon mammatum* (Wahl.) Mill. (syn. *H.*

Fig. 1. (A) Hypoxylon canker (*H. mammatum*) of aspen (*Populus tremuloides*). (B) Stem breakage on aspen at site of Hypoxylon canker.

Fig. 2. Perithecia of *Hypoxylon mammatum*.

pruinatum (Klotz.) Cke.) includes the following genera of woody plants: *Acer, Alnus, Betula, Carpinus, Fagus, Picea, Populus, Pyrus, Salix, Sorbus*, and *Ulmus* (32). The fungus is generally considered a disease-inducing pathogen mainly of *Populus tremuloides* Michx., *P. grandidentata* Michx., *P. alba* L., and *P. tremula* L. Early reports of Hypoxylon cankers on *P. balsamifera* L. have led to some confusion as to whether balsam poplar is a host. Hybrid poplar clones may be affected by Hypoxylon canker if one of the parent lines is susceptible.

Miller's (32) broad species concept raises questions of the biological relationships between saprobic and pathogenic forms as well as the ability of the fungus to transfer between hosts. Inoculation of *P. tremuloides* with isolates from *Salix* has demonstrated pathogenicity (17). Pathogenicity of the fungus from the other nonpoplar hosts has not been established, and there is no information on the role of these substrata in the inoculum pool for poplars.

The geographic range of Hypoxylon canker of aspen is more restricted than that of the host or the fungus. In North America, the disease is prevalent throughout the northeast, the Great Lakes region, and the northwestern prairies. Incidence is fairly low in the central Rocky Mountains, but the disease is noticeably absent in the northern Rocky Mountains and in Alaska despite abundance of aspen and collection of the fungus (9,29). Until very recently, the disease was thought to be restricted to North America even though the fungus has been reported from northern Europe and the European *P. alba* and *P. tremula* are known to be susceptible. Current evidence suggests that the disease has been present in the Alps for at least 25 years (36).

In summary, *H. mammatum* has a wide geographic and saprobic host range and a somewhat more restricted pathogenic range. In addition, the pathogenic forms of the fungus vary in morphology and in virulence (23). Further study of the biological relationships and pathogenic capabilities of *H. mammatum* from various sources is needed to understand this highly variable fungus.

Disease Cycle

Ascospores are assumed to be the source of inoculum, but no one has been able to develop a reliable inoculation technique using ascospores. Research efforts have focused on factors affecting spore germination in an attempt to identify the proper conditions for infection.

Rogers and Berbee (38) described the ascospore stage as developing in the vicinity of conidial production. Perithecia produced in a stroma (Fig. 2) can liberate spores for years from both standing and fallen trees (20). Ascospores are ejected whenever the stroma is moistened and the bark temperature is above freezing (43), and continuously moistened stroma liberates spores on a 12-hour cycle (20). Spore trapping and observations of smoke movement patterns in aspen stands have shown that slow wind velocities result in rapid vertical distribution of ascospores, whereas high wind velocities produce limited vertical but extensive horizontal distribution (20). These studies and others have demonstrated that ascospore inoculum abounds in and near affected stands.

The role of conidia in the life cycle of *H. mammatum* has not been established. Rogers and Berbee (38) suggest that conidia function as spermatia, even though they can be germinated on artificial media, but question their colony-forming abilities. Conidial structures form as early as 3 months after mycelial inoculations in wounded bark and develop on pillars (Fig. 3) from a dense mat of hyphae between the cortex and periderm (38). A number of authors have described the imperfect stage of *H. mammatum* on the host and in culture, but only recently have two different anamorphs—a nodulosporium type and a geniculosporium type—been recognized in culture (G. S. Gilbert and C. J. Wang, *unpublished*).

Bier (9) began studies of the inoculum and infection court that many investigators have continued. He noted that cankers were often associated with bark injuries, but he was unable to induce cankers with either ascospores or conidia. Gruenhagen (24) obtained infection with ascospore inocula on wounded bark, but other investigators have not been able to duplicate his work. Bier and Rowat (10) may have found the key to inducing cankers with spore inocula by desiccating stem cuttings of *P. trichocarpa* Torr. & Gray to reduce the water content of the bark. This inoculation technique has not been tested with aspen, although recovery by Anderson and French (4) of *H. mammatum* from stem sections maintained in the greenhouse may be an example of this effect of reduced water content.

Observation of naturally occurring cankers suggests possible sites of infection. Manion (30) found most infections on aspen in central New York State to be associated with 1- or 2-year-old lateral branches (Fig. 4), some of which showed no injury. Anderson et al (6) found that 95% of the cankers in their test plantations in south central Minnesota were associated with branch galls caused by the insect *Saperda inornata* Say (Fig. 5). Some have associated *S. calcarata* Say with stem cankers (5), whereas others have suggested that *S. calcarata* is not involved (9). *Oberea schaumii* LeC. is another insect associated with infection (9,33). In Wisconsin, cicada (*Magicicada septendecim* (L.)) oviposition scars were

Fig. 3. Conidial pillars of *Hypoxylon mammatum*. (Reprinted with permission from *Tree Disease Concepts* by Paul D. Manion, ©1981, Prentice-Hall, Inc., Englewood Cliffs, NJ).

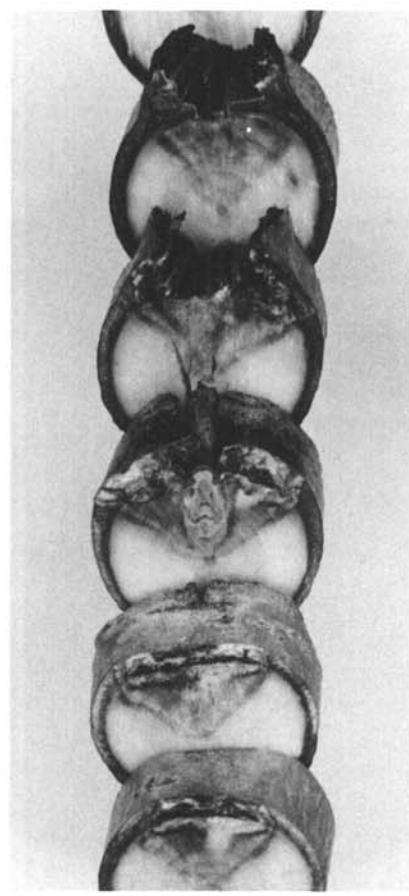


Fig. 4. Cross section of Hypoxylon canker of aspen associated with a second-season lateral branch on a 4-year-old stem 2 cm in diameter. Mottled bark and xylem tissues and interruption of the third annual ring suggest invasion during the third growing season.

an important site of infection (34). These represent valid observations made in different locations and at different times. The differences observed within and among locations (5) suggest that broad assumptions on one infection court can be misleading. Although the infection courts may vary considerably, there should be some common features that set the stage for infection.

The inability to artificially initiate infection with spore inoculation of wounds has prompted the suggestion that infected tissue from diseased trees is disseminated directly by insects (5) or by downy woodpeckers (*Picoides pubescens* Linnaeus) feeding on insects (35). The actual role or relative importance of ascospsores, conidia, or infected tissue in the dissemination and initiation of *H. mammatum* infection is clearly not resolved.

Host Defenses to Infection

Spore germination and inoculation studies have disclosed inhibitory compounds in aspen that could be effective in combating invading fungi, including *H. mammatum*. French and Oshima (19) separated aspen bark into four layers and found that the phellem stimulated, and the green layer and secondary phloem inhibited, ascospore germination. Hubbes (26) identified pyrocatechol in aspen bark as a component inhibitory to ascospore germination. He also demonstrated that pyrocatechol content of trees on different sites varied, as did the content in the bark at different locations on the trees. A variety of compounds inhibitory to *H. mammatum,* including other phenolics and various glycosides, have been found but not purified and characterized (28). Flores and Hubbes (14) found phytoalexin-like materials, partially characterized as phenolic glycosides, that form in aspen wood in response to infection by *H. mammatum*.

One of the several obstacles to accepting these data as demonstrating effective chemical defenses of aspen to infection by *H. mammatum* is that the inhibitory properties of the compounds depend on the test medium (28). Another is that the inhibitory qualities of the bark depend on the sterilization method (18). Solving these problems requires completing the chemical identification of the various compounds and quantitating their existence in aspen tissue to show effective levels in the tree.

Toxic Properties of Pathogen Metabolites

The possibility that *H. mammatum* produces a toxin or toxins that play a critical role in the pathological process was first suggested in 1964 by Hubbes (27), who showed that diffusible substances capable of passing through a dialysis membrane were produced by the growing fungus and were capable of inhibiting wound callus formation in the host. Attempts to isolate and purify the toxic substance, called mammatoxin, (21,39,40) showed that several compounds were produced that caused responses in bioassays (Fig. 6). These compounds were separable by chromatography and partitioning into various organic solvents. Fungal isolates differed in the kinds of compounds produced (21). Culture age also affected the kinds and amounts of compounds produced (40). To date, no one has succeeded in purifying and identifying any toxic metabolite.

Evidence supporting the role of these metabolites in pathogenesis is primarily their host selectivity (15,39,40), although nonspecific metabolites were also detected (40). Other evidence includes the extraction of compounds with toxic activity from cankered aspen tissue and the similarity of the bark collapse response of stems to toxin preparations and to the material from natural cankers (39). This evidence is circumstantial, and genetic studies on pathogenicity, virulence, and toxin production by *H. mammatum* are needed to establish the role of these compounds in disease development.

Several experimental results are contrary to what would be expected of pathotoxins. Schipper (39) reported that an avirulent conidial isolate produced little detectable toxic activity but also noted that another isolate previously shown to be virulent produced little activity. Griffin et al (22,23) found an isolate that had lost virulence capability after years of subculturing but retained toxin-producing ability. Aspen clonal variation in responses to the metabolites (15,22) showed a discontinuous variation in sensitivity to the toxic materials, with highly sensitive clones, moderately sensitive clones, and highly tolerant clones. Sensitivity to the toxins, however, was not correlated positively with various measures of susceptiblity of the aspen clones to the disease. French (15) found no correlation between the lengths of cankers produced by inoculating single ascospore isolates of the fungus and the sensitivity of the aspen clones to the toxic metabolites. In French's study, the aspen clones were inoculated on their natural sites, so site factors were not controlled. Griffin and Manion (22) controlled site variation and obtained a negative correlation between canker length and toxin sensitivity, the opposite of what one expects of a pathotoxin. They suggested that the toxin bioassay response could be related to the abilities of the aspen clones to produce a resistance response, rather than a measure of toxins per se in the culture filtrate preparations. The aspen leaf bioassay clearly gives responses to compounds other than pathotoxins, as indicated by the response to metabolites of nonpathogenic fungi (39). The complexities associated with the number and variability of toxic compounds produced and the relative nonspecificity of the bioassays will make this a difficult problem to solve.

Environmental Interactions

The role of environmental factors in the incidence and impact of Hypoxylon canker has been extensively investigated and described. Unfortunately, most of the descriptions are based on limited sampling and minimal statistical testing. The observations and analysis of Anderson and Martin (5) for Minnesota and of Bruck and Manion (11) for New York provide some statistical testing of various factors. The discouraging complexity of the problem was pointed out by Anderson (1) when he suggested that some of the interpretations made 12 years earlier on 211 plots were less certain when the number of plots was increased to 469.

Some of the complexity arises from the observation that there is less infection in older stands. This contrasts with the observation that infection rate does not vary significantly with size or age of the trees (5). Possible explanations may be that the relationship of disease incidence to mortality is not constant with age and that stands reaching old age are genetically superior with respect to canker resistance. In any case, single observations of stands do not necessarily characterize the potential disease incidence—a time-dependent interaction of site with host and pathogen genotypes.

In addition, these surveys (1,5,11) point out the well-recognized inconsistency of relating canker incidence to site index. Site index, a measure of tree height in relation to age, is an imperfect measure of the quality of aspen sites. Bruck and Manion (11) found very little difference in site index among stands with widely varying moisture, soil chemical properties, and soil fertility. The imperfections of site index as a measure of site quality for aspen and the problems with properly measuring disease potential are two of many reasons for the inconsistencies in relating site index to Hypoxylon canker incidence.

Higher canker incidence has been associated with open stands, but this relationship may be less certain than most have reported (1). Increased sunlight and air movement in open stands were suggested as favoring infection (5). Among 63 paired sample plots of edge trees and interior trees, canker incidence was higher in edge trees (1). Open stands have more edge trees than interior trees. Bruck and Manion (11) showed a relationship of stand density to such site factors as soil moisture and nutrients. These were correlated with canker incidence and therefore may be related to stand density.

Because the significance of and the basis for the relationship of Hypoxylon canker incidence and stand density are

clearly unknown, the management implications of adjusting stand density through thinnings are also unknown. Anderson (1) indicated that thinning studies in aspen generated inconsistent results but also reported on one large-scale test in which mortality and infection rates were significantly higher on thinned plots than on unthinned plots. Thinning of a 10-year-old aspen sucker stand resulted in Hypoxylon canker-induced mortality of 23.6% of the residual stand, whereas only 14.4% of the unthinned stand was killed by Hypoxylon canker at age 25 years (3). The thinned stand was reduced below optimum stocking by Hypoxylon canker-induced mortality, but tree diameters and cordwood volume were greater than those of the unthinned stand. On the basis of these studies, recommendations concerning disease control by thinning are inappropriate.

The relationship of Hypoxylon canker incidence to stand composition is also confusing. Anderson suggested that Hypoxylon canker was less prevalent in pure aspen stands than in a mixed forest, but 12 years later the relationship was less certain (1). Archambault (7) reported a higher incidence of disease in pure stands than in mixtures in Quebec. The problem with these comparisons is the confounding relationship of stand density and possible differences related to the species composition of the mixture.

A concept that water stress affects Hypoxylon canker development originated from an inverse relationship of rainfall and newly infected field-grown trees reported by Day and Strong (13), the work by Bier and Rowat (10) on bark turgor in relation to infection by facultative parasites, and the inoculation experiments on greenhouse-grown seedlings by Bagga and Smalley (8). Bruck and Manion (11) further demonstrated that canker incidence in the field was higher in sites with low moisture availability. A possible physiological explanation for this phenomenon is the commonly observed overproduction of proline by water-stressed flowering plants (25). A preliminary examination showed that the proline content of aspen increased dramatically under water stress (D. H. Griffin, *unpublished*). Also, the colony diameter growth rates of *H. mammatum* isolates were stimulated much more by proline than by other amino acids used as nitrogen sources (D. H. Griffin and K. Quinn, *unpublished*). We have not yet examined the effect of proline and other amino acids on ascospore germination, but the relationship of water stress-stimulated proline production, proline-stimulated mycelial growth rates, and enhanced canker development under water-stressed conditions is an interesting avenue for further investigation.

These examples show some of the problems in interpreting environmental interactions and Hypoxylon canker. The studies ignored the genetic component and the interaction between genetic and site components and also ignored regional differences, even though such differences are obvious even within a state (1). Differences in stand development are also being ignored. For example, some stands on abandoned farmland are of recent seed origin with vegetative expansion into clones. Others are well-established forest clones that have developed after logging and/or fires. The site conditions and genetic selection of these stands differ, and so may the response to disease. In addition, variation in clone size may affect interpretation of data from plots involving one to many clones. Conclusions relative to environmental impacts on Hypoxylon canker should take into account some of these differences and do not necessarily need to be universally applicable.

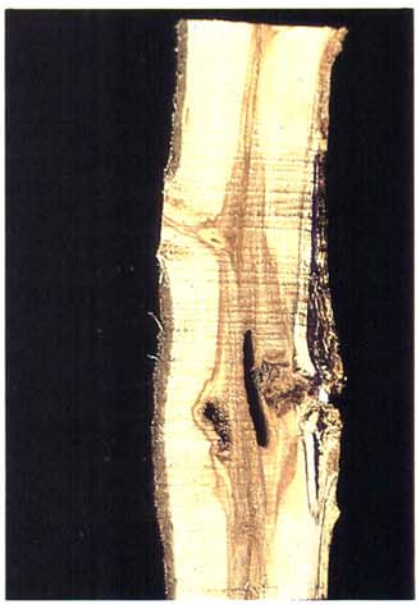


Fig. 5. Section through a young Hypoxylon canker associated with a *Saperda inornata* gallery. Mottled bark on the right side is characteristic of invasion by *H. mammatum*.

Fig. 6. Leaf bioassay of *Hypoxylon mammatum* toxins from culture filtrates, in which a sixfold dilution series is being tested on leaves of three clones.

Fig. 7. Aspen disease-resistance test of full-sib families. Mortality rate associated with 20 years of natural infection by *Hypoxylon mammatum* and *Cryptosphaerla populina* is 10% for the family row on the left (flagged in orange) and 90% for the family row on the right (flagged in blue).

Genetics of Disease Resistance

Different levels of sophistication have been used to identify and characterize genetic mechanisms of disease resistance. At the simplest level, clonal variation observed in the field suggests moderate genetic control of resistance (12). At the other extreme, in vitro testing of tissue-cultured cotyledons with toxic fungal metabolites has identified variation that may relate to resistance or susceptibility (42).

Inoculations with mycelium have been used to demonstrate genetic variation in clonal and half-sib family tests. Both the trees and the isolates varied significantly in three main responses: canker elongation, death of the inoculated branch, and wound callus formation (16,23,41). These studies very likely bypass mechanisms of pathogenic interaction occurring in the infection court but probably reflect host-pathogen interactions once the canker has been established. Valentine et al (41) found that the three responses were heritable to varying extents and suggested that the criteria could be used in a selection and breeding program.

Natural canker incidence in plantations of families from controlled crosses reveals differences in the progeny in relation to the parentage (6). In our aspen genetics plantation in New York (Fig. 7), we are evaluating disease increase and mortality rates and the lag periods from planting to first incidence and to first mortality for full-sib families from crosses of randomly selected parent trees. High rates of disease increase have not correlated with mortality, indicating that some families show degrees of tolerance

to the impacts of Hypoxylon canker. No families have combined long lag periods and low rates of increase; the least diseased families had either a long lag period or a low increase rate. Both general and specific combining abilities for the measures of disease are evident in the population of parents being tested.

The potential for genetic improvement has been demonstrated in limited tests on selected sites. Before extensive application of disease resistance can be recommended, large-scale tests on many sites must be conducted. We recently established over 50 test plantations throughout New York, using more than 100 open-pollinated seed collections from families with low disease incidence in our genetics field station plantings. The interactions of site and genotype will be evaluated to make recommendations for future intensive aspen fiber production systems.

Impact of Hypoxylon Canker and Other Problems of Aspen

Hypoxylon canker kills an estimated 1–2% of aspen annually (1). Marty (31) attempted to estimate the economic impact of Hypoxylon canker in the Lake States by considering the relationship of disease, growth, and harvest. In Minnesota, eastern Upper Michigan, and southwestern Wisconsin, where growth exceeds harvest, the impact of the disease was slight. In other areas of the Lake States, however, the impact was estimated at $4.4 million per year at the time of harvest. The 1972 study (31) may not reflect the market today and into the future, but the procedure is clearly appropriate.

Hypoxylon canker is not the only problem on aspen. Wood decay and stain probably have the most important impact on aspen production (2). In a recent survey of aspen cankers in Colorado, Juzwik et al (29) found *Phibalis pruinosa* (Ell. & Ev.) Kohn & Korf, *Cryptosphaeria populina* (Pers.) Sacc., and *Ceratocystis fimbriata* Ell. & Halst. on 93, 83, and 80% of the plots, respectively, but no *H. mammatum*. Sooty bark, caused by *P. pruinosa*, was the leading cause of mortality, and Cryptosphaeria canker was more important than previously thought.

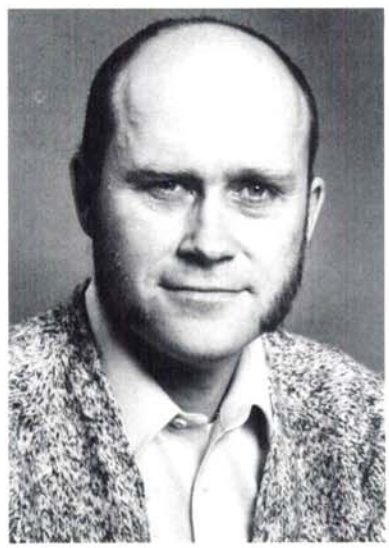
In New York, we are just beginning to recognize the importance of Cryptosphaeria canker (Fig. 8). In a 20-year-old plantation, *C. populina* and *H. mammatum* each accounted for about 12–14% of the mortalities. An additional 9% of the trees died with both diseases, for a total canker-associated mortality of about one-third of the stand.

Fig. 8. Perithecia of *Cryptosphaeria populina* protruding from the bark of a dead aspen.

Outlook

Sixty-five years of research on Hypoxylon canker of aspen has generated a number of questions for future research:

1. What are the pathogenic capabilities of *H. mammatum* from different substrata and why is Hypoxylon canker absent in the northern Rocky Mountains and Alaska?
2. What are the specific conditions associated with infection and how do they relate to insect activity and other unknown factors?
3. Do readily measurable natural defense mechanisms exist that could be used to understand infection and breed for resistance?
4. What are the chemical identities of the toxins and what is their role in the disease process?
5. What are the roles of moisture stress and other environmental factors in disease development and can these be manipulated to minimize losses caused by Hypoxylon canker?
6. Are there simple methods for identifying disease-resistant aspens and can the information gained be used to improve existing stands and to reduce disease losses in future intensive fiber production plantations?
7. Because solutions to the Hypoxylon canker problem could inadvertently increase other problems, the present and future roles of the many other diseases



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and pests of aspen must also be considered.

The years of investing time and money in Hypoxylon canker and aspen disease research have not produced dramatic changes in aspen management. The economic return on research investment at this point is difficult to justify. The effort could possibly be termed basic research with no immediate payoff expected, but actually most of the research is better described as applied. What, then, is the justification?

We see the payoff for past and continued research on Hypoxylon canker and other diseases of aspen coming in the near future. As long as we have surplus fiber resource from massive land resources and can tolerate long hauling costs, forest tree disease research will be difficult to justify economically. Forest pathology research in a surplus world provides basic knowledge and is helpful to some by providing an appropriate epitaph for problems that develop. However, as available land resources for fiber production diminish and hauling costs dictate that the products be produced within certain limits of the processing plants, then forestry will shift from gathering to producing and managing resources. Production and management can go through major instability cycles of feast and famine or, with proper information, can move quickly to supply a reasonably stable commodity flow. We see past and future research providing the basis for developing a stable, intensive fiber-producing system based on aspen.

Acknowledgments

This work was supported by grants from the McIntire-Stennis Cooperative Forestry Research Program of the U.S. Department of Agriculture and from the St. Regis Corporation (now Champion International).

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