

# Effects of Fertilization on Hypoxylon Canker of Trembling Aspen

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## ABSTRACT

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Aspen (*Populus tremuloides*) on two sites in Michigan were fertilized in May 1977, then inoculated with *Hypoxylon mammatum* in June; canker incidence and length were recorded in August and October. Incidence was highest and average length greatest in August for both sites, although the relationship among fertilizer treatments varied. Canker incidence was not independent of fertilizer treatment in August but was in October. There was no significant difference between incidence level in August and October on either site, even though the number of cankers declined for nearly all treatments. Canker length declined from August to October for all treatments except K, NK, and PK, but the decrease was not significant. No significant differences in canker incidence or length and fertilizer treatments between sites were observed. N fertilizer favored Hypoxylon infection and canker development during the summer, whereas P and K treatments restricted canker incidence and development.

Trembling aspen (*Populus tremuloides* Michx.) is widely distributed and comprises the largest wood volume in the Lake States (12). Hypoxylon canker, caused by *Hypoxylon mammatum* (Wahl.) Mill. (= *H. pruinaum* [Klot.] Cke.), is the most serious disease of trembling aspen in the Lake States and accounts for a yearly loss of 8.4 million m<sup>3</sup> of wood. This mortality approaches the net annual aspen growth of 9.3 million m<sup>3</sup> (17).

Aspen mortality and prevalence of Hypoxylon canker have been correlated with many stand and site variables (1-4,7,13,14,16). Early studies by Gruenhagen (14) and Lorenz and Christensen (16) indicated aspen growing on "poor" quality sites showed a greater incidence of canker infection than those growing on "good" sites. Conversely, later investigators (1,3,13) found no relationship between canker incidence and site quality, site index, or tree vigor.

Bagga and Smalley (5) reported that fertilized, moisture-stressed greenhouse-grown aspen cuttings had a lower canker incidence than unfertilized, nonmoisture-stressed controls, but this relationship has never been tested in the field. Because operational fertilization of forest stands in the Lake States may increase in the future, we initiated a field study to determine the effects of various fertilizer

treatments on the incidence and development of Hypoxylon canker on young aspen.

## MATERIALS AND METHODS

Two naturally occurring 4-yr-old aspen clones resulting from clear-cutting of mature aspen stands in Ontonagon County, Michigan, were selected in the spring when time of leaf flush, flowering, leaf morphology, and bark characteristics could be used for clone delineation (8). Clone uniformity was necessary because clones vary in growth response to fertilizers (21) and in susceptibility to disease (20). The first stand (site 1) was located on a coarse sand (Rosseau series) and the second (site 2), on a loamy fine sand (Wallace series). Both soils were classed as typic haplorthods. Rainfall and temperature data for the study area were obtained from the U.S. Forest Service, Kenton Ranger Station, approximately 16 km from the study sites.

A randomized block fertilizer trial, with blocking done by site, was established in a uniform part of each stand. Each block contained nine treatment plots each covering 232 m<sup>2</sup>. Fertilizer treatments applied to each block included N, P, K, NP, NK, PK, NPK, and Ca; the ninth plot served as a control. Fertilizer rates were 225 kg/ha of N as NH<sub>4</sub>NO<sub>3</sub>, 112 kg/ha of P as triple superphosphate, 112 kg/ha of muriate of potash, and 2,224.5 kg/ha of lime. Fertilizers were broadcast by hand in May 1977 before leaf initiation.

Before fertilization, soil physical and chemical properties were determined for each site. With a 2.5-cm diameter soil probe, 21 sample cores were randomly collected within each treatment plot to a

depth of 1 m. Cores were separated by horizon and randomly grouped into three composite samples of seven samples per treatment plot. Soils were analyzed for texture, moisture-holding capacity, organic component, exchangeable, K, Ca, Mg, available P, total N, and available N as NH<sub>4</sub> and NO<sub>3</sub>.

During June 1977, 10 trees per fertilized treatment (90 trees per site) were randomly selected and inoculated with *H. mammatum* at a height of 1 m. The culture was isolated from a nearby stand of naturally infected aspen and was identified by J. D. Rogers of Washington State University, Pullman.

The bark of each tree was surface-disinfected with a 70% solution of ethyl alcohol and bruised with a hatchet (14). A 12-mm<sup>2</sup> circular plug was removed from the bark through to the xylem, and a slightly smaller plug from a 10-day-old *H. mammatum* culture grown on 2% malt agar was inserted. An equal number of control trees at each site were similarly inoculated with sterile 2% malt agar plugs. All plugs were covered with three to four layers of Parafilm to prevent tissue drying. At the end of 6 wk and 14 wk, each inoculated tree was visually inspected for canker incidence. If infection was observed, vertical canker length was measured and reisolation of *H. mammatum* attempted.

One-way analysis of variance was done to determine treatment effects on canker incidence; a chi-square test was used to determine if the number of cankers was significantly different at 6 wk and 14 wk. A *t* test with unpaired plots was used to determine if canker lengths were significantly different at 6 wk and 14 wk.

## RESULTS

The relationship between *H. mammatum* incidence, fertilizer treatment, and measurement time varied (Table 1). By August, 6 wk after inoculation, the incidence of cankers on site 1 was highest with fertilizer treatments that included N, with the exception of NK. Incidence was lowest with P, K, or Ca treatment; the incidence was lower with these treatments than in the control. On site 2, treatments with the highest incidence were NK and Ca and those with the lowest were NPK, P, and K; incidence was higher in the control than with the latter three treatments. On both sites, cankers developed on approximately half of the

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trees.

By October, 14 wk after inoculation, the total number of cankers decreased for all treatments on both sites with the exception of the P treatment on site 1, which remained the same, and the PK treatment on site 1, which increased by one. The incidence was highest with the PK treatment on site 1, followed by the N and NPK treatments, and lowest with the Ca and K treatments. On site 2, cankers were not evident with the NPK treatment; incidence was highest with the N treatment.

One-way analysis of variance indicated that canker incidence was not independent of treatment in August but was in October. Chi-square analysis showed no significant difference between the incidence in August and that in October, even though the number of cankers declined for nearly all treatments. Further, no significant difference in canker incidence and fertilizer treatments between sites was noted.

Trends in average canker length were similar to those for canker incidence, declining between August and October (Table 2). A *t* test for unpaired plots run for each treatment showed that the decline in canker length from August to October was not significant. One-way analysis of variance done for each measurement period showed no significant difference in average canker length between treatments for either August or October. Likewise, no significant difference was noted in canker length and fertilizer treatments between sites.

Analysis of soil samples from each site did show differences between sites, with a general low fertility level for both. Soil on site 1 was of coarser texture and had lower nutrient levels and moisture-holding capacity than that on site 2. However, moisture content in the major rooting zone (A<sub>2</sub> and B<sub>21</sub> horizons) never reached the permanent wilting point throughout the observation period on either site. During July and August, the U.S. Forest Service weather station at Kenton recorded more than 23 cm of rain, which was approximately 130% above the previous 10-yr average.

## DISCUSSION

Although not statistically significant, N fertilization seemed to favor Hypoxylon infection during the summer, whereas P and K treatments restricted incidence. The same pattern was observed in the results of average canker length from the two sites and may be due to the nutrient imbalance caused by fertilizing with only one element. Generally, canker incidence was higher and canker length was longer with the combination fertilizer treatments than with the P, K, or N treatment. An overabundance of N in relation to P and K increases the supply of amino acids relative to that of carbohydrates (18). This may increase tissue succulence

**Table 1.** Relationship between fertilizer treatment and Hypoxylon canker incidence in trembling aspen clones

Site	Month	Number of trees with cankers, according to treatment									
		N	P	K	NP	NK	PK	NPK	Ca	Control	Total
1	August	8	3	4	6	4	6	8	2	5	46
	October	4	3	2	3	3	7	4	1	3	30
2	August	6	3	3	6	7	5	2	8	4	44
	October	5	2	2	4	4	4	0	4	2	27

**Table 2.** Average lengths (mm) of Hypoxylon cankers on trembling aspen clones according to fertilizer treatment

Month	Site	Treatment									
		N	P	K	NP	NK	PK	NPK	Ca	Control	
August	1	22	8	7	21	10	20	24	5	17	
October	2	21	8	9	18	26	14	8	19	8	
	Average	21.5	8	8	19.5	18	17	16	12	12.5	
August	1	13	8	6	13	12	30	13	3	12	
October	2	21	6	7	12	16	12	0	12	5	
	Average	17	7	6.5	12.5	14	21	6.5	7.5	8.5	

(which presumably facilitates pathogen entry), favor the production of complex nitrogen sources required by the pathogen, or reduce the level of some metabolite produced by the plant that inhibits the parasite (15).

High levels of P and K tend to decrease disease susceptibility by altering the N balance in plants (19). We found, however, that plots with high levels of P and K (PK treatment) in relation to N had both a high disease incidence and above-average canker length at the end of the growing season. This may have been influenced by differences in the natural susceptibility of the two clones that were tested.

Disease incidence and canker length data for October were not as striking as those for August and did not corroborate the greenhouse results of Bagga and Smalley (6), who reported that, under similar soil moisture levels, unfertilized plants were more susceptible than fertilized plants. Two reasons are postulated for these differing results. In past studies of Hypoxylon canker, investigators frequently applied massive amounts of inoculum to test trees to assure infection. We wanted to insure that the host would become infected and yet have the potential to overcome the pathogen as the various fertilizer treatments altered the tree's physiological balance. Consequently, we used a small amount of inoculum to best elucidate fertilizer treatment effects.

Lack of host moisture stress also may account for some of our differing results. Other investigators (6,13) have found that moisture stress in the plant tends to promote infection. In studies with various ascomycete bark cankers, Bier (9-11) reported that low bark turgidity level is influenced to a large degree by the amount of water available in the soil. Bruck (13), working with Hypoxylon canker in New York, found that any

factor contributing to soil moisture availability and retention showed a strong negative correlation to disease incidence. The strongest relationship in his study was total moisture received during the growing season, and he further concluded that canker incidence was not influenced by a single variable but rather by an array of interacting environmental factors, including site fertility. Our sites had abundant moisture throughout the infection period; presumably, the lack of moisture stress made the sample trees less susceptible to infection.

Another factor bearing on our results was that the trees were young aspen suckers and probably were still interconnected through the parental rootstock. Nutrient translocation between sprouts on different fertilizer plots was possible, therefore, and could have masked the treatment effects expected for independently growing seedlings.

Our field study has not supported substantial reduction of Hypoxylon canker incidence in young aspen by fertilization. Susceptibility to the disease can be altered by fertilization, but plant moisture stress during a given year, aspen clonal variation, and other factors may negate or confound fertilization effects.

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