The Development of Hypoxylon Canker of Populus tremuloides: Role of Interacting Environmental Factors

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ABSTRACT

Any factor which contributed to host-moisture stress increased susceptibility to the pathogen. Air relative humidities (RH) higher than 50% were not favorable for canker development and maximum canker development occurred in plants subjected to combinations of low RH and water stress. Disease development was poorest on plants grown in soils with high water-holding capacities. Nonfertilized plants were more susceptible than plants fertilized regularly.

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Aspen (*Populus tremuloides* Mich.) mortality and prevalence of Hypoxylon canker have been correlated in one way or another with stand conditions and site characteristics. Povah (14), Bier (7), Lorenz and Christensen (13), Gruenhagen (11) and Anderson (2) found fewer cankers in older stands than in younger stands. Gruenhagen (11) and Lorenz and Christensen (13) found a higher canker incidence on trees growing on poorer sites than on those on good sites. R. L. Anderson (2) found no correlation between canker incidence and tree vigor, height of the soil water table, or the tree's previous site history. Day and Strong (10), Povah (14), and Schreiner (16) reported greater canker incidence on trees in open stands than on those in dense stands. Gerald Anderson (1), however, found that cankers developed and enlarged more rapidly on shaded aspen than on those exposed to full light.

Aspen remain highly susceptible to attack by *Hypoxylon pruinatum* (Klot.) Cke. from June through August, according to the inoculation studies of Rogers (15). Sasaki (unpublished manuscript on file at Dept. of Plant Pathology, Univ. of Wisconsin, Madison) using inoculation techniques and materials different from Rogers' found that susceptibility reached its peak during May and June. Wood and French (19) found that cankers increased in length more from late May through early August than during other times of the year. In Minnesota, annual canker development varied with the particular year, and elongation averaged 25 cm in 1959 compared to 38.4 cm in 1960 (17). Bier (8) reported that dormant cuttings of poplar could be successfully inoculated with *H. pruinatum* when bark moisture content remained low. Bagga and Smalley (3, 4) reported increased canker development on greenhouse-grown aspen under moisture stress.

The present investigations determined the influence of air temp, relative humidity (RH), available soil moisture, the soil type, and the moisture present at the inoculum site on disease development.

MATERIALS AND METHODS.—Aspen plants used in greenhouse and growth chamber studies were derived from root cuttings taken from naturally cankered trees at the University of Wisconsin Arboretum, Madison. Plant materials and techniques for growing rooted aspen cuttings have been described previously (4). Rooted cuttings were grown in 10.16-cm diam clay pots containing methyl bromide-fumigated "U.C. soil mix" (5). Soil fertility was maintained by weekly applications of Hyponex® (Hydroponic Chemical Co., Copley, Ohio) prepared fertilizer (available nutrients: 7% N, 6% P, 19% K). Greenhouse temp were adjusted for 21 C night and 24 C day with a photoperiod of 16 h maintained with supplemental incandescent illumination. Relative humidity of the air in the greenhouse during the study period ranged from 38 to 65%. Growth chambers were adjusted to the prescribed temp with a photoperiod of 16 h under 21,520 lux of 1/3 "Gro-lux" and 2/3 "Cool-white" fluorescent lights (Sylvania Co., Danvers, Mass.).

The fungus for inoculation was isolated from naturally infected host tissue by the tissue plate method and pure cultures were maintained in storage at 4 C on malt agar slants. In most of the inoculations, unless otherwise specified, plant stems were surface disinfected with 70% ethyl alcohol and wounded 17 cm above the soil line. Wounds consisted of 7.0-mm tangential cuts which exposed the xylem. Mycelial blocks about 5 mm in diam

cut from 2-wk-old malt agar cultures were placed between the bark and wood, and the wounds were covered with sterile cheesecloth and Sealtex Latex Bandage® (Sealtex Co., Chicago, Ill.).

The degree of disease development on inoculated plants was expressed as the number of plants which developed cankers and the lengths of the cankers after a specified period. Presence of *H. pruinatum* in the induced cankers was confirmed by reisolation.

RESULTS.—*Relative humidity.*—To study the effect of RH at inoculated wounds on infection, 30 greenhouse-grown plants inoculated with mycelium (3), were divided into three groups of 10 plants each. In one group, the points of inoculation were wrapped with moist cotton, covered with cheesecloth and sealed with latex bandage; in a second group, the inoculation sites were covered with cheesecloth and latex bandage; in the third group, the inoculation wounds were covered with cheesecloth only. For each one of the three treatments, five wounded, noninoculated, but otherwise similarly treated, trees served as controls. All of the plants were watered to field capacity every fifth day to provide susceptible stressed plants as previously described (3).

No infections resulted either in the noninoculated plants, or in inoculated plants whose wounds were left exposed to the air through cheesecloth. In contrast, cankers developed on the plants with sealed wounds with or without moist cotton. Thus, infection of susceptible greenhouse-grown aspens in air naturally ranging between 38 and 65% RH resulted only when moisture loss from the inoculated wound was prevented.

A more critical experiment was devised to determine the effects of high humidity at the exposed wound on subsequent canker development in plants grown in growth chambers. After inoculation, wounds on the plants were covered with either cheesecloth or latex bandage. Half of the plants, 10 from each treatment, were incubated in a plant growth chamber at 28 C and 100% RH, and the other half were incubated in a chamber at 28 C and 50% RH. All of the plants were watered to field capacity every third day during the 15-day experiment.

At 100% RH, 20% of the inoculated plants became cankered from both the sealed and nonsealed wounds. At 50% RH no infection occurred when the wounds were left exposed, but when the inoculated wounds were sealed with latex bandage 60% of the plants developed cankers.

In another study, 20 four-mo-old plants in 10.16 cm diam pots were grown at 28 C in growth chambers with atmospheric moisture maintained at 90% RH. Another 20 plants were grown in a chamber at 50% RH. Soil moisture in half of the pots at each RH was maintained continuously at field capacity. Field capacity was maintained by means of a subirrigation system utilizing an absorbent cotton and cheesecloth wick connected to a water source. The remaining plants were watered only when they showed wilting symptoms. All plants were inoculated with mycelium.

Maximum canker development was observed in plants subjected to the combination of low RH and drought. Only a limited number of plants developed cankers at 50% and 90% RH when soil moisture was maintained at field capacity (Table 1). When four of the noncankered plants from this treatment were later transferred to a growth chamber at 50% RH and watered only on wilting, three of them developed running cankers in 1 wk. The four plants left under the original conditions remained symptomless.

Temperature.—Previous workers determined the optimum temp for the growth of *H. pruinatum* (12), but no studies have reported the effect of temp on the disease itself. Eighty 4-mo-old rooted aspen cuttings were wound-inoculated on stems and grown in growth chambers at eight different temp (4 to 32 C). Five plants with noninoculated stem wounds at each temp were used as controls. Relative humidity in the growth chambers was regulated at 60-70% with photoperiod of 12 h and plants were watered only at wilting.

Maximum canker development occurred at 28 C, although cankers developed at all temp between 12 and 32 C (Fig. 1). After 10 days, all the inoculated plants at 20, 24, and 28 C developed cankers. No cankers developed on noninoculated plants.

Three months after inoculation, 12 symptomless plants, six from a growth chamber at 4 C and six from a chamber at 8 C, were transferred to 28 C. Before these plants were moved to the higher temp, the latex bandage was removed from the wounds to check for incipient canker development. The wounds of three plants from each of these two cool growth chambers were resealed with latex bandage, while wounds of the remaining three plants were left unsealed.

Running cankers developed within 2 wk on two out of the three plants in which the wounds were resealed and transferred to higher temp. The plants at 28 C in which the

TABLE 1. Effect of different soil moisture levels and atmospheric humidities on development of Hypoxylon canker in aspen at 28 C, 2 wk after inoculation

Relative humidity (%)	Soil moisture maintained at field capacity		Plants watered at wilting	
	Plants cankered (No.)[b]	Average canker[a] lenth (mm)	Plants cankered (No.)[b]	Average canker[a] length (mm)
50	2	12	10	50
90	2	12	10	28

[a]Canker length: LSD($P = 0.05$) = 9.4.
[b]Ten plants were inoculated for each treatment.

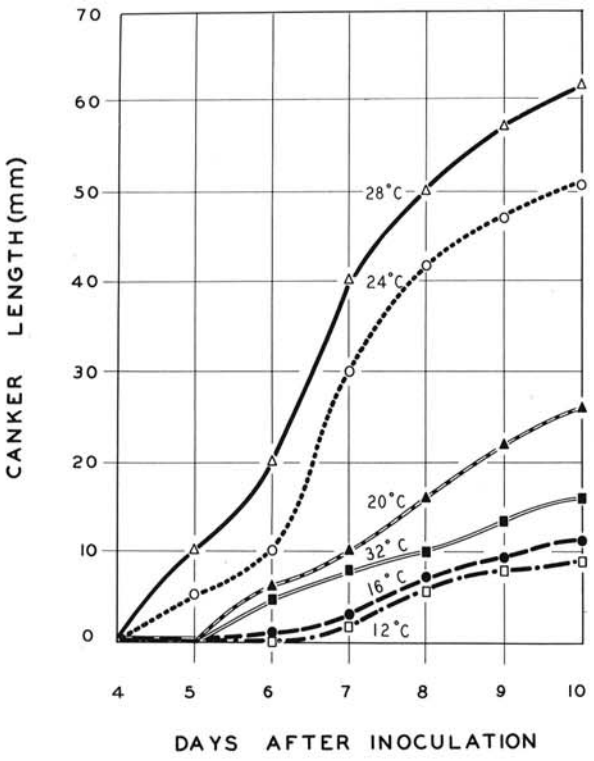

Fig. 1. Effect of temp on canker development on growth chamber grown aspens. Each point represents the average canker length on 10 inoculated plants. Plants were watered at wilting.

wounds were not resealed and the plants left at 4 and 8 C remained symptomless. Thus, inoculum on the symptomless plants at 4 and 8 C remained viable without penetration. If penetration had occurred at the lower temp, the replacement of latex at 28 C would have not been necessary.

Interacting atmospheric factors and soil moisture.—Temperature, RH, and soil moisture as single variables were found to affect canker development. An experiment was designed to study the combined effect of these factors on infection and canker development. Eighty, 3-mo-old plants (ca. 64 cm tall) grown in growth chambers were wound-inoculated on stems. Forty additional plants with noninoculated stem wounds were used as controls. The plants were equally distributed in four growth chambers adjusted to the following atmospheric conditions: temp 24 C, RH 50%; temp 24 C, RH 90%; temp 28 C, RH 50%; and temp 28 C, RH 90%. Ten of the 20 plants in each growth chamber were watered to field capacity every day, while the remaining plants were watered only at wilting. After 10 days, cankered plants were counted and canker lengths were measured.

Canker development was most frequent and rapid in the group of plants subjected to the combination of low RH, high temp, and moisture stress. Minimum canker development occurred in the plants which were watered daily and exposed to high RH and low temp. Canker development in the plants under moisture stress in chambers with 90% RH at 24 C and 28 C was about the same as in those watered daily at 24 C and 28 C with 50% RH (Table 2). No cankers developed on the noninoculated check plants.

Plant nutrition.—To study the effect of availability of nutrients on canker development in moisture stressed plants under controlled conditions, 20, two-mo-old plants were watered twice weekly with Hyponex fertilizer at 2.5 g/liter of water for 60 days prior to inoculation. Twenty similar plants were not fertilized and these became stunted after 30 days and in 2 mo had developed small, yellow leaves. Ten inoculated plants from each fertility group were watered to field capacity daily, whereas the other ten plants were watered every fourth day.

Plants under moisture stress without added fertilizer exhibited maximum canker development. Fifteen days after inoculations 90% of the nonfertilized plants under moisture stress developed cankers averaging 58 mm long. Only 20% of the plants fertilized and watered daily developed cankers with an average canker length of 5 mm (Table 3). The experiment was repeated, with similar results.

TABLE 2. Influence of temperature, relative humidity (RH) and moisture stress on the development of Hypoxylon canker 10 days after inoculation

	24 C		28 C	
Treatment	Cankered plants (No.)[a]	Canker length (mm)[b]	Cankered plants (No.)[b]	Canker length (mm)[b]
Watered daily				
50% RH	4	24	6	41
90% RH	3	10	5	23
Watered on wilting				
50% RH	6	36	8	62
90% RH	5	24	5	37

[a]Ten plants were inoculated for each treatment.
[b]Canker length: LSD($P = 0.05$) = 10.8.

TABLE 3. Effect of fertilization on susceptibility of greenhouse grown aspens grown under two water treatments[a]

Treatment	Watered daily		Watered every fourth day	
	Cankered plants (No.)[b]	Average canker lenth (mm)[c]	Cankered plants (No.)[b]	Average canker length (mm)[c]
Not fertilized for 2 mo	3	10	9	58
Fertilized	2	5	4	25

[a]Temperature and relative humidity during the experimental period were 18-33 C and 30-100%, respectively.
[b]Ten plants were inoculated for each treatment.
[c]In the sixty days prior to inoculation, plants were watered twice weekly with a 2.5 g/liter solution of a commercial fertilizer (Hyponex-available nutrients: 7% N, 6% P, 19% K).

Soil type.—To study the influence of soil types with different water holding capacities, 16 plants were grown in each of the following: 100% sand; one part sand to three parts peat; equal parts sand and peat; three parts sand to one part peat; 100% peat; field soil (plano-silt loam). Soil fertility was maintained by twice-weekly applications of fertilizer (2.5 g/liter Hyponex). Inoculations were made when the plants were approximately 64-cm tall. Half of the inoculated plants grown in each soil type were watered to field capacity daily and the remaining plants were watered to field capacity every fourth day. These schedules of watering were started 7 days prior to inoculation and were continued during the experiment.

To determine the moisture-holding capacities of the six soils, 375 cm^3 of each soil in 500-ml graduated cylinders were watered and allowed to drain. Samples of each soil were then weighed, and oven dried at 95 C to constant weight. The field capacities of the six soils are indicated in Table 4.

After 20 days, the disease development was most severe in the plants under water stress and least in plants under daily watering (Table 4). This was generally true of all the soil mixtures used in this experiment, although disease development in peat was limited regardless of the water treatment. The greatest difference in canker development in response to water treatment was observed in inoculated plants grown in sand. Only half of the inoculated plants watered daily developed cankers, averaging 24 mm, whereas 87% of the plants watered every fourth day developed cankers which averaged 58 mm in length.

DISCUSSION.—Apparently any factor which contributed to host moisture stress increased susceptibility to the pathogen. Relative humidities higher than 50% were unfavorable for canker development. Maximum canker development resulted in plants subjected to combinations of low RH and water stress.

It has been suggested that infection may occur during the dormant season when bark injuries could remain receptive to fungus invasion longer than during the growing season (9). This hypothesis was supported both by the idea that dormant trees with their relatively low moisture content may be more favorable for canker development (9), and also by the fact that ascospore inoculum is present in the air at this time (18). On the other hand, ascospore germination has been shown to be best at higher temp (28 to 32 C) and very limited at temp below 16 C. Berbee and Rogers (6) were unable to produce cankers by artificial inoculation using mycelium as inoculum during the dormant season, although they obtained cankers from inoculations during the growing season. The results obtained in the present studies tend to support the theory that infection in nature takes place during the growing season, probably during periods of water stress. In growth chamber inoculations, the temp range for canker development was 12-32 C. Infection did not take place below 12 C though inoculum survived 3 mo. Kramer and Kozlowski (12) stated that in addition to low bark turgor level in the dormant season, the water content also decreases during the summer to a minimum

TABLE 4. Canker development on inoculated plants grown under water plants grown under water stress on different soil mixtures[a]

Substrate and watering schedule	Plants cankered (No.)[b]	Average canker length (mm)[c]
All sand (Fc 31%)[c]		
Watered every fourth day	7	58
Watered daily	4	24
3 sand + 1 peat (Fc 28%)		
Watered every fourth day	6	49
Watered daily	4	21
Field soil (Fc 46%)		
Watered every fourth day	6	28
Watered daily	5	22
2 Sand + 2 peat (Fc 60%)		
Watered every fourth day	4	28
Watered daily	3	23
1 sand + 3 peat (Fc 84%)		
Watered every fourth day	4	23
Watered daily	2	14
All peat (Fc 48%)		
Watered every fourth day	3	17
Watered daily	2	10

[a]Fc = Field capacity. Temperature and relative humidity ranged from 22 to 28 C and 50 to 95%, respectively.
[b]Ten plants were inoculated for each treatment.
[c]Water treatment: LSD ($P = 0.05$) = 27.8. Soil type: LSD ($P = 0.05$) = 13.2; ($P = 0.01$) = 17.5.

just before leaf fall as a result of heavy loss of water in transpiration. Thus, it is also possible that infection occurs during the summer, when the temp for ascospore germination is favorable, and at times when host water content is also low enough to favor infection.

Disease development on inoculated plants grown in different soils in these studies was considerably altered by different water treatments. Plants grown in sand showed the greatest disease development, while plants grown in peat had the least canker development. These results differed from Anderson's (2), who reported no correlation between soil type and canker development. In general, the effect of different water treatment on canker development on plants grown in different soil mixtures followed similar patterns. For the plants grown in all soil types, the greatest canker development was observed on the plants under water stress. However, more pronounced differences in disease development were observed on the water stressed plants grown in soils with the lower water holding capacities. It is probable that sudden water stress in the field would increase the susceptibility of trees and this might account for the cyclic occurrence of new infection as reported by Day and Strong (10).

Nonfertilized plants were more susceptible than plants fertilized regularly. Gruenhagen (11) and Lorenz and Christensen (13) reported more infection on poorer (and, no doubt, less-fertile) sites. On the other hand, Anderson (2) reported the absence of any relationship between canker development and site index. The microclimatic factors prevailing in a region at a certain time may greatly influence the severity of disease, which may account for these contradictory reports. Thus canker development is influenced by available soil moisture, by temp, atmospheric RH, and host vigor. Availability of soil moisture appears to be the most important factor influencing aspen susceptibility; fewer infections were obtained even in unfertilized plants if they were watered adequately. However, under similar soil moisture levels unfertilized plants were more susceptible than fertilized plants.

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