FACTORS AFFECTING CYTOSPORA CANKER OCCURRENCE ON ASPEN[1]

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Abstract. Experiments were designed to determine how moisture content of aspen trees is related to infection and expansion of *Cytospora* cankers, whether *Cytospora chrysosperma* propagules are found on or in aspen bark, and if there is variation in virulence among *C. chrysosperma* isolates. Cankers were significantly larger on drought-stressed trees than on nonstressed trees. In the spring, wounds on drought-stressed, potted aspen trees, *Populus tremuloides*, were susceptible to infection by *C. chrysosperma* for at least 10 days after wounding. Wounds on nonstressed trees were susceptible for about 4 days. Resistance to canker initiation, as measured by the number of expanding cankers, was expressed about 7 days after wounding on stressed trees. Resistance in nonstressed trees was observed within 2 days. *Cytospora* was not found on asymptomatic trees as an inner bark inhabitant but was found on bark surfaces. Virulence of *C. chrysosperma* isolates was significantly different among isolates from 8 different hosts and 5 isolates from aspen when inoculated on aspen.

Quaking aspen *(Populus tremuloides)* is the most widely distributed tree species in North America, comprising more than 25% of the commercial forest in Colorado (11). Aspen also is now one of the most commonly planted shade trees in eastern Colorado. *Cytospora chrysosperma* is a facultative wound pathogen, causing cankers in 97% of Colorado's native aspen stands (7) and considerable mortality in urban plantings. Besides aspen, the fungus affects many other broadleaf hosts. Christensen (5) reported that *Cytospora* caused necrosis of surface-disinfested, uninoculated cuttings of several species. Based on these findings, he concluded *Cytospora* may exist within apparently healthy bark of poplar, willow, and other species.

Numerous observations and experiments with *Populus* spp. link canker expansion to tree stress (3,4,6,8,9,12). Defoliation stress was coupled to canker expansion (9) in rooted *P. deltoides* cuttings, but most information about tree stress and *Cytospora* canker involves drought. *Cytospora* cankers were common on *P. deltoides* growing on droughty soil, but cankers were not observed on trees growing on deep soil that retained moisture well (12). In another study, *P. deltoides* cuttings had more cankers, and wounds were susceptible for a longer period as moisture stress increased (4). Affiliation of cankers with stressed trees, or on particular sites in a tree, was correlated with low moisture levels in those trees or sites (3).

The relationship of stress with infection of aspen by *C. chrysosperma* in the western United States is not well understood, nor is it known if *Cytospora* is a bark epiphyte or endophyte, or if isolates vary in virulence. Thus, our objectives were to determine how moisture content in potted aspen trees is related to infection and expansion of *Cytospora* canker, if *C. chrysosperma* spores or hyphae are found on or in aspen bark, and if there is variation in virulence among *C. chrysosperma* isolates.

Material and Methods

Duration of wound susceptibility. Three experiments were performed to determine how long wounds on trees remain susceptible to infection under drought stress. One experiment (I) was conducted from September through October, and the other experiments were conducted from February through March (II) and from April through May (III). Seed-propagated, 1.5 m to 2.0 m tall, approximately 2.5 cm diameter, 4- to 5-year-old aspen trees were grown in 19 L black plastic pots in a mixture of 50% topsoil, 25% sand, 20% peat moss, and 5% composted wood chips and cow manure. Trees were maintained at 21°C to 30°C in a greenhouse for the duration of the experiments. Half of the trees were watered to runoff daily. Stressed trees in experiment I were given 500 mL of water per week. In experiments II and III, stressed trees were given approximately 250 mL water, rather

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than the 500 mL per week, when they appeared wilted or when water potential (WP) measured less than –2.0 MPa, so that the plants' water potential would be more stable. The differential in WP between treatments was maintained from 1 week prior to wounding until conclusion of the experiments. Predawn WP of a leaf from each tree was measured with a pressure bomb (PMS Instrument Company, Corvallis, Oregon). Measurements were made twice weekly in experiment I and on alternate days in experiments II and III.

The *C. chrysosperma* isolate was obtained from *P. deltoides* growing in Fort Collins, Colorado, and was maintained on potato-dextrose agar (PDA) plates at 24°C to 30°C in dark. Mycelium on agar discs (8 mm in diameter) was cut aseptically from 7-day-old cultures and used as the inoculum source.

Wounds were made on day 0 at 15 cm intervals along the stems after bark was wiped with 70% ethanol. Longitudinal, 16 mm long wounds extending just into the xylem were made with a disinfested cold chisel on all trees. In experiment I, 10 stressed and 10 nonstressed trees were each wounded 6 times on day 0. Each tree was then inoculated at one of the wounds at 0, 2, or 7 days after wounding. Agar disks were applied as controls to the other 3 wounds at the same time as inoculations were made. In experiment II, 22 stressed and 22 nonstressed trees were inoculated at 2, 4, 5, and 6 days after wounding to determine the duration of wound susceptibility suggested to be between 2 and 7 days in experiment I. Because the duration of wound susceptibility was apparently longer in this spring experiment, a third experiment was initiated. In experiment III, 12 stressed and 12 nonstressed trees were inoculated on days 0, 2, 7, 10, or 14 days after wounding. After inoculation, wounds were wrapped with parafilm for 2 days. Locations of wounds to be inoculated on a specific day were randomized. To determine if *C. chrysosperma* was present in the inoculated wounds, samples were taken at all wound margins 3 weeks after inoculation.

Canker length, defined by bark discoloration, was measured 2 weeks after inoculation (Table 2). For each experiment, mean canker length of stressed versus nonstressed trees was compared by a Student's t-test.

***Cytospora* in/on bark.** To determine if *C. chrysosperma* is a bark inhabitant, 29 asymptomatic trees in 3 natural aspen stands in the Red Feather Lakes area of Roosevelt National Forest in northern Colorado were sampled monthly from May 1986 through April 1987. One stand was about 1.6 km from the other two, which were about 100 m apart. At each sampling time, bark was swabbed with a 1:9 solution of sodium hypochlorite:water followed by swabbing with 70% ethanol and allowed to dry. One 9 mm diameter bark sample (phloem only) per tree was removed immediately with a sterile cork borer and placed bark side up on PDA. This was a test of *Cytospora* presence in bark, since we assumed *Cytospora* outgrowth, if present, would originate from beneath the bark surface because these samples were surface disinfested. A second bark sample, not surface disinfested, was removed from an area 2 cm to 3 cm below the first sample site. This sample was placed bark side down on PDA. This was a test of the presence of *Cytospora* on the bark, since we assumed *Cytospora* outgrowth would originate from the bark surface because these samples were not surface disinfested. All samples were incubated at 24°C to 30°C in a lab for 2 to 14 days.

Virulence of *Cytospora*. Two experiments were conducted to assess the virulence of *C. chrysosperma* isolates on excised aspen branches. Aspen branches approximately 1.5 cm in diameter at the base were excised from trees from several groves in the Red Feather Lakes area. Branches were placed in plastic bags and transported on ice to the laboratory. Branches were placed upright in plastic bags with their bases in distilled water and refrigerated until they stopped gaining weight. In experiment I, we assessed the relative virulence of 8 isolates, 1 isolate obtained from each of 8 *Populus* and *Salix* species (Table 1). One wound was inflicted longitudinally with a cold chisel on 15 to 22 branches per isolate at sites previously wiped with 70% ethanol. Inoculum consisted of mycelium on agar disks (8 mm diameter) cut aseptically from 1-week-old PDA cultures. After inoculation, wounds were wrapped with parafilm

Table 1. *Cytospora chrysosperma* isolates used in experiments involving infection and canker expansion on *Populus tremuloides*.

Isolate number	Source[1]	Location	Host[2]
1	c	Fort Collins, CO	PT
2	ca	Fort Collins, CO	PD
3	c	Durango, CO	PA
4	c	Durango, CO	PN
5	c	Fort Collins, CO	SA
6	c	Fort Collins, CO	PS
7	a	Crow Hill, CO	PT
8	c	Aztec, NM	PW
9	c	Aztec, NM	PAC
10	c	16 km E. of Red Feather Lakes, CO	SA
11	ca	Red Feather Lakes, CO	PT
12	c	Wolf Creek Pass, CO	PT
13	c	Red Feather Lakes, CO	PT

[1]All isolations were made in 1986. a = single ascospore; c = single conidium; ca = canker.

[2]PA = *Populus angustifolia;* PAC = *P. acuminata;* PD = *P. deltoides* cv Siouxland; PN = *P. nigra;* PS = *P. sargentii;* PT = *P. tremuloides;* PW = *P. wislizenii;* SA = *Salix amygdaloides.*

for 3 days. Inoculated branches were placed upright with their bases in distilled water in translucent plastic bags and incubated at 24°C to 30°C in a lab. Canker length was measured 2 or 3 weeks after inoculation. For experiment II, we compared 5 *C. chrysosperma* isolates obtained from 5 different *P. tremuloides* trees by inoculating 11 aspen branches per isolate for a total of 55 branches. Analysis of variance of mean square-root-canker length and LSD (P = 0.05) analyses were utilized.

Results

Duration of wound susceptibility. Duration of wound susceptibility was similar for stressed and nonstressed trees in the fall experiment (I) but not in the spring experiments (II and III). In experiment I, susceptibility lasted between 2 and 7 days (Table 2). Cankers developed on all wounds inoculated at 0 and 2 days. No cankers developed on control wounds. In experiment II, susceptibility of stressed trees lasted longer than susceptibility of nonstressed trees (Table 2). Only a few wounds on nonstressed trees were susceptible 2 days after wounding, whereas wounds on stressed trees remained susceptible through 6 days. Experiment III refined and confirmed the duration that wounds

Table 2. Effect of drought stress on duration of wound susceptibility to *Cytospora chrysosperma* of greenhouse-grown aspen trees.

Experiment/Seasion[1]	Days after wounding when inoculum applied	Cankers[3] Stress	Cankers[3] No Stress
I Fall	0	10/10	10/10
	2	10/10	10/10
	7	0/10	0/10
Mean canker length (mm)		47.2 a[2]	18.0 b
II Spring	2	15/22	3/22
	4	19/22	2/22
	5	17/22	0/22
	6	8/22	0/22
Mean canker length (mm)		24.5 a	12.0 b
III Spring	0	12/12	12/12
	2	11/11	3/12
	7	3/6	0/10
	10	5/7	0/11
	14	0/4	0/11
Mean canker length (mm)		117.4 a	26.6 b

[1]Experiment 1 = September–October, 1986; Experiment II = February–March, 1987; Experiment III = April–May, 1987.

[2]Mean canker length = mean of canker sizes measured 2 weeks after inoculation. Values within same experiment followed by different letters are significantly different (P = 0.01) based on Student's t-test.

[3]Number of cankers resulting from number of inoculated wounds. Some wounds could not be used. Number following the slash (/) is the number of wounds used.

remain susceptible (Table 2). Stressed trees had wounds that were susceptible for 10 to 14 days after wounding, and nonstressed trees had susceptible wounds for only 4 days after wounding. Since there was only one fall experiment, we cannot determine if the duration of wound susceptibility is less in the fall as suggested by our experiment. Mean canker size for stressed trees was significantly larger than canker size on nonstressed trees on all experiments (Table 2). Mean WP over the entire experiment for stressed and nonstressed trees, respectively, in these 3 experiments were:

Experiment I: –1.57 MPa, and –0.32 MPa
Experiment II: –1.50 MPa, and –0.30 MPa
Experiment III: –1.46 MPa, and –0.47 MPa

***Cytospora* in/on bark.** *Cytospora* was isolated 38 times from the 349 samples assayed for the pathogen on the bark (Table 3). Thirty-six of the isolates were obtained from samples collected

Table 3. Monthly isolations of *Cytospora chrysosperma* from aspen bark in natural aspen stands.

Month[1]	% of samples with *Cytospora*[2] On bark surface	Within bark
January	0.0	0.0
February	0.0	0.0
March	0.0	0.0
April	0.0	0.0
May	3.4	0.0
June	13.8	0.0
July	20.7	0.0
August	31.0	0.0
September	17.2	0.0
October	20.7	0.0
November	20.7	0.0
December	3.4	0.0
Total	*10.8*	*0.0*

[1]Sampling was begun in May 1986, and continued through April 1987.

[2]Twenty-nine isolations were made each month from bark surfaces, and 29 isolations from within bark, for a total of 349.

from June through November. One isolate was collected in May and one in December. *Cytospora* was never isolated from the 349 samples assayed for presence of the pathogen in the bark.

Virulence of *Cytospora*. We found that isolates of *Cytospora* from different hosts caused significantly different canker sizes on inoculated aspen branches, as did different isolates from different aspen trees (Tables 4 and 5). Interestingly, isolates 11 and 13, both collected in the Red Feather Lakes area, caused significantly different average canker sizes.

Discussion

There were 3 effects of drought on *Cytospora*-caused infection and canker development on aspen trees (Table 2). First, fewer cankers occurred on nonstressed trees. Second, greater expansion of cankers occurred on stressed than nonstressed trees. Third, the duration of wound susceptibility on aspen trees apparently was related to the water status of the trees. Resistance to infection of wounds in aspen may require at least 2 to 6 days, and usually more, to limit canker formation if trees are stressed. In contrast, resistance mechanisms limited canker formation within 48 hours in nonstressed trees in the 2 spring experiments

Table 4. Mean canker size on excised aspen branches inoculated with *Cytospora chrysosperma* isolates from 8 host species.

Host species (isolate no.)[1]	Mean canker length (mm)[2]
Populus angustifolia (3)	2.2 a
Populus acuminata (9)	2.2 a
Populus wislizenii (8)	3.2 ab
Populus deltoides cv. Siouxland (2)	3.9 bc
Populus sargentii (6)	4.1 bcd
Populus nigra v. *italica* (4)	4.4 bcd
Salix amygdaloides (5)	4.8 cd
Populus tremuloides (1)	5.2 c

[1]Isolates are described in Table 1.

[2]Mean canker length = mean of canker sizes measured 2 weeks after inoculation. Means followed by different letters are significantly different based on an LSD ($P < 0.5$), $n = 15$.

Table 5. Mean canker size on excised aspen branches inoculated with 5 *Cytospora chrysosperma* isolates from aspen.

Location (isolate no.)[1]	Mean canker length (mm)[2]
Crow Hill (7)	2.2 a
Red Feather Lakes (13)	2.4 a
Wolf Creek Pass (12)	2.7 a
Red Feather Lakes (11)	5.2 b
Fort Collins (1)	6.1 b

[1]Isolates are described in Table 1.

[2]Mean canker length = mean of canker sizes measured 2 weeks after inoculation. Means followed by different letters are significantly different based on an LSD ($P < 0.5$), $n = 11$.

(Table 2) but not in the fall experiment. The time required for resistance to reach a sufficient level in stressed and nonstressed trees is consistent with the time frame of periderm formation (1,2).

Christensen's results (5) led him to suggest that *C. chrysosperma* could be found within apparently healthy bark. Schoeneweiss (10) found bark colonization by *C. kunzei* some distance from the point of wounding and inoculation, where no necrosis or obvious bark injury occurred. However, we could not confirm these observations on aspen because *C. chrysosperma* was not isolated from within apparently healthy bark, only from its outer most surface (Table 3).

We found virulence differences among isolates obtained from different host species and within a

host species. Because we used only 1 isolate from each host or aspen tree, any differences in canker sizes can be assumed to be a result of variation in the fungal population and not related to the isolates' host. Thus, aspen planted in or out of the species' normal range might, therefore, be infected by the pathogen from other species. This aspect of *Cytospora* canker management needs further study before we can make recommendations.

In summary we found that drought stress favors larger cankers and increases the duration wounds are susceptible to infection; *Cytospora* does not seem to be an inhabitant within bark, but can be isolated from bark surfaces; *Cytospora* isolates from one host, apparently, can infect another host species; and there is variation in virulence among *Cytospora* isolates from different host species and also from a species. Thus, suppression of *Cytospora* canker can be improved by preventing wounds, preventing drought, and removing infected tissue (inoculum sources) from all hosts in the urban environment. Preventing severe fluctuations in soil moisture by the use of mulch, precise irrigation scheduling, proper planting techniques, and planting aspen in landscapes compatible with their soil moisture tolerances, will prevent predisposing stresses. Utilizing a pressure bomb is probably the easiest and most direct measure of plant water status and can be easily utilized in the field.

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