Effects of Environmental Stress on the Development of Cytospora Canker of Aspen

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ABSTRACT

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Greenhouse, field, and laboratory studies examined the role of selected environmental stresses on the development of Cytospora canker of aspen trees. In greenhouse studies, we examined the resistance of aspen to *Cytospora chrysosperma* after exposure to drought, flooding, or defoliation. Drought-stressed trees had larger cankers than control trees, whereas flooded trees did not. Water potential of trees was a significant covariant that explained variation in canker size. Severely defoliated trees (75 to 100%) had larger cankers than nondefoliated control trees or trees with 50% defoliation. Carbohydrate content of roots of defoliated trees was significantly less in 100% defoliated trees than in 75 and 50% defoliated trees. Canker size on field-inoculated aspen and cottonwood (cv. Siouxland) was related inversely to tree water potential. Peak susceptibility to canker expansion occurred when water potential dropped below –1.6 MPa. Relative turgidity was not associated with canker size. In vitro growth of *C. chrysosperma* was affected positively by decreasing osmotic- and matrix-based water potential until water potentials were lowered to –1.0 MPa. Below –1.0 MPa, fungal growth was affected negatively.

Quaking aspen (*Populus tremuloides* Michx.) is one of the most widely distributed forest tree species native to North America (15). In Colorado and Utah, aspen comprises more than 25% of commercial forests (15). Aspen also is planted widely as an ornamental in the intermountain west. Throughout its distribution, aspen is prone to numerous root, stem, and foliage diseases. The most common canker disease of aspen in Colorado is caused by *Cytospora chrysosperma* (Pers.:Fr.) Fr. Hinds (13) reported Cytospora canker present in 97% of native aspen stands sampled in Colorado.

C. chrysosperma usually is considered a facultative parasite, which attacks trees when their resistance is lowered by predisposing environmental stress (9,14). Specific stresses that are important in predisposing aspen to canker pathogens are not known. Three stresses important in other diseases induced by facultative parasites and that are common in urban and native forests are drought, flooding, and defoliation (25). Aspen is particularly prone to defoliation because it is host to a large number of leaf-feeding insects and foliar pathogens (14,16). Because wide ecological distribution of aspen exposes them to extremes of high and low soil water content, these abiotic stresses may be important in predisposing aspen to infection by Cytospora, as they are with other aspen diseases such as Hypoxylon canker (*Hypoxylon mammatum* (Wahlenberg) J.H. Miller (8).

Threshold levels of predisposing drought stress for some tree species afflicted with facultative pathogens like *C. chrysosperma* are reported. Schoeneweiss (24) reported that European white birch (*Betula pendula* Roth) infected by *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not. was predisposed by –1.2 MPa of water potential stress. Bertrand et al. (1) found significantly larger cankers caused by (*Cytospora leucostoma* (Sacc.)) on French prune (*Prunus* spp.) trees on dry growing sites with tree water potential values under –1.5 MPa than on trees growing on well-watered sites. Levels of drought stress experienced by aspen and cottonwood (*Populus deltoides* Bartram ex March. cv. Siouxland) in nature, however, were not examined.

Some pathogenic fungi grow better or infect more effectively at lower water potential and thus may cause more damage to host plants that are drought stressed (7,10–12,20). Conversely, the growth of some fungi declines with decreasing water potential (12). One method for examining the response of fungi to water potential stress is by the addition of various osmotica and matrica (including polyethylene glycol [PEG], various sugars, sugar alcohols, and salts) to liquid and solid media (7,12,19,21).

Our objectives for this study were to (i) determine if drought, flooding, or defoliation stresses increase the severity of Cytospora canker on aspen; (ii) determine if field-grown aspen and cottonwood are predisposed to infection by water stress near the thresholds previously reported (1,23); (iii) test the reliability of two methods of determining the moisture status of trees, relative turgidity (2) and water potential, with a pressure chamber (27); and (iv) assess the effect of osmotically and matrically reduced water potential on in vitro growth of *C. chrysosperma*.

MATERIALS AND METHODS

Plant material. For drought and flooding experiments, 3-year-old aspen seedlings (open pollinated) were grown in 18.9-liter black plastic pots in a glass greenhouse with a temperature range of 20 to 32°C. The soil mix contained 25% sand, 20% peat, 5% composted manure and sawdust, and 50% local clay loam. One-year-old aspen seedlings used in defoliation experiments were grown in 3.8-liter black plastic pots in a glass greenhouse with the same soil mix as above.

Trees used in the field study were 4- to 8-year-old aspen and 7-year-old Siouxland cottonwood planted at the Colorado State University research farm, Fort Collins. Trees did not receive supplemental irrigation after they were established. At study initiation (1987 to 1988), the aspen and cottonwood were, respectively, 1.5 to 2 m and 14 to 16 m tall, and 4 to 6 cm and 10 to 15 cm in diameter at 1.4 m.

Fungal isolates. *C. chrysosperma* isolate 89-1, originally from an aspen canker in Fort Collins, was used in all drought and flooding experiments in year 1. For all drought and flooding experiment replicates in year 2, isolates 89-1 and 89-2, originally from a cottonwood located in Fort Collins, were used. Both isolates were used in defoliation, field inoculation, and in vitro water potential studies. One-week-old cultures were used to inoculate trees. For

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culture maintenance, we used a yeast-malt (YM) agar (17) containing 3 g of yeast extract, 3 g of malt extract, 10 g of maltose, and 5 g of peptone per liter of distilled water.

Drought and flooding stresses. Two stress treatments and one control, each with eight trees per treatment, were undertaken. In year 1, the experiment was performed three times (inoculations on 2 June, 17 July, and 29 August), utilizing one *C. chrysosperma* isolate. In year 2, the experiment was repeated two more times, on 30 June and 29 July, with two isolates. Drought stress was achieved by withholding moisture from the trees until they reached a predawn water potential of –1.5 to –2.0 MPa. Water potential was measured (27) on two leaf petioles every other day with a pressure chamber. After 7 days under the stress regime, trees were inoculated. After this, whenever trees were more stressed than –1.5 MPa, they received 250 ml of water. This watering–drying cycle was continued until the conclusion of the experiment, 4 weeks after inoculation.

Flooding stress was achieved by placing trees in a tank of tap water so that the entire soil profile and approximately 5 cm of the stem were submerged. The tank was covered with aluminum foil to limit algae growth. Flooded trees were inoculated 48 h later because initial stress occurs within 24 h (18). The final treatment consisted of inoculated controls, where trees were watered adequately every second or third day until runoff occurred. Water potentials of control trees were maintained between –0.5 and –1.0 MPa. Average water potential of the trees after inoculation were –1.9, –0.4, and –0.5 MPa for the drought, flood, and control treatments, respectively.

Table 1. Water potential of polyethylene glycol (PEG) and mannitol test solutions

	Mean water potential[a]
PEG (%)	
0.0	0.27 ± 0.02
8.4	0.51 ± 0.05
15.6	0.74 ± 0.06
20.0	1.03 ± 0.11
23.0	1.39 ± 0.08
25.3	1.58 ± 0.10
27.2	1.85 ± 0.09
30.1	2.20 ± 0.08
34.3	3.30 ± 0.12
Mannitol (%)	
0.0	0.22 ± 0.01
1.3	0.52 ± 0.03
2.2	0.75 ± 0.04
3.3	0.99 ± 0.05
4.7	1.22 ± 0.04
5.8	1.49 ± 0.07
7.1	1.69 ± 0.09

[a] Water potential (MPa) ± standard error was measured with a dew point hygrometer. Measured means are an average of four replicates from three performances of the experiment (n = 12).

In drought and flooding experiments, 2.54-cm-long wounds of a mashing-lacerating type were made through the bark to the sapwood with a flame-sterilized cold chisel. The bark surrounding the wounds was surface-disinfested with 70% ethanol before and after wounding. In year 1, two wounds per tree were located 20 cm apart, with the lowest wound 15 cm above the soil line. Eight trees were utilized for each treatment. Wounds were placed 90° clockwise around the stem from one another. A 1-cm-square block of mycelium and agar was placed on one wound and sterile agar on the second wound. Both were covered with commercial wax film. The two isolates used the second year were randomly placed on two of three wounds, and sterile agar was placed on the third. At the conclusion of all experiments, isolations were made from the margins of cankers on half of the trees.

Defoliation stress. We utilized three stress treatments (50, 75, and 100% defoliation) and one control treatment, each with 10 trees inoculated with one of two isolates of *C. chrysosperma*. Each isolate was assigned randomly to five of the 10 trees in each treatment. Defoliation was achieved by removing leaves with a razor blade. Since a defoliation period of 3 to 4 weeks (25) is required to cause significant stress, defoliation regimes were maintained by removing new leaves for 4 weeks be-

Table 2. Percentage of field inoculations resulting in canker expansion, water potential values, and relative turgidity for *Populus tremuloides* (aspen) and *P. deltoides* (cottonwood) trees inoculated with *Cytospora chrysosperma*

	Aspen				Cottonwood			
	Successful inoc.[a] (%)	MPa[b]	Relative turgor Bark	Relative turgor Wood	Successful inoc. (%)	MPa	Relative turgor Bark	Relative turgor Wood
April	17	–0.8	76.2	84.4	17	–0.6	65.1	70.3
May	17	–0.7	...[c]	...[c]	17	–0.9	71.0	69.8
June	58	–1.6	86.9	81.7	42	–0.7	72.3	67.3
July	92	–2.1	...[c]	...[c]	83	–2.0	67.8	66.6
August	100	–2.5	85.3	77.8	100	–2.2	67.1	66.4
September	100	–2.5	...[c]	...[c]	100	–2.9	66.7	66.1
October	...[c]	...[c]	83.1	82.5	...[c]	...[c]	65.6	64.0
r[d]		–0.99*	0.79	–0.99		–0.93*	0.32	–0.93*

[a] Each value is the percentage of successful inoculations out of four on three different trees (12 total inoculations). The inoculations were made between the first day and the fifth day of each month.
[b] Each pressure bomb reading is the mean of three leaves on four branches from each of three different trees made on the first day of each month, n = 36.
[c] Data not taken.
[d] Pearson correlation coefficient between MPa, relative turgor (bark and wood), and % successful inoculations; * = significant correlation at $P = 0.05$.

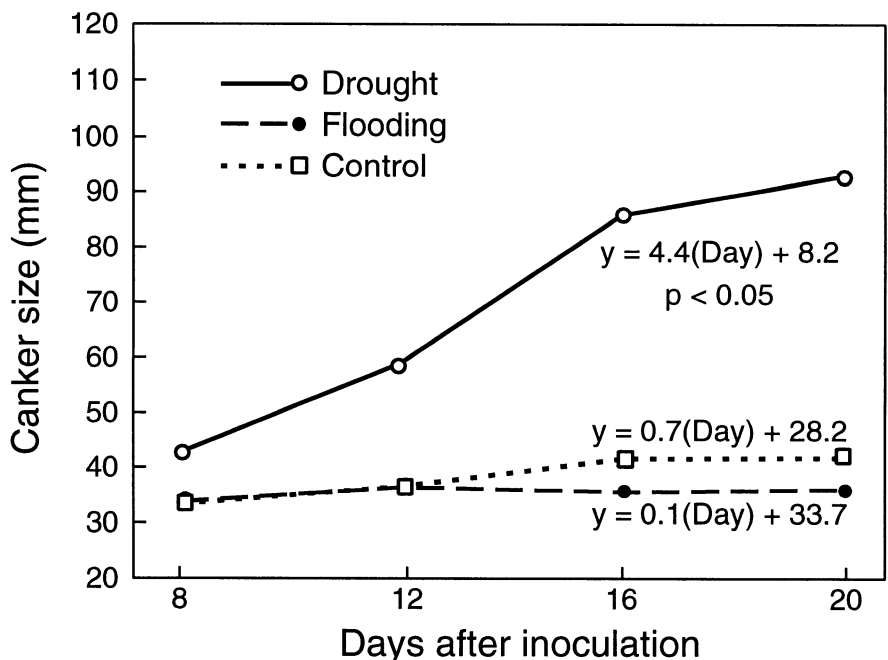

Fig. 1. Average canker size on *Populus tremuloides* inoculated with *Cytospora chrysosperma* and subjected to drought or flooding. Each mean represents 56 cankers: one wound per tree in year 1 and two per tree in year 2, eight trees per treatment, and three repetitions in year 1 and two in year 2 of the experiment. Data from two different isolates and two different years are pooled.

fore inoculation. Experiments were conducted four times, with two repetitions early in the growing season (1 June and 3 July) and two late in the growing season (14 August and 17 September). Inoculations of defoliated trees were made the same way as for the drought and flooding experiments except that only one wound was made per tree with a smaller (1.3 cm) chisel. Uninoculated controls were not used in these experiments.

Carbohydrate analysis. Total nonstructural carbohydrate (TNC, % dry weight) of root samples was determined by acid extraction in 0.2 N H_2SO_4 for 60 min, followed by analysis (26). Duplicate extractions and titrations were performed randomly on all samples, with dextrose and blank standards in each run. Root samples consisted of 20 g (dry weight) of woody root tissue from each of four trees from each treatment in drought, flooding, and defoliation stress experiments. Root samples were washed in distilled water, dried for 4 days at 70°C, and milled through a 1-mm-mesh screen.

Field inoculations. Relative turgidity (RT) of wood and bark was measured on the first day of each month for 7 months for field-grown cottonwood, and the first day of every other month for aspen. Determinations were made between 0500 and 0700 hours (14). Water potential measurements of leaves, taken with a pressure bomb, were made between 0500 and 0700 hours on the first of each month. Water potential measurements of leaves were made prior to excising branches for relative turgidity measurements.

Inoculations were made at each date on the main stem of three aspen trees, and on a major branch subtending the branch used in pressure chamber measurements for three cottonwood trees. Four wounds were made with a 2.54-mm-wide chisel on the three aspen and cottonwood trees every month from April to September. The upper and lower two wounds on each tree received isolate 89-1 and 89-2, respectively.

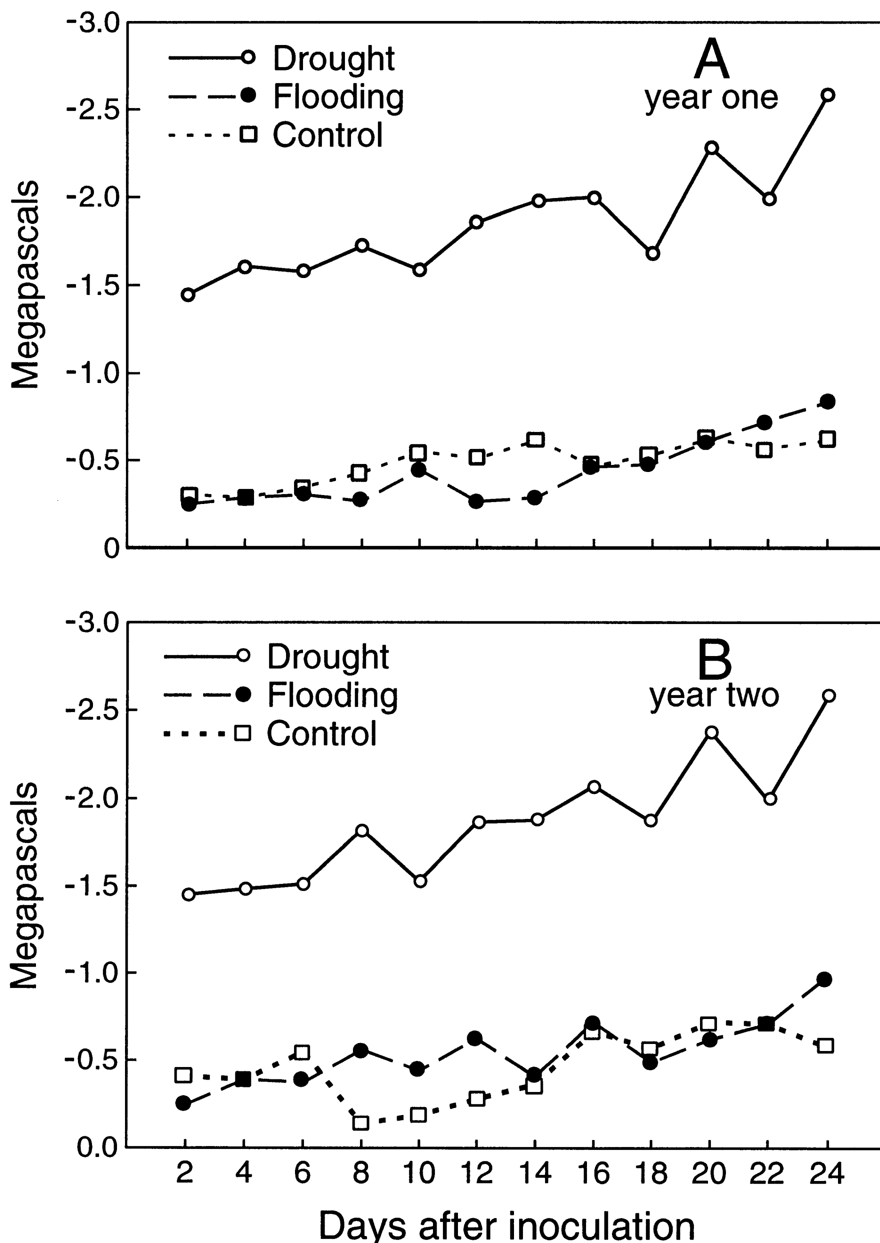

Fig. 2. Average pressure chamber values for *Populus tremuloides* trees infected with *Cytospora chrysosperma* and subjected to drought or flooding. In (**A**) (year 1), each mean represents 48 measurements: two leaves per tree, eight trees per treatment, and three repetitions of the experiment. In (**B**) (year 2), each mean represents 32 measurements: two leaves per tree, eight trees per treatment, and two repetitions of the experiment.

In vitro water potential studies. Two sets of three experiments each were performed to compare the response of *C. chrysosperma* to matrically and osmotically lowered water potential with its best carbon source, maltose, (17) and sucrose, a sugar commonly found in bark. In the first set of experiments, YM medium without agar was used with 10 g of either maltose or sucrose. To produce lower water potential matrically, the medium was amended with various concentrations of PEG (Table 1) and autoclaved (29).

To examine the effects of osmotic water potential depression on *C. chrysosperma* growth, mannitol was used as an osmoticum in the second set of three experiments. The same base medium was used, except sucrose was lowered to 5 g. An increase in growth of *C. chrysosperma* at low levels of water potential stress was noted in these mannitol experiments. Consequently, experiments were conducted with lower concentrations of PEG to determine if the same trend existed in PEG-based medium. In all experiments, a 3-mm agar plug of *C. chrysosperma* was transferred to each of four 250-ml flasks containing 125 ml of media per treatment. A sterile agar plug transferred to one flask per treatment served as a control. In all experiments using PEG, controls without sucrose or maltose were used to determine if PEG was a carbon source for the fungus. All experiments were conducted three times. Flasks were placed on a rotary shaker at 21°C under ambient laboratory lighting for 10 days.

Water potential determination. Water potentials of the liquid media were determined with a dew point hygrometer prior to addition of the fungus. Measurements of all media with the hygrometer were made at 21°C after calibrating the instrument with NaCl solutions of known water potential (11). The range of water potential for test media was –0.22 to –3.3 MPa (Table 2).

Data collection. For greenhouse studies, canker size was recorded as the sum of horizontal and vertical dimensions of discolored bark. Cankers were measured at 4-day intervals starting 8 days after inoculation. In field experiments, cankers were not measured but were recorded as expanding

or not expanding. Growth of *C. chrysosperma* in test media was evaluated by measuring total dry weight of colonies after 10 days. Flask contents were vacuum filtered through no. 4 Whatman filter paper previously dried at 100°C for 3 days. Fungal material on the filter paper was rinsed twice with hot water to remove excess PEG before dry weights were obtained.

Data analysis. Canker size was compared among stress treatments (drought, flooding, and defoliation) by regression analysis. Because canker size did not differ by isolate, isolates were pooled in the analysis. All computations were performed by the regression procedure in SPSSX (28). Analysis of variance of total nonstructural carbohydrates was performed using SPSSX. Analysis of covariance was used to analyze the relationship of canker size and average water potential at 20 days after inoculation using SPSSX.

In the field study, water potential and RT data were analyzed by analysis of variance, and means were compared with Fisher's protected LSD. An analysis of the association between pressure chamber values and percentage of successful inoculations (cankers that expanded) was run using Minitab (20).

Fungal dry weights for each liquid culture experiment were analyzed statistically by analysis of variance with the SPSSX programs (27). Weights from control flasks were analyzed in a separate analysis of variance. Weights of the residue on filter paper from noninoculated flasks were not significantly different among treatments. Polynomial linear regression, with the level of water potential stress as the independent variable and dry weight as the dependent variable, was performed with Minitab (20).

RESULTS

Drought and flooding stresses. Cytospora cankers were significantly larger ($P = 0.05$) at each measurement date after day 8 on trees grown under drought stress conditions compared with cankers on adequately watered control trees (Fig. 1). Canker size on trees under the flooding treatment was not significantly different from the controls at any measurement date (Fig. 1). Water potentials of drought-stressed trees were significantly ($P = 0.05$) lower than control (nonstressed) or flooded trees (Fig. 2). At 20 days after inoculation, water potential values were well-correlated with canker size and explained enough of the variation that, when water potential was used as a covariant, mean canker sizes were not significantly different from canker sizes on nonstressed or flooded trees. Noninoculated, drought-stressed trees did not show any wilting, dieback, or other symptoms. Mortality of 12 to 50% of the noninoculated flooded trees and inoculated flooded trees occurred after 5 weeks of continuous flooding. Mortality was not caused by cankers since they had not girdled the trees.

Total available carbohydrate of roots was not significantly different ($P = 0.05$) from the control for either drought- or flood-stressed treatments. All cankers sampled yielded the pathogen from isolations when experiments were terminated.

Defoliation stress. Trees with 100% defoliation had a significantly greater rate (3.2, $P = 0.05$) of canker expansion than found on control trees (Fig. 3). Trees with 75% defoliation had a significantly greater rate (2.4, $P = 0.05$) of canker expansion than control trees. The 50% defoliation did not affect canker size.

TNC of roots of 100% defoliated trees (7.9% carbohydrate per dry weight) was significantly ($P = 0.05$) lower than TNC of the other three treatments. TNC of roots from 50% (12.0%) and 75% (12.1%) defoliated trees was not significantly different from that of control trees (11.5%). Also, no significant differences occurred in TNC between experiments conducted early in the growing season (June and July) and those conducted late in the growing season (August and September). TNC of roots was a significant covariant for the 100% defoliated trees, but not for 50 and 75% defoliated trees. Noninoculated defoliated trees did not have symptoms different from those seen on inoculated trees.

Field inoculations. Water potential of aspen decreased markedly from May through July (Table 2). Cottonwood followed a similar trend, with a decrease in water potential from June to July and another large decrease from August to September. A strong negative association for both aspen and cottonwood ($r = -0.99$ and -0.93, respectively) occurred between water potential measured with a pressure chamber and percentage of successful inoculations (Table 2).

Few inoculations of either host were successful until June (Table 2). Successful inoculations increased from 17% in May to 58% in June on aspen, and from 17% in May to 42% in June on cottonwoods. During June, water potential of aspen lowered to –1.6 MPa. Water potentials of aspen stayed below this level for the rest of the growing season. Water potentials of cottonwood exhibited a similar change in July, lowering to –2.0 MPa. In July, successful inoculations increased to 91% for aspen and 83% for cottonwood; in August, both increased to 100%.

Relative turgor (RT) of both aspen and cottonwood bark and wood followed trends similar to but not equal to water potential during the growing season (Table 2). For aspen and cottonwood, the highest RT came in April for wood and in June for bark. Bark RT always lagged behind wood RT in early summer. No significant correlation occurred between RT and percentage of successful inoculations except for wood RT in cottonwoods, but the relationship was negative (Table 2).

In vitro studies. Growth of *C. chrysosperma* was affected by the culture medium's water potential and was dependent on the type of water potential-lowering agent utilized. A decrease in matrically controlled (with PEG) water potential significantly inhibited pathogen growth in liquid culture solutions with a water potential lower than –1.00 MPa (Fig. 4A). A

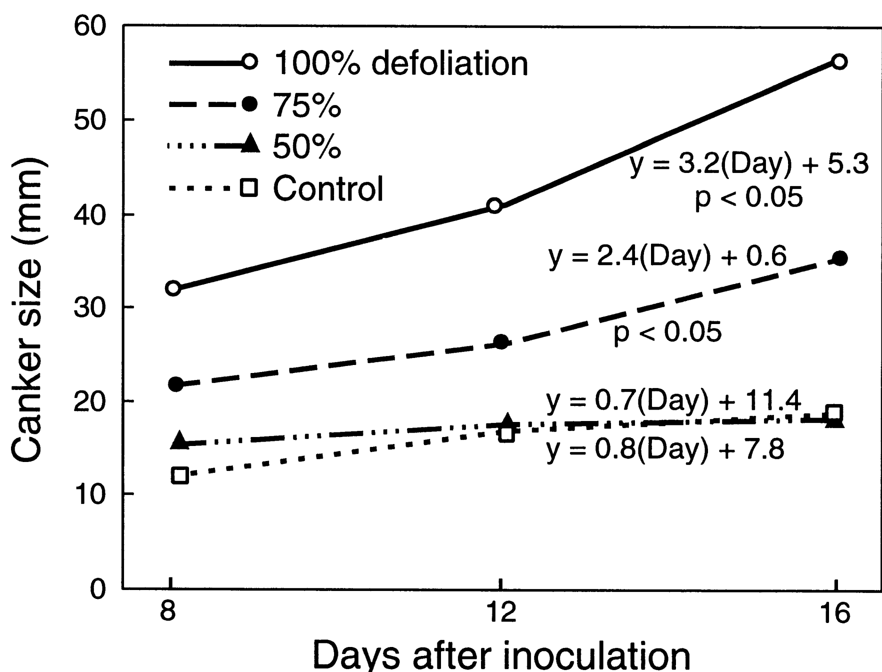

Fig. 3. Average canker size on *Populus tremuloides* inoculated with *Cytospora chrysosperma* and subjected to defoliation stress. Each mean represents 40 cankers: one canker per tree, 10 trees per treatment, and four repetitions of the experiment. Data from two different isolates were pooled.

linear trend (P = 0.01) of decreasing growth with decreasing water potential occurred for both isolates with maltose as the carbon source (Fig. 4B). A slight quadratic trend (P = 0.01) was found using PEG and sucrose (Fig. 4A), with peak growth at 1.0 MPa. Regressions also showed a linear trend (P = 0.01) below −1.0 MPa for PEG with sucrose. When a smaller range of water potential stress (0 to 1.8 MPa) was imposed on *C. chrysosperma* with mannitol, a quadratic trend (P = 0.01) was revealed, with growth stimulated by osmotic stress to –0.9 MPa then depressed below –0.9 MPa (Fig. 5A). Higher order polynomial effects were not significant for any experiment. The regressions (Fig. 5B) for *C. chrysosperma* in response to water potential reduced matrically with lower concentrations of PEG were similar in revealing quadratic trends (P = 0.01) at low levels of water potential stress (–0.27 to −1.0 MPa).

A difference in growth response occurred between the two carbon sources (Figs. 4 and 5). Fungal growth with sucrose was significantly greater (P = 0.05) than with maltose only at the greatest stress level (–3.0 MPa). In all experiments with PEG, control treatments without a primary carbon source (sucrose or maltose) had significantly (P = 0.05) less growth than treatments with a carbon source.

DISCUSSION

Larger cankers formed on drought-stressed aspen than on nonstressed trees. Some drought-stressed trees were killed by girdling cankers. This was not unexpected for this pathogen–host system because similar responses are know for other hosts (5,21,23,25,30). In contrast, flooding produced no effect on canker size but did cause mortality of 12 to 50% of the trees. Mortality was not caused by cankers, because the trees were not girdled.

Larger cankers developed on aspen experiencing 75 to 100% defoliation than on control trees. Thus, instances of severe defoliation may predispose trees to damage by *C. chrysosperma*. Several insects, including tent caterpillars, the large aspen tortrix, aspen leaf tier, and three species of geometrid moths can defoliate aspen in the central Rocky Mountains (16). Also, several fungal foliar pathogens, including *Marssonina populi* (Lib.) Magnus, *Ciborinia* spp., and *Melampsora medusae* Thuem., can cause severe defoliation of aspen (14).

The association of drought and defoliation with larger cankers should be considered when managing the health of aspen in urban and natural ecosystems. Preventing severe fluctuations in soil moisture by the use of mulch, precise irrigation scheduling, proper planting techniques, and the planting of aspen in landscapes compatible with their soil moisture tolerances will prevent the predisposing stresses of flooding and drought. Even though flooding stress did not increase canker size, it did cause the death of many of the trees after the treatment ended. Therefore, soil flooding should be avoided. Utilizing a pressure bomb is probably the easiest and most direct measure of plant water status and can be easily performed in the field. Defoliation by insects and foliar pathogens can be prevented by selecting resistant clones, modifying environmental conditions conducive to the pest, and applying pesticides.

Defoliation reduces carbohydrate content of plants by reducing photosynthetic area. Water stress is also known to affect plant carbohydrate content (25). We did not find any effect of drought, flooding, or 50 and 75% defoliation on carbohydrate content of roots. Thus, the stress treatments probably were not severe or long enough to affect carbohydrate content of roots.

Pressure chamber readings were strongly associated with canker establishment on aspen and cottonwood (r = 0.99 and 0.93, respectively), whereas RT was not related. For example, the month when aspen showed its peak RT for bark was when canker incidence increased. In their work with *C. leucostoma* on French prune, Bertrand et al. (1) also found canker incidence correlated well with pressure chamber measurements but not with RT.

Overall, our study substantiates earlier findings on poplars (5) that trees were not susceptible to Cytospora canker unless they experienced substantial stress. Aspen and cottonwood reached peak susceptibility to Cytospora canker when water potential dropped below –1.6 MPa. These data support our findings from greenhouse tests that showed drought-stressed trees exhibited larger cankers than adequately watered controls.

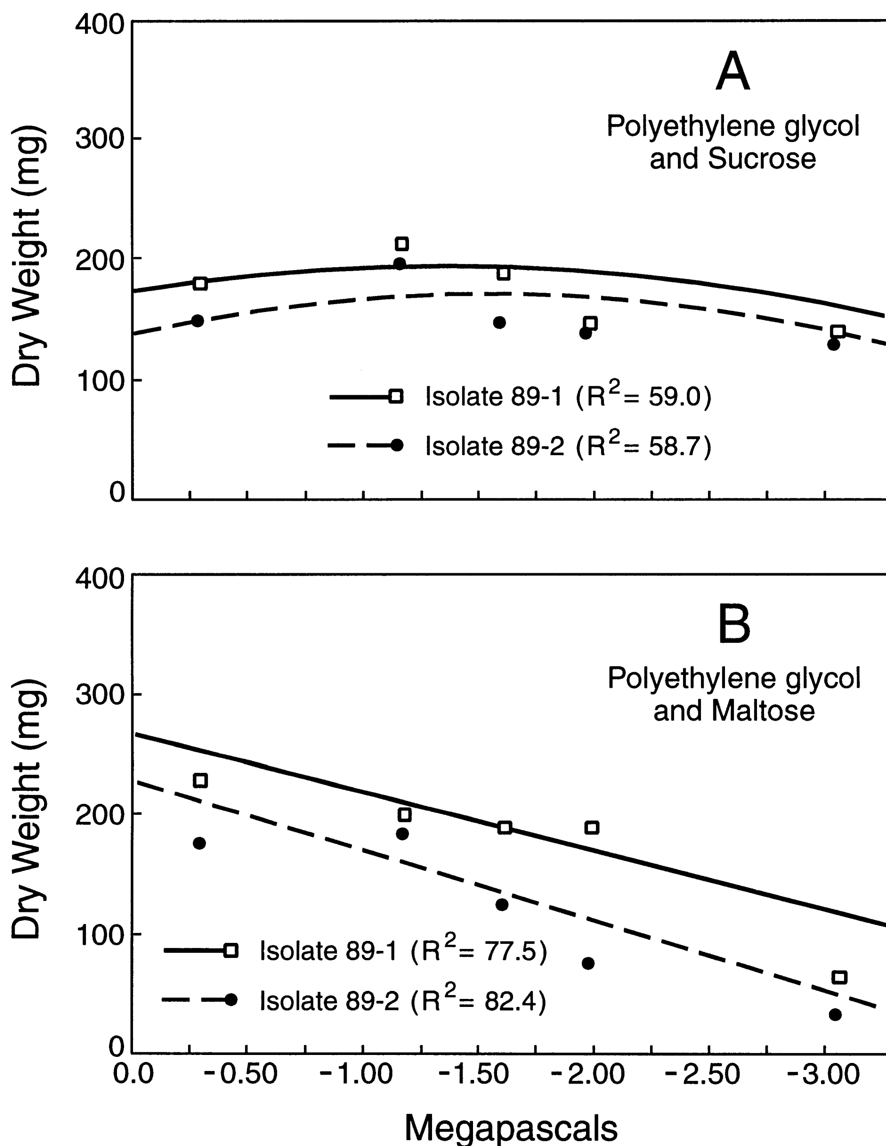

Fig. 4. Regression of mycelial dry weight on water potential (0 to 3.0 MPa) for two isolates of *Cytospora chrysosperma* grown in liquid culture. **(A)** Water potential was lowered using polyethylene glycol, with sucrose as the primary carbon source. **(B)** Water potential was lowered using polyethylene glycol, with maltose as the primary carbon source. Each mean represents nine dry weights: three flasks per treatment and three repetitions of the experiment.

It is unclear whether increased susceptibility of drought-stressed trees is due to a stimulation of pathogen growth by lowering of water potential, as exhibited by the spruce pathogen *Cytospora kunzei* Sacc. (21), or to a weakening of host defensive reactions by environmental stress, or to both factors. Profer (21) reported maximum growth of *C. kunzei* at –1.4 to –1.5 MPa, which is in the range of stress that predisposes spruce to Cytospora canker. In our study, the optimal growth for *C. chrysosperma* was around –1.0 MPa. This growth optimum was not within the range of water potential normally considered stressful to aspen. *C. chrysosperma* growth was inhibited at water potential values higher (less negative) than reported to inhibit many other fungi (6,7,10–12,19). Drought-stressed trees (water potential in the range of –1.4 to –3.2 MPa) had significantly larger cankers than did well-watered trees. This water potential still supports *C. chrysosperma* growth. Thus, we hypothesize that, because *C. chrysosperma* growth in vitro is reduced by increased (more negative) water potential, cankers were larger under drought stresses because host defenses were weakened rather than because of direct stimulation of pathogen growth. Additionally, the fact that two different, presumably unrelated, types of stress (drought and defoliation stresses) were both correlated with canker size suggested that different types of stress may cause a similar host response. Previous researchers found that in trees, drought stress slows host resistance mechanisms such as periderm production (22), rate of lignification, and reaction zone size (4). Other researchers found defoliation induces numerous physiological changes in trees, including changes in the rate of amino acid synthesis and levels of phenolic substances (25).

The growth of *C. chrysosperma* in response to water potential was similar when controlled osmotically with mannitol or matrically with PEG (Figs. 4 and 5). If growth responds similarly to water potential changes with different osmotica and matrica, then specific solute effects can be disregarded. However, it does appear that the growth of *C. chrysosperma* is depressed by PEG. The matrix nature of PEG may affect growth (7), and PEG is known to reduce oxygen content of liquid culture solutions (19), which could affect fungal growth. However, the concentrations used in these experiments should not be high enough to restrict oxygen content to inhibitory levels (19). Another possibility is that mannitol could stimulate fungal growth by serving as a carbon source.

Our findings are of interest to nursery managers and arborists who wish to prevent Cytospora canker. Thus, barring other stresses, if aspen and cottonwood are kept adequately watered and severe defoliation is prevented, the incidence of Cytospora canker should be low.

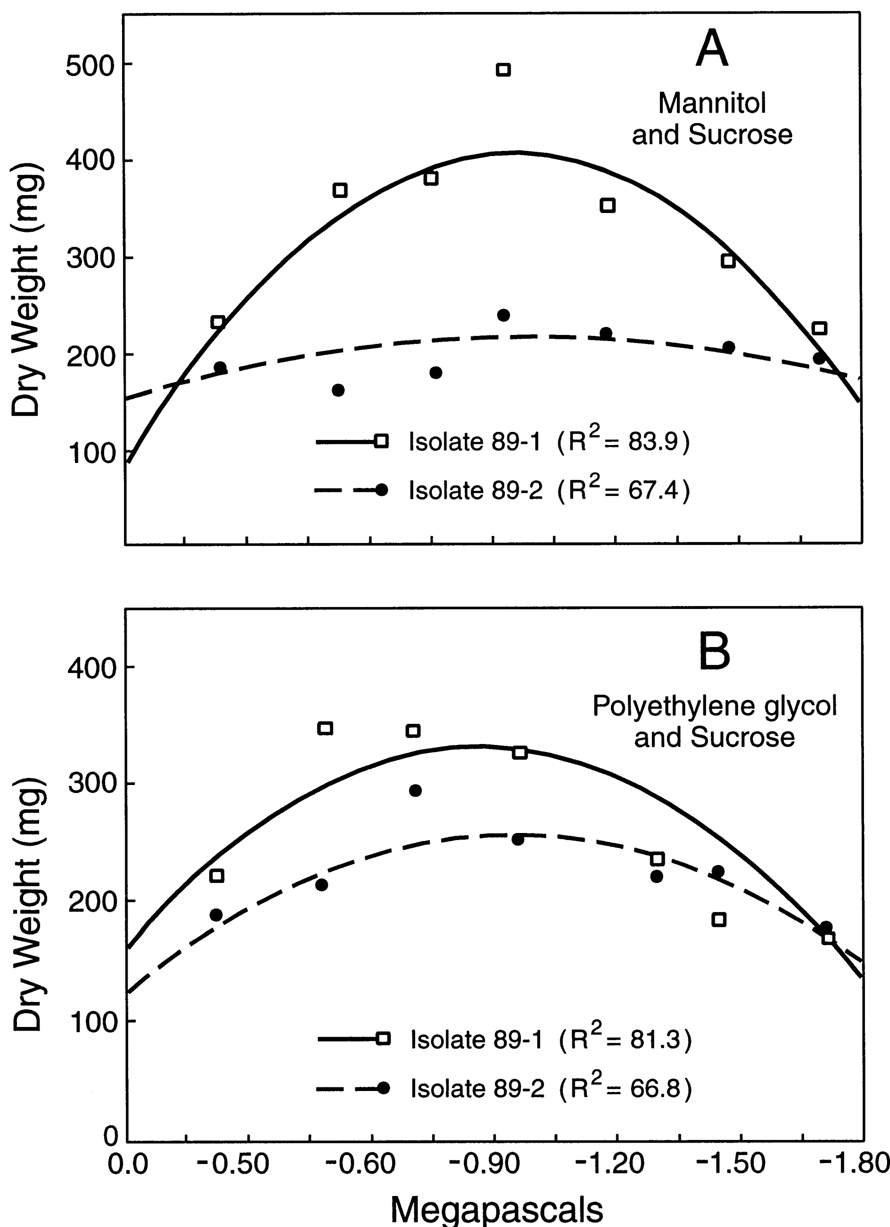

Fig. 5. Regression of mycelial dry weight on water potential (0 to 1.8 MPa) for two isolates of *Cytospora chrysosperma* grown in liquid culture. **(A)** Water potential was lowered using mannitol, with sucrose as the carbon source. **(B)** Water potential was lowered using polyethylene glycol, with sucrose as the carbon source. Each mean represents nine dry weights: three flasks per treatment and three repetitions of the experiment.

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