Drought Predisposition to Cytospora Canker in Blue Spruce

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ABSTRACT

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Wound-inoculated stems of potted 5-yr-old Colorado blue spruce transplants subjected to drought stress became predisposed to attack by a conidial isolate of *Cytospora kunzei*. Bark cankers appeared on stems with a plant water potential of −20 and −30 bars. No cankers formed on unstressed stems or on stems of plants subjected to freezing stresses of −20 and −30 C. The pathogen colonized wood tissues of stressed and unstressed stems to the same extent but formed necrotic cankers only on the bark of drought-stressed plants. These results support the hypothesis that drought stress is the main factor in the predisposition of spruce to Cytospora canker. A selective medium was developed for isolation of *C. kunzei*.

Colorado blue spruce, *Picea pungens* Engelm., particularly selections with long silver-blue needles that are often grafted onto rootstock of Norway spruce, *P. abies* (L.) Karst, is one of the most highly valued conifers for landscape plantings in the north temperate zone of the United States (5). Although *P. pungens* is a hardy, relatively stress-tolerant species, the stem-canker fungus *Cytospora kunzei* Sacc. var. *picea* Waterman (perfect stage *Valsa kunzei* Fr. var. *kunzei* Waterman) causes extensive girdling and premature death of the lower branches. Trees are rarely killed by the disease (10), but the removal of cankered branches destroys the shape and beauty of landscape specimens. *Cytospora kunzei* also attacks Norway and white spruce, *P. glauca* Moench, but is most destructive on Colorado blue spruce.

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Although this disease has been recognized for many years, some confusion remains as to the source of inoculum and the environmental conditions affecting disease development (5). Jorgensen and Cafley (4) and others (10) have reported successful inoculations with mycelia, ascospores, and conidia. Kamiri and Laemmlen (5), however, were able to obtain infection with cultures derived from ascospores but not from conidia. They hypothesized that ascospores are the infective inoculum and suggested that conidia may serve as gametes in the hybridization of perithecial initials. They also reported that stem wounds were a prerequisite for infection because inoculations of needles and unwounded stems were unsuccessful.

Many species of *Cytospora*, including *C. kunzei*, are believed to be weak or nonaggressive parasites that attack plants predisposed by environmental stresses (2,4,5). The most common stresses associated with predisposition in woody plants are drought and freezing (8). No reports have appeared in the literature demonstrating a relationship between freezing stress and Cytospora canker. Several workers have suggested drought as a predisposing factor based on field observations. Jorgensen and Cafley (4) measured growth rings in diseased and healthy white and Norway spruce from shelterbelts in Ontario. They reported a correlation between growth reduction and canker initiation and hypothesized that trees with poorly developed root systems were predisposed by drought stress to Cytospora canker. Kamiri and Laemmlen (5) subjected potted blue spruce seedlings inoculated with monoascospore and monoconidium cultures to drought stress by reduced watering. Cankers appeared sooner and canker incidence was higher in drought-stressed plants than in well-watered controls. They concluded that for disease development, trees must be weakened by stress such as drought. Their studies did not include measurements of plant water status; therefore, the level of drought stress required for predisposition was not determined.

This study was undertaken to assess the role of water and freezing stresses as predisposing factors in Cytospora canker of spruce under conditions of controlled and measured stresses. Because the *Valsa*, or ascospore, stage appears to be rare on spruce in Illinois, inoculations were made with cultures derived from conidia to test the hypothesis of Kamiri and Laemmlen (5) that the *Cytospora*, or conidial, stage does not produce disease, even on plants under predisposing stress.

MATERIALS AND METHODS

Host plants. Bare-rooted 5-yr-old transplants of *P. pungens* were obtained from a commercial nursery and potted in 5.75-L containers in a 1:2:2 soil-sand-peat mix. Plants were watered daily and

fertilized every 2 wk by injecting soluble fertilizer in irrigation water at the rate of 2.5 ml of a solution containing 25% N as NO_3 + 25% K as K_2O/L of irrigation water applied to soil saturation. Experimental plants were grown for 2 mo, at which time the root systems were well established.

Inoculum. A conidial isolate of *C. kunzei* was obtained by removing with a sterile needle a small mass of conidia from a dissected pycnidium from a naturally occurring canker on a Colorado blue spruce in Urbana, IL. Cultures were grown on potato-dextrose agar (PDA) at 25 C under continuous fluorescent light to promote sporulation. The fungus was identified as *C. kunzei* on the basis of spore size and sporophore characters (10). The inoculum was prepared by mixing a 21-day-old culture of the fungus with sterile water in a blender. Inoculation wounds were made 8, 16, and 24 cm above the soil line (plant height about 40 cm) by boring horizontal holes through stems with a sterile 1-mm drill bit. Inoculum was injected into the holes with a syringe and wounds were wrapped with Teflon tape to prevent drying and contamination.

Drought stress. Ten plants were placed in a growth chamber maintained at 24 C and 30% RH, with a 16-hr photoperiod at 120W/m^2 of combined incandescent and cool-white fluorescent light. Water was withheld from eight plants, whereas two controls were watered daily for 10 days. Plant water potentials (Ψ_w) were monitored with a pressure chamber. After 10 days, Ψ_w of stressed plants were about −20 + 3.0 bars, and after 14 days they were −30 + 0.7 bars. Control plants had Ψ_w of −2.5 + 0.2 bars. Stressed and control plants were inoculated and placed under equilibrium conditions in a humidity cabinet as described by Schoeneweiss (6). After a 48-hr stabilization period, plant Ψ_w were remeasured and the plants were incubated for 7 days. The Ψ_w were again measured and the plants were transferred to the greenhouse and watered. Changes in plant Ψ_w during incubation were less than 5%.

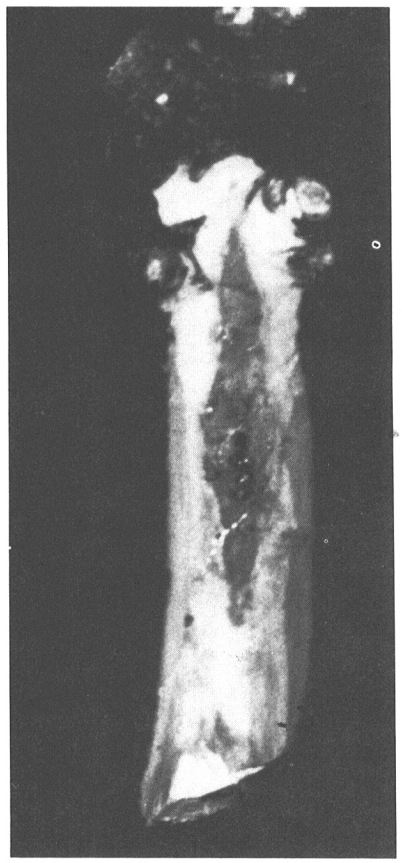

Fig. 1. The stem of a Colorado blue spruce drought stressed to −30 bars plant water potential and inoculated with *C. kunzei*. The bark cortex has been removed to show the typical bark canker surrounding the inoculation point 30 days after inoculation.

Freezing stress. Plants were placed outdoors in a screenhouse in early September to induce dormancy. After the spruce had been exposed to several light frosts but were not fully acclimated by subfreezing temperatures, the plants were inoculated and frozen in a programmed freezer, as described by Schoeneweiss and Wene (9). When stem temperatures monitored with thermocouples reached −20 or −30 C and had remained at these temperatures for 30 min, the plants were removed and thawed overnight at 0 C. The control plants were held at 5 C. All plants were then transferred to the greenhouse.

Assessment of stress predisposition. Stems were examined for canker formation 30 days after inoculation. Because Cytospora cankers on spruce are inconspicuous except for profuse resin exudation (4), the outer bark cortex was removed with a razor blade, as described by Waterman (10), so that necrotic areas could be measured (Fig. 1). Maximum canker diameters were recorded.

According to Schoeneweiss (7), criteria used to evaluate the effects of stress on disease susceptibility should include the relative growth rate and/or distribution of the pathogen in the host. This evaluation is usually accomplished by culturing serial sections of host tissue on nutrient media to determine the extent of colonization from inoculation points. The isolation of *C. kunzei* in culture from spruce stems was hindered by the presence of extensive microflora in the bark and wood tissues; *C. kunzei* grows slowly in culture and is quickly overgrown by more vigorous contaminants. To overcome this problem, several antibiotics and fungicides were screened for selective activity against contaminants by incorporating the materials in nutrient agar. A medium containing 39 g of Difco PDA, 0.1 g of chloroamphenicol, 0.1 g of streptomycin sulfate, and 0.3 g of ethazol (Truban) in 1 L of water acidified to a pH of 5.5 with dilute HCl gave the best results without affecting growth of *C. kunzei*. The bark was removed aseptically at the cambium and serial sections of wood, starting at inoculation points, were plated on the selective medium to determine the extent of wood colonization.

RESULTS

Drought stress. Bark cankers formed on all stems of plants subjected to drought stress, whereas no cankers were observed on controls (Table 1). No evidence of streaking or necrosis of wood tissues was observed.

When data on canker diameters and the extent of wood colonization were tested by analysis of variance, there were no significant differences between heights of inoculations on stems. Therefore, data from inoculation points within treatments was pooled and analyzed by Duncan's new multiple range test for treatment means. In drought-stressed plants, canker diameters were significantly greater ($P = 0.05$) at −30 bars Ψ_w than at −20 bars (Table 1). The extent of wood colonization at −30 bars was also significantly greater than at −20 bars. Although there was a considerable increase in wood colonization at −20 bars

Table 1. Bark canker size and extent of wood colonization by *Cytospora kunzei* in stems of Colorado blue spruce subjected to predisposing drought or freezing stress

Treatment	Replicates (no.)	Stress level	Bark canker diam. (mm)	Colonization of wood (mm)
Water stress				
Check	8	−2.5[x]	0.0 a[y]	45.0 a
Wilted	12	−20.0[x]	25.3 b	61.6 ab
Wilted	12	−30.0[x]	66.7 c	83.3 c
Freezing stress				
Check	8	5.0[z]	0.0	45.0 a
Frozen	12	−20.0[z]	0.0	46.7 a
Frozen	12	−30.0[z]	0.0	57.5 a

[x] Plant water potentials in bars.
[y] Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using Duncan's multiple range test.
[z] Temperatures (C).

over that in controls, the difference was not significant because of high plant to plant variation.

Freezing stress. No bark cankers appeared on plants frozen to −20 or −30 C (Table 1). *C. kunzei* colonized wood tissues to some extent in all treatments, but differences between treatments were not significant.

DISCUSSION

In this study, cankers formed on Colorado spruce transplants subjected to drought stress but not on those subjected to freezing stress, the two most common environmental stresses predisposing woody plants to attack by canker fungi (8). Schoeneweiss (7–9) presented data from many investigations with controlled drought and freezing stresses and hypothesized that the predisposition of woody stems to weak canker fungi involves threshold levels that must be exceeded. The levels of stress imposed in this study were beyond threshold levels for predisposition in most woody species (8). Because no cankers formed on unstressed control plants, these results confirm the hypothesis (5) that drought stress is the main factor predisposing spruce to Cytospora canker.

The conidial isolate of *C. kunzei* used in this study formed cankers on drought-stressed stems; thus, conidia may serve as the infective inoculum in stem wounds of plants subjected to predisposing drought stress. Data from this study and that of Kamiri and Laemmlen (5) indicate that both conidia and ascospores may serve as primary inoculum. The relative importance of ascospores versus conidia in the epidemiology of spruce canker under field conditions awaits further investigation.

Cytospora canker of spruce appears to be a bark-canker disease, as opposed to many other stem-canker diseases of woody plants that cause discoloration and necrosis of xylem tissues. Although the fungus was recovered from wood tissues some distance from inoculation points, even in unstressed control plants, necrotic canker areas were restricted to bark tissues of drought-stressed plants. Bier (1) theorized that drought-stress predisposition to facultative parasites occurs when the bark moisture content falls below a critical level. Hodges and Lorio (3) reported an increase in sugars and a decrease in starch in the inner bark of loblolly pines under moisture stress, which might be involved in water-stress predisposition (7). The relation of the level and composition of carbohydrates in the inner bark of spruce to canker susceptibility seems worthy of investigation.

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