Dose-Response Relationships of Five Conifers to Infection by Conidia of *Gremmeniella abietina*

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**ABSTRACT**


Seedlings of five coniferous species were tested for their relative susceptibility to an isolate of the European strain of *Gremmeniella abietina*. In June 1979, seedlings of each species were sprayed to runoff with distilled water or with suspensions containing 10^2, 10^3, 5 \times 10^3, 10^4, or 10^5 conidia per milliliter. Percent infection was determined in 1980, and survivors plus some replacement seedlings were reinoculated in June of that year. Effects of the second inoculation were determined in 1981. In both years, the order of susceptibility was *Pinus resinosa* > *P. strobus* > *Picea glauca* and *P. abies*. Disease incidence in *Pinus sylvestris* was not significantly different from *P. strobus* in 1980 but was much higher in 1981. Low disease incidence in *P. sylvestris* in 1980 was attributed to reduced shoot growth (thus, fewer potential infection courts) after transplanting in 1979. For each *Pinus* species, increased inoculum resulted in increased disease. Disease incidence was so low in *Picea* spp. at all inoculum levels that no relationship of inoculum dose to disease incidence could be identified. Where *G. abietina* was found on *Picea* spp., it may have been a weak parasite or saprobe.

Scleroderris canker kills red (*Pinus resinosa* Ait.) and Scots (*P. sylvestris* L.) pines in New York (14,19). The European strain of the pathogen *Gremmeniella abietina* (Lager.) Morelet (anamorph *Brunchorstria pinea* (Karst.) Höhn) predominates in the state (8) and differs from other strains in North America in that it rarely produces ascospores, it kills large as well as small trees, and it has a host range that includes conifers in at least five genera (19). Conidia are produced whenever mature pycnidia are moistened, and they are liberated by splashing rain to be carried in raindrops or aerosols (17,18). Conidia germinate best in the presence of free water, so prolonged periods of high humidity and moisture on the plant surface are assumed to favor germination (4).

On *Pinus nigra* var. *austriaca* (Hoess.) Asch., and presumably other trees, the pathogen gains ingress by penetrating scales of long or short shoots or buds, breaching the periderm, and invading the cortex when the host is dormant (10,15). Infection is most likely to occur on the most recent year's growth, where the pathogen can survive for at least one growing season as an epiphyte or saprobe while awaiting conditions favorable for ingress (10). Trees inoculated in the spring may show symptoms that same year, but symptoms usually do not appear until early the following year. Infected seedlings may be dead by the end of the second growing season (9,18).

Some conifers proven susceptible to *G. abietina* in New York are important components of forests in the southern and western United States, and severe losses are feared if the pathogen is introduced into those areas (D. D. Skilling, unpublished). New York state law thus prohibits transport of any light. The medium was a modification of the European strain of the pathogen. One effect of the legislation is to regulate movement of cut Christmas trees because diseased trees may produce inoculum for at least 7 mo after they are cut (11).

The list of known hosts for *G. abietina* is long (4), but species and provenances vary in susceptibility (3,5,13,16,19). The principal species managed for Christmas trees in New York are Scots pine, eastern white pine (*Pinus strobus* L.), white spruce (*Picea glauca* (Moench) Voss), balsam fir (*Abies balsamea* (L.) Mill), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Of these, Scots pine is regarded as highly susceptible to Scleroderris canker, but the others seem to have resistance that deteriorates only when inoculum concentrations are exceptionally high (19). The objective of this study was to quantify relative susceptibility of conifers to *G. abietina* when exposed to various inoculum doses.

We anticipated that results of this effort could provide plant health regulatory officials with additional data on which to base future legislation.

**MATERIALS AND METHODS**

Seedlings of six coniferous species were planted in 30 plots (1.5 \times 2.5 m) in a field near Harrisville, NY, in May 1979. Each plot contained one row of 25 3-yr-old seedlings of each of the following species: red pine, Scots pine, eastern white pine, white spruce, Norway spruce (*Picea abies* (L.) Karst.), and Douglas-fir. All seedlings had been grown in the state nursery near Saratoga Springs, NY, from seeds collected in New York. The plots were arranged in two parallel rows of 15 and were oriented so that the center of each plot in the first row was 9 m from the edge of a stand of mature red pine. The center of each plot in the second row was 12 m from the stand. Some trees in the stand had dead lower branches that bore pycnidia of *G. abietina*, but disease was generally scattered and infrequent. On an evening in June 1979, 3 wk after planting, seedlings in each plot were sprayed to runoff with distilled water or an aqueous suspension containing 10^2, 10^3, 5 \times 10^3, 10^4, or 10^5 conidia per milliliter. Conidia were from a 3-wk-old culture of *G. abietina* grown at 18°C under continuous light. The medium was a modification of one originally developed by Bruck et al (1), but we substituted V-8 juice for lima bean extract. Our isolate of *G. abietina* was from a nearby infected red pine and was identified as the European strain by serological comparisons (7). Inoculum was applied with a Chapman No. 152 sprayer (R. E. Chapman Mfg. Works, Inc., Batavia, NY) pressurized to 1.4 atm, and the volume (about 2 L/plot) of suspension applied was ample to wet each seedling to runoff. Each treatment was replicated five times in a complete block design. Temperature and relative humidity during and after inoculation were recorded on a hygrothermograph. Airborne inoculum from nearby infected trees that could supplement our applications was monitored with Vaseline-coated microscope slides held horizontally on wire supports. One slide was placed in the center of each plot, and nine slides were placed in files of three, 0, 3, and 6 m from the edge of the red pine stand. Slides were changed and conidia were counted weekly from May through September. When symptomatic seedlings (those with...
needs discolored from short shoots outward, premature abscission, and discolored cambium) were found in the plots in 1980, they were counted, rogued, and buried. This was done to prevent production of conidia within the plots. Occasionally, symptomatic shoots were collected, moistened, and incubated in polyethylene bags at 10 C for 48-96 hr. Emergence of G. abietina pycnidia during that treatment confirmed presence of the pathogen and ensured correct interpretation of symptoms.

On an evening in June 1980, survivors of 1979 inoculations were sprayed with water or conidial suspensions as before. Each plot was treated with the same inoculum dose applied in 1979, and deposition of conidia from nearby infected trees was monitored as before with Vaseline-coated slides throughout the 1980 growing season. In addition, a separate but nearby planting of six plots of 5-yr-old red pines, each plot with 50 trees planted 6 wk earlier, was inoculated. This was necessary because many red pines inoculated with high inoculum doses in 1979 became diseased and were rogued. Each new plot received one of five inoculum doses or water. Symptoms on red and Scots pines from 1980 inoculations were recorded as they appeared in 1981. However, all white and Norway spruce from three replicates of plots that received 0, 5 X 10^2, and 10^3 conidia per milliliter were harvested, moistened, and incubated at 10 C in polyethylene bags for 48-96 hr. While still moist, they were examined microscopically for pycnidia of G. abietina. If one pycnidium was found on a tree, the tree was recorded as diseased.

In both years, weather conditions during and for 24 hr after inoculation were favorable for conidial germination. Deposition of dew was high, cloud cover was thick, temperatures ranged from 12 to 22 C, and the seedlings remained moist for at least 24 hr.

RESULTS

Percent infection reported for 1980 was based on populations of 20-25 individuals per species per replicate. However, because diseased seedlings were rogued and destroyed in 1980, correspondingly fewer trees were examined in 1981. Percent infection for red pine in 1981 at inoculum dosages of 5 X 10^1, 10^2, and 10^3 was based on a single plot of 50 trees for each dosage.

The seedlings were carefully examined on several occasions in 1979, but no symptoms were found. Premature abscission of 1979 needles was first observed on red and Scots pines in February 1980. At that time, cambial tissue on portions of shoots where abscission occurred was necrotic, and G. abietina was readily isolated from such tissue. On the other hand, discoloration of basal ends of needles, a more easily observed and equally reliable symptom of disease on pines, was not evident until early May, and disease assessment was deferred until then. Symptoms developed similarly in 1981. Disease incidence in Scots pine inoculated in 1980 was related to log (conidial concentration) by linear regression, and all other treatments were best related by second-order equations as determined by the method of least squares.

Red pine was clearly the most susceptible species to G. abietina (Fig. 1). Disease incidence in plots with the lowest inoculum dose, 100 conidia per milliliter, averaged 61 and 97% of the seedlings in 1980 and 1981, respectively. Other pines tolerated exposure to higher inoculum doses in both years, and white pine consistently had an apparent minimum dosage threshold between 10^2 and 10^3 conidia per milliliter. When bark was removed from symptomatic red and Scots pines, most had extensive necrosis of vascular cambium and the disease was obviously lethal. Cambial necrosis and other symptoms on white pines, however, were confined to shoot tips and never progressed to the stem. Diagnostic symptoms or signs were not observed on white or Norway spruce in 1980, but a few pycnidia were found on small, lower, dead twigs of both species in 1981. No more than three pycnidia were found on any spruce, and most bore only one. Of white spruce receiving 0, 5 X 10^2, or 10^3 conidia per milliliter, the average percentage of trees with pycnidia was 0, 5, and 0, respectively. Percent infection of Norway spruce at the same inoculum doses averaged 2, 2, and 12, respectively. Because so many Douglas-fir seedlings died after transplant in both years, this species was not considered further.

Conidia were found on some Vaseline-coated microscope slides in the plots each week of sampling in both years (Fig. 2). In most cases, ambient inoculum was of little consequence, except when it was sufficient to cause disease in red pine, which averaged 20% in 1981.

DISCUSSION

The incidence of Scleroderris canker in populations of red, Scots, and white pine seedlings increased as inoculum dosage increased. Dorworth observed the same trend when he varied inoculum dosage of the North American strain of G. abietina applied to red pine (6). Differences in measurement of inoculum deposition and retention unfortunately precluded direct comparison between his experiments and ours.

Different species of pines also varied in their susceptibility to the disease. Red pine was most susceptible, followed by Scots and white pine (in that order). Disease incidence in red and Scots pine was higher in 1981 than in 1980, but it was similar in white pine for both years. That may have been due in part to differences in recovery of the trees after planting. At the time of inoculation in 1979, 3 wk after planting, white pine shoots were about one-third to one-half elongated, whereas most Scots and red pine shoots were less than one-fourth elongated. In 1980, the trees had been in place for over 1 yr, except for replacement red pines planted 6 wk earlier. Shoots of all trees were two-thirds to three-fourths elongated when inoculum was applied. Current year's shoots are assumed to be sites for most G. abietina infection (9), and longer shoots on red and Scots pines in 1980 may account for increased disease the following year.

![Fig. 1. Effect of inoculum concentration of Gremmeniella abietina on incidence of Scleroderris canker in 1980 (A) and 1981 (B). Regression lines for all but red pine in 1981 are based on means of six inoculum doses ranging from 0 to 10^2 conidia per milliliter and five replicates per dose. Correlation coefficients (r) range from 0.92 to 0.98 at P = 0.01.](image)

![Fig. 2. Mean number of Gremmeniella abietina conidia per square millimeter per week trapped on Vaseline-coated slides in the centers of the plots (9 and 12 m from a stand of mature red pines). Means are calculated over a 13-wk period from May through September. Solid bars = 1979; open bars = 1980.](image)
In 1981, our decision to record every spruce seedling at least one pycnidium as diseased may have resulted in erroneously high values for disease incidence. Pycnidia were abundant on pines, but extensive examination was required to locate them on spruce. Those on spruce were always found on small, dead twigs near bases of crows, but there were many more similar twigs bearing no signs of the pathogen. We saw no evidence that the pathogen would proceed into the main stem. Pomerleau (12) postulated that midsummer frost killed many pine shoots and that the saprophytic activity of _G. abietina_ thereafter was misinterpreted as pathogenicity. This may be the case for our spruce; shading and crowding from more rapidly growing pines could have been the primary cause of twig death.

Drift of inoculum into our plots from adjacent, mature pines contributed little to the overall incidence of disease except in red pine, where it caused some disease in two replicates in 1980 and all five replicates in 1981. Mean values above 0 for disease incidence in water-sprayed Scots, white pines, or spruce resulted from disease occurrence in only one of five replicates. Because plots that received the highest inoculum dose were adjacent to water-only plots in some blocks, accidental drift during inoculation may have contributed to disease in these plots. We saw no reproduction of the pathogen, and we were many more similar twigs bearing no signs of the pathogen. We suspected that they pose little threat as vehicles for spread of the pathogen. Eastern white pine also appears to have considerable resistance to _G. abietina_ and only becomes infected when exposed to inoculum doses comparable to those found immediately adjacent to diseased red or Scots pine.

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**LITERATURE CITED**