A *Phomopsis* Species Associated with Nonlethal Adelgid Galls on Upper Crown Branchlets of Red Spruce in West Virginia

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ABSTRACT

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A twig necrosis of dominant and codominant red spruce was associated with previously formed nonlethal galls of the eastern spruce gall adelgid in high-elevation stands of West Virginia. A *Phomopsis* sp. was isolated from 14 of 43 (33%) dying adelgid galled twigs. Subsequent seedling inoculations produced cankers in 14 of 48 (29%) attempts; the fungus was consistently reisolated from symptomatic tissues. These studies add to the interpretations of continuing health appraisals of red spruce in the eastern United States.

The health status of red spruce (*Picea rubens* Sarg.) has been intensively studied within the high-elevation forests of the Appalachian Mountains (7,11-15). These studies investigated numerous abiotic and biotic stressors in attempts to explain perceived changes in red spruce health and productivity. Surveys of high-elevation red spruce in the early 1980s used visual rating systems to evaluate foliar discoloration and needle loss, but little effort was given to carefully diagnose the causes of the observed symptoms (22). Numerous subsequent investigations have focused on the role of air pollutants as potential incitants of the generalized symptoms of discoloration and needle loss (1,10,11,16,20).

Other etiological agents of red spruce crown symptoms have also been investigated (15). Mielke et al (13) reported that Cytospora canker, caused by *Leucocytospora kunzei* (Sacc.) Z. Urban (syn. *Cytospora kunzei* Sacc), was present in 78% of declining red spruce trees in high-elevation stands of West Virginia. More recent reports include the role of the conifer swift moth (*Hepialus gracilis* Crote) as a root feeding and boring insect leading to injury of root systems of high-elevation red spruce in the Northeast (8). Bergdahl et al (5) has subsequently reported colonization of the *H. gracilis*-incited root wounds by pathogenic fungi. In addition, extremes in winter temperatures and associated freeze injuries have also been implicated in causing symptoms in red spruce (6). Thus, multiple agents may be acting alone or in concert in causing the observed deterioration of red spruce on a site-specific basis.

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In our investigations of natural red spruce stands in West Virginia for crown health and soil and foliar nutrient status (2,4), we used visual crown ratings for discoloration and needle loss as previously described (14). Crown symptoms and foliar nutrient status were evaluated in 39 natural stands in West Virginia between 2 July and 14 November 1990. The crown symptoms most frequently observed in nine dominant or codominant trees per stand were foliar yellowing and loss of older needles. Few symptoms were observed on most trees with mean class values of 5% discoloration and 10% defoliation. However, red spruce with considerable discoloration (30%) and defoliation (60%) were occasionally observed (4).

While climbing trees for collection of foliage from the topmost portions of the crown for foliar nutrient analysis, our climber noted branchlets with symptoms of needle discoloration and dying associated with previously formed but nonlethal galls of the eastern spruce gall adelgid (*Adelges abietis* (Linnaeus)) (Fig. 1). The purpose of this investigation was to examine the dying galls for possible pathogenic fungi leading to twig canker formation in the uppermost branches of red spruce. These studies may explain a portion of observed symptoms of crown browning and thinning commonly reported on red spruce growing at high elevations.

MATERIALS AND METHODS

Sample collection. Three plots of red spruce from within those previously surveyed (2) within the Monongahela National Forest were selected for study in the fall of 1991. These plots were located within stands 0251, 0342, and 0356, respectively, as designated on maps of red spruce stands provided by the USDA Forest Service Forest Health Monitoring Program, Morgantown, West Virginia. Binoculars were used to determine the presence of twig injury caused by the eastern spruce gall adelgid (3). Trees with symptoms of gall injury were climbed, and at least three branches were sampled to obtain a minimum of five previously formed adelgid galls on which needles had only recently begun to die in 1991. Field sampling was conducted on 24 October. Branch samples were placed in an ice chest for transportation to the laboratory, where they were maintained at 2 C prior to further observations and fungal isolations.

Samples were observed in the laboratory between 2 and 5 November. Length of galls, condition of needles, number of galls per branch, and age of gall by twig whorl were recorded.

Isolations. Upon completion of observations, all needles were mechanically removed from the galled internode. Bark was cleaned by gentle scrubbing with a stiff nylon brush under running tap water to remove surface debris and lichens. Twigs were rolled across paper towels soaked with 95% ethanol and flame-sterilized. The outer bark was removed with a sterile scalpel to expose the underlying inner bark and cambial layer, and the length and shape of necrotic tissues were recorded. Wood chips were removed with a sterile scalpel from the margin between necrotic and healthy tissue and placed in the center of a petri dish containing 2% malt extract agar (MEA). Culture dishes were coded to twig, branch, tree, and plot. Cultures were incubated at room temperature (approximately 21-23 C) under 24-hr fluorescent lighting for 21 days.

Inoculum preparation and inoculations. A *Phomopsis* sp. was isolated and identified with the assistance of N. G. Wenner, senior research assistant in the Department of Plant Pathology at the Pennsylvania State University. Inoculum was prepared by growing the *Phomopsis* sp. in pure culture on MEA. The cultures were washed with sterile distilled water, and conidia and mycelial fragments were dislodged with a sterile brush. A hemacytometer was used to adjust spore concentrations to approximately 6,000/ml. On 4 June 1992, 40 red spruce seedlings were removed from the greenhouse where they had been held to break dormancy; the new growth was approximately 3-4 cm long. Five droplets of spore suspension inoculum were placed on one emerging shoot of each of 20 seedlings, and a sterile pin was used to puncture the twig surface through each

inoculum droplet. Identical procedures with sterile water droplets provided wounds used as controls on a separate emerging shoot of each seedling. A second wounding and inoculation method involved making a 0.5-cm slit through the bark of new shoots with a sterile scapel parallel to the twig axis, then placing a 1-mm cube of media containing *Phomopsis* sp. mycelia, spores, and pycnidia directly into the wound on each of 20 trees; control wounds were treated with sterile agar on a separate emerging shoot.

Four additional trees were used for inoculations of the 1991 stem tissues by means of similar slit wounds and cubes of inoculum; control wounds were also made on each seedling. In this instance, moistened sterile cotton was positioned over each of the four wounds per seedling to hold the inoculum in place, and a strip of Parafilm was used to secure the cotton.

All seedlings were placed into a growth chamber held at 15–17 C and 85–90% RH with a 9-hr daily photoperiod for 5 wk. Seedlings were watered weekly. Observations for symptoms were made weekly, with reisolations made after 3 wk from a seedling showing symptoms associated with a pinpricked inoculated wound. All other reisolations to confirm infections took place after 5 wk on 10 July, using 2% MEA.

Fig. 1. Swollen twigs caused by nonlethal galls of the eastern spruce gall adelgid on branchlet of red spruce.

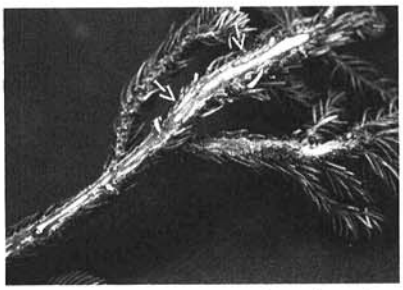

Fig. 2. Outer bark removed to show inner bark necrosis extending beyond the original eastern spruce gall adelgid attack loci in a branchlet of red spruce. Arrows indicate extent of original attack.

RESULTS

No difficulty was encountered in locating older adelgid galls with browning needles during the resurvey of the red spruce stands; three symptomatic trees were easily found in each of the three stands. A total of 48 adelgid galls were examined across the nine trees, with gall ages ranging from 7 (oldest) to 1 yr prior to the 1991 sampling (Table 1). Average length of twig necrosis associated with galled areas was 6.8 cm, with 90% of all galls exhibiting inner bark necrosis (Table 1). Symptoms included small (<1 mm in diameter) necrotic lesions, elongated streaks of necrosis running beyond the gall, and a more extensive and generalized necrosis of the inner bark extending beyond the actual gall dimensions (Fig. 2). Often, necrosis first appeared on the lower surface (underside) of the galled area. Both acropetal and basipetal needle necrosis was found but was always associated with the area of gall formation. The same *Phomopsis* sp. was isolated from 14 of 43 (33%) of the sampled twigs (Table 1).

Both inoculation methods resulted in 25% of the seedlings becoming infected. Symptoms of twig droop and lesion development were evident 3 wk after inoculation; no additional infections were evident at the time (5 wk) of attempted reisolations for *Phomopsis* sp. from symptomatic tissues. One control wound (scalpel slit) also resulted in a symptomatic twig. *Phomopsis* sp. was isolated from lesions on nine of 10 symptomatic shoots; the fungus was not isolated from the symptomatic wound on the control shoot.

Inoculations made on the 1-yr-old shoots resulted in four of eight inoculated wounds becoming infected. *Phomopsis* sp. was reisolated from all inoculated wound sites 5 wk after inoculation. Symptoms included needle and twig droop, followed by necrosis.

DISCUSSION

Although we did not attempt to conduct a disease incidence survey for the latent necrosis of previously formed adelgid galls, we had no difficulty in finding the symptoms when we revisited several red spruce stands (2). In addition to those selected for climbing and sampling, numerous trees appeared symptomatic by binocular observation. We initially expected to isolate *L. kunzei* from the symptomatic tissues (12), but after *Phomopsis* sp. was identified, we turned our interest toward determining its potential pathogenicity.

Inoculations were successful 25% of the time on newly emerging shoots and 50% of the time on 1-yr-old stem tissues. Thus, it appears as if *Phomopsis* sp. is capable of entering and colonizing previously formed adelgid galls in the higher crowns of red spruce. These results are consistent with pathogenicity reports for other *Phomopsis* spp. on various species of spruce (9,17), but we found no reports of association of this fungus with adelgid galls. Although we are certain of the *Phomopsis* identification because of the presence of alpha and beta spores in culture, identification of the species will be difficult because the current taxonomy of the genus is confused (21).

The mechanisms of latent infection were not investigated, and neither were the possibilities of inoculum development at the topmost crown cankers with subsequent spore dispersal and potential infections taking place within the lower crown branchlets. However, our findings are important in the attempts to understand the current and ever-changing health status of red spruce in the eastern United States (1,10,16,18,19). Our finding of a *Phomopsis* sp. asso-

Table 1. Number of adelgid galls, year of adelgid attack, length of galls, presence of necrotic inner bark, and frequency of *Phomopsis* isolations from galled tissues in red spruce within high-elevation stands in West Virginia

Plot	Tree	Adelgid galls	Year(s) of formation[a]	No. with inner bark necrosis	Average length of necrosis (cm)	Frequency of *Phomopsis* isolation[b]
1	1	5	1986–90	4	7.8	1/4
	2	6	1988	5	7.3	2/5
	3	5	1988–90	3	8.6	1/3
2	1	5	1986–88	5	8.7	2/5
	2	5	1986	4	9.7	2/5
	3	5	1984–88	5	7.0	1/5
3	1	7	1988–90	7	3.1	1/6[c]
	2	5	1990	5	4.4	2/5
	3	5	1988–90	5	4.2	2/5
Totals		48		43 (90%)	$\bar{x} = 6.8$	14/43 (33%)

[a] Inclusive of all gall formation; samples collected 24 October 1991.
[b] With one exception (tree 2, plot 2), fungal isolations were made only from galls with necrotic inner bark.
[c] One of the seven necrotic galls was not cultured because tissue integrity was lost during dissection and sterilization.

ciated with eastern spruce gall adelgid suggests that an important interaction is taking place that leads to delayed branchlet dieback in the upper crowns of high-elevation red spruce in West Virginia.

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