PINE WILT AND PITCH CANKER OF VIRGINIA PINE IN SEED ORCHARDS

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Abstract.--Decline and mortality of Virginia pines in seed orchards is emerging as a major problem. Pitch canker, pine wilt, and declining root systems appear to be responsible for the mortality. Factors that cause a stress in Virginia pines in seed orchards contribute to the increased susceptibility of trees to these diseases. Research is needed to determine the role each of the diseases plays in tree mortality, to ascertain the influence management practices have on disease incidence, and to develop control stratagies.

Additional keywords: Fusarium moniliforme var. subglutinans, Bursaphelenchus xylophilus, root rot, wounds, resistance, control

Virginia pine (Pinus virginiana Mill.) occurs naturally from central Pennsylvania and New Jersey southward to Alabama (Box and Foil 1968), and is grown for pulpwood on 25 to 35-year rotations throughout its natural range. However, this species is known for persistent limbs which makes it undesirable to pulpwood cutters (Williston and Balmer 1980). Conversely, these characteri tics plus short, dark green needles, make it suitable for Christmat trees (Box and Foil 1968). Plantings of Virginia pine Christmas trees in the Southeastern United States have increased dramatically since 1970, and the species is now a serious competitor in regional markets (Murray et al. 1981; Utz and Balmer 1980)

At present there are approximately 235 acres of Virginia pine seed orchards in the southern United States (Anonymous 1982), with some orchards supplying genetically improved seed to the Christmas-tree market (Chandler 1979). The seed orchard environment differs considerably from those under which the species has been grown in the past, and that environment is proving conducive to the emergence of new insects and disease problems and the resurgence of old ones (Gibson and Jones 1976; Shea 1971). Two previously endemic diseases of this species, pitch canker and pine wilt, currently threaten Virginia pines grown in seed orchards.

The pinewood nematode, Bursaphelenchus xylophilus (Steiner & Buhrer) Nickle, causes a wilt disease of pines (Nickle 1981). Although the disease is epidemic on pines in Japan, it is apparently endemic in the United States (Dropkin et al. 1981). The nematode is vectored primarily by longhorn beetles (Coleoptra: Cerambycidae) that emerge from dead trees carrying the dauer larvae. When the beetles feed on healthy trees, the resulting wounds provide points of entry for the nematode larvae. The nematodes mature and reproduce rapidly in the resin canals, reducing oleoresin flow, arresting transpiration, and causing

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chlorosis and death within 3 months after initial infection. The beetles oviposit in dying trees, and the life cycle of the nematode, closely coordinated with the beetle, is repeated (Dropkin et al. 1981; Wingfield et al. 1982). By December 1980, the pinewood nematode has been recovered from Virginia pines in Illinois, Indiana, Maryland, South Carolina and Virginia (Dropkin et al. 1981).

Pitch canker, caused by Fusarium moniliforme Sheld. var. subglutinans Wr. & Reink. (Kuhlman et al. 1978), was first reported on Virginia pine in 1946 (Hepting and Roth). Virginia pine is now ranked as one of the most susceptible species to this disease (Dwinell 1978; Barrows-Broaddus and Dwinell 1979). The cankers, which occur primarily on the bole and main branches, are sunken and characterized by heavy pitch flow and by resin soaking of the underlying wood (Hepting 1954). The causal fungus can invade any fresh wound, regardless of cause or location (Blakeslee et al. 1980; Dwinell and Barrows-Broaddus 1981).

PINE WILT

In the past 18 months, we have been asked to examine two Virginia pine seed orchards in which the trees showed a sudden loss of normal leaf coloration, turned a bright rust red, and rapidly died. Initially, the problem had been diagnosed as pitch canker. Although the symptoms of pitch canker were apparent, and the causal fungus was isolated, the incidence of pitch canker in the orchards could not alone explain the sudden and extensive mortality. Furthermore, the slight degree of resin-soaking of the sapwood beneath the cankers was atypical of pitch canker on Virginia pine (Hepting 1954). In the past, mortality of Virginia pines in seed orchards caused by pitch canker has averaged 4-5% per year (Dwinell and Barrows-Broaddus, unpublished). As of December 1982, mortality averaged 30% at the Champion International (Newberry, S. C.) and Hammermill (Selma, Al.) seed orchards.

Trees were exacavated at both locations because the sudden decline of Virginia pine suggested that the problem might be in the root systems. At the Hammermill orchard, where Virginia pine is used as the root stock, three of the four declining trees that were excavated had deteriorated root systems. The root systems exhibited nonspecific, general decay. Symptoms and signs of root rots caused by Heterobasidion annosum (Fr.) Bref. (white, stringy rot) or Verticicladiella spp. (resin-soaked, dark stain) were absent. Tissue samples from the roots were plated on 2% malt extract agar and media selective for Fusarium (Agrawal et al. 1973) and Heterobasidion annosum (Kuhlman and Hendrix 1962). An array of saprophytic fungi were isolated on all media. The root system of a fourth declining Virginia pine appeared to be normal and showed little evidence of decay.

The Virginia pines at the Champion International seed orchard are grafted on loblolly (P. taeda L.) root stock. Although the root decay observed at the Hammermill orchard was not observed at the Champion Orchard, resin-soaked lesions on the main roots were readily apparent. Root tissue isolation from these lesions yielded F. oxsporum Schlecht. (53%), F. moniliforme var. subglutinans (35%), and F. solani (Mort.) Sacc. (12%) on the Fusarium-selective media; but on Heterobasidion annosum - selective media and 2% malt extract agar, only saprophytes were recovered.

In both seed orchards, chainsaw sections through the bole at several heights revealed extensive blue stain. Since the sudden decline of these trees was similar

to symptoms of pine wilt (Dropkin et al. 1981; Wingfield et al. 1982), and t pinewood nematode was first found in association with blue-stain in longleaf pine (P. palustris Mill.) (Steiner and Buhrer 1934), bole samples were brought to the laboratory and assayed for the pinewood nematode. Large numbers of nematodes were extracted from these samples using a pie pan technique (Cairns 1960). The nematodes matched the description of Bursaphelenchus xylophilus (Steiner & Buhrer 1934; Nickle et al. 1981). Although B. xylophilus often colonize in all parts of infested pine trees (Dropkin et al. 1981), none were recovered from root systems of the grafted Virginia pines we excavated. We found only saprophytic nematodes in deteriorated root systems of the trees we excavated.

In January 1983, two rapidly declining Virginia pines were observed in progeny test at the Baldwin State Forest near Milledgeville, Ga. Tissue s from bole sections cut at 18 inch intervals with a chainsaw from these trees yielded as many as 852 pinewood nematodes/g sapwood fresh wt (x = 352). This was the first report of the pinewood nematode in Georgia (Dwinell and Barrow Broaddus 1983).

To determine the pathogenicity of the Alabama population of B. xylophilus, we inoculated 2-year-old seedlings of Virginia and loblolly pines grown in plastic flats (33 x 13 x 11 cm) which contained a mixture of soil, pine bark, and sand (2:1:1 v:v:v). Near the top of each stem, the bark was removed, and a moistened plug of cotton infested with 5,000 nematodes from a population increased on cultures of Botrytis cinerea (Dropkin et al. 1981) was attached with foil.

Each of four flats contained 12 seedlings. Four loblolly seedlings had been inadvertently planted among these four trays of Virginia pines and were not able to compete satisfactorily. Within 1 month these four seedlings began to decline. With the onset of mortality, serial sections cut from the seedling were placed in 10 ml of sterile water in test tubes for 48 hrs. The number of nematodes extracted averaged 16 per cm of stem tissue. The greatest concentration of nematodes was near the base of the seedlings (120 nematodes/cm). In contrast, only 2 of 20 Virginia pines died. The Virginia pines, however, were more vigorous than the loblolly pines at the time of inoculation. A better to of pathogenicity of pinewood nematode might have been accomplished on stressed Virginia pine seedlings.

Biotic and abiotic factors that stress conifers apparently predispose them to infection by the pinewood nematode (Dropkin et al. 1981; Adams Wingfield et al. 1982). The 1981 drought in South Carolina probably contributed to the increased susceptibility of Virginia pines at the Champion International seed orchard. In Alabama at the Hammermill orchard, root pruning produced by subsoiling in the summer of 1982 wounded root systems and increased moisture stress in these trees, probably contributing to the deterioration of the root systems and infestation of the trees by B. xylophilus. Cultural activities that wound roots increase the risk of root disease development (Horner and Alexander 1983). Pitch canker in seed orchards may also contribute both directly and indirectly to the pine wilt problem. On main stems, these cankers adversely affect translocation, which probably increases susceptibility to infection by B. xylophilus. Also since this nematode is mycophagous (Dropkin et al. 1981), it

it might feed on the pitch canker fungus, F. moniliforme var. subglutinans.

Whether or not the pinewood nematode is a primary pathogen in seed orchards is open to question. As Wingfield (1983) recently noted, the nematode may be transmitted to dead and dying trees without necessarily being the primary cause or their death. In a study on jack pine (Pinus banksiana Lamb.) infected with dwarf mistletoe, Burnes et al. (1983) found that cerambycid beetles and B. xylophilus were secondary colonizers of declining trees.

The decline of Virginia pines in seed orchards is a complex problem because of the number of biotic and abiotic factors involved. Research is needed to elucidate the role of the pinewood nematode in declining pines in seed orchards in the South.

PITCH CANKER

F. moniliforme var. subglutinans, like many canker-producing organisms, requires a wound as an infection court. Routine seed orchard management practices such as branch pruning, mowing, and cone removal create wounds for the entry of the pathogen. Weather-related injuries caused by wind, ice, and hail may also serve as entry points. Kelley and Williams (1982) suggested that wounds on loblolly pines caused by Hurricane Frederic in September 1979 provided infection courts for the pitch canker fungus, resulting in an epidemic in one loblolly pine seed orchard in Alabama. A tornado probably caused intensification of pitch canker in another Virginia pine seed orchard managed by Kimberly-Clark at Coosa Pines, Al. (Dwinell and Barrows-Broaddus 1981). The development of cankers in branch crotches of Virginia pines at the Hammermill seed orchard probably resulted from heavy ice accumulation on the trees during an ice storm in 1982.

In July 1981 we established a study in a Virginia pine progeny test at Coosa Pines, Al. to determine if isolates of the pitch canker pathogen varied in their ability to invade Virginia pines. Two isolates each of F. moniliforme var. subglutinans recovered from cankers on Virginia (D-25; D-26) and shortleaf (P. echinata Mill.) (D-29; D-30) pines were used to inoculate 20 11-year-old Virginia pines. The trees were wounded with a hammer and chisel by removing a 3 cm^2 area of bark at 1.3 m above ground to expose the cambium. Ten replicate wounds (2 per tree) were sprayed to run-off with 10^6 conidia/ml suspensions of each isolate. Five trees served as wounded, but uninoculated controls. After 14 months, treated sections of the boles from each of the 25 trees were brought to the laboratory for analysis. The outer bark was removed and the length, width, and area of each canker were measured. At the point of the original wound, each bole section was cut in cross-section with a bandsaw to permit the measurement of the extent of resin-soaking of the sapwood. Tissue samples from the edges of the cankers were plated on a Fusarium-selective medium (Agrawal et al. 1973).

All trees inoculated with the pitch canker fungus exhibited main stem cankers after 14 months. Although cankers caused by isolate D-26 from Virginia pine were generally smaller than those caused by other isolates, differences in canker size among isolates were not statistically significant (Table 1). Over all treatments, canker length averaged 32.2 cm and width averaged 7.8 cm. The

area of resin-soaked wood (11.4 cm^2) represents about 11% of the area of t bole. The pitch canker fungus was reisolated from 65% of the cankers. Isolations from control trees showed little evidence of natural infection by the pitch canker fungus. Wounds on these trees showed signs of healing after 14 months. The time of the year (July) that wounds were made on control tre may have accounted for this lack of natural infection. In a study on the epidemiology of pitch canker in a loblolly pine seed orchard, Kuhlman et al. (1982) found that fall and winter inoculations were more successful than tho in spring and summer. Inoculation of wounds on slash pine with the pitch c fungus in September was more favorable to the growth of the pathogen than we inoculations in June (Barrows-Broaddus 1983).

Table 1. Dimensions of pitch canker on Virginia pines in a progeny test are 14 months after inoculation with four isolates of F. moniliforme var. subglutinans

Isolates		Canker size[a/]			Area of resin-soaked wood[b/]
No.	Pine host	Length (cm)	Width (cm)	Area (cm^2)	(cm^2)
D-25	Virginia	37.2	8.9	296.9	13.4
D-26	Virginia	25.2	5.9	133.2	5.4
D-29	Shortleaf	30.7	8.1	222.2	14.4
D-30	Shortleaf	35.6	8.1	257.4	12.5
Mean[c/]		32.2*	7.8*	227.4*	11.4*
Control		4.3	1.8	7.3	0

[a/] Data based on five trees/isolate

[b/] Cross-sectional area through the point of wounding

[c/] * Denotes means for treatments are significantly different from control means ($P \geq 0.01$)

Since circumstantial evidence from a previous study (Dwinell and Barrows-Broaddus 1981) indicated that the pitch cankers on slash pine could be initiated by infection through branch stubs, fresh branch stubs were created with clippers and sprayed with a spore suspension of an isolate (D-25) of F. moniliforme var. subglutinans on five additional Virginia pine trees in the Coosa Pines area. Al 10 of the branch stubs (2 per tree) that had been inoculated became infected and were resin soaked. In 4 stubs, the pathogen grew into the bole from the stub, causing a resinous canker to develop on the main stem adjacent to the inoculated branch stub.

DISEASE CONTROL

The lack of information on various aspects of the disease cycle of both pitch canker and pine wilt has delayed the development of suitable control measures in Virginia pine seed orchards. Since wounds are a prerequisite for the initiation of pitch canker, we suggest that management practices be modified to reduce the wounding of trees. The practice of root pruning to increase seed production should be re-evaluated because the damage causes stress on trees and predisposes them to attack by longhorn beetles and subsequent infestation by the pinewood nematode. Root pruning also increases the risk of root disease caused by pathogenic fungi.

Disease control in seed orchards should also include: (1) establishment of orchards on suitable sites, (2) adjusting fertilizer schedules to avoid over-fertilization, and (3) controlling insect vectors (Blakeslee et al. 1980). For example, Fraedrich and Witcher (1982), stated that excess nitrogen increased pitch canker severity on loblolly, slash, and Virginia pines. Barnett and Thor (1978) found that Virginia pine trees growing on poorly drained sites were more susceptable to pitch canker than those on well drained sites.

Since individual clones within species vary greatly in their susceptibility to infection by F. moniliforme var. subglutinans (Dwinell et al. 1977; Dwinell and Barrows-Broaddus 1981; Kelley and Williams 1982; Kuhlman et al. 1982), it may be possible to select for resistance to pitch canker disease. Barnett and Thor (1978) suggested that resistance to pitch canker was controlled by either dominance or epistatic gene action. Barrows-Broaddus (1983) observed in a greenhouse study on Virginia pine that, when seedlings were inoculated with F. moniliforme var. subglutinans, certain pine progeny exhibited partial resistance to disease development.

Seed orchard establishment and maintenance are expensive. The threat of serious losses from pine wilt and pitch canker diseases requires that we develop means to successfully control these two diseases to avoid considerable economic losses in the future.

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