Variation in Susceptibility to the Pitch Canker Fungus Among Half-Sib and Full-Sib Families of Virginia Pine

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Portion of a Ph.D. thesis submitted by the senior author to North Carolina State University.

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We thank Sharon V. Lumpkin and Sheila G. Allgood for technical assistance; and Louis Olivier and Alan Brannen of the Kimberly-Clark Corporation for supplying the survey data and for technical assistance.

This research was partially supported by a grant from the Georgia Forestry Commission.

Accepted for publication 1 November 1983.

ABSTRACT

Barrows-Broaddus, J., and Dwinell, L. D. 1984. Variation in susceptibility to the pitch canker fungus among half-sib and full-sib families of Virginia pine. Phytopathology 74: 438-444.

A survey for pitch canker among ramets of eight clones in a seed orchard of Virginia pine (*Pinus virginiana*) in Alabama indicated that the incidence of disease (0–52% ramets with cankers) was related to specific clones. When seedlings of half-sib familes from these clones were inoculated with *Fusarium moniliforme* var. *subglutinans*, the causal agent of pine pitch canker, the ranking correlation of the families for susceptibility to the pathogen (based on 2 yr of trials) to the seed orchard survey averaged 0.77. In an 11-yr-old progeny test in Alabama, new shoots on full-sib families from four of the seed orchard clones were inoculated with *F. moniliforme* var. *subglutinans* for two consecutive years. The most susceptible family ($\bar{x}$ = 83%) was related genetically to one of the most susceptible clones from the survey (52%) and a moderately susceptible ($\bar{x}$ = 42%) family from the greenhouse trials. Ranking of susceptibility among the other families, however, was not consistent between years and did not match the survey or the greenhouse trials. Inoculated shoots in greenhouse and field trials initially developed resinous lesions and purple discoloration of the shoot. Sporodochia (1–3 mm in diameter) and microscopic sporodochia (0.06–0.2 mm in diameter) were observed on dead shoots. In the field, dead shoots became defoliated. Prior to needle drop, hyphae were observed on the surfaces of the needles, and microscopic sporodochia were observed emerging from the epidermis adjacent to the needle fascicles. Histological examination of surviving shoots from inoculated seedlings and branches indicated that the formation of a periderm in the cortex and reaction parenchyma in the xylem was a factor in delaying invasion by the pathogen.

Additional key words: colonization rate, resin ducts.

Since 1974, pitch canker has become severe on seed orchard trees of slash pine (*Pinus elliottii* Engelm. var. *elliottii*) plantings in Florida (12) and on slash, loblolly (*P. taeda* L.), longleaf (*P. palustris* Mill.), shortleaf (*P. echinata* Mill.), and Virginia (*P. virginiana* Mill.) pine seed orchard trees across the southeastern United States (10,13). The causal organism, *Fusarium moniliforme* Sheld. var. *subglutinans* Wr. & Reink. (24), can invade any fresh wound, regardless of cause or location (10).

Where relative levels of susceptibility among *Pinus* species have been quantified, chiefly by inoculation trials on 1-yr-old seedlings, Virginia pine ranks as the most susceptible species (4,8). In natural stands, disease progression on host trees is rapid on leaders, branches, and main stems where sunken cankers characterized by heavy pitch flow and by resin soaking of the underlying wood are

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observed (15).

Virginia pine is grown for pulpwood on 25- to 35-yr rotations, but the persistent branching pattern of the species restricts its usefulness for some forest products (6,32). From the standpoint of Christmas tree production, however, the numerous branches are considered an asset, and since the mid-1970s, plantings of Virginia pine for the Christmas tree market have dramatically increased in the southeastern United States (25,31).

Pitch canker poses a threat to the Virginia pine/Christmas tree industry not only because of the high susceptibility of the species to the disease, but also because of the way the trees are managed for market. Wounds on the root collar from mowing for weed control and on the stem from pruning branches to shape the tree could be potential infection courts for the pathogen. Fraedrich and Witcher (14) observed that cankers on Virginia pine seedlings fertilized with N in combination with P, K, or both are significantly longer than those on seedlings receiving no N. With expanded planting of Virginia pine across the South, many trees will be planted on marginal sites. Barnett and Thor (3) noted that site variation contributes to the incidence of pitch canker in a 6-yr-old progeny test of Virginia pines where disease is greater on poorly drained sites.

Control of the disease may depend in part upon selecting and breeding trees for resistance. Incidence of pitch canker in southern pine seed orchards varies with specific clones (13,21,23). When seedlings of half-sib families of slash and loblolly pines from seed orchard clones are inoculated in greenhouse trials (9), a range of susceptibility may be demonstrated.

A source of genetically improved seedlings of Virginia pine for the Christmas tree producer is a seed orchard owned by the Kimberly-Clark Corporation, Coosa Pines, AL. Approximately half of the 27 clones originally grafted have been designated as superior for Christmas trees (7,32).

We report here the incidence of pitch canker on eight clones of superior Virginia pines in the Kimberly-Clark seed orchard, the range of susceptibility of the half-sib families in greenhouse trials, and the susceptibility of full-sib families of trees in a progeny test. Shoots in inoculated and control treatments were examined histologically to observe disease progression in the tissues and to evaluate the part that anatomical responses to injury and infection among the families play in resisting invasion by the pathogen. Rates of fungal colonization along shoots of inoculated seedlings and trees were also measured.

MATERIALS AND METHODS

Greenhouse tests. Seeds from open-pollinated cones were collected from eight clones (12-32, 12-18, 12-33, 12-44, 12-20, 12-22, 12-27, and 12-14) of Virginia pine at the Kimberly-Clark seed orchard. The seeds were stratified for 30 days and then planted separately in flats containing wetted vermiculite. When the hypocotyls were ~2.5 cm long, the seedlings were transplanted into plastic flats (33 × 13 × 11 cm) containing a mixture of fumigated soil, pine bark, and sand (2:1:1, v/v). Fourteen seedlings were planted in each of 15 flats for each half-sib family. The flats remained in a greenhouse for 1 yr prior to the first treatment in May 1981. The experiment was repeated on surviving seedlings in May 1982.

Virulent isolates of *F. moniliforme* var. *subglutinans* were recovered from typical pitch cankers on loblolly pine in North Carolina and on Virginia pine in Alabama. The two isolates were grown on lima bean agar (Difco Laboratories, Detroit, MI 48232) for 7 days on a 12-hr photoperiod at 24 C. Conidia were washed from the culture plates with sterile deionized water. The number of conidia per milliliter was adjusted to 150,000 as measured with a Coulter electronic particle counter (model ZBI; Coulter Electronics, Inc., Hialeah, FL 33010). Two-month-old shoots on the 1-yr-old host plants were treated by placing a 1-μl droplet of inoculum on the stem and then puncturing the epidermis through the droplet with a sterile hypodermic needle. Seedlings in control treatments were wounded by needle puncture of the epidermis through a 1-μl droplet of sterile deionized water. All seedlings in each flat received a single treatment. The two isolates plus a control constituted the study treatments.

After inoculation, the seedlings were placed in a mist chamber at 20 C for 24 hr and then moved to a greenhouse bench where the flats were arranged in a randomized complete block design with five replications per treatment per half-sib family.

Field tests. Eleven-year-old trees in a full-sib progeny test at Coosa Pines, AL, were chosen for field trials in June 1981 and 1982. Progeny came from seed orchard clones 12-32, 12-27, 12-18, and 12-44 with 12-20 as the pollen source.

The two isolates of *F. moniliforme* var. *subglutinans* used in the greenhouse trials were grown on lima bean agar for 7 days with a 12-hr photoperiod. The epidermis on new shoot growth on branches 1.5–6.0 m above ground level was punctured ~1 mm deep with a sterile dissecting needle, and a small piece of aerial mycelium from the culture plate was placed in the puncture. Shoots in control treatments were wounded ~1 mm deep with a sterile dissecting needle. Each tree received all of the study treatments; 20 shoots, 10 per isolate were inoculated, and 10 shoots were wounded controls. In 1981, four trees of each full-sib family were treated. In 1982, six trees of each full-sib family were similarly treated.

Histological procedure. In 1981, after each of six intervals (1, 4, 12, 21, 35, and 56 days after inoculation) five seedlings per treatment were selected at random from each half-sib family in the greenhouse. After each of three intervals (12, 35, and 56 days) four shoots per treatment of each full-sib family in the progeny test were likewise collected. Shoots from seedlings and branches were sampled for histological examination and culturing according to the diagram in Fig. 1. The inoculation point "c" was located on new shoot growth 2.5 cm above the node. Five sections, each ~5 mm long, were taken above the inoculation point and five sections starting with the inoculation point were taken down to the node. Alternate sections were fixed in FPA (40% formaldehyde-propionic acid-ethanol [5:5:90, v/v]) (20) and cultured on a Fusarium-selective medium (1) to confirm colonization by the pathogen.

After 56 days, the remaining shoots from seedlings and branches were rated for shoot mortality and the development of active or inactive lesions. Lesions were rated as active or inactive on the basis of resin production. Those that continued to exude resin were rated as active. They usually girdled >50% of the stem, and adjacent

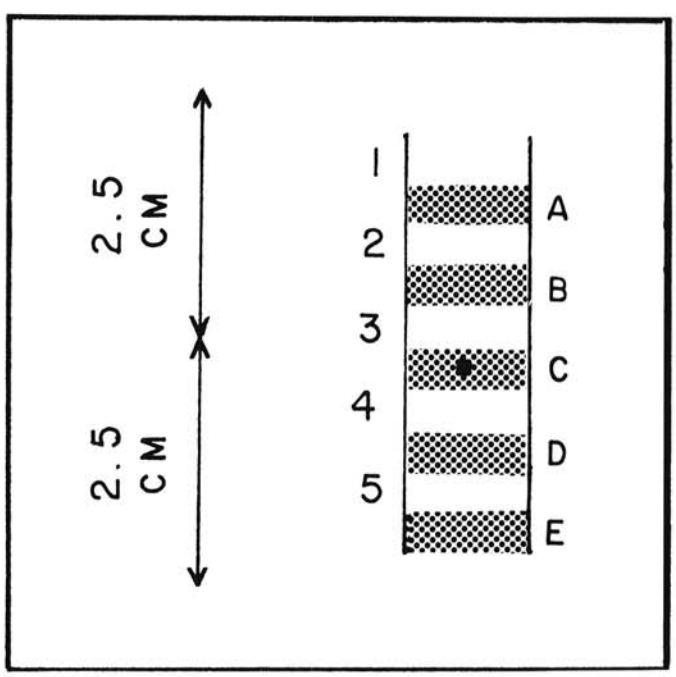

Fig. 1. Sampling design for Virginia pine shoots wounded in control and inoculated treatments. Shaded areas were fixed for histological examination. Clear areas were plated on a Fusarium-selective medium. The location of needle puncture is at C and the node is at E.

tissues had purple discoloration. Inactive lesions no longer exuded resin, tended to girdle <50% of the stem, and had no purple discoloration beyond the margins of the lesion. At the end of the experiment, inactive lesions were sampled for histological examination and tested for the presence of active pathogens as previously described.

In 1982, shoots from greenhouse and field trials were rated for mortality and lesion development 56 days after inoculation. Samples were then collected for histological examination and for colonization by the pathogen as previously described.

Fixed tissue sections were dehydrated in a graded series of *t*-butyl alcohol solutions and embedded in Paraplast + (Scientific Products Div., American Hospital Supply Corp., Evanston, IL 60201) by standard methods (20). The paraffin blocks were stored in softening solution (10% glycerin-1% sodium lauryl sulfate) (2) for 2 wk to 2 mo in a refrigerator. Transverse and tangential sections 8–12 μm thick were cut, mounted on glass slides with Haupt's adhesive (20), and stained with Triarch's quadruple stain (Triarch Inc., Ripon, WI 54971). Stained sections were examined with a Leitz microscope and photographed on Kodak technical pan film 2415 with an attached Wild MP5 515 camera.

Transverse sections from control treatments on stained slides were measured for resin duct size and number of resin ducts per square millimeter. There were five replications on each slide from the greenhouse studies, and four on each slide from the field tests.

Percent shoot mortality was subjected to analysis of variance, Duncan's multiple range test, and rank correlations (26,28), the data from the slides, to analysis of variance, and Duncan's multiple range test (16,26).

TABLE 1. Pitch canker survey on eight seed orchard clones of Virginia pine, Kimberly-Clark Corporation, Coosa Pines, AL[z]

Clone	Ramets surveyed (no.)	Ramets cankered (%)
12-32	23	52
12-33	53	52
12-18	13	46
12-20	38	36
12-22	36	33
12-44	25	28
12-27	30	16
12-14	5	0

[z]Survey conducted by company staff, September 1979, on trees 14–18 yr old.

RESULTS

In 1979, the staff of the Kimberly-Clark Corp. surveyed the Virginia pine seed orchard (trees 14 to 18 yr old) for incidence of pitch canker (bole cankers and crown dieback). Of the eight clones evaluated in this study, clones 12-32, 12-33, and 12-18 had the highest disease incidence, and clones 12-44, 12-27, and 12-14 had the lowest disease incidence (Table 1).

Greenhouse trials. There was a significant difference in percent dieback of inoculated shoots between years (Table 2). Overall shoot mortality was higher in 1982 (28–65%) than in 1981 (26–48%). Ranking correlation among the eight families for relative susceptibility, however, was high at 0.94, $P = 0.01$. Ranking correlations of the greenhouse trials to the seed orchard survey were 0.68 (1981) and 0.86 (1982), $P = 0.05$. There was no difference between the two isolates of *F. moniliforme* var. *subglutinans* that were used, and the above values represent pooled data. The three most susceptible families for both years, based on percent shoot dieback, were 12-18, 12-33, and 12-44. The three least susceptible families were 12-22, 12-27, and 12-14. Symptomatology of inoculated shoots was similar to that previously described for Virginia pine (3,8). No wounded controls developed shoot mortality.

Growth rate of *F. moniliforme* var. *subglutinans* along

TABLE 2. Percent shoot mortality and lesion development in eight half-sib families of Virginia pine inoculated with *Fusarium moniliforme* var. *subglutinans*[x]

Family no.	1981[y] Trees inoculated (no.)	1981[y] Lesions Inactive	1981[y] Lesions Active	1981[y] Shoot mortality[z] (%)	1982 Trees inoculated (no.)	1982 Lesions Inactive	1982 Lesions Active	1982 Shoot mortality[z] (%)
12-18	86	47	5	48 a	60	27	8	65 a
12-33	88	59	2	39 ab	57	23	21	52 ab
12-44	89	50	8	42 a	65	47	6	47 abc
12-32	84	56	7	37 ab	50	34	18	47 bc
12-20	89	56	7	37 ab	58	48	9	39 bc
12-22	89	62	3	35 ab	56	31	39	30 bc
12-27	86	65	12	23 b	50	62	8	30 bc
12-14	90	63	11	26 b	56	67	5	28 c

[x]Data were pooled for two fungus isolates.
[y]New shoot growth on the same group of seedlings was inoculated in May 1981 and May 1982. Fewer seedlings were available for inoculation in 1982 due to destructive sampling the previous year for colonization data and histological examination.
[z]Means within each year followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.01$. There is a significant difference between years (Student's *t*-test) in percent shoot mortality. Ranking correlation of relative susceptibility among families is 0.90.

TABLE 3. Percent shoot mortality and lesion development in four full-sib families of Virginia pine inoculated with *Fusarium moniliforme* var. *subglutinans*[x]

Family no.	1981 Laterals[y] inoculated (no.)	1981 Lesions Inactive	1981 Lesions Active	1981 Shoot mortality[z] (%)	1982 Laterals[y] inoculated (no.)	1982 Lesions Inactive	1982 Lesions Active	1982 Shoot mortality[z] (%)
12-32 × 12-20	80	5	20	75 a	120	9	0	91 a
12-27 × 12-20	80	42	8	50 ab	120	27	0	73 b
12-18 × 12-20	80	55	9	36 b	120	20	0	80 ab
12-44 × 12-20	80	65	2	33 b	120	18	0	82 ab

[x]Data were pooled for two fungus isolates.
[y]Twenty laterals per tree were inoculated, 10 per isolate, with *F. moniliforme* var. *subglutinans*. Ten laterals per tree were wounded for control treatments. In 1981, four trees per family were treated. In 1982, six trees per family were treated.
[z]Means within each year followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.01$.

inoculated stems sampled in 1981 was similar for all families. Initially, the fungus was confined to the area adjacent to the inoculation point (Fig. 1). Twenty-six days after inoculation, the fungus was recovered in all positions except the first. By day 56, it was recovered from position 1 in half the families. It appeared that colonization from the inoculation point was slightly more extensive and rapid below (toward the node) than above the inoculation point. Latent buds at the node directly below the inoculation point had enlarged by day 56 in all families. Inactive lesions sampled on 80 seedlings at the end of the experiment yielded isolates of the pathogen from lesion tissue occurring only in positions 3 and 4, and from asymptomatic tissue in positions 1, 2, and 5.

Field trials. There was no difference between the two isolates of *F. moniliforme* var. *subglutinans* that were used, and the following values represent pooled data. There was a significant difference between years in percent mortality of inoculated lateral shoots (Table 3). Overall shoot mortality was higher in 1982 (73–91%) than in 1981 (33–75%). The most susceptible family for both years, based on percent shoot mortality, was 12-32. Each year, different families were the least susceptible. In 1981, 12-18 and 12-44 had the lowest percent shoot mortality (36 and 33%, respectively). In 1982, however, 12-27 with 73% shoot mortality was lower than those of the other three families, but differed significantly only from 12-32. In 1981, no wounded controls died. In 1982, 10% of the wounded controls in families 12-32, 12-44, and 12-18 died from tip moth (*Rhyacionia* sp.) infestation. All of the shoots killed by tip moth were cultured on a Fusarium-selective medium, and *F. moniliforme* var. *subglutinans* was isolated from 83%. No wounded controls in family 12-27 died or had symptoms of tip moth infestation and the pathogen was not present in cultured samples from these shoots.

Fusarium moniliforme var. *subglutinans* colonized all sample positions by day 12. Percent colonization was higher at day 12 in families 12-18 and 12-27 than in families 12-32 and 12-44, but decreased from days 35 to 56. Percent colonization increased in 12-32 and 12-44 from days 12 to 35 with a decrease by day 56. At the end of the experiment, 32 shoots with small, apparently inactive lesions, found only in positions 3 and 4, were sampled. Although the pathogen was recovered from inactive lesion tissue, asymptomatic tissue in positions 1, 2, and 5 also yielded positive cultures.

Symptoms on inoculated shoots in the field initially were identical to those observed on shoots inoculated in the greenhouse (development of resinous lesions accompanied by purple discoloration of the shoot). The shoots in field inoculations, however, in many instances had complete needle loss by day 35. Although infection rarely progressed past the first node below the inoculation point on shoots inoculated in the greenhouse, by 56 days the dead areas on inoculated shoots in the field had extended to one to three nodes. On dead shoots, hyphae were observed growing out of the bark, and sporodochia were fruiting in fascicle scars.

Histology of wounded controls. In both greenhouse and field samples, a single layer of reaction parenchyma as described by Jewell et al (19) began to form in the xylem 12 days after wounding. By days 21 and 35, the reaction parenchyma was formed, and the tracheids between the reaction parenchyma and cambium were enlarged and atypically oriented (Fig. 2A). Where callus development was most pronounced, the cambium was disorganized and the cortex had ruptured. Callus was frequently observed being generated by the reaction parenchyma, forming thick layers extending through the cortex, which tended to slough off necrotic tissue from the wound (Fig. 2B).

Families in control treatments differed significantly in the size of their cortical resin ducts (Table 4), but not in the total number of resin ducts per square millimeter. In 1981, among the half-sib families grown in the greenhouse, 12-32 had the largest diameter resin ducts (0.204 mm) and 12-27 had the smallest (0.147 mm). Data for 1982 were not analyzed because of missing replications. Among the full-sib families in the field in 1981 and 1982, 12-32 had the largest diameter (0.264 and 0.227 mm) and 12-27 the smallest diameter (0.173 and 0.164 mm) resin ducts, respectively.

Histology of inoculated wounds. At the inoculation point, invasion by the pathogen radiated toward the pith in all families as previously described on Virginia pine seedlings (4). The most rapid invasion occurred preceding periderm formation (Fig. 2C) 4 to 12 days after inoculation.

Invasion by the pathogen vertically above and below the inoculation point 12–35 days after inoculation was in the phloem (Fig. 2D), in the inner cortex, and inside the resin canals. Invaded resin ducts became necrotic and adjacent cortex parenchyma was stimulated to cell division and radial cell orientation (Fig. 2F). However, the subsequent formation of callus around the necrotic resin ducts and in the cortex next to the phloem, frequently did not contain a second radial invasion by the pathogen across the cambium then along the rays to the pith (Fig. 2G).

By 35–56 days after inoculation, the pathogen invaded the first node below the inoculation point. Tracheids in the vascular traces of emerging buds that were continuous with the main stem at the node served as barriers against continued downward invasion by the pathogen present in the cortex. In other areas of the stem, however, the pathogen was able to bypass the tracheid barriers through invasion down the pith and through the parenchyma connecting the infected cortex of the stem and the emerging buds. Branch buds emerging from infected nodes were healthy if periderm separated the connecting parenchyma from the diseased cortex in the node. Where invasion by the pathogen was at least temporarily resisted (usually the first node below the inoculation point in seedlings and the first to third node in the branch shoots), all parenchymatous tissues below infected areas had converted to callus (Fig. 2H).

Resin ducts in the cortex were frequently filled with hyphae throughout their entire length. In addition to the sporodochia (1–3 mm in diameter) observed in fascicle scars, microscopic sporodochia (0.06–0.2 mm in diameter) were also observed in ruptures on the epidermis of stems, buds, and axils of stems and emerging branch buds.

Shoot dieback on loblolly and slash pine is characterized by the reddening of needles on infected branches (13,14). Affected shoots on inoculated Virginia pine branches, however, dropped all red needles by the end of the experiment. Within 12 days after inoculation, hyphae from the cortex of diseased shoots invaded adjacent healthy needles. Needle traces arising from infected stems were also invaded by the pathogen. By day 35, dissolution of parenchymatous tissues in the needle fascicles had occurred.

TABLE 4. Diameter of resin ducts in the cortex of Virginia pines in four full-sib families of 11-yr-old trees in Alabama and eight half-sib families of 1-yr-old seedlings in the greenhouse artificially wounded and sampled 12 days later

	Diameter of resin ducts (mm)	
Family	1981	1982
Alabama[y]		
12-32 × 12-20	0.264 a	0.227 a
12-44 × 12-20	0.224 b	0.213 b
12-18 × 12-20	0.196 c	0.199 c
12-27 × 12-20	0.173 d	0.164 d
Greenhouse[z]		
12-32	0.204 a	···
12-33	0.181 b	···
12-18	0.174 bc	···
12-44	0.171 bcd	···
12-14	0.161 cde	···
12-20	0.158 de	···
12-22	0.156 de	···
12-27	0.147 e	···

[y] Means followed by the same letter within each column are not significantly different according to Duncan's multiple range test, $P = 0.01$. Each number represents an average from one slide with four (1981) and six (1982) shoot replications per slide, six resin ducts measured per shoot.

[z] Means followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$. Each number represents an average from one slide, five stem replications per slide, six resin ducts per stem. Data for 1982 were not analyzed because of missing replications.

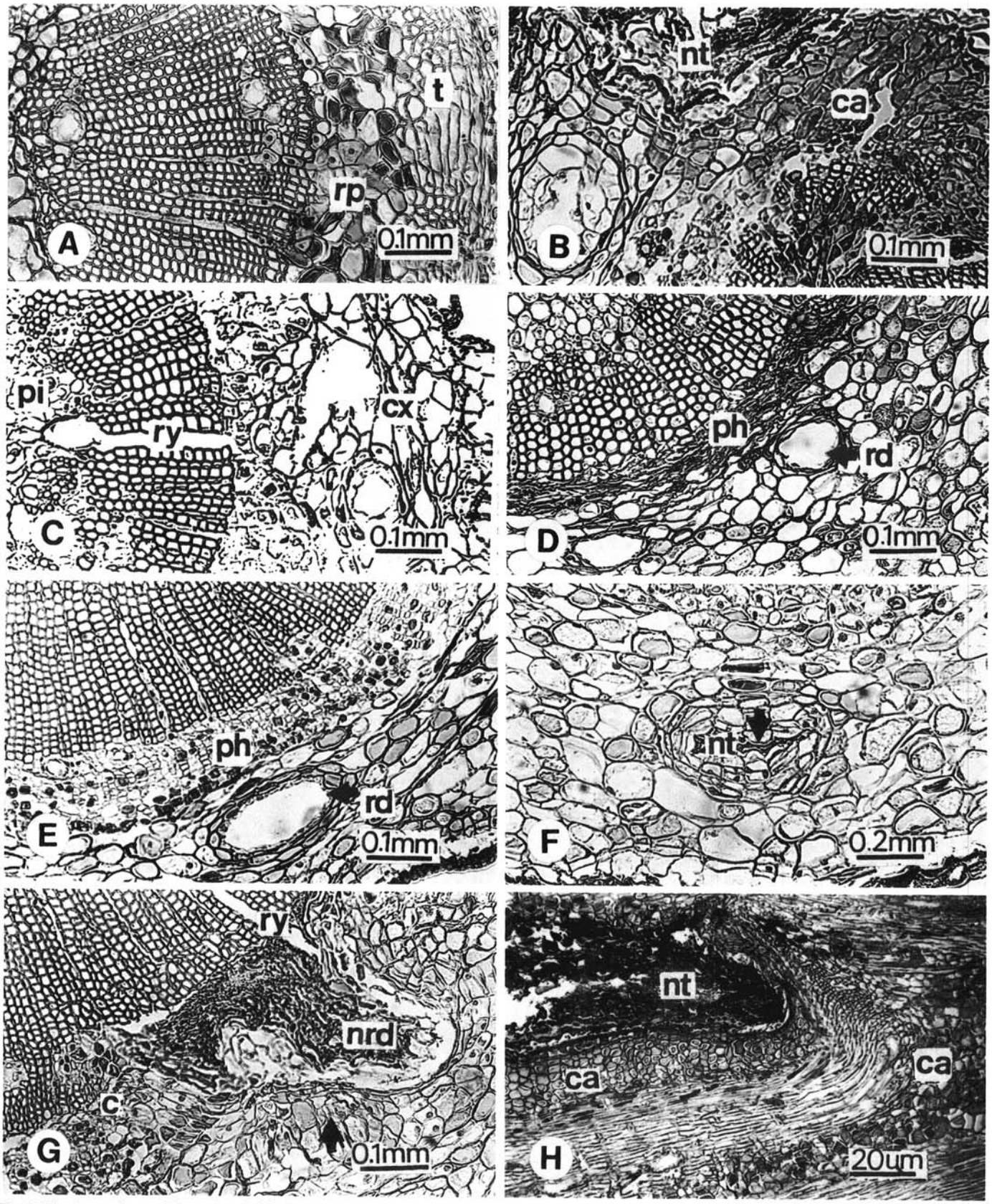

Fig. 2. Histology of wounds inoculated with *Fusarium moniliforme* var. *subglutinans* on the shoots of 1-yr-old seedlings and branches on 11-yr-old trees of Virginia pine. **A,** All shoots in wounded controls formed a layer of reaction parenchyma (rp) in the xylem with the tracheids (t) between the reaction parenchyma and cambium enlarged and atypically oriented. **B,** Callus (ca) generated by the reaction parenchyma formed layers that extended through the cortex to slough off necrotic tissue (nt) from the wound. **C,** In inoculated wounds, destruction of parenchymatous tissues proceded radially from the inoculation point across the cortex (cx) through the rays (ry) to the pith (pi). **D,** Invasion by the pathogen vertically above and below the inoculation point through the phloem (ph), inner cortex, and resin ducts (rd) caused collapse of these tissues as compared to **E,** a nonwounded control. **F,** An invaded resin duct became necrotic (nt) with adjacent cortex parenchyma stimulated to cell division (arrow) and radial cell orientation. **G,** The formation of callus (arrows) adjacent to the necrotic resin duct (nrd) frequently did not contain a second radial invasion by the pathogen across the cambium (c) along the rays (ry) to the pith. **H,** Where invasion by the pathogen was at least temporarily resisted in the node, all parenchymatous tissues below infected areas (nt) had converted to callus (ca). Figs. **A–G** are transverse sections and **H** is a tangential section.

Hyphae were visible in the gaps left by disintegrated cortex and resin ducts (Fig. 3A). Prior to needle drop, hyphae colonized the surfaces of dead needles, and microscopic sporodochia were observed emerging from the epidermis adjacent to the fascicle (Fig. 3B).

The formation of anatomical barriers to ingress observed in all inactive lesions was similar to those previously described in Virginia, slash, loblolly, and pond (*P. serotina* Michx.) pine seedlings inoculated with *F. moniliforme* var. *subglutinans* (3). Families 12-14 and 12-27 in the greenhouse (1981 and 1982) and 12-44 and 12-18 in the field (1981) had a high percent of inactive lesions. These were characterized by similar callus development in the pith radially opposite the inoculation point as was observed in the nodes where disease progression was arrested. Vertical spread of hyphae above and below the inoculation point was delimited by callus in the inner cortex as compared to periderm layers two to six cells thick observed in active lesions.

DISCUSSION

Relative susceptibility of half-sib families of Virginia pine seedlings in the greenhouse to *F. moniliforme* var. *subglutinans* was similar for two successive years of tests, and the ranking agreed with that of the disease survey on the parent seed orchard clones. Performance of full-sib families of 11-yr-old trees in a progeny test had mixed results. Family 12-32 was highly susceptible in both greenhouse and field trials. Family 12-27, however, gave disappointing results in the field trials considering its ranking of low susceptibility on inoculated seedlings and in the seed orchard survey. Families 12-18 and 12-44 of high susceptibility in the greenhouse were low in the field in 1981 and high in 1982. Most of the ramets in clone 12-33, which was highly susceptible in the greenhouse trials, have been rogued from the seed orchard because of the high incidence of dieback due to pitch canker.

Numerous sporodochia were observed fruiting from fascicle scars on dead shoots in both the greenhouse and field. Sporodochia are also commonly observed on infected slash pine branches in Florida (5) and in loblolly pine seed orchards (21). This study, however, is the first report of microscopic sporodochia observed on dead needles attached to infected shoots in the field. Histological examination of these needles revealed extensive internal and surface colonization by hyphae. These needles were subsequently shed by the trees into the litter where they possibly continued to serve as an inoculum source in the disease cycle.

Resistance observed in Virginia pines to the pitch canker pathogen in this study was based on the formation of anatomical barriers against invasion by the pathogen. Similar barriers in response to wounding alone have been observed in seedlings of southern pines (4,27). Responses to parasitic injury were also similar to those observed on slash pine resistant to *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (17–19) and white (*P. strobus* L.) pines resistant to *C. ribicola* J. C. Fisch. ex Rabenh. (22,29). Apparently the types of anatomical barriers formed in response to injury in pines are generalized and differences among trees occur in response time and quantity of barriers formed.

A symptom of pitch canker is excessive resin flow from the canker and resin-soaking of the underlying tissue. Virginia and slash pines have larger cortical resin ducts than the less susceptible loblolly and pond pines (4). Among the families of Virginia pine examined in this study, there was also a significant difference in the size of the cortical resin ducts. The high-to-moderately susceptible family (based on field and greenhouse tests), 12-32, had the largest ducts, and the least susceptible families (based on greenhouse tests), 12-14, 12-20, 12-22, and 12-27, had the smallest.

Resin ducts adjacent to an inoculated wound soon became blocked with tylosoids. True (30) postulated that parenchymatous tissues, such as tylosoids, formed in response to hyphal ingress contain abnormally large amounts of starches and oils, and the host is stimulated to provide additional food and food-containing cells that can be used by the pathogen. Once inside the cortex, the pitch canker fungus appears to frequently use the tylosoid-filled resin ducts as portals for vertical spread of the pathogen beyond the inoculation point, and then as a reservoir for its hyphae, after the tissues of the duct have been degraded. Large ducts with their potential for providing extra food for the pathogen could be a liability to the host.

In the field, where severity of wounding and inoculum potential vary, resistance based on anatomical barriers could be bypassed when environmental conditions favor rapid and extensive colonization by the pathogen of host tissue. Environmental factors not encountered when screening for resistance in the greenhouse, such as insect vectors, could also affect performance in the field. The outbreak of pine tip moth in Alabama in 1982 may have been partially responsible for the increase in shoot dieback over 1981. Dwinell and Barrows-Broaddus (11) observed a low correlation between shoot mortality of inoculated loblolly pine families in the greenhouse and shoot dieback of the parent clones in the field. In this study, shoot mortality of Virginia pine families in the greenhouse matched pitch canker incidence on parent clones, but only partially matched shoot mortality in the progeny test.

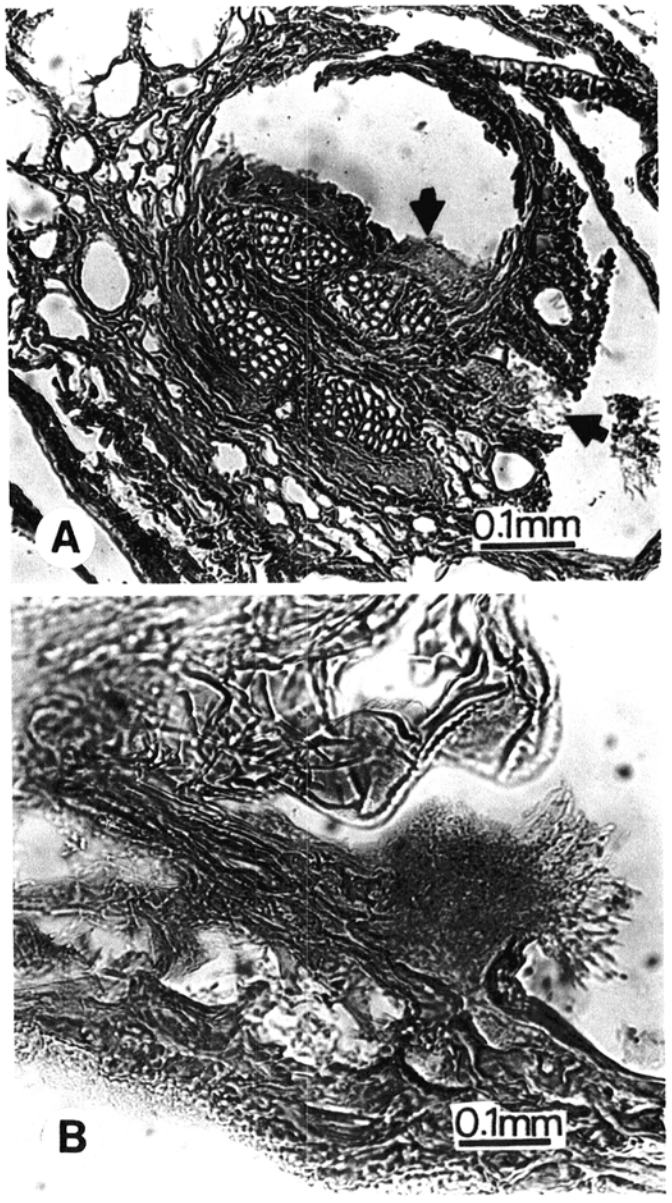

Fig. 3. Histology of Virginia pine needles infected with *Fusarium moniliforme* var. *subglutinans*. **A,** By day 35, parenchymatous tissues in infected needle fascicles were degraded and hyphae (arrows) were visible in cavities left by disintegrated tissues (transverse section). **B,** Microscopic sporodochium on a needle adjacent to the fascicle observed prior to needle drop (tangential section).

This study demonstrates that the incidence of pitch canker is related to specific clones in Virginia pines and that evaluation of progeny seedlings in the greenhouse can identify those clones with partial resistance to the disease. Control in Christmas tree plantations, however, will depend upon combining partial resistance with careful stand management, such as avoiding overfertilization and marginal planting sites, minimizing pruning wounds by genetic selection for form, and controlling insect vectors to reduce predisposition of the host to infection by the pathogen.

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