Histopathology of *Fusarium moniliforme* var. *subglutinans* in Four Species of Southern Pines

Jane Barrows-Broaddus and L. D. Dwinell

Plant pathologist and principal plant pathologist, USDA Forest Service, Southeastern Forest Experiment Station, Forestry Sciences Laboratory, Carlton Street, Athens, GA 30602.

Portion of a Ph.D. thesis by the first author. North Carolina State University.

Mention of a trade name or proprietary product does not constitute a guarantee or warranty of the product by the USDA or imply approval of it to the exclusion of other products that may also be suitable.

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

Accepted for publication 17 November 1982.

ABSTRACT

Barrows-Broaddus, J., and Dwinell, L. D. 1983. Histopathology of *Fusarium moniliforme* var. *subglutinans* in four species of southern pines. Phytopathology 73:882-889.

New shoots formed on 1-yr-old Virginia (*Pinus virginiana*), slash (*P. elliotii* var. *elliotii*), loblolly (*P. taeda*), and pond (*P. serotina*) pines were wounded and inoculated with *Fusarium moniliforme* var. *subglutinans*, the causal agent of pine pitch canker. Seedlings were sampled for histological examination and for colonization by the pathogen 1, 4, 12, 26, 35, and 56 days after inoculation. After 56 days, the remaining trees were rated for shoot mortality and lesion development. Active lesions on Virginia and slash pines had more continuous resin flow and more extensive purple discoloration along the stem than those on loblolly and pond pines. *Fusarium moniliforme* var. *subglutinans* rapidly grew intercellularly through the cortex of all host species. The pathogen attacked parenchyma cells throughout the stem, causing gaps in the cortex, rays, and pith. Infection in the cortex of Virginia and slash pines tended to be diffuse and was not delimited by boundary tissues such as a periderm, but in loblolly and pond pines, infected areas were bounded by a periderm, which created the appearance of a discrete lesion. Disease progression was arrested in the first node below the inoculation point where the fungus was contained in cortical tissue surrounded by stem and lateral bud tracheids. Virginia and slash pines had a higher percentage of resin ducts forming tylosoids than loblolly and pond pines. More layers of regenerative parenchyma were observed in the xylem in inactive lesions of loblolly and pond pines than in Virginia and slash pines. Lignification of pith cell walls was observed in all species, but was more prevalent in inactive lesions in loblolly and pond pines. Primordial sporodochia were observed in Virginia pine in axils and sheaths of buds emerging directly below a dead shoot.

Pitch canker, first reported by Hepting and Roth (16) in 1946 on Virginia pine (*Pinus virginiana* Mill.), is a serious disease of pines in the southern USA. Since 1974, the disease has become severe on slash pine (*P. elliotii* Engelm. var. *elliotii*) plantings in Florida (13) and slash, loblolly (*P. taeda* L.), longleaf (*P. palustris* Mill.), shortleaf (*P. echinata* Mill.), and Virginia pine seed orchard trees across the southeast (12,14).

The disease is characterized by resinous cankers on the trunk, branches, and shoots of susceptible pine species. The underlying wood in bole and branch cankers is resin soaked, often to the pith. Crown dieback, the common symptom on planted slash pine in Florida (13) and loblolly pine in seed orchards in North Carolina and Mississippi (14), results from the development of lesions on succulent shoots.

The causal agent of pitch canker is *Fusarium moniliforme* Sheld. var. *subglutinans* Wr. & Reink. (22), whose hosts also include many agronomic and horticultural crops (9). A wound, regardless of cause or location, provides an infection court, which is required by the pathogen (12). Although *F. moniliforme* var. *subglutinans* generally attacks pines 17 yr of age or older (26), Barnard and Blakeslee (3) reported that the pathogen also causes death of slash pine seedlings in nurseries. Miller and Bramlett (23) found that *F. moniliforme* var. *subglutinans* is also associated with damage to strobili, cones, and seed in slash and loblolly pine seed orchards.

Pines vary in susceptibility to the pathogen both among and within species. Dwinell (10) inoculated 1-yr-old seedlings and rated shortleaf and Virginia pines highly susceptible, slash pine intermediate, and loblolly pine more resistant to infection. Dwinell and Barrows-Broaddus (11) observed wide ranges in percent shoot mortality among inoculated half-sib families within both slash and loblolly pines. Inoculated pond pine (*P. serotina* Michx.) seedlings support a moderate percentage of resinous lesions with slight or no subsequent shoot mortality (L. D. Dwinell, *unpublished*).

The basis for variation in susceptibility to the pathogen appears to be internal and not due to the absence of infection courts. Kuhlman et al (21) observed that inoculation in a loblolly pine seed orchard, where clones were previously rated for susceptibility to pitch canker, was more successful on susceptible than on resistant and intermediate trees. Most infections occurred on the youngest tissue, but no important relationships were found between disease susceptibility and shoot phenology or mineral content of the foliage. These data indicate that seedling inoculations could be used to test for resistance to the pathogen.

We report here on histopathology in the seedlings of four pine

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

species of varying susceptibility to *F. moniliforme* var. *subglutinans*. The objectives of this study were: to describe seedling response to wounding with and without inoculation by *F. moniliforme* var. *subglutinans*, to quantify anatomical and histochemical differences among pine species in response to injury and infection, and to measure and compare rates of fungal colonization along stems of inoculated seedlings.

A preliminary report on histopathology of inoculated slash and loblolly pine seedlings has been published (4).

MATERIALS AND METHODS

Pine culture and inoculation. Seeds of Virginia, slash, loblolly, and pond pines from bulk field collections were planted separately in flats containing wetted vermiculite. When the hypocotyls were approximately 2.5 cm long, the seedlings were transplanted into plastic flats (33 × 13 × 11 cm) that contained a mixture of fumigated soil, pine bark, and sand (2:1:1, v/v). Fourteen seedlings were planted in each of nine flats for each species. Flats were kept in a greenhouse for 1 yr prior to treatment.

Two isolates of *F. moniliforme* var. *subglutinans*, whose virulence was confirmed in previous pathogenicity tests, were originally recovered from typical pitch cankers, one from loblolly pine in North Carolina and the other from Virginia pine in Alabama. The 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 10^6 as measured with a Coulter electronic particle counter. Two-month-old shoots on the 1-yr-old host plants were inoculated with a hypodermic needle through a 1-μl droplet of inoculum. Seedlings in control treatments were wounded by a hypodermic needle 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 three replications per treatment per species.

Histological procedure. After each of six intervals (1, 4, 12, 26, 35, and 56 days), three seedlings per treatment of each species were randomly 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 samples, each approximately 5 mm long, were taken above the inoculation point, and five samples starting with the inoculation point were taken down to the node. Alternate samples were fixed in FPA (40% formaldehyde-propionic acid-ethanol [5:5:90, v/v]) (20) for histological examination or cultured on a *Fusarium*-selective medium (1) to confirm colonization by the pathogen.

After 56 days, the remaining 288 seedlings were rated for shoot mortality and development of active or inactive lesions. Lesions were rated as active if they girdled at least 50% of the stem and continued to exude resin. They were rated as inactive if they girdled less than 50% of the stem and no longer exuded resin. At the end of the experiment, inactive lesions were sampled for histological examination and for colonization by the pathogen as previously described.

Fixed tissue segments 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 in a refrigerator. Transverse and tangential sections 8–10 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).

Histochemical tests (18,25) were conducted on selected tissue sections to detect the following chemical constituents: pectin (iron absorption and ruthenium red methods), lignin and wound gum (orcinol and phloroglucinal reactions), and cellulose (zinc-chloriodide reaction).

Stained sections were examined with a Leitz microscope and photographed with an attached Wild MP5 515 camera and Kodak technical pan film 2415.

Transverse sections of control stems on Triarch-stained slides were measured for resin duct size, number of resin ducts per unit area, and percent resin ducts with tylosoids. In sections from inoculated stems, areas of reaction zones formed in response to pathogen ingress were measured. Three slides that contained sections taken in the area of needle puncture were chosen for each species at each sampling date. Three replications were on each slide. Measurements were taken from three sections of each replication.

Transverse sections on orcinol- and phloroglucinol-stained slides were observed for lignification of pith cell walls in both control and inoculated seedlings as previously described. The total number of lignified pith cell walls was counted on five sections for each replication on each slide.

Reaction zones formed in response to infection were counted on inactive lesions sampled 56 days after inoculation. On Triarch quadruple-stained slides, data were taken on three transverse sections for each replication on each slide.

Percent shoot mortality and the data from the slides were subjected to analysis of variance and Duncan's multiple range test (27).

RESULTS

Shoot mortality and symptomatology. Mortality of inoculated shoots averaged 37% for Virginia, 33% for slash, 17% for loblolly,

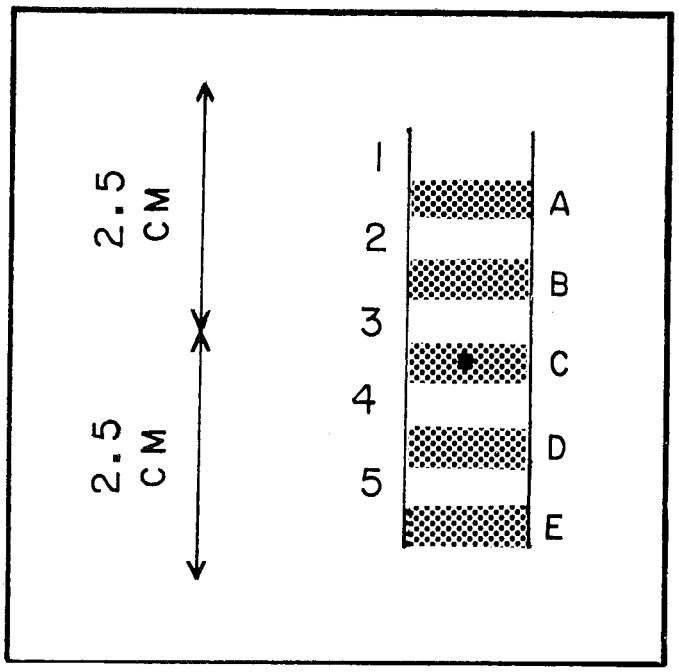
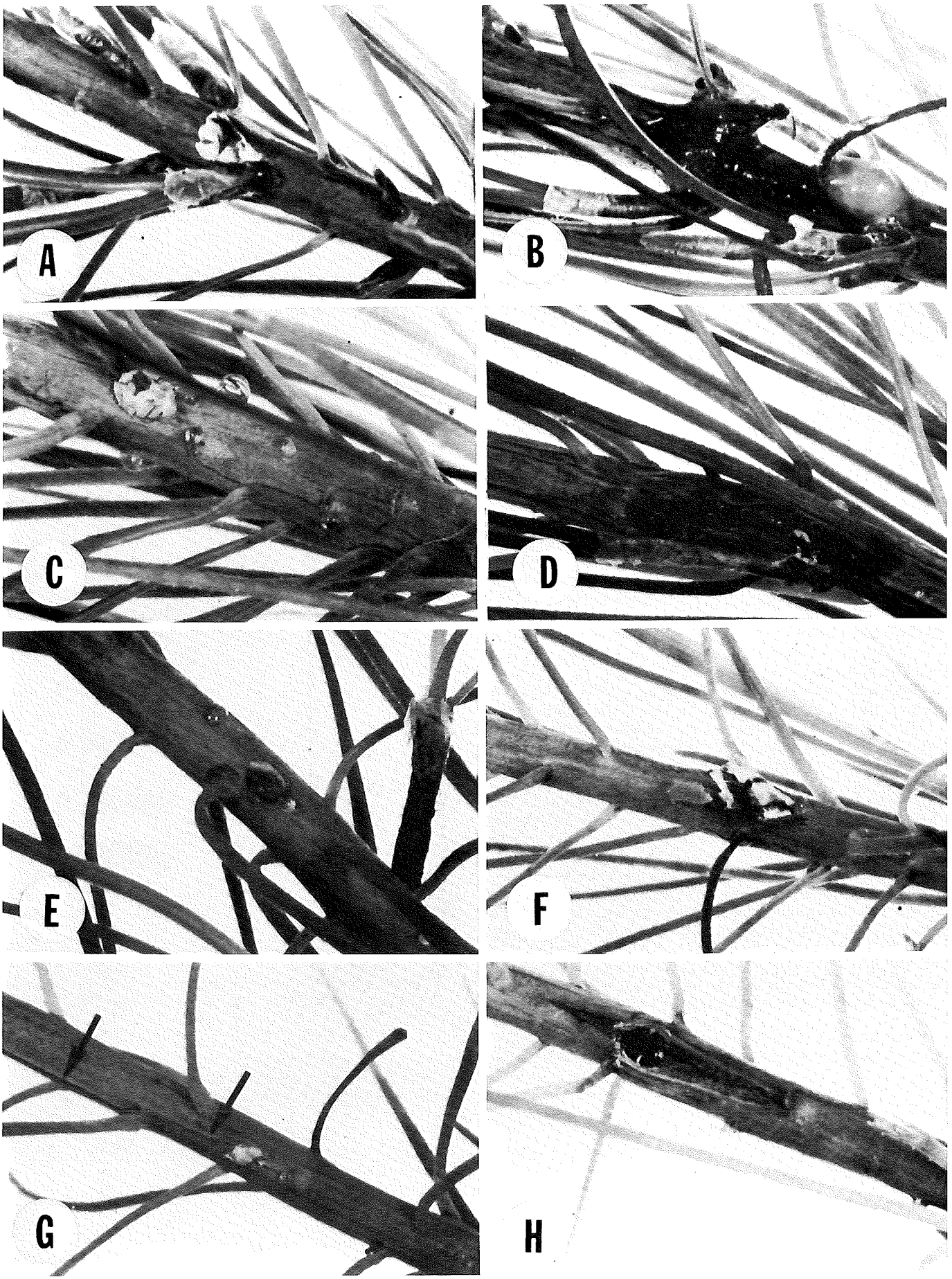


Fig. 1. Sampling design for pine seedlings wounded in control and inoculated treatments. Lettered shaded areas were fixed for histological examination. Numbered clear areas were plated on a Fusarium-selective medium. The location of the needle puncture is at C and the node is at E.

TABLE 1. Shoot mortality and lesion development in four southern pine species inoculated with *Fusarium moniliforme* var. *subglutinans*[y]

Pinus species	Trees inoculated (no.)	Lesions		Shoot mortality (%)[z]
		Inactive	Active	
P. virginiana	49	45	18	37 a
P. elliotii	46	37	30	33 a
P. taeda	52	67	16	17 b
P. serotina	49	88	2	10 b

[y] Data were pooled for two fungus isolates.
[z] Means followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.01$.


A
B
C
D
E
F
G
H

and 10% for pond pine (Table 1). There was no difference between the two isolates of *F. moniliforme* var. *subglutinans* used, and the above values represent pooled data. No wounded control shoots died.

The percentage of inoculated shoots with active lesions ranged from 2.0 for pond pine to 30 for slash pine. Conversely, the formation of inactive lesions was 88% on pond pine and 37% on slash pine (Table 1). In general, inoculated Virginia and slash pines had more continuous resin flow and more extensive purple discoloration along the stem than inoculated loblolly and pond pine seedlings (Fig. 2).

The appearance of the small lesions formed on the wounded controls was similar to that of many of the inactive lesions on inoculated stems. The control wounds initially exuded resin that soon formed a dry crust over the lesion. In pond pine, numerous fissures were observed along the stems where cork layers formed (Fig. 2).

Colonization of inoculated stems. Growth rate of *F. moniliforme* var. *subglutinans* along the stem was similar for all species (Table 2). The fungus was generally confined to the area adjacent to the inoculation point. *Fusarium moniliforme* var. *subglutinans* was not recovered from stem pieces in position 1 until day 56. It appeared that colonization from the inoculation point was slightly more extensive and rapid downward (towards the node) than upward from the inoculation point. Latent buds at the node directly below the inoculation point had enlarged by day 56 in all species. Inactive lesions sampled on 24 seedlings at the end of the experiment yielded isolates of the pathogen from lesion tissue occurring only in segments 3 and 4.

Histology of wounds. All species responded similarly to wounding. Tylosoids formed inside resin ducts adjacent to the wound (Fig. 3A). Throughout the cortex, resin first accumulated in affected cells which soon disintegrated. Necrotic cells in the cortex were delimited by a periderm layer. A reaction zone in the xylem formed directly distal to the wound. This zone was comprised of parenchymatous cells, which appeared to be regenerative (Fig. 3C). Wound reaction zones were observed in slash, loblolly, and pond pine seedlings 12 days after wounding, but were not observed in Virginia pine until the next sampling on day 26. In the pith, scattered parenchyma cells deposited lignin in their walls as determined by the phloroglucinol-orcinol histochemical tests (Fig. 3B). This reaction was observed in loblolly and pond pine seedlings at 12 days and in all species at 26 days. There was no statistically significant difference among the species over time because of the variation in the number of lignified cells from stem to stem.

The kinds of pines studied differed significantly in diameter of

Fig. 2. A–H. Comparison of seedlings of four pine species wounded as controls and inoculated with *Fusarium moniliforme* var. *subglutinans*. **A,** Virginia, **C,** slash, **E,** loblolly, and **G,** pond pines artificially wounded in control treatments. Wounds initially exude resin that soon forms a crust over the lesion. In pond pine, numerous fissures (arrows) form along the stem and fill with cork layers. **B,** Virginia, **D,** slash, **F,** loblolly, and **H,** pond pines wounded and inoculated with the pitch canker fungus. In general, inoculated Virginia and slash pines had more continuous resin flow and more extensive purple discoloration along the stem than inoculated loblolly and pond pine seedlings (×2.3).

TABLE 2. Colonization by *Fusarium moniliforme* var. *subglutinans* at each location[x] along stems of four southern pine species over six time intervals[y]

Time (days)	Colonization (%): *P. virginiana* stem segment:[z]					*P. elliottii* stem segment:					*P. taeda* stem segment:					*P. serotina* stem segment:				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	0	0	0	17	0	0	0	33	17	0	0	0	0	0	0	0	0	17	0	0
4	0	0	0	50	0	0	0	0	83	0	0	0	0	50	17	0	0	0	33	0
12	0	0	67	67	0	0	0	67	83	0	0	0	50	83	17	0	0	67	83	17
26	0	0	67	83	17	0	0	83	100	0	0	0	67	100	17	0	0	17	100	17
35	0	0	67	67	50	0	0	67	83	0	0	0	67	67	17	0	0	83	33	17
56	0	33	50	83	83	33	33	83	83	83	17	83	100	100	83	33	50	83	100	67

[x] Refer to Fig. 1 for explanation of stem segment location numbers.

[y] Data were pooled for both fungus isolates. Each location represents percent colonization calculated from six replications. No colonization occurred in control treatments with three replications for each time for each species.

[z] Inoculation was in a puncture wound between stem segments 3 and 4. Each segment was 5 mm long and each of these were separated by a 5-mm segment that was taken for histological examination.

TABLE 3. Average diameter of resin ducts in cortex and percent total resin ducts with tylosoids in four southern pine species artificially wounded and sampled over six time intervals[x]

Variables and *Pinus* species	Time (days) 1	4	12	26	35	56	Mean
Resin ducts in cortex (avg. diam., mm)[y]							
P. virginiana	0.155 bc	0.153 bc	0.156 bc	0.178 ab	0.184 a	0.164 ab	0.165
P. elliottii	0.150 bcd	0.139 cd	0.138 cd	0.146 bcd	0.128 de	0.139 cd	0.140
P. taeda	0.105 fg	0.113 ef	0.105 fg	0.109 efg	0.106 efg	0.106 efg	0.107
P. serotina	0.108 efg	0.100 fg	0.102 fg	0.092 fg	0.091 g	0.094 fg	0.098
Resin ducts with tylosoids (%)[z]							
P. virginiana	4.8 fg	2.5 jkl	12.7 b	12.9 b	12.1 b	14.4 a	9.9
P. elliottii	0 m	8.6 d	12.8 b	10.3 c	10.9 c	12.0 bc	9.1
P. taeda	2.9 ijk	2.0 kl	0 m	3.6 ghij	4.3 gh	6.2 e	3.2
P. serotina	0 m	1.5 l	5.7 ef	3.8 ghi	1.9 kl	3.2 hijk	2.7

[x] Means followed by the same letter for average diameter resin ducts in cortex and for percent total resin ducts with tylosoids are not significantly different according to Duncan's multiple range test, $P = 0.01$.

[y] Each number represents an average based on three slides, with three stem replications per slide, six resin ducts measured per stem.

[z] Each number represents an average based on three slides, three stem replications per slide, three transverse sections per stem.

resin ducts in the cortex, in percent of resin ducts in both cortex and stele that formed tylosoids (Table 3), and in number of resin ducts per square millimeter in transverse section. Averages over all sampling dates for diameters (in millimeters) of cortical resin ducts were: Virginia, 0.163; slash, 0.140; loblolly, 0.107; and pond, 0.098. Slash, loblolly, and pond pines averaged 4.0–4.7 resin ducts per square millimeter, but Virginia pine had significantly more at 5.8 per square millimeter. Percent of resin ducts with tylosoids increased over time for all species. Virginia and slash pines formed significantly more tylosoids than loblolly and pond pines. Percent resin ducts with tylosoids averaged over all time was 9.9 and 9.1 for Virginia and slash, and 3.2 and 2.7 for loblolly and pond pines, respectively.

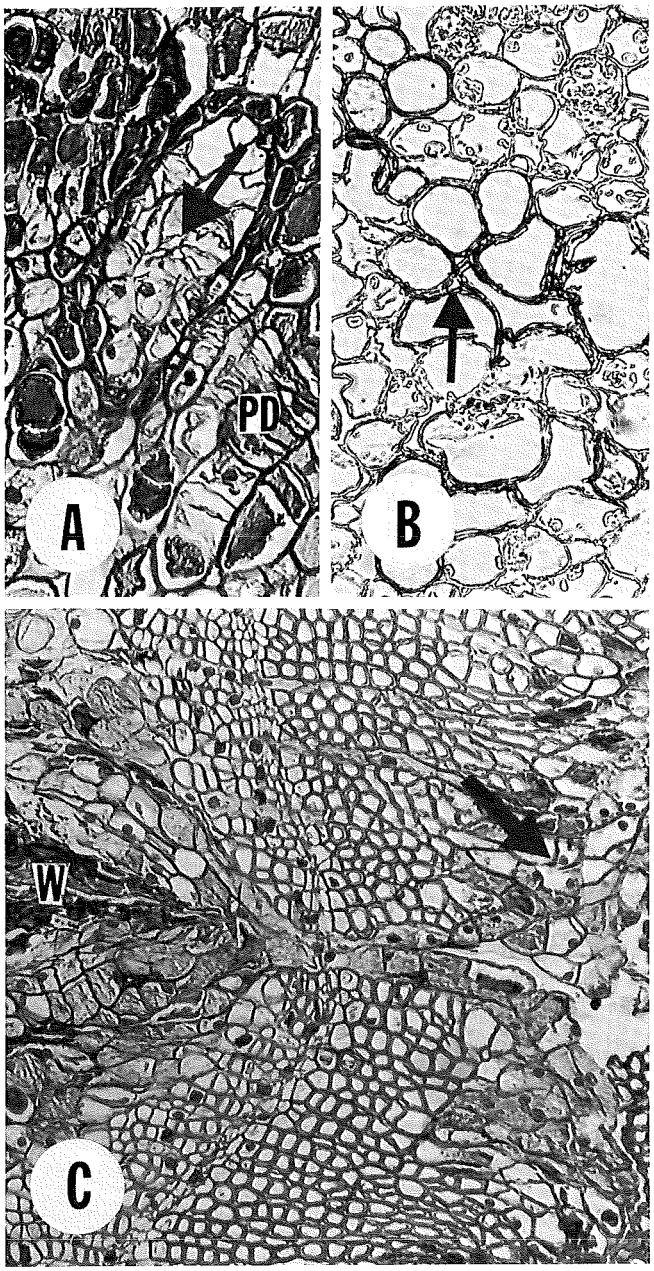


Fig. 3. Histology of artificial wounding in shoots of four kinds of southern pines. Virginia, slash, loblolly, and pond pine seedlings responded to wounding in a similar manner. **A,** A resin duct adjacent to a wound in the cortex of Virginia pine is filled with tylosoids (arrow). PD = periderm (×1,180). **B,** In the pith, scattered parenchyma cells (arrow) deposited lignin in their walls as determined by phloroglucinol-orcinol histochemical tests (×1,180). **C,** A reaction zone comprised of parenchymatous cells (arrow) formed in the xylem directly behind the wound (W) and appeared to have a regenerative function (×1,180). All photographs are of transverse sections.

Histology of inoculated wounds. The pattern of reaction to pathogen ingress was similar in all species. Some individuals of all species died rapidly before any wound response tissue had a chance to form, but rapid death was most prevalent in Virginia pine.

F. moniliforme var. *subglutinans* rapidly grew intercellularly through the cortex. Cells accumulated resin before they became necrotic and disintegrated. Generally infection in the cortex of Virginia and slash pines was diffuse and not delimited by boundary tissues such as a periderm (Fig. 4A). Resin ducts in affected areas were filled with tylosoids. In loblolly and pond pine seedlings, infected cortical areas tended to be bounded by a periderm, which created the appearance of a discrete lesion (Fig. 4B), and only resin ducts in the immediate vicinity of the lesion were affected. Both diffuse and discrete lesions could be found in the cortex of all four species, but discrete lesions were more common in loblolly and pond pines.

The pathogen attacked parenchyma cells throughout the stem producing gaps in cortex, rays, and pith. Where tissue had disintegrated, hyphae were readily observed (Fig. 4F). Hyphae were especially numerous in cavities left by disintegrated cortical resin ducts and rays. Xylem tracheids became plugged with resin, but the fungus did not appear to attack these cell walls. The formation of peridermal layers in the cortex and the reaction zone of parenchyma cells in the xylem did not stop the progression of infection; parenchyma-type cells appeared to be particularly vulnerable to attack by the fungus. In small resin ducts in the xylem, epithelial cells lining the ducts and associated ray parenchyma became hypertrophied before being degraded by the pathogen (Fig. 4C–D).

In all four species, disease progression was usually arrested at the node below the inoculation point, the node being defined as the location where the current season's growth was separated from that of the previous year's and where new branch buds arose from the stem. Where shoot mortality occurred, the stem was necrotic in the upper portion of the node, and the pathogen progressed through the connecting parenchyma cells into the new buds there. Continued ingress down the node by the pathogen appeared to be physically halted by the tracheids in the vascular traces of emerging buds which were continuous with the main stem. In tangential section, the fungus was often contained in cortical tissue surrounded in a "V" formed by the stem and lateral bud tracheids (Fig. 4G). Buds emerging below this area were free of the pathogen.

Primordial sporodochia were formed on the surfaces of dead shoots in the axil of the stem and emerging branch buds (Fig. 4E). Meristems of necrotic buds also contained small sporodochia nestled deep in the sheath.

Histology of resistance mechanisms. The formation of inactive lesions in response to inoculation with *F. moniliforme* var. *subglutinans* was observed in all species. The pathogen remained viable in the stem at the inoculation point but did not penetrate far into tissue above and below the limited area of the visible lesion.

Histological examination of these lesions revealed that seedlings resisted severe infection by forming several layers of regenerative parenchyma, by sloughing off infected cortex, and by lignification

TABLE 4. Resistance mechanisms in inactive lesions formed on four species of southern pines 56 days after inoculation with *Fusarium moniliforme* var. *subglutinans*[x]

Pinus sp.	Lignified cell walls in pith (no.)[y]	Layers of regenerative parenchyma (no.)[z]
P. virginiana	23.2 c	1.82 b
P. elliottii	25.7 bc	1.43 b
P. taeda	57.0 a	2.93 a
P. serotina	49.4 ab	2.42 ab

[x] Means followed by the same letter within each column are not significantly different according to Duncan's multiple range test, $P = 0.01$.

[y] Each number represents the average of three slides, three stem replications per slide, five transverse sections per replication.

[z] Each number represents the average of three slides, three stem replications per slide, three transverse sections per replication.

of pith parenchyma. These responses were observed in all species, but loblolly and pond pines formed significantly more lignified cell walls in the pith and more layers of regenerative parenchyma cells than Virginia and slash pines (Table 4). Many of the lignified pith cells began to look like tracheids and probably functioned as such to aid in water transport after normal xylem tracheids became plugged with resin and other metabolites (Fig. 5A). In the xylem, orientation of tracheids changed in advance of the fungus so that in transverse section, tracheids were oriented as in tangential section (Fig. 5B). Layers of regenerative-type parenchyma formed on the

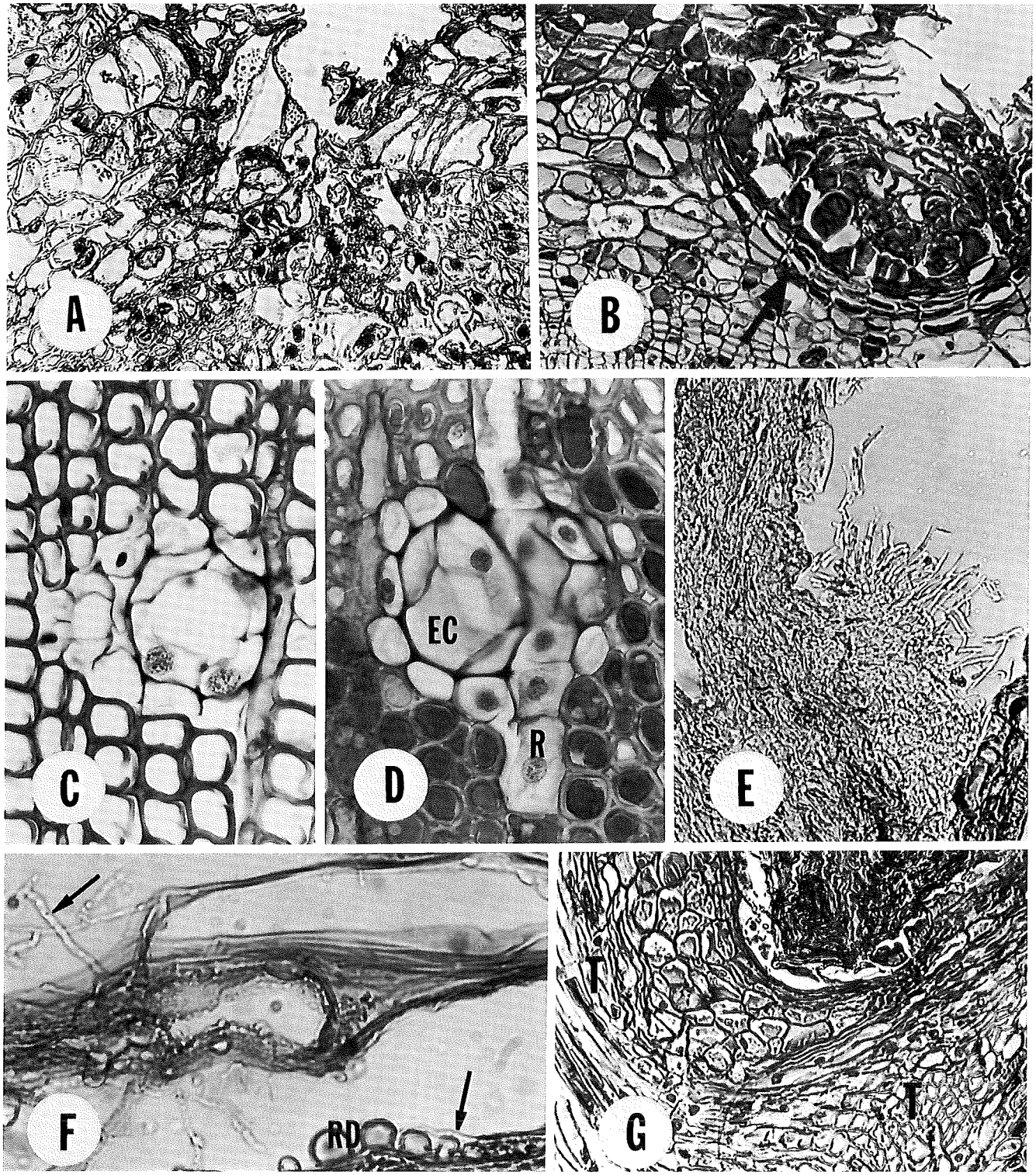


Fig. 4. Histology of shoot wounds inoculated with *Fusarium moniliforme* var. *subglutinans* in four kinds of southern pines. **A,** Generally infection in the cortex of Virginia and slash pines was diffuse and not delimited by a periderm (×1,180). **B,** In loblolly and pond pines, infected cortical areas tended to be bounded by a periderm (arrows) giving the appearance of a discrete lesion. **C,** Example of a normal resin duct in the xylem (×2,950). **D,** In inoculated stems, the epithelial cells (EC) lining the ducts and the ray parenchyma (R) associated with them became hypertrophied before they were degraded by the pathogen (×2,950). **E,** Primordial sporodochium formed in the sheath of a necrotic bud emerging from the first node below the inoculation point (×1,180). **F,** Hyphae (arrows) grew intercellularly and could be observed after tissues had disintegrated. RD = resin droplets (×2,950). **G,** In the node, disease progression was frequently contained in cortical tissue surrounded by tracheids (T) from the stem and lateral bud (×1,180). Photographs A–D are of transverse sections, and E–G are of tangential sections.

side of the stem opposite the advancing front of the fungus and provided new tissues for continued maintenance of the stem even after the pathogen had destroyed response tissues formed in the pith (Fig. 5C). Diseased tissues above and below the inoculation point were progressively delimited by periderm in the cortex and eventually sloughed off (Fig. 5D).

DISCUSSION

Virginia and slash pines were more suceptible to *F. moniliforme* var. *subglutinans* than were loblolly and pond pines. Previous inoculation tests conducted by the authors (5,11) on greenhouse-grown seedlings of Virginia, slash, and loblolly pines resulted in higher shoot mortality than was observed in the present study, especially for Virginia pine (48–100 vs. 37%). Dwinell (10) observed in inoculated seedlings that Virginia pine is the most susceptible, slash intermediate, and loblolly pine most resistant to infection. In another study, he found that percentages of shoot mortality for pond pine vary from none to low, depending upon the isolate used for inoculation (L. D. Dwinell, *unpublished*).

Symptomatology of the inoculated seedlings was similar to that observed by Dwinell (10). In slash, Virginia, and shortleaf pines, he observed reddish-purple discoloration of the stems, and the fungus was recovered at least 12 mm above and below the inoculation point. In loblolly, distinct lesions developed at the inoculation point, and the fungus was recovered only from the lesion and the tissue immediately adjacent to it.

Colonization patterns of stem pieces were similar for all species. Generally, the percentage of fungus recovery was higher proximal to than distal from the inoculation point. Hepting (15) observed in

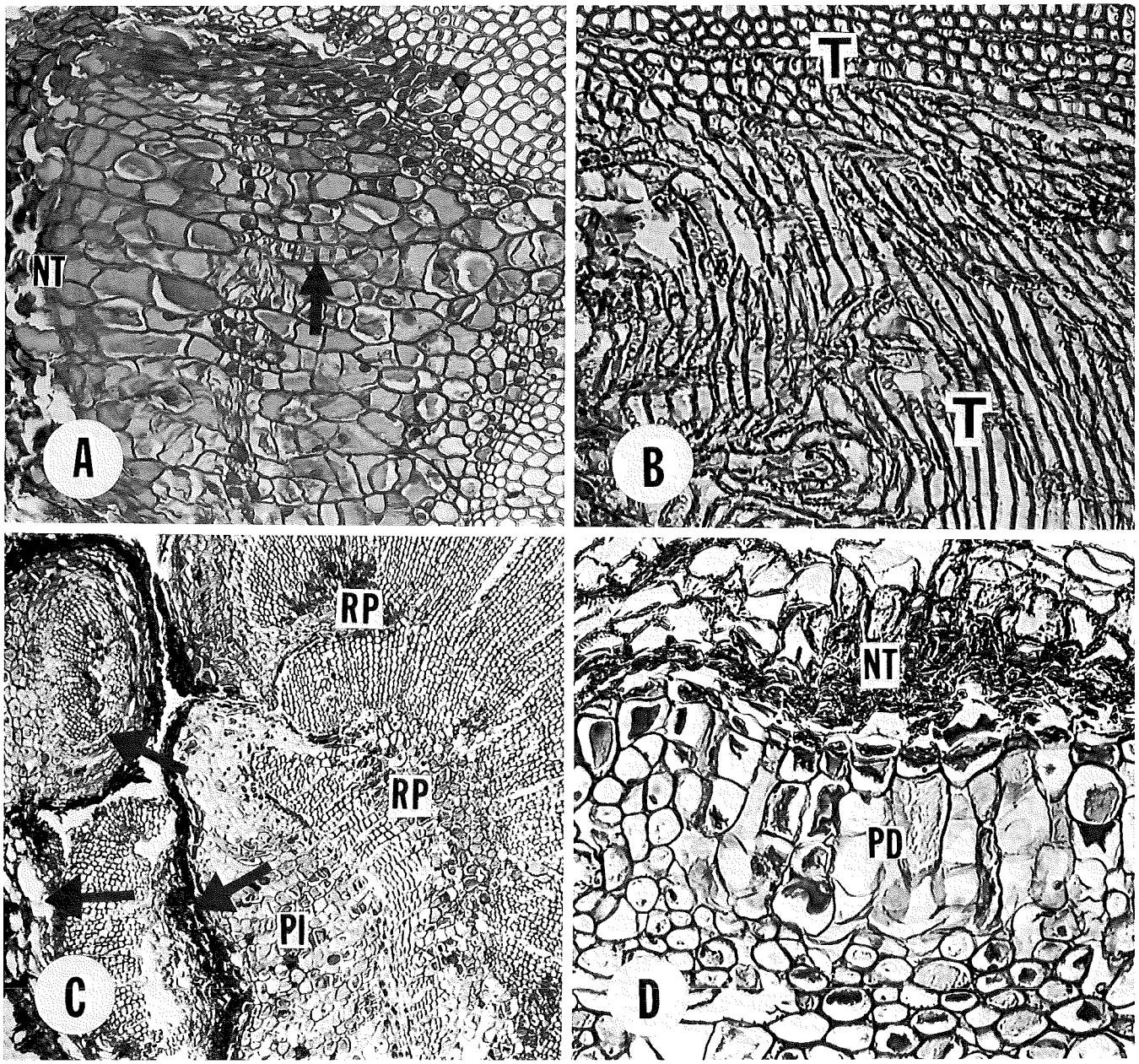


Fig. 5. Histology of resistance mechanisms in four kinds of southern pines inoculated with *Fusarium moniliforme* var. *subglutinans*. **A,** Tracheidlike cells (arrow) are laid down from parenchyma in the pith and probably function for continued transport after xylem tracheids have become plugged with resin and other metabolites. NT = necrotic tissue (×1,180). **B,** Orientation of xylem tracheids (T) changed in advance of the fungus so that in transverse section, they were oriented as in tangential section (×1,180). **C,** Seedlings resisted infection by sloughing off infected areas including the cortex (arrows), by laying down tracheidlike cells in the pith (PI), and by forming layers of regenerative parenchyma (RP) in the xylem in advance of the invading fungus (×472). **D,** In the cortex of pond pine 10 mm above the inoculation point, a layer of periderm (PD) delimits the diseased, necrotic tissue (NT) which is being sloughed off (×1,180). All photographs are of transverse sections.

inoculated Virginia pine trees that the fungus spreads parallel to the long axis of the stem further below than above the inoculated wound. By the end of our experiment, stem pieces in all five positions for all species, except Virginia pine, had at least 17% colonization. Loblolly and pond pines with their limited lesions and lower percent shoot mortality probably had asymptomatic colonization. *Fusarium moniliforme* var. *subglutinans* also colonizes strobili and cones of slash and loblolly pines (23) and gladiolus corms (6) without producing observable symptoms. Since the inactive lesions in all the inoculated pines supported viable hyphae, these pines possibly harbor the pathogen until environmental conditions permit a disease outbreak.

Resin ducts responded to wounding and infection with tylosoid formation and hypertrophy of epithelial cells. Generally, the formation of resin ducts and resin production in response to wounding are considered to confer protection of pines against infection (17,24). The pitch canker fungus, however, readily attacked resin ducts and the tylosoids filling them. The fungus then proliferated in the cavities left in the stem after the ducts were degraded. For this disease, resin production did not appear to be a protective advantage, and loblolly and pond pines, which had smaller ducts and lower percentage tylosoid production, were less susceptible to the pathogen.

Microscopic, primordial sporodochia were observed on dead shoots near the node, in the axils of buds and in bud sheaths in Virginia pines. We have only rarely observed sporodochia on inoculated, greenhouse-grown seedlings, or on diseased Virginia, slash, shortleaf, and loblolly pine in seed orchards (*unpublished*). Blakeslee et al (7), however, reported that sporodochia visible to the unaided eye occur routinely on naturally infected, small-diameter branches in slash pine plantations in Florida. Macroscopic sporodochia are also observed on diseased slash pine seedlings in commercial nursery beds (3). If microscopic sporodochia are also present in diseased pines, they may be a significant source of inoculum, especially when the environment is conducive for sporulation.

Inoculated shoots that died were killed back only to the node below the inoculation point. Dwinell (10) also reported that shoot death does not proceed past a node when Virginia, slash, shortleaf, and loblolly pine seedlings are inoculated with the pitch canker fungus. In the present study, histological examination revealed that the fungus was present in the upper portion of the node and that buds originating there were infected. In crown dieback of slash pines in Florida, new shoots in the spring are frequently killed by cankers that were initiated the preceding fall (13). Dormant infections in nodal areas on laterals could harbor viable hyphae able to colonize new shoots when the growing season resumes.

In general, reponse to injury, whether as an artificial wound or ingress by the pathogen, was similar for all pines tested. Speirs et al (28) also observed this uniformity of response in comparing wounded 6-wk-old slash pine seedlings to rust resistance zones formed in response to infection by *Cronartium quercuum* (Berk) Miyabe ex Shirai f. sp. *fusiforme*. Shoots of seedlings inoculated with the pitch canker pathogen either rapidly died or formed active or inactive lesions. Species differed in the percentages of each type of reaction. The formation of regenerative parenchyma and the atypical orientation and appearance of tracheids observed more frequently in the inactive lesions has also been observed on slash pine seedlings in association with resisted infections by *C. quercuum* f. sp. *fusiforme* (19,28). Regenerative parenchyma formed in seedlings infected with the pitch canker fungus, however, was readily degraded by the pathogen.

Variation in susceptibility to pitch canker has been observed among inoculated slash (8,11) and loblolly (11) pine seedlings and among naturally exposed slash and loblolly pines in seed orchards (12). The variation in the degrees of anatomical responses to injury and infection described in the present study may help locate individual seedlings resistant to pitch canker.

LITERATURE CITED

1. Agrawal, S. C., Khare, M. N., and Kushwara, L. S. 1973. A selective medium for isolation and quantitative estimation of *Fusarium* in soil. Sci. Culture 39:555-556.
2. Alcorn, S. M., and Ark, P. A. 1953. Softening paraffin-embedded plant tissues. Stain Technol. 28:55-56.
3. Barnard, E. L., and Blakeslee, G. M. 1980. Pitch canker of slash pine seedlings: A new disease in forest tree nurseries. Plant Dis. 64:695-696.
4. Barrows-Broaddus, J. B. 1981. Histopathology of *Fusarium moniliforme* var. *subglutinans* in slash and loblolly pine seedlings. (Abstr.) Phytopathology 71:858.
5. Barrows-Broaddus, J., and Dwinell, L. D. 1979. Variation in virulence of diverse sources of *Fusarium moniliforme* var. *subglutinans* on Virginia and loblolly pine. (Abstr.) Phytopathology 69:525.
6. Barrows-Broaddus, J., and Dwinell, L. D. 1980. Decay and colonization of gladiolus corms by the pine pitch canker fungus. Phytopathology 70:847-850.
7. Blakeslee, G. M., Kratka, S. H., Schmidt, R. A., and Moses, C. S. 1978. Sporodochia of the pitch canker fungus (*Fusarium moniliforme* var. *subglutinans*) as found in diseased slash pines in Florida. Plant Dis. Rep. 62:656-657.
8. Blakeslee, G. M., and Rockwood, D. L. 1978. Variation in resistance of slash pine to pitch canker caused by *Fusarium moniliforme* var. *subglutinans*. (Abstr.) Phytopathol. News 12:207.
9. Booth C. 1971. The genus *Fusarium*, pp. 127–129. Commonw. Mycol. Inst., Kew, Surrey, England.
10. Dwinell, L. D. 1978. Susceptibility of southern pines to infection by *Fusarium moniliforme* var. *subglutinans*. Plant Dis. Rep. 62:108-111.
11. Dwinell, L. D., and Barrows-Broaddus, J. 1979. Susceptibility of half-sib families of slash and loblolly pine to the pitch canker fungus, *Fusarium moniliforme* var. *subglutinans*. (Abstr.) Phytopathology 69:1-A4.
12. Dwinell, L. D., and Barrows-Broaddus, J. 1981. Pitch canker in seed orchards. Pages 234–240 in: Proc. Sixteenth South. For. Tree Improv. Conf., May 1981. Virginia Polytech. Inst. and State University, Blacksburg.
13. Dwinell, L. D., and Phelps, W. R. 1977. Pitch canker of slash pine in Florida. J. For. 75:488-489.
14. Dwinell, L. D., Ryan, P. L., and Kuhlman, E. G. 1977. Pitch canker of loblolly pine in seed orchards. Pages 130–137 in: Proc. Fourteenth South. For. Tree Improv. Conf., June 1977. Univ. of Florida, Gainesville.
15. Hepting, G. H. 1954. Gum flow and pitch-soak in Virginia pine following *Fusarium* inoculation. U.S. Dep. Agric., For. Serv. Stn. Pap. 40. 10 pp.
16. Hepting, G. H., and Roth, E. R. 1946. Pitch canker, a new disease of some southern pines. J. For. 44:742-744.
17. Jane, F. W. 1956. The structure of wood, pp. 81–104. MacMillan, New York.
18. Jenson, W. A. 1962. Botanical histochemistry. W. H. Freeman & Co., San Francisco, CA. 408 pp.
19. Jewell, F. F., and Speirs, D. C. 1976. Histopathology of one- and two-year-old resisted infections by *Cronartium fusiforme* in slash pine. Phytopathology 66:741-748.
20. Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill, New York. 523 pp.
21. Kuhlman, E. G., Dianis, S. D., and Smith, T. K. 1982. Epidemiology of pitch canker disease in a loblolly pine seed orchard in North Carolina. Phytopathology 72:1212-1216.
22. Kuhlman, E. G., Dwinell, L. D., Nelson, P. E., and Booth, C. 1978. Characterization of the *Fusarium* causing pitch canker of southern pines. Mycologia 70:1131-1143.
23. Miller, T., and Bramlett, D. L. 1978. Damage to reproductive structures of slash pine by two seed-borne pathogens: *Diplodia gossypina* and *Fusarium moniliforme* var. *subglutinans*. Pages 347–355 in: Proc. Flowering and Seed Development in Trees: A Symposium. U.S. Dep. Agric., For. Serv., Southeastern Forest Expt. Stn., New Orleans.
24. Mirov, N. T. 1967. The genus *Pinus*, pp. 349-395. Ronald Press, New York.
25. Rawlins, T. E., and Takahashi, W. N. 1952. Technics of Plant Histochemistry and Virology. National Press, Millbrae, CA. 125 pp.
26. Schmidt, R. A., and Underhill, E. M. 1974. Incidence and impact of pitch canker in slash pine plantations in Florida. Plant Dis. Rep. 58:451-454.
27. Snedecor, G. W., and Cochron, W. G. 1978. Statistical Methods. Iowa State University Press, Ames. 593 pp.
28. Spiers, D. C., Walkinshaw, C. H., and Jewell, F. F. 1977. Rust resistant reaction in juvenile slash pine simulated by wounding. (Abstr.) Proc. Am. Phytopathol. Soc. 4:141.