Incidence and Histology of Stem-Girdling Galls Caused by Fusiform Rust

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ABSTRACT

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Isolates of *Cronartium quercuum* f. sp. *fusiforme* were found to produce a high incidence of lethal stem-girdling galls in certain families of slash pine. This girdling, one of many fusiform rust disease processes, was studied because it appeared to be associated with families of pines that had been selected for their putative resistance to fusiform rust. Stem-girdling galls with a bulbous swelling above a constriction on the stem were found to disorganize the tissues of rust-infected trees much more severely than typical galls tapered at both ends. Infected seedlings with stem-girdling galls grown in a greenhouse died within 1 yr; those grown in the field died after several years. Statistical differences within families and single-gall rust isolates were significant in some greenhouse experiments. Family differences were also significant for stem-girdling galls in some field plantings but not others; this inconsistent relationship was undoubtedly due to significant variation among replications.

Additional keywords: *Pinus elliottii* var. *elliottii*.

Fusiform rust (*Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Hedgc. & N. Hunt) Burdsall & G. Snow), the most destructive disease of southern pines, affects pines in many ways. Slash pine (*Pinus elliottii* Engelm. var. *elliottii*) seedlings die quickly when their terminal shoots are infected (13,17). Rust galls on older pines usually begin on the branches and then expand to the stem. The tree may recover or live for many years.

When rust galls form on the trunk of a slash pine after the infection has extended from the branches to the stem, the infection may cause a bulbous swelling above a constriction in the stem. Whether these stem-girdling galls occur on seedlings or older trees, they eventually cause death. Formation of stem-girdling galls on older trees can have a great economic impact on commercial tree production because infected trees may survive for years, using nutrients and competing with healthy trees, only to die before harvest.

Susceptible pine families often have high early mortality as seedlings, dying from galls that infect their terminal shoot (13). When resistant families are infected, branch galls tend to develop. Although infected trees from resistant families may survive longer, stem-girdling galls possibly will develop. When this happens, the advantage of using resistant trees can be offset by delayed mortality. Even the healthy trees that are harvested will be affected because of the effects of competition from the tree with stem-girdling galls.

Our first observations of large numbers of stem-girdling galls on slash pines were in a study of resistant slash pines at the U.S. Department of Agriculture Forest Service greenhouse in Gulfport, MS. These stem-girdling galls appeared to be the result of an incompatible reaction between pines and fusiform rust isolates, resembling the stem-tissue changes that are found in graft incompatibilities (1,2,8,10,11,16). The tissue damage in graft and pollen incompatibilities and stem-girdling galls include whorled cells and necrotic areas (18) that interfere with water and nutrient transport.

In this study we describe the occurrence and pathology of stem-girdling galls that formed on inoculated slash pines grown in the greenhouse. We also compare the incidence of stem-girdling galls caused by nine isolates of the pathogen among several slash pine families. Our objective was to determine the potential of stem-girdling galls to kill trees when family and inoculum are varied.

MATERIALS AND METHODS

Field tests. For the field comparison, the frequency of stem-girdling galls was observed on slash pines near Greenwood, FL, and Savannah, GA. At Greenwood, there were four replications of 10-tree row plots for each of 43 open-pollinated slash pine families. The number of stem-girdling galls was counted on all trees having stem galls at age 5 yr; findings were confirmed at 6 and 8 yr. Identities and mortalities of the pine families at age 8 yr were presented earlier (13). This Florida planting appeared to have been infected between the first and third growing seasons.

For the Savannah field tests, three replications of 10-tree row plots were observed for trees from 14 commercial slash pine families and two controlled bulk seed sources. Four families were rust resistant (20% or less of plantings from these families would develop fusiform rust infections). Four other families were rust susceptible (more than 40% of plantings from these families would develop fusiform rust infections). The remaining six families were intermediate in resistance (20–40% of plantings would develop fusiform rust infections). One of the bulk seed sources was susceptible; the other was intermediate. All observations were made just before the sixth growing season. These trees appeared to have been infected during the third or fourth growing season.

Greenhouse tests. Seeds for fusiform rust-resistant slash pine seed lots were selected from Forest Service (FS) and University of Florida (3) seed orchards; susceptible seed lots from these sources were included as controls. At Gulfport, the slash pine families included the 15 open-pollinated families described by Walkinshaw and Bey (14). In addition, seeds from the following open- and control-pollinated slash pines were tested for incidence of stem-girdling galls: Florida Cooperative trees 179-55, 293-55, 70-56, 79-56, 316-56, 346-56, 28-60, and B-210; FS trees 8-4, 8-7, 9-2, 18-27, J-1-3, La-11, and W-1-20; 16 crosses with FS female parent 8-7; 13 crosses with FS female parent 18-27; and susceptible FS control pines 18-62. A Mississippi bulk seed lot also was tested.

The inoculation of open-pollinated families at Gulfport used the nine single-gall isolates described by Walkinshaw and Bey (14). The design of three replications of eight seedlings per family per isolate was repeated for eight experiments. Control-pollinated crosses of pines 8-7 and 18-27 were inoculated with each of the two single-gall inocula: MS-15 and LA-7. These two field isolates were among the nine used for the open-pollinated families. The inoculations included three replications of 12 seedlings per family per field isolate. Inoculation procedures were identical for open- and control-pollinated slash pine families. Basidiospore concentration was held between 12 and 18 spores/mm square of surface.

Growing conditions were the same for all families. The percentage of stem-girdling galls was tabulated 6 and 9 mo after inoculation.

A test also was run at the Resistance Screening Center (RSC) at Asheville, NC, to insure that stem-girdling galls were not a result of the inoculation method used at Gulfport. Family and field isolate effects on occurrence of stem-girdling galls were measured. Pine families used at RSC were the FS La-11 and the Florida Cooperative's C-71, 179-55, 25-61, and 357-56. A susceptible bulk seed lot was used as a control. The five pine families used at the RSC were highly resistant and open pollinated. Twelve single-gall field isolates of rust were collected in Jackson County, FL, and used to inoculate the seedlings. Three replications of 10 seedlings per field isolate per pine family were inoculated according to the RSC standardized techniques (7,13). Basidiospore inoculum concentration was 20,000 spores/ml of inoculum.

Analysis of variance on families treated as fixed effects was performed on greenhouse and field data. Families were arranged in a randomized design with three or four replications.

For histological studies, stem-girdling gall specimens were taken from slash pines 6, 9, and 12 mo after greenhouse inoculation from three areas: the tapered region above the bulbous swelling, the zone of maximum swelling, and the constricted zone below the swelling. Specimens were fixed, dehydrated, embedded, and stained as described previously (12). Sections were cut 12 to 15 m thick. A 1% w/v stain of fast green, rose bengal, and acridine red in 50% ethyl alcohol was used to observe fungi in the tissues.

RESULTS

Field observations. The incidence of stem-girdling galls varied greatly among pine families in field progeny tests. In Georgia, the number of stem-girdling galls differed among families (significance probability = 0.003). The means for percentage of galls found to be stem-girdling galls were 5.0, 15.4, and 11.2 for the resistant, intermediate, and susceptible families, respectively. In Florida, 43 slash pine families were examined, and the percentage of galls that were stem-girdling galls ranged from 0 to 38. Main effects of families could not be specified because of significant variation in the replications. Ten of the 43 families that qualified for breeding in rust control programs are listed in Table 1. The mean percentage of stem-girdling galls for families in this resistant group was 21 compared with 22 for susceptible families. Family effects on percentage of stem-girdling galls were not significant in either group.

Greenhouse incidence. Stem-girdling galls devastated certain pine families in the greenhouse at Gulfport. The stem-girdling galls appeared as early as 3 mo after inoculation, and the majority formed by 6 to 9 mo. For example, stem-girdling galls were 43% of the total galls among specimens of one family 6 mo after inoculation and 90% 9 mo after inoculation (Table 2). Typically, stem-girdling galls in the greenhouse had rapid linear growth (8 mm per month) until 6 mo; then growth slowed to 4 to 6 mm per month. Most seedlings with stem-girdling galls died between 9 and 12 mo after inoculation, whereas seedlings with galls tapered at both ends lived 4 to 5 yr.

Figure 1 shows a stem-girdling gall on a 9-mo-old greenhouse seedling; Figure 2 shows one on a 5-yr-old tree. In the greenhouse, stem-girdling galls killed virtually all seedlings on which they appeared within 1 yr of inoculation. Although growth of these seedlings generally was not stunted 6 mo after inoculation, these seedlings became severely chlorotic between 9 and 12 mo after inoculation, and the stems below the gall became too weak to support the plant. Seedlings with galls tapered at both ends were not chlorotic or weak and survived beyond 1 yr.

Greenhouse isolate effects. Production of stem-girdling galls in the greenhouse at Gulfport was closely related to the nine isolates used for inoculation. Analysis of variance with a fixed model (eight families and five isolates) showed that isolate effects were significant (significance probabilities = 0.0001, 0.0001, 0.004, and 0.004) in four of eight experiments. One isolate was common to all experiments. The overall number of stem-girdling galls and the total number of galls were not correlated (r^2 = 0.0 to 0.4) among the nine field isolates.

Comparison of isolate MS-15 with LA-7 on 48 slash pine families (two isolates and three replications) showed that MS-15 caused fewer stem-girdling galls (mean = 10%) than LA-7 (mean = 36%). These means were significantly different at the 1% level. This difference in isolate effects also occurred when virulent isolate FL-3 (14) was compared with MS-15. High numbers of stem-girdling galls also were caused by another isolate (FL-3) described by Walkinshaw and Bey (14).

The isolate effect also was indicated, although not significant, at the RSC, where slash pine families were inoculated with the isolates from Florida. With these 12 isolates, percentage of stem-girdling galls ranged from 0 to 26%. Isolate effects were not statistically significant (f = 1.4), whereas family effects were significant at the 1% level.

Family effects in the greenhouse. Because incidence of stem-girdling galls in the field varied among families, we investigated

TABLE 1. Percentage of infection and stem-girdling galls on slash pines after the sixth growing season at Greenwood, FL[a]

Pine family	Percentage of trees galled	Percentage of galled trees with stem-girdling galls
C-184	33	38
C-71	45	8
C-200	47	17
C-201	32	38
25-61	40	28
C-163	73	23
C-213	53	17
41-62	25	0
M-601	58	22
C-115	42	22

[a] Means for four replications of 10 trees. The most resistant 10 families of the 43 observed are listed from highest to lowest rust resistance rank as assigned by the University of Florida Cooperative Forest Genetics Research Program. These families have rust index scores adequate for breeding. The r^2 for percent infection versus percent stem-girdling galls for the 43 families was 0.0.

TABLE 2. Incidence of stem-girdling galls presented as total percentage of galls in rust-infected seedlings inoculated and maintained 9 mo in a greenhouse at Gulfport, MS[a]

Experiment 1		Experiment 2	
Family	Percentage of stem-girdling galls[b]	Family	Percentage of stem-girdling galls[b]
8-7 × 293-55	62	8-7 × 18-14	85
8-7 × 18-62	95	8-7 × H-7	82
8-7 × La-11	75	8-7 × 294-55	40
8-7 × 357-56	87	8-7 × 357-56	86
8-7 × H-28	17	8-7 × 9-2	40
8-7 × 21-58	62	8-7 × 52-56	69
8-7 × 211-55	87	8-7 × 6-56	90
8-7 × 5-56	47	8-7 × 5-56	63
18-27 × 293-55	15	18-27 × 18-14	6
18-27 × 18-62	0	18-27 × H-7	6
18-27 × La-11	0	18-27 × 294-55	4
18-27 × 357-56	0	18-27 × 9-2	44
18-27 × H-28	82	18-27 × 52-56	18
18-27 × 21-58	0	18-27 × 6-56	0
18-27 × 211-55	4		

[a] Slash pines 8-7 and 18-27 are U.S. Department of Agriculture Forest Service trees near Gulfport, MS. Inoculum was from a single gall in an unimproved slash pine plantation near Bogalusa, LA. Growth conditions and inoculations were identical to those described by Walkinshaw and Bey (14). Three replications of 12 seedlings were used.

[b] The analysis of variance mean squares of experiment 1 and experiment 2 for the model were 3,852 and 2,750, respectively, and for the error they were 665 and 314, respectively. Family effects had significant probabilities of P = 0.0001 in both experiments.

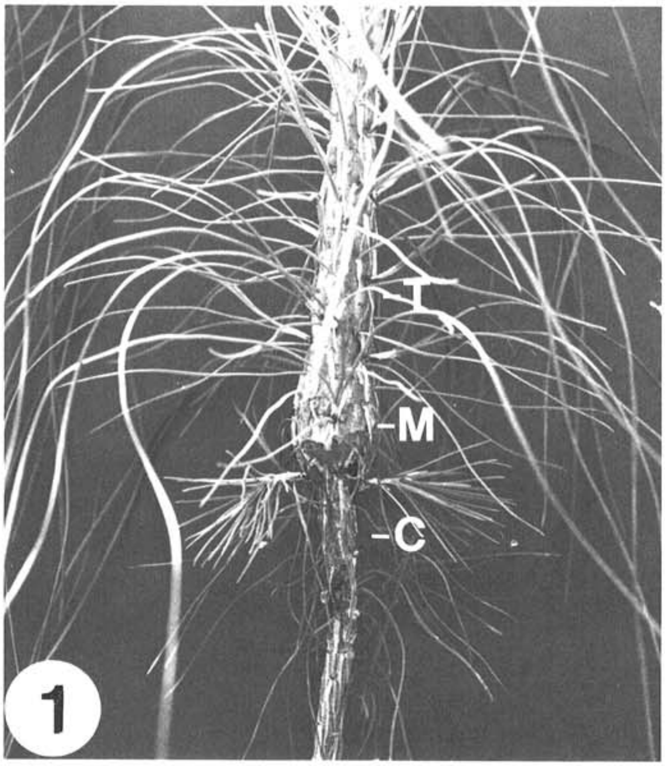
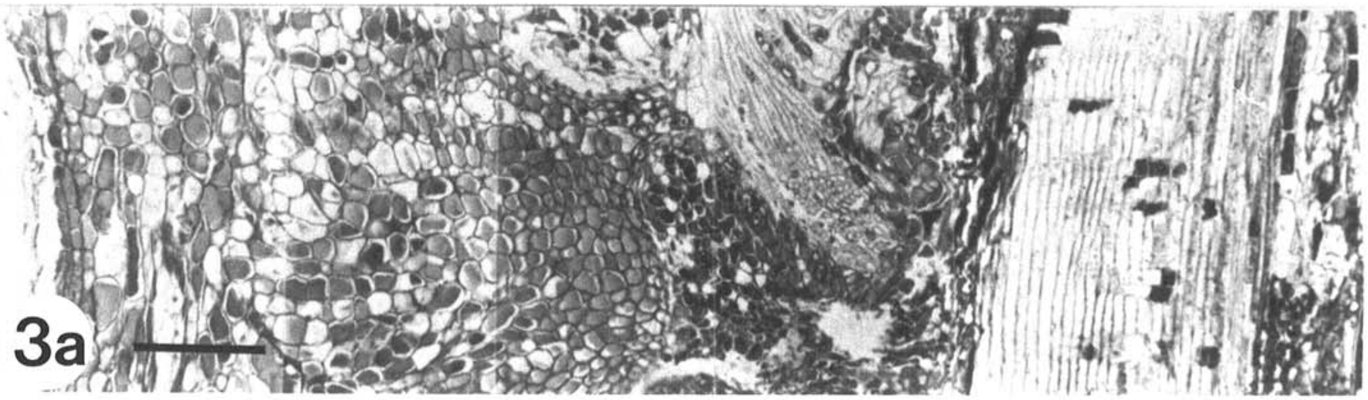


Fig. 1. Nine-month-old slash pine with a stem-girdling gall. Histological observations were made in the tapered upper zone (T), zone of maximum swelling (M), and constricted basal zone (C). The two adventitious shoots mark the point of maximum cambial disorganization.

Fig. 2. Developing stem-girdling gall on a 5-year-old slash pine in the field.



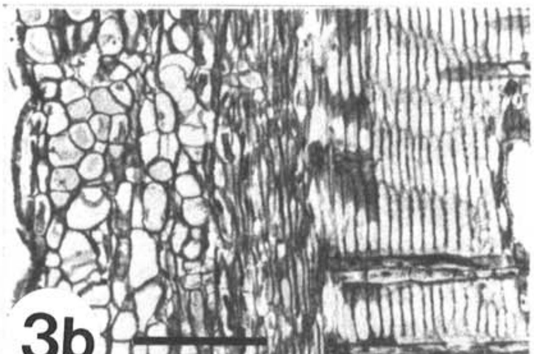


Fig. 3. Comparison of the cortex and xylem in: **A,** a stem-girdling gall, and **B,** a healthy seedling. The cortex is three times wider in the gall and has massive cellular disorganization 7 mo after inoculation. Bar = 200 μm.

family effects in the greenhouse. In eight experiments with open- and control-pollinated seedlings from more than 50 field-resistant families, family effect on incidence of stem-girdling galls was significant in five of eight inoculations. The main effect of family for two of these five inoculations is questionable because family × inoculum interaction was significant. Slash pine 8-7 formed more stem-girdling galls than any other pine family (Table 2). Slash pine 18-27, with similar field resistance to 8-7 (4), had an average of 14% stem-girdling galls compared with 68% for 8-7.

Histology of stem-girdling galls. Overgrowth of the stem galls in greenhouse and field trees indicated massive disorganization of tissues. To verify this assumption, the internal structures of 6- to 12-mo-old galls were examined. Disorganized tissues were divided into three zones for histological study: the tapered apical region, the middle zone that exhibited maximum swelling, and the constricted basal zone. The degree of disorganization was readily apparent by a comparison of stem-girdling gall tissue (Fig. 3A) and normal tissue (Fig. 3B); note the whorled formation and proliferation of affected cells in the stem-girdling gall tissue.

The tapered upper region of the stem-girdling gall (Fig. 4A) contained dense starch granules in cortical and ray parenchyma cells. Fungal hyphae in this zone often were dense and growing actively, as they were in galls tapered at both ends. However, tissue in the upper part of the taper did not contain the fungus, and the cambial region was not disorganized. Transverse sections through the zone of maximum swelling contained numerous cells that resembled callus (Fig. 4B) and abundant fungal hyphae, but no cambium and xylem fibers. Tissue in the constricted zone contained large numbers of necrotic cells and cells filled with tannin or other ergastic materials (Fig. 4C). Tissues were disorganized, and the cambium was distorted or missing. Fungal hyphae were numerous or dying. For comparison, a transverse section through the cambial region of a typical gall is shown in Figure 5. Note hyphae (arrows) and the tissue organization. Details on the anatomy of typical galls on slash pine are provided by Jewell and Speirs (5) and Jewell et al (6).

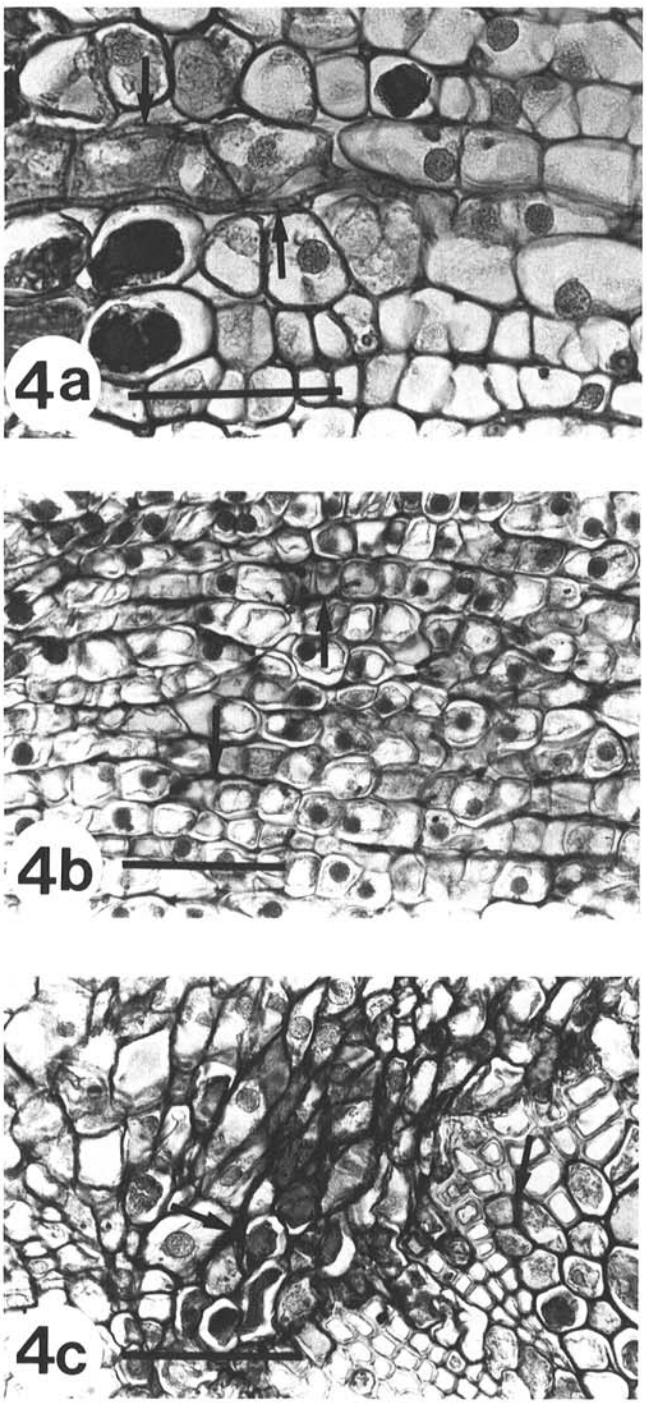


Fig. 4. Histology of: **A,** tapered upper zone, **B,** zone of maximum swelling, and **C,** constricted basal zone. Note the fungus hyphae (arrow) in the tapered upper zone. Rapidly dividing callus cells with prominent nuclei characterize the zone of maximum swelling. Necrosis and lack of an identifiable cambium are markers of the constricted zone. Bar = 200 μm.

DISCUSSION

Although occurrence of stem-girdling galls clearly appears to vary among families in the field, the interaction of family by location and family by replication may nullify family effects. The interactions are compounded by large variations in initial infection among replications and between locations.

Stem-girdling galls readily formed on slash pine seedlings in greenhouses at Gulfport and at the RSC. Because methods of inoculation and spore densities differed markedly at these locations, the formation of stem-girdling galls appears unrelated to inoculation methods. Transition from typical galls (those tapered at both ends) to stem-girdling galls occurred after 3 mo at both locations.

Greenhouse infection of many pine families led to the death of the seedlings within 1 yr. Thus, growth of select pathotypes may be stopped by early death of their host. Alternatively, many trees with small galls, by surviving longer, may give virulent pathotypes time to multiply. This possibility needs to be considered in the development of resistant pine families to control fusiform rust.

Results of variation in frequency of stem-girdling galls on slash pine families agree with those of Layton (9), who found that family effects were significant ($P = 0.05$) in greenhouse inoculations. The frequency values we obtained for slash pine family 8-7 were higher than for any of Layton's families. Moreover, family 8-7 had the highest number of stem-girdling galls in our study. This pine exhibits a high coefficient of variation for percentage of seedlings exhibiting galls when inoculated with a number of field isolates (14).

On the other hand, families such as 18-27 generally vary little among inocula in the percentage of galls formed and had a low percentage of stem-girdling galls. Our previous work (4) strongly suggests that these two families, 18-27 and 8-7, which are about equal in field resistance, have different compatibilities with the rust isolate. In family 8-7, the fungus has a greater chance of forming an incompatible reaction that results in a stem-girdling gall. In family 18-27, field isolates cause more typical galls and significantly fewer stem-girdling galls.

Starch accumulation in stem-girdling galls parallels that reported for incompatible grafts in citrus seedlings (15). In stem-girdling galls, the complete disorganization originates in the gall rather than in healthy tissue. First, abnormal cambial activity

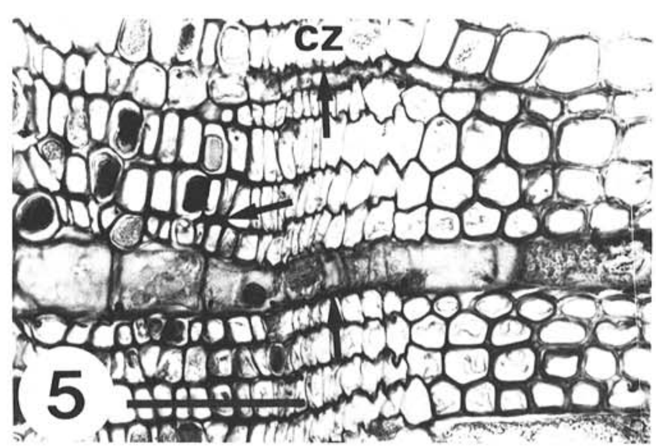


Fig. 5. Transverse section of a 9-mo-old typical gall sectioned at the area of maximum swelling. Anatomy was described in detail by Jewell et al (6). Note the difference in cell organization in the cambial zone (CZ) in this gall compared with the stem-girdling galls shown in Figures 3A and 4. Bar = 200 μm.

appears to cause the tissue to become disorganized. Then, in both the swollen and constricted areas of stem-girdling galls, the cambium becomes a mass of undifferentiating cells incapable of producing normal fibers. Numerous wound-callus cells at the maximum swelling interfere with normal translocation.

In this study we found that the incidence of stem-girdling galls can be high in certain family × inoculum combinations in the greenhouse. Both susceptible and highly resistant slash pine families can have a high or low incidence of stem-girdling galls. The incidence of stem-girdling galls in resistant families varied widely, but certain inocula seemed to promote their development. Because stem-girdling galls kill older slash pine trees in the field, specialists in tree breeding should avoid developing families that concentrate the genes that enhance the formation of stem-girdling galls. Detection of these genes is easier in the greenhouse because inocula cannot be controlled in field tests.

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