Histopathology of One- and Two-Year-Old Resisted Infections by Cronartium fusiforme in Slash Pine

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ABSTRACT


Anatomical resistance to Cronartium fusiforme 1 and 2 years after artificial inoculation of slash pine seedlings 5-7 weeks of age was expressed by localized zones of dark-staining living and nonliving tanninized host cells. Resistance-zones were delimited by atypical vertical (reaction) parenchyma. Extent of zones depended on age of the host sample, with greatest expression occurring in 1-year-old infected seedlings. A regression of zones in 2-year-old samples, in some cases to only remnants remaining deep in the xylem next to the pith, indicated the probability of complete recovery with increased host age and growth. Hyphe of C. fusiforme were limited to the resistance-zones and were considered viable in bordering reaction parenchyma. Although hyphe were common in the tanninized cells of the resistance-zone, hyphe appeared to be nonfunctional.

Additional key words: pathological anatomy, Pinus elliottii var. elliottii, rust resistance.

Evidence of resistance and its variability in slash pine, Pinus elliottii var. elliottii Engelm., to Southern fusiform rust (caused by Cronartium fusiforme Hedge. and Hunt ex Cumm.) has been well documented. Mainly, the evidence was based on macroscopic examination of seedlings 6-12 months after inoculation in which the absence, presence, shape, and size of galls were evaluated (9, 16, 18). Some of the test seedlings bore galls atypical in size and shape for the species of pine or rust involved, whereas other seedlings classed as galled at 6 months had no obvious sign of galls at 12 months. These were considered to have “recovered”. An earlier report of apparent rust recovery in slash and loblolly (P. taeda L.) pines (15) was substantiated by additional observations of tissue reactions considered to be anatomical mechanisms of the pine host for resistance to C. fusiforme (6).

Subsequent histological studies of slash pine seedlings from rust-resistant parents 1 and 2 years after inoculation in part have confirmed previous findings (6, 12), but variances with these reports also were observed.

MATERIALS AND METHODS

The tissue examined was from wind-pollinated progeny of two slash pine parents reported resistant or potentially resistant to fusiform rust (5, 14). The seedlings had been inoculated at 5-7 weeks of age using the technique of Snow and Kais (17) with inoculum originating from typical galls on slash pine. Samples were taken 1 and 2 years after inoculation from seedlings classed either as galled, recovered (inactive), or small (atypical) galls. The samples were taken from the stem area that was previously inoculated (infection area). Three to five seedlings of each classification were examined. All seedlings used had borne stem or foliar symptoms typical of C. fusiforme infection (4). Tissue was processed and stained in an osceillin BB and aniline blue schedule for microscopic examination by previously reported techniques (7, 8).

RESULTS

Cronartium fusiforme was found to be or to have been established, at least to some degree, in all the seedling collections examined.

Samples with resistance-zones.—Stem samples from 1- and 2-year-old infection areas on seedlings classified as recovered or having small (atypical) galls appeared, macroscopically, to be rust-free, except for certain irregular stem characteristics in the infection area, including slight-to-severe stem crook with no swelling, small swellings, bark cracking and/or discoloration, and resin exudation [Fig. 1-(A, B)]. Transverse sections of the affected stem areas revealed one or more necrotic-appearing areas in the bark and/or xylem [Fig. 1-(C, D)]. Frequently, symptoms on these seedlings resembled damage resulting from attack by insects of the genus Pityophthorus. The necrotic areas when observed microscopically, were somewhat similar to the restricted reaction areas previously reported in the xylem of individual slash pine seedlings expressing resistance to C. fusiforme (6). Present observations show considerably more host tissue involved in the resistant reaction than formerly reported, and for clarity the term “resistance-zone” will be used to identify this tissue. Often this zone extended from the pith to the epidermis, or apparently
Fig. 1- (A, B). Slash pine stem samples exhibiting resisted Cronartium fusiforme infections. A, B) External stem characteristics indicating resistance 1 year after infection (×4). C, D) Macro-transverse sections with internal necrotic characteristics of 1- and 2-year-old resisted infections, respectively (×6). E, F) Micro-transverse view of the necrotic areas of C, D. Note difference in radial extent of abnormal (a) and normal (n) phloem in E and inward bending of host cambium in F (×18).
Fig. 2-(A to F). Photomicrographs of slash pine showing the resistance-zones and their cellular components. A, B) Respectively, transverse and longitudinal views of resistance-zones originating at the pith, with B showing leaf tracelike development (×40). C) Transverse view of dark-staining reaction parenchyma at periphery of resistance-zone. Note tracheidlike cell files (×150). D) Longitudinal view of dark-staining multi-type reaction parenchyma cells in xylem (×70). E) Transverse view of reaction parenchyma with radial and vertical intercellular hyphae (×320). F) Tanninized (T) area of resistance-zone with nonfunctional hyphae (h) and haustoria (ha) and thick-walled ray parenchymalike cells. N = normal tissue (×200).
was present only in the cortex or in the xylem at various distances from the pith tissues [Fig. 1-(E, F)]. In the latter case, where individual cortical and xylem resistance-zones were radially opposite, they were separated by host xylem free of the type of tissue associated with the resistance zones. This occurrence was uniformly observed in the 2-year-old infections, whereas continuous zones from pith to cortex were common in the 1-year-old infections. Regardless of sample age, the resistance-zones, particularly the xylem zones, were found to originate in the outer cell layers of the pith, which were portions of the metaxylem present in the primary plant body at time of inoculation [Fig. 2-(A, B)]. Origin of resistance-zones was most obvious in longitudinal sections in which zone tissue extended laterally and vertically at an oblique angle from the point of initiation at the pith into secondary xylem (Fig. 2-B), often continuing into the outer cortex. Transverse serial sections through similar tissue areas showed resistance-zones apparently originating at various radial distances. Exclusive use of transverse sections was found to be misleading in relation to the origin of resistance-zones, particularly if only a portion of a sample was examined.

A striking feature of resistance-zones was their sharp delimitation from adjacent normal host tissue by a series of mostly dark-staining parenchyma-like and/or tanninized cells (reaction parenchyma), often in radial files resembling normal cellular structuring of pine tracheids [Fig. 2-(A, C)]. In samples with the resistance-zone continuous from the interior xylem through the cortex, the cambial cylinder was interrupted for the width and vertical extension of the resistance-zone in contact with host cambium (Fig. 1-E). This was common with 1-year-old infections, whereas in 2-year-old samples an inward curvature of apparently typical cambium was observed opposite a cortical and/or xylem zone, indicating previous injury to, or interruption of, the cambium in that particular area with subsequent restoration of normal cambial activity (Fig. 1-F).

Complete “tannization” of groups of cells, which were considered nonliving, was common in portions of the resistance-zones, whereas other portions contained cells that appeared to have been living (functional) parenchyma at fixation, but had much darker-staining cell contents than normal pine parenchyma (Fig. 1-E). Tanninized cells were usually centrally located in the resistance-zone or at its outer limits, and were surrounded, in transverse section, by a somewhat wedge-shaped mass of the dark-staining parenchyma (Fig. 1-E). Initially, utilizing transverse sections exclusively, this type of parenchyma was considered similar to the “reaction (vertical) parenchyma” described in rust galls on slash and shortleaf pine (6). However, longitudinal sections revealed not only vertical reaction parenchyma, but also areas of small and nearly isodiametric parenchyma cells (Fig. 2-D). These cells could be mistaken for vertical reaction parenchyma in transverse view only. Thus, reaction parenchyma of resistance-zones is composed of different types of parenchyma cells not just vertical parenchyma as previously reported (6).

Although there was an apparent lack of an organized tissue system for continuance of radial and vertical production of cells in resistance-zones, sufficient lateral extension of the zone occurred to more or less keep pace with the normal growth pattern of the host. This suggested cambium-like or meristematic activity by certain of the reaction parenchyma cells. This feature resulted in little, if any, noticeable effect on the usual cylindrical development of the stem. Some flattening of the host stem in the area of the zone was observed in 1-year infections, but this was much less evident in the 2-year samples [Fig. 1-(C, D)].

Although establishment of *C. fusiforme* had occurred in samples studied, rust was found to be confined to the resistance-zone, except in one instance in transverse view where the rust fungus was observed one to two cells beyond the zone border. This was not considered significant. In the zone tissue, *C. fusiforme* hyphae were abundant in the reaction parenchyma and, due to staining response, were considered viable. Both radial and vertical intercellular hyphal growth had occurred in the zones. Vertical hyphal orientation was particularly evident in the reaction parenchyma of the host as observed in transverse section (Fig. 2-E). This feature of *C. fusiforme* growth in pine has not been previously emphasized.

Haustoria, usually abundant in typical *C. fusiforme* gall tissue in slash pine (7), often were difficult to observe, and were considered scarce in relation to the abundance of hyphae present. Possibly, haustoria were missed due to the unusually dark-staining parenchyma cell contents, but this is considered unlikely. Hyphae and haustoria were abundant in tanninized areas of the zone, but from their staining characteristics and general appearance were considered to be nonfunctional. Degeneration and hypertrophy of the fungus was evident, but hyphae and haustoria were usually quite recognizable (Fig. 2-F), as reported previously for this and other pine-gall rusts in similar tissue (7, 8, 10). In tanninized tissues, hyphae and haustoria were apparently affected by the same xylene-insoluble substance that caused tannization of host cells, the host and parasit had similar staining reactions. Origin or purpose of tannin production in the host is uncertain, as other reports have indicated (8, 19, 21). This tissue usually is limited in extent and sharply delimited from adjacent host cells (Fig. 2-F). Possibly, this is a type of initial defense response of the host to the establishment of *C. fusiforme*, which resulted in stimulation of certain meristemlike cells of the host.

The tannin-infused areas contained many cells that resembled ray parenchyma in outline, but lacking nuclei or normal cell contents. These cells had abnormally thick walls (Fig. 2-F). Cell dissolution was noted in deep xylem tannin areas, and was frequent near the cambium, as well as in the cortex. This dissolution probably resulted from splitting and tearing produced by lateral and vertical growth pressures from the surrounding normal host tissue [Fig. 1-(E, F); 2-(A, B, F)].

A major feature of 2-year-old infections not present in 1-year-old resisted infections was the radial separation of resistance-zones by the usual type of pice xylem tissues—tracheids and xylem rays—and re-establishment of the complete cambial cylinder. A reduction in size of the zone area occurred in relation to the total area of the host stem [Fig. 1-(E, F)]. Usually, these xylem tissues were not typically oriented. This was particularly obvious with tracheids formed early in the growth process that resulted in separation of the resistance-zones into xylem and cortical areas in the 2-year-old samples. In transverse
sections, several rows of tracheids had been laid down with their long axis horizontal rather than parallel to the vertical axis of the stem. This development was initiated at the outer edge of the deep xylem zone. Tissue orientation slowly approached normality as lateral growth progressed, presumably from gradual restoration of typical cambial development and activity. Subsequent tracheid development approached or reached the characteristic vertical alignment in the stem (1, 2) although size, shape, and radial alignment of these cells often was atypical for a considerable distance toward the restored vascular cambium (Fig. 3-A). Atypical orientation and appearance of tracheids indicates a similar abnormality in their precursor cells. Since no definable cambium was present to form the atypical tracheids in the former area of the resistance-zone, we concluded that a substitute type of meristematic system had been active for their formation. The result of atypical cell deposition by the host was to bury the inner portion of the resistance-zone deep in the xylem where it would be preserved indefinitely in a nonfunctional state, as would any remaining portions of C. fusiforme present in the inner zone tissue.

Resistance-zones often extended through the host phloem and cortex to the epidermis. These zones normally were surrounded by a well-defined periderm, consisting mainly of tanninized cells with peripheral reaction parenchyma [Fig. 3-(A, B)]. Frequently, primary tissues were found embedded in outer portions of cortical zones, which indicated that initiation of the resistance-zone occurred in the area of primary tissue formation (the pith area) [Fig. 3-(B, C)]. Often these zones were located immediately beneath, or had been forced by normal cortical growth, through the host epidermis. Previously, similar observations have been made for slash pine infected with C. fusiforme, and other pine-gall rust combinations, but interpretation of function and development of the periderm and initiation of cortical areas of infection differed from the present work (11, 12, 20). This development, more common in the 2-year-old infections, resulted in sloughing-off the cortical tissue (Fig. 3-D). Frequently, in transverse sections, resistance-zones appeared to be isolated in the cortex and not to have a corresponding xylem zone present (Fig. 3-B). However, examination of sections of stem portions below the isolated cortical zones would reveal an isolated xylem-zone obliquely below, but on the same radius of the stem as, the cortical zone. This strongly suggests an upward and outward movement of the cortical zone during its separation from the xylem-zone, much as leaf traces form in pine (1, 2).

Hyphae and haustoria of C. fusiforme were observed in tissues of the resistance-zones in the cortex. The fungus was considered nonfunctional in tanninized cells of the zone and viable in the peripheral reaction parenchyma and xylem zones. Of particular interest was the apparent potential for intra-cortical growth of C. fusiforme observed in limited areas of the outer reaction parenchyma of certain samples (Fig. 3-E). Whether this hyphal growth would result in re-establishment of C. fusiforme in the normal host tissue is uncertain.

Unusual cellular characteristics were observed in phloem adjacent to resistance-zones of certain of the 1- and 2-year-old samples. Although, C. fusiforme was not observed in the abnormal phloem areas, the cellular characteristics of these areas, more frequent in the 1-year samples, were similar to gall-phloem described for this rust on slash pine (7): hypertrophy, increase in number of phloem parenchyma, and increased radial thickness of phloem (Fig. 1-E). The abnormal phloem often extended up to 1 mm radially along the cambium away from the zone and graded into a normal phloem configuration for slash pine (Fig. 1-E). There is a suggestion from these observations that the abnormality was formed in advance of, or in the absence of direct contact with, the fungus. Previously, abnormalities in slash pine have been reported only in direct relation to the presence of hyphae of C. fusiforme (7).

In both 1- and 2-year-old samples, where considerable tissue dissolution had occurred in resistance-zones associated with cambial-cortical areas, concentrations of highly active parenchymalike cells were observed at the edges of these zones [Fig. 3-(F-H)]. Cells were uniformly thin-walled, irregular in size and shape, and with little or no intercellular space. Overall appearance of these cellular areas was similar to callus-culture cells of slash pine (22), and their function in relation to the resistance-zones appears to be to form a protective callus over the host tissue that was exposed by the disintegration of the resistance-zone. Callus development was most pronounced in areas where the host cambium was absent or badly disorganized and the cortex and epidermis were ruptured [Fig. 3-(F, G)]. Callus cells usually underlay the periderm that bordered the resistance-zone and appeared to be initiated mainly by parenchyma of xylem or phloem rays, resin ducts, or cortex. However, callus cells often were observed being generated by the reaction parenchyma that bordered the resistance-zone; this indicated a meristematic capability of at least certain of these unusual parenchyma cells [Fig. 3-(F, G)]. Proliferation of callus cells exerted a pressure on portions of the periderm bordering the zone causing it to tear, and particularly on the phellem which commonly was crushed [Fig. 3-(G, H)].

Samples without resistance-zones.—Samples from galled seedlings had tissue configurations that were considered characteristic for susceptible slash pine (7). Without exception, gall tissue proximal to pith was delimited by one-to-several cell layers of vertical reaction parenchyma and characterized by hypertrophy and hyperplasia of host cells outward through the cambium and phloem into the cortex. Tissue sampled from typical rust galls showed no host reaction (other than a few instances of very limited cortical necrosis) that would prevent or restrict the normal development of C. fusiforme, even though the seedlings were from resistant parents.

Stem samples from two seedlings with 2-year-old infections and classed as galled were of interest in that microscopic examination revealed that a considerable portion of the stem was normal slash pine tissue, whereas the remainder of the stem was characteristic of C. fusiforme gall tissue [Fig. 4-(A-D)]. Distinct separation of gall and normal tissue was obvious. Even though restricted in area, gall tissue had apparently developed typically (7). Other than vertical reaction parenchyma separating the gall and normal tissue, no extravagant
Fig. 3-(A to H). Photomicrographs of xylem and cortical features involved in recovery of resisted *Cronartium fusiforme* infections in slash pine. A) Transverse section showing resistance-zone (rz) in cortex and xylem separated by typically (t) and atypically (at) oriented xylem tracheids (×40). B) A resistance-zone, which was pushed through the epidermis by normal radial host growth, in the outer cortex. Note primary tissue (p) in zone (×48). C) Enlarged portion of B showing the remnants of primary needle tissue (p) buried in the zone (×70). D) Cortical cavity caused by loss of a 2-year-old resistance-zone, the remnants of which remain buried in the deep xylem tissue. Note inward bending of cambium and first annual ring (×18). E) Viable *C. fusiforme* hyphae (h) extending from reaction parenchyma into normal cells of the cortex (×150). F, G) Meristematic cells (callus) forming to heal wound in cambial area and inner cortex caused by a resistance-zone. Note meristematic activity of reaction parenchyma (r) (×30). H) Enlarged portion of G (×73).

Production of the multitypes of reaction of parenchyma associated with resistance-zones in sibling progeny was observed. Unusual off-center location of pith indicated a considerable differential between normal and gall tissue in radial and tangential activity of the cambium.

**DISCUSSION**

Expressions of anatomical resistance, characterized as resistance-zones, were recognizable features of 1- and 2-year-old *C. fusiforme* infections in some progeny of two rust-resistant slash pine parents. Resistance-zones in the

Fig. 4-(A to D). Transverse sections of unusual samples from two progeny rated "galled". A, B) Sections of 2-year-old stem gall showing relation of gall (g) to normal (n) stem tissue. Note offset pith (p) in A (×6). C, D) Sections showing sharp delimitation of gall from normal stem tissue (×18).
samples studied were basically uniform in the type or
color of abnormal host stem tissue developed in the
zone, although some age-difference variations were
observed in their overall appearance. Progeny of the two
parent trees did not differ in resistance reaction, nor were
types of reaction to infection identifiable as has been
reported for C. fusiforme and other pine gall rusts (3, 12).
Apparently, development of resistance-zones prevented
the concurrent growth of C. fusiforme and the pine host
that is considered characteristic of this and other conifer
rusts (7, 10, 13).

In general, C. fusiforme was limited in extent within
resistance-zones; some areas were heavily colonized and
other areas of the same zone had little or no obvious rust
genus development. Of interest was the apparent
viability of the fungus and its relation to reaction
parenchyma bordering the portion of the resistance-zone
associated with host cambium interruption common in the
1-year-old infections (Fig. 1-E, 2-E). Hyphae were
observed among the zone cells both radially and
vertically, indicating at least some growth of the fungus in
conjunction with development of the reaction
parenchyma. Typically, such hyphal growth is
accompanied by numerous haustoria in the affected host
parenchyma cells of rays, resin ducts, phloem, and cortex.
However, development of haustoria in the reaction
parenchyma that were examined was limited in relation to
the amount of hyphae present and to the nearly uniform
mass of parenchymalike cells of the zone. The reaction
parenchyma present probably was unsuited for haustorial
formation. It would follow that because the usual
nutrient-absorbing capability (haustoria) of the fungus
was reduced, vigor and abundance of hyphae would be
adversely affected. In general, this was true in the reaction
parenchyma except for localized tissue areas in which
hyphae were observed that were considered (from their
staining and morphological features) to have the
capability of growth and survival even though haustoria
were limited in number. This host-parasite relationship
suggested the possibility that C. fusiforme in the restricted
environment of the resistance-zones may assume, at least
partially, a saprophytic-type relationship in the zone
rather than the strictly parasitic association considered
characteristic for this rust species. Such a hypothesis
might explain the apparent inability of the rust to extend
significantly into host tissue beyond the limits of the
reaction parenchyma bordering the resistance-zones.
Further conjecture would be that the stimulation
considered necessary for extension of C. fusiforme and
other pine rust (7, 8, 13), was prevented due to absence of
characteristic host cambium and/or its derivatives in the
zone area.

The differences observed between 1- and 2-year-old
resisted C. fusiforme infections in the slash pine progeny
that were studied are concluded to be a regression of the
resistance-zone with increased host age and growth.
Tissues of the zone have limited regenerative capabilities
and are simply overgrown by the more uniform and
continuous normal tissue production of the host. It is
further concluded that, with continued normal growth
and development, resistant progeny of the host would
completely recover from C. fusiforme infection. The only
remnant of the initial infection would be the remains of
the resistance-zone buried deep in the xylem.

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