Histopathology of Fusiform Rust-Inoculated Progeny from (Shortleaf × Slash) × Shortleaf Pine Crosses

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ABSTRACT

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Progeny from (shortleaf × slash) × shortleaf pine crosses, inoculated at age 6 wk with fusiform rust, were sampled and examined histologically 1 yr following treatment. Similar uninoculated progeny were used as controls. No consistent reaction of the host progeny to the rust pathogen was observed. In general, three distinct host-parasite relationships were identified: typically susceptible, with host characteristics typical of the rust gall anatomy of a susceptible pine-fusiform rust interrelationship; pseudoresistant, with the host expressing normal tissue deposition and anatomical features (resistance zones) considered limiting to the advance of the pathogen, but from which it egressed to establish typical gall-forming tissue in the host; and resistant, with evidence of pathogen establishment in the pith area of the host followed by the formation of normal-appearing tissues through the xylem to the cortex area and no indication of pathogen viability evidenced in the affected host tissue. This third type of host reaction suggests that reliable resistance to fusiform rust can be obtained by backcrossing certain southern pines.

Shortleaf pine (*Pinus echinata* Mill.) has been acknowledged to be highly resistant to *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (1), the southern fusiform rust pathogen, and thus was considered a promising source of fusiform rust resistance in southern pine-breeding programs (3,4,7). However, susceptibility to fusiform rust was subsequently reported for progenies from crosses of shortleaf pine with slash pine (*P. elliottii* Engelm. var. *elliottii*), a normally rust-susceptible species (5,9,10). Histopathological studies of fusiform rust-infected (galled) progenies from such crosses reported anatomical host reactions of two types: typically susceptible and pseudoresistant (13). Apparently, the presence of fusiform rust-resistant shortleaf parentage (theoretically 50%) in the progeny was not sufficient to interfere with the establishment and gall-forming process of the rust pathogen.

The purpose of this research was to study the effect of fusiform rust resistance in shortleaf pine progenies of shortleaf × slash hybrid parents backcrossed to shortleaf pine. Progenies of this type, theoretically a mix of 0.33% slash pine and 0.67% shortleaf pine, possibly would express anatomical reactions to fusiform rust infection differing from the reactions previously reported for F_1 progenies of shortleaf × slash and slash × shortleaf pine (13).

MATERIALS AND METHODS

Progeny from four shortleaf × slash hybrid parents pollinated with bulked shortleaf pollen were inoculated, at the age of 6 wk, with *C. q.* f. sp. *fusiforme*, as described previously (8). Control progeny were of like seed sources and age but were uninoculated. Tissue was sampled 1 yr after inoculation, from five controls and 10 each of progeny with typical fusiform rust galls, atypical galls, and no perceptible gall development. Of the inoculated progeny, only those exhibiting initial symptoms of rust infection were sampled (8). Tissue processing for light microscopy was by previously reported techniques (13,17,18). Entire samples were serially sectioned at 15 μm. Staining was by a revised schedule with orseillin BB and aniline blue (17): the samples were prepared with xylol, two times, to remove paraffin; graded ethyl alcohols (ETOH) from 100 to 35%, 15 sec each; 0.5 or 1.0% orseillin BB (C.I. 26670) in 3% aqueous acetic acid, 24 hr; 50% ETOH, to destain until no color ran from the slide; 70, 95, and 100% ETOH, 15 sec each; 0.25 or 0.5% water-soluble aniline blue (C.I. 42755) in Methyl Cellosolve, 5–10 min; 100% ETOH, to rinse; 100% ETOH, 10 sec; and Vaughn's solutions (24), 15–30 sec each, to rinse and clear; xylol, overnight; and mount in Canada balsam.

RESULTS

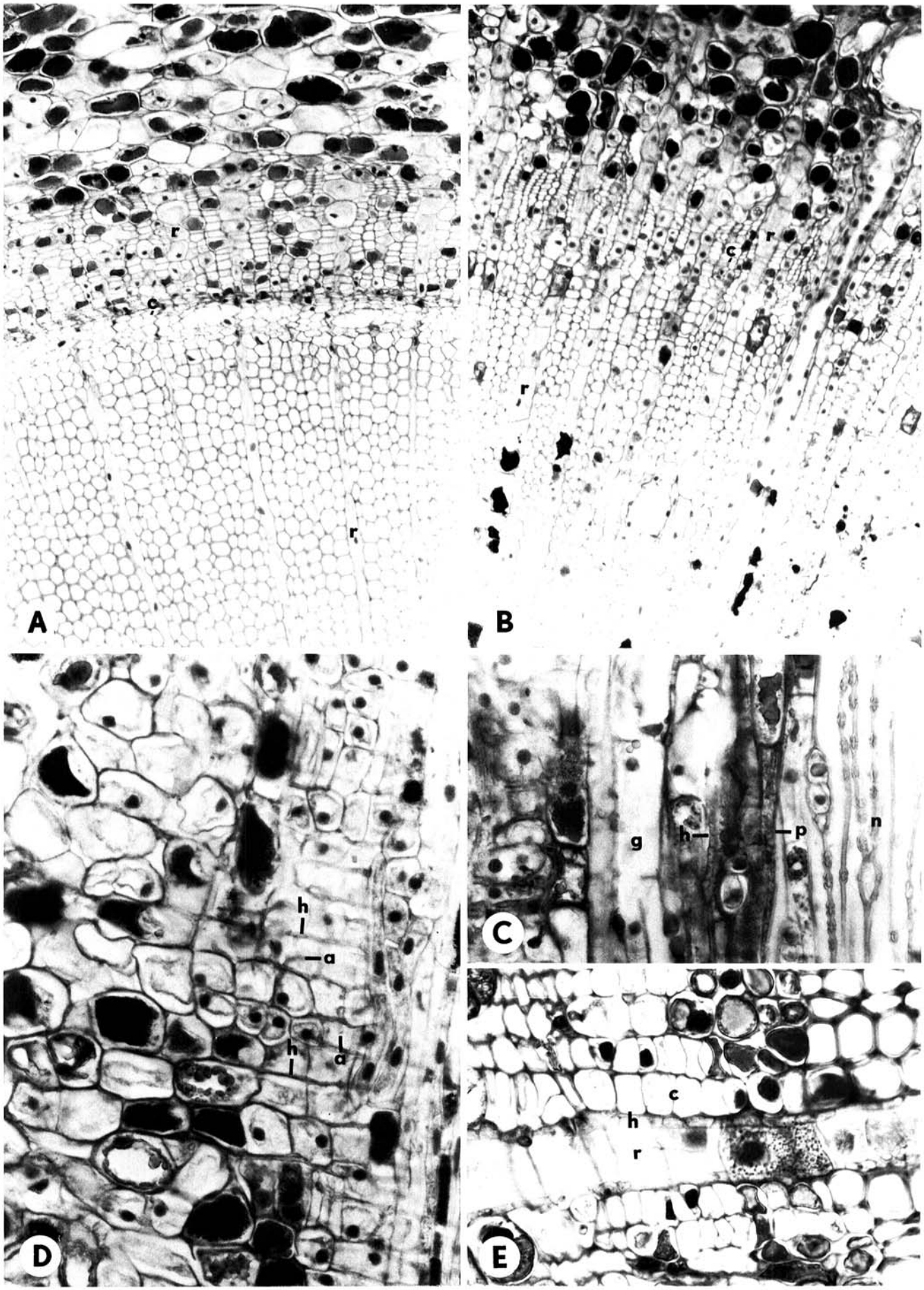
Tissue samples of uninoculated progeny revealed anatomical features considered typical of *Pinus* (2), and no pathological abnormalities were observed (Fig. 1A).

Inoculated progeny exhibited one of three distinct host tissue reactions: typically susceptible, pseudoresistant, and resistant. Although the reaction types are similar to previous results (13,19), the tissues of the present study revealed specific characteristics unique to the backcross progeny.

Typically susceptible reaction. The typically susceptible reaction was observed in fusoid galls typical of those caused by the fusiform rust pathogen, with anatomical characteristics similar to those of gall tissue of loblolly (*P. taeda* L.) and slash pine (6,8,17). Compared to the controls, gall tissue exhibited higher (having more cells tangentially) and more numerous xylem and phloem rays, xylem tracheids atypical in length and shape, an increased number of phloem parenchyma cells, hyperplasia in the cortex, and little or no interruption of the cambial cylinder (Fig. 1A and B). Reaction parenchyma (17) defined the inner extent of penetration by the pathogen and abnormalities of host tissue in the xylem (Fig. 1C). Seldom did the pathogen occur in the pith. Whorls or tracheids and rays, common in shortleaf pine galls of *C. q.* f. sp. *echinatae* (1) and in interspecies progeny with shortleaf in the parentage (7,18), were infrequent.

Hyphae and haustoria were well established in the gall tissue and appeared typical of fusiform rust (6,17) (Fig. 1D). Pathogen contact with the secondary cambium, necessary for continued gall development (17), was exhibited frequently around the circumference of the gall (Fig. 1E). There was no evidence, in the

Fig. 1. A and B, Transverse sections of rust-free (control) and typically susceptible progeny 1 yr after rust inoculation, respectively. In B, note the increase in ray cell size, the increase in the number of rays (r) in the xylem and phloem, and the absence of disruption of the cambial cylinder (c) (×122). **C,** Reaction parenchyma (p) in the xylem, separating gall tissue (g) from normal host tissue (×248). **D,** Typical intercellular hyphae (h) and intracellular hyphae (a) in the host tissue (×248). **E,** Hyphae (h) in contact with the cambial area (c) along a phloem-xylem ray (r) (×520).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samples examined, of incompatibility of the pathogen with the host tissue.

Pseudoresistant reactions. Macroscopically, in galls of the pseudoresistant type, only portions of the circumference of the stem were swollen. Often, these galls exhibited a flat surface extending into swollen areas and then normal-appearing stem tissue.

Internally, pathological tissue was associated with the flattened and swollen areas of the stem. Tissue abnormalities, usually wedge-shaped zones separated by areas of normal pine tissue, extended from the outer cortex into the xylem (Fig. 2A). These zones were characterized by cell tanninization, peripheral periderm-like cells, dark-staining parenchyma and parenchyma-like cells within and bordering the pathological tissue, and interruption of the secondary cambium for the radial and longitudinal extent of the zone. A sharp demarcation of pathological and normal tissue existed. The zones appeared similar to resistance zones in slash pine (15,16,20) but with less cell tanninization and tissue regression.

Occasionally, the pathological zone appeared isolated in the cortex by a periderm. However, as evidenced by hyphal extension from the zone, past the periderm and into adjoining tissue, pathogen establishment probably occurred prior to differentiation of the periderm, or the pathogen simply extended past or through the periderm barrier. Frequently, sclerenchyma differentiated between the pathological zone and the periderm, indicating a potential sloughing-off of the zone (Fig. 2B). Other samples exhibited less inhibition of the pathogen, by inadequate periderm formation, which apparently permitted hyphal extension from the zone into the inner cortex, phloem, and cambium (Fig. 2C). Similar findings for slash and white pine (*P. strobus* L.) exist (12,20,23).

Tissues associated with the swollen areas of the stem, frequently adjacent to the pseudoresistance zones, exhibited, in general, anatomical features similar to those of the typically susceptible samples. However, unusual anatomical features of the host and pathogen were also observed. One feature was the tanninization of the xylem ray parenchyma immediately under the secondary cambium (Fig. 2D). Often several cells per ray were affected. Intercellular hyphae of the pathogen, associated with the tanninized cells, appeared normal and functional. Haustoria were rarely observed in the tanninized cells but possibly were obscured by the tannin deposition. Viable hyphae occasionally extended along phloem rays to the cambial area and then (judged by staining differences) appeared nonviable along the corresponding ray in the xylem (Fig. 2E). Frequently, dark-staining files of vertical parenchyma cells, similar to reaction parenchyma (17), were observed in newly developed xylem (Fig. 3A). These parenchyma cells were apparently differentiated for a short time by the pathogen-invaded cambium, as more typical cellular deposition occurred between these vertical parenchyma and the host cambium (Fig. 3B). The pathogen was observed extending distally to the inner extent of the vertical parenchyma (Fig. 3B), an unusual feature for reaction parenchyma, which normally delimits the innermost extent of the pathogen in host tissue (13,17,18).

Viable, nonviable, typical, and atypical hyphae of the rust pathogen were prominent in the pathological tissue (Fig. 3B). The viability of hyphae was based on past experience and staining differentials (11,13,14,16). The pathogen became established in parenchyma cells initiated by a proliferation of cells bordering the pathological tissue zones in both the cortex-phloem and the xylem of the host, which produced files of parenchyma cells. These cells, some callus-like, responded meristematically to differentiate additional parenchyma radially and tangentially. This occurred frequently where the zone interrupted the secondary cambium (Fig. 3C and D). Similar results have been reported for slash pine (12,16).

Resistant reactions. Intensive microscopic examination of samples from progeny that were macroscopically rust-free 1 yr after inoculation revealed that the pathogen had been temporarily established in certain progeny. This was evidenced by cellular abnormalities in needle trace bases adjacent to the pith (Fig. 4A and B), which frequently could be traced to remnants of a primary or cotyledon needle base at the stem surface (Fig. 4A). These needle remnants exhibited heavy tanninization of host cells, some of which contained tanninized and nonfunctional haustoria, indicating an initial establishment of the pathogen (Fig. 4C). Immediately beneath the needle bases, sclerenchyma had developed, indicating a potential for the sloughing-off of the needle base tissue (Fig. 4A). Limited indications of host cell abnormality associated with fusiform rust infection were exhibited on the outer cortex of certain samples (Fig. 4A). No evidence of the pathogen was observed in these cortical tissues. However, the needle trace bases in the pith area did exhibit evidence of the pathogen and host cell abnormalities. Ray parenchyma cells were larger in size and number than the normal. Abnormal tracheids were present, and hyphae of the pathogen were evident but appeared degenerate (Fig. 4B and D). The affected tissue was very limited in extent, both radially and tangentially, and coincided with the limited presence of the degenerate hyphae. Normal and near-normal host issue was observed from the abnormality through the xylem, cambial area, phloem, and cortex (Fig. 4A).

DISCUSSION

The present results indicate considerable variation in the reaction to fusiform rust by the backcross progeny observed. The reaction ranged from typical gall development (susceptible reaction) to indications of host resistance. These observations appear to substantiate previous research concerning F_1 interspecies southern pine progenies with shortleaf pine as one parent (13,19).

The typically susceptible reaction exhibited by certain backcross progeny was surprising. Even though two thirds of the cross producing these individuals was fusiform rust-resistant shortleaf pine, the anatomical expression of the tissues was very similar to that reported for rust galls on fusiform rust-susceptible slash and loblolly pine (6,17). No evidence of host tissue regression or other tissue abnormalities indicative of attempts by the host to resist establishment or extension of the pathogen was observed (16). In contrast to other findings (13,18) whorls of tracheids and ray cells in the gall xylem were absent; the significance of their absence is unknown. Likewise, no unusual morphological features of hyphae or haustoria of the pathogen were evident. Such abnormalities suggest the pathogen is under stress of some type (14). The host anatomy indicated continued gall development with potential mortality of the progeny.

The pseudoresistant samples exhibited anatomical characteristics similar, for the most part, to host responses reported previously for F_1 southern pine hybrids (13), but specific differences were observed. The tanninization of ray parenchyma in the xylem immediately beneath the cambial area indicates a possible incompatibility between the host and the pathogen. As the ray parenchyma near the pith (which is the earliest formed) usually appeared normal for gall tissue (17,18), the tanninization of similar cell types differentiated later (proximally to the cambium) on the host indicates a possible incompatibility of the host and the pathogen. Stimulation of cambial initials by the presence of the pathogen has been proposed as the cause of the characteristic gall anatomy of *C. q.* f. sp. *echinatae* and *C. q.* f. sp. *fusiforme* (17,18). Thus, if an atypical relationship arose in the gall cambium, subsequent cell deposition would, in time, possibly reflect abnormalities. This might explain the development of the tanninized ray parenchyma. Whether these parenchyma cells were

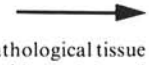
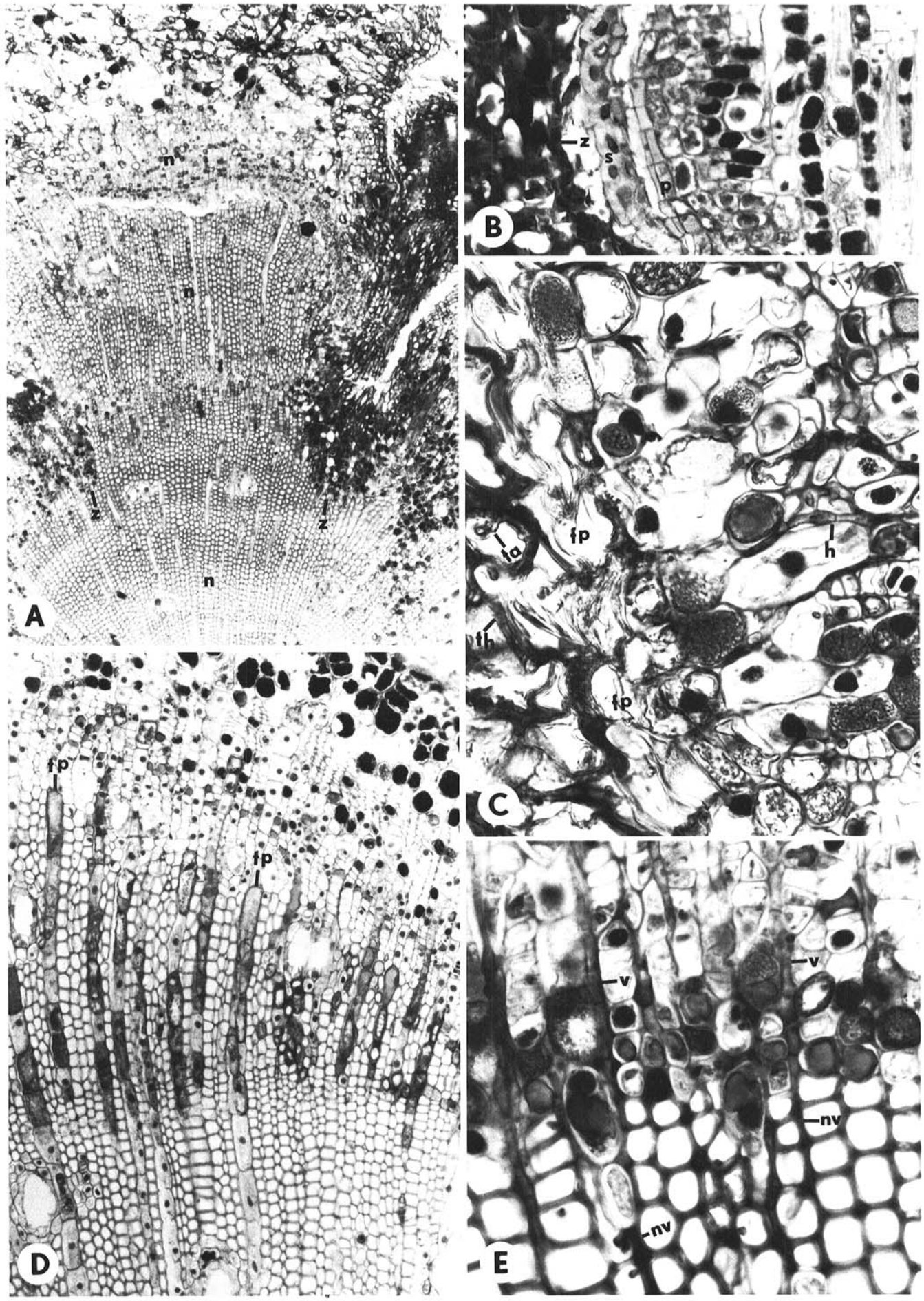


Fig. 2. A, Transverse view of two wedge-shaped zones of pathological tissue (z) separated by normal host tissue (n) (×50). **B,** Periderm (p) and sclerenchyma (s) beneath the cortical zone of pathological tissue (z) (h) from the pathological zone into the cortical area. Note the tanninized haustorium (ta) and tanninized hyphae (th) in the pathological zone (×520). **D,** Tanninized ray parenchyma (tp) in the xylem (×122). **E,** Viable hyphae (v) and nonviable hyphae (nv) along rays from phloem to xylem (×520).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functional (viable) or would survive any length of time is unknown. By conjecture, such gall areas might eventually cease to function, and with host survival these areas would be covered by or buried in normal host tissue, in a healing process similar to that described for slash pine (15,16). Further, the deposition of several files of reaction (vertical) parenchyma by the gall cambium and the change of hyphal condition at the gall cambium appear to be additional evidence of host-pathogen incompatibility. These hyphal and host cell changes suggest a series of host cambial stimulations to or by the pathogen, rather than the uniform formation of typical gall tissue following the differentiation of reaction parenchyma, as previously reported (17,18). The present samples also exhibited

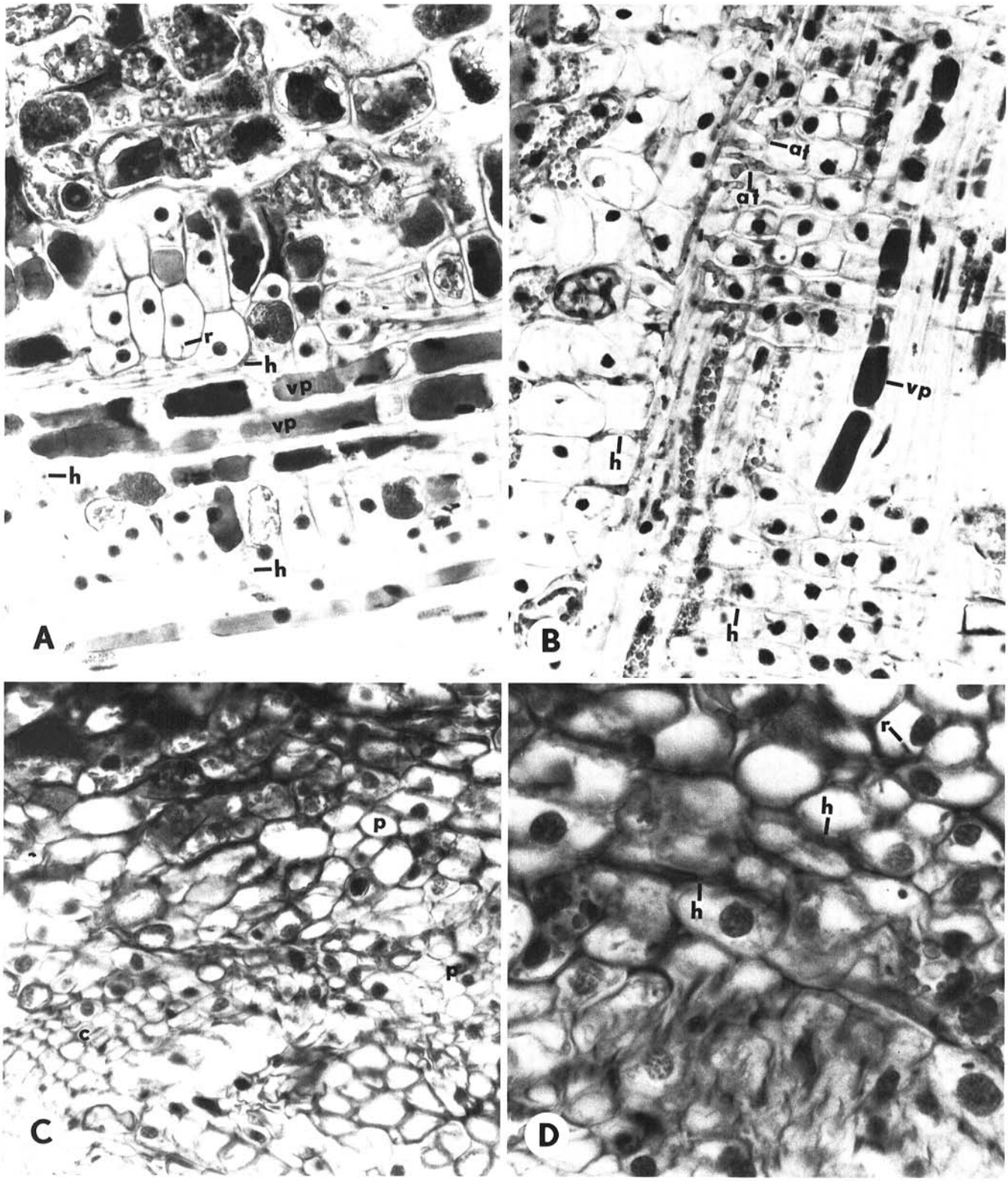

Fig 3. A, Longitudinal view of vertical parenchyma (vp) differentiated in the cambial area. Note hyphae (h) and haustoria (r) above and below the vertical parenchyma (×248). **B,** Longitudinal view of typical hyphae (h) and atypical hyphae (at) of the rust pathogen. Note the vertical parenchyma (vp) (×248). **C,** Transverse view of parenchyma cells (p) differentiated at the border of a pseudoresistance zone where the cambial cylinder (c) is interrupted (×248). **D,** Hyphae (h) and haustoria (r) in parenchyma bordering the pseudoresistance zone (×520).

evidence of an interruption of the intercalary growth of the pathogen in and along the host cambium, considered necessary for typical gall development (17,18). The potential growth and survival of galls with these characteristics is uncertain. Regardless, the pseudoresistant samples do indicate the need for careful examination for atypical stem configurations of progeny tested for rust resistance. If pseudoresistant individuals are missed in progeny tests, the evaluation of cross or parent performance might

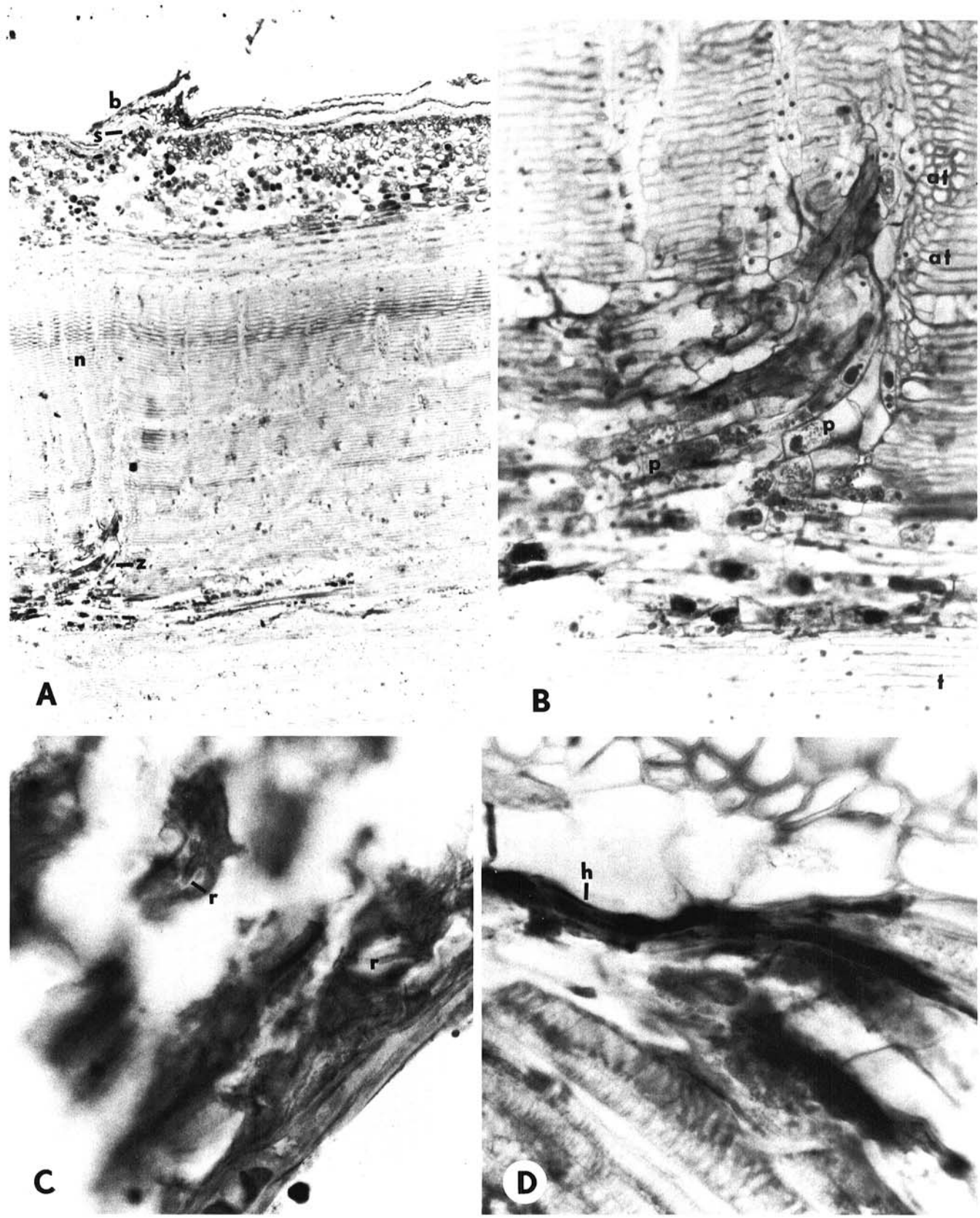

Fig 4. A, Longitudinal view of progeny indicating resistance. Note the needle base (b) and the pathological zone (z) at the base of the needle trace, separated by normal host tissue (n); also note the deposition of sclerenchyma (s) beneath the needle base (×32.5). **B,** Enlargement of the pathological zone in A, exhibiting enlarged parenchyma cells (p) and abnormal tracheids (at) at the base of the needle trace. Note the normal tracheid (t) (×520). **C,** Tanninized haustoria (r) in the remnant of a needle base (×520). **D,** Degenerate hyphae (h) in the pathological zone in A and B (×520).

be invalid, and individuals with potentially active rust galls might be introduced into valuable plantings, seed orchards, etc.

The samples exhibiting resistance to the rust pathogen were of particular interest. That the pathogen was established in the host early in the life of individual progeny is evidenced by the presence of the rust and associated host tissue abnormalities near the pith, at needle trace bases, and at the corresponding needle remnants on the stem. The progeny plant body, when inoculated at 6 wk of age, was principally primary tissue, with cotyledons, primary needles, and their respective needle traces an integral part of the plant (22). In general, rust infection of the primary foliage results in the establishment of the rust in the hypocotyl by way of needle traces (11,14,21). In resistant progenies, infection and pathogen establishment occurred, and abnormal cellular configuration was initiated. At this point, apparently for unknown reasons, the pathogen either ceased to function or ceased to stimulate the differentiation of atypical (gall) tissue in the host. The pathogen degenerated, and typical tissue then formed by radial and longitudinal host growth until sample collection. Typically, fusiform rust gall development is principally dependent on primary or secondary cambial contact and stimulation by the pathogen (17,21). Thus, because of infection of the progenies during their primary tissue stage, the breakdown of the gall-forming process in the host-pathogen relationship probably occurred in the primary cambium area (22). Subsequent development of the secondary cambium, with its derivatives, was free of pathogen stimulation and resulted in the formation of typical host tissue. This growth pattern effectively buried the pathogen and pathological tissue deep in the host stem cylinder. Figure 4A and B demonstrates this conclusion. Also, no indications of potential escape or growth of the pathogen (12), characteristic of the pseudoresistant samples, was observed or expected, because of the anatomical features exhibited by the resistant samples.

In general, the present observations indicate that backcrossing shortleaf × slash pine hybrids to shortleaf pine can produce individual progenies expressing effective resistance to fusiform rust. A restrictive incompatibility, of unknown origin, apparently arises between the host and the pathogen, preventing the usual gall-forming process associated with fusiform rust and its pine host. In addition, the present work again suggests, in support of earlier findings (13,19), that simply infusing shortleaf pine into the hybridization of southern pine does not ensure resistance to fusiform rust. Some resistance is gained by such breeding, but close scrutiny of progenies is necessary to evaluate symptom and pathogen development to properly utilize progeny test results.

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