Histopathology of the Brown Spot Fungus on Longleaf Pine Needles

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Research conducted in the School of Forestry, Louisiana Tech University, under the McIntire-Stennis Cooperative Forestry Research Program, and in cooperation with the U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station, Gulfport, MS, via Cooperative Agreement 19-81-33.
Accepted for publication 28 December 1982.

ABSTRACT

Jewell, F. F., Sr. 1983. Histopathology of the brown spot fungus on longleaf pine needles. Phytopathology 73:854-858.

The brown spot fungus, *Scirrhia acicola* (Dearn.) Siggers, affected only the mesophyll cells in bar spot type lesions on needles of longleaf pine, *Pinus palustris* Mill. The affected mesophyll cells were collapsed and flattened in a lattice pattern. Hyphae of *S. acicola* in symptomatic tissue were sparse and were not observed in host tissue adjacent to or beyond the symptomatic area. The amount of host tissue damage was far out of proportion to the presence of the pathogen. The production of a toxin by *S. acicola* is proposed as a cause for the extensive damage to the host mesophyll. In contrast, profuse hyphal development and cellular deterioration were noted in all tissues of host needles presumably killed by *S. acicola*. Spore-bearing structures of *S. acicola* developed in the outer mesophyll and at maturity were somewhat erumpent and split the epidermis. Conidia were four-celled, olive to light brown, curved, and pointed at one end but somewhat blunt at the other. Perithecia were present only on dead tissue. Ascospores were hyaline, were two-celled with one nucleus per cell, and exhibited no oil globules. Fungi consistently associated with needles infected by *S. acicola* were a species of *Fumago*, present on symptomatic areas but not on dead tissue, and species of *Lophodermium* and *Pestalotia*.

Additional key words: Dothidiaceae, *Hypoderma,* Phyllacoraceae, *Systremma*

Brown spot needle blight, caused by *Scirrhia acicola* (Dearn.) Siggers, is the major disease of longleaf pine, *Pinus palustris* Mill. (4,11,13). Research on the etiology, the mode of infection, and the taxonomy of the causal organism have been published (1,2,4,5,9,12–14). However, little is known of the effect of *S. acicola* on the tissues of the affected host needles, other than a brief report on the pathological anatomy of bar-spot symptoms of *S. acicola* on longleaf pine (6).

This article reports anatomic changes in the tissues of mature, fasicled needles of field-grown longleaf pine infected by *S. acicola*, and the general morphology of the fruiting structures of this fungus.

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MATERIALS AND METHODS

Living and dead needle samples were collected from infected 2- to 4-yr-old "grass stage" seedlings of longleaf pine (Fig. 1A) during

1981 in Harrison County, MS. Samples collected in March and May included green needles with bar spot symptoms (Fig. 1B) and needles bearing ascervuli. Samples of necrotic distal portions of needles with green basal areas and dead needles were taken in December. Approximately 10–20 individual samples were collected for each of the tissue types from 15 longleaf pine seedlings. Where possible, needle samples 1 yr of age were collected. In addition, only bar spot samples considered to be fully developed were selected. No collections were made at this time for study of the chronological development of the bar spot or other symptom types associated with *S. acicola* on longleaf pine. In the latter case, the specific identity of these symptom types is questioned by the author. Check material consisted of five samples from individual needles free of visible brown spot disease from each of 10 longleaf pines. Samples for paraffin sectioning were processed and examined by light microscopy as previously reported (7). Fresh mounts, prepared in distilled water or in lactophenol-aniline blue, were used to supplement observations on spore characteristics from needle tissue incubated in moist chambers for 24–72 hr.

RESULTS

Transverse sections of healthy longleaf pine needles closely resembled those described by Harlow (3) (Fig. 2A).

Bar spot symptoms. Microscopically, the initial significant observation was the limited or localized effect of the pathogen on the host tissue. This occurred primarily in the mesophyll of the lesions, where mesophyll cells had collapsed, producing large intercellular spaces (Fig. 2B and C). In longitudinal view, the affected tissue presented an open, latticed appearance (Fig. 2C). The cellular configuration was initially considered the result of dissolution of the cell contents by *S. acicola*. However, observations revealed the cells had actually become flattened by collapsing around their nuclei at right angles to the long axis of the needle (Figs. 2C, 3A and B). The cell contents, including chloroplasts, were destroyed or rendered nonfunctional by displacement or compaction in the cell (Fig. 3B). The loss of chloroplasts may explain the development of the yellow color typical of the *S. acicola* bar spot symptom. Also, the retention of the integrity of the cell walls of the affected mesophyll possibly accounts for the diseased needles remaining rigid and not bending or breaking at the symptom area on the green needles.

In most symptomatic needles examined, the effect of *S. acicola* was limited to the mesophyll. The endodermal cell layer, vascular system, hypodermis, and epidermis were usually not affected (Fig. 3A and B). Wolf and Barbour (16) noted no apparent invasions of the vascular tissues by *S. acicola*. The only effect of the pathogen on tissues other than the mesophyll was the slight dissolution of the outer wall of individual endodermal cells proximal to the affected mesophyll, but this was infrequent. Hypodermal cells rarely exhibited disease effects even when in direct contact with collapsed mesophyll cells (Fig. 3A and C). A similar relationship was observed in mesophyll tissues at the edges of the symptom areas where sharp delimitation of affected and unaffected cells occurred (Fig. 3A and C). A completely collapsed cell often directly adjoined an apparently normal mesophyll cell. Usually, partially collapsed mesophyll cells separated the symptom area from the unaffected tissue (Fig. 3C).

The relationship of hyphae of *S. acicola* and the tissues of the symptom areas was unusual. In general, as suggested by Verrall (15), hyphae were not abundant and, in many samples, were very difficult to observe, even in areas of considerable mesophyll damage (Fig. 3A and C). When observed, hyphae were intracellular in mesophyll cells or were in the spaces formed by the collapsed mesophyll cells.

Hyphae were abundant in localized portions of the symptom area where sporulation of *S. acicola* occurred. Here, large, dark, short-celled hyphae were massed in the outer mesophyll and under the epidermis forming a partially erumpent, spore-producing stroma. As stromata matured, sufficient conidia were produced to

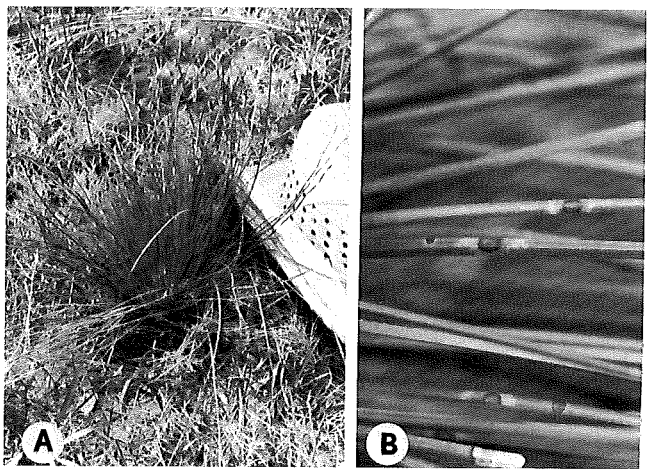


Fig. 1. Longleaf pine. **A,** Grass stage seedling; **B,** needles with bar spot symptoms of *Scirrhia acicola*.

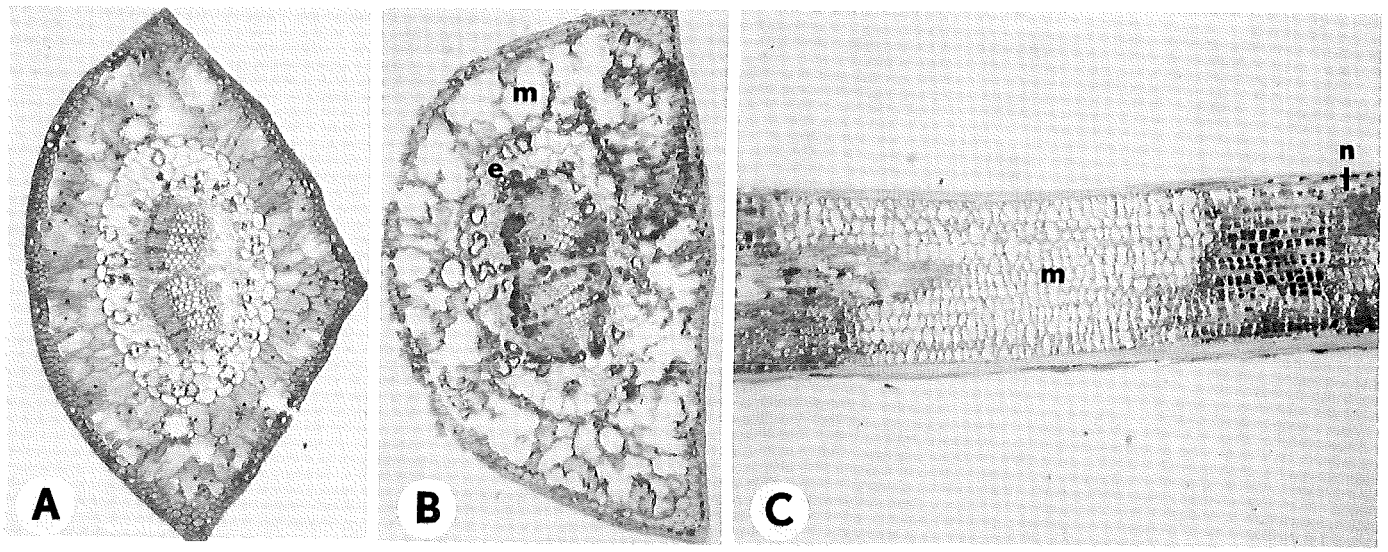
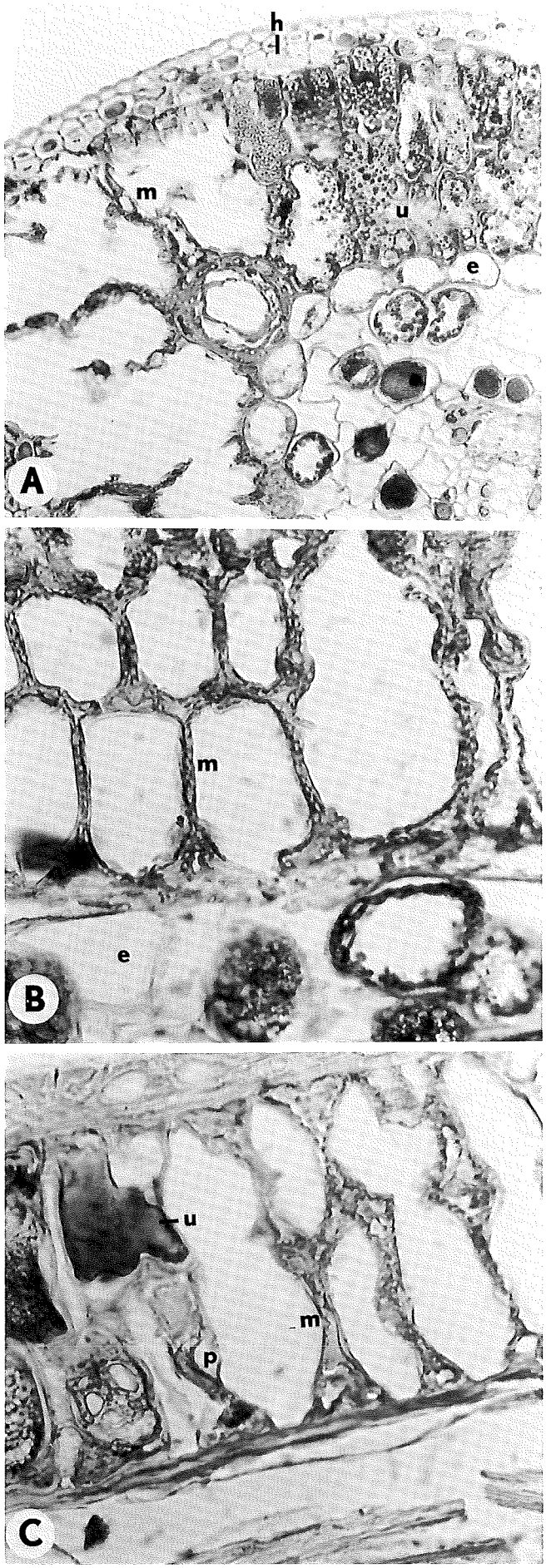


Fig. 2. Longleaf pine needle. Transverse sections of **A,** normal appearance and **B,** area with symptoms of *Scirrhia acicola*, with collapsed mesophyll (m) and unaffected endodermis (e) (×57); and **C,** longitudinal section through symptomatic area showing the lattice appearance of the collapsed mesophyll (m) in contrast to the normal (n) (×40).



split the epidermis (Fig. 4A). Stromata appeared to remain covered at maturity except for the epidermal split. This agrees with the findings of Siggers (13). The conidia were acicular, curved, olive to light brown, usually somewhat pointed at the apical end and blunt at the base, and distinctly segmented (Fig. 4F). This agrees with previous descriptions of these spores (2,13,15), except that mature conidia were usually four-celled and rarely three- to five-celled (Fig. 4F). This was true of spores from field collections as well as from moist-chamber incubations.

The perfect stage of *S. acicola* was not observed in the samples of green symptom-bearing needle tissue collected in March or May.

A species of *Fumago* was frequently associated with symptom areas of *S. acicola*. A similar report exists for loblolly pine, *P. taeda* L. (8). The *Fumago* sp. observed on longleaf pine commonly plugged stromata, and from these loci, the large, black hyphae spread over the needle surface. Sporulation of *Fumago* sp. was common on the needle surface.

Dead needle tissue. Dead needle tissue supported fruiting bodies of fungi other than *S. acicola*. The most common were species of *Lophodermium* and *Pestalotia*. A species of *Hypoderma* was observed infrequently. *Fumago* sp., common on the green symptom-bearing needles, was not observed on the dead needles.

The dead needle tissues exhibited many of the same characteristics as tissue with bar spot symptoms. The major difference was that the endodermis, transfusion tissue, and vascular elements of the dead tissue all exhibited deterioration, not just the mesophyll. In addition, fungal hyphae were prevalent and plentiful throughout the tissues. It was not possible to identify all hyphae as those of *S. acicola*.

Sporulation, both sexual and asexual, by *S. acicola* was common on the dead needle tissue, particularly after incubation in moist chambers. Paraffin sections of field samples of dead tissue revealed that the stromata of *S. acicola* were initiated in the outer portions of the mesophyll just under the epidermis, where large, short-celled, dark hyphae congregated (Fig. 4A–C). These hyphae apparently invaded the space between the hypodermis and epidermis to form the stromata. As hyphae were sparse in cells and intercellular spaces adjoining the stromata, the origin of the stromatal hyphae is uncertain. Observation of mature stromata showed separation of hypodermal cells with a few of these cells being pushed (cast) down toward the mesophyll (Fig. 4A). Conidia observed on these stromata were similar to those from green symptom-bearing tissue.

Perithecia of *S. acicola* were observed only on the dead needle tissue. Sectioning of field samples revealed that the perithecia were formed on the outer surface of their stromata and at maturity split and lifted the epidermis, which remained intact over the perithecia (Fig. 4B and C). Often the epidermis split to form parallel longitudinal slits and formed a strip of epidermal tissue over the perithecia (Fig. 4C), as reported by Siggers (13). As described by Dearness, asci (= *Oligostroma acicola*) (1) from moist-chamber incubations were wide at the base and tapered toward the tip (Fig. 4D). There were eight ascospores with one, usually terminal, ascospore per ascus (Fig. 4D). The ascospores from field and moist-chamber samples were hyaline, two-celled with a rounded terminal end and a sharply tapering base (Fig. 4E). No oil globules were observed. However, each cell of the two-celled ascospore contained a nucleus (Fig. 4E). As reported (13), frequently conidia were produced on the same stroma as perithecia.

DISCUSSION

The reaction to infection by *S. acicola* of the longleaf pine needles examined was considered unusual. This was most evident in areas of bar spot symptoms in the green tissue, where the

Fig. 3. Longleaf pine needle with symptoms of fungal infection. **A,** Transverse view showing collapsed mesophyll (m), unaffected mesophyll (u), hypodermis (h), and endodermis (e) (×200). Longitudinal views of **B,** latticelike collapsed mesophyll (m) adjoining unaffected endodermis (e) (×400) and of **C,** adjoining collapsed (m), partially collapsed (p), and noncollapsed (u) mesophyll (×400).

pathogen effect was limited to the mesophyll cells. These cells had collapsed, not by dissolution or degradation of the cell contents and cell walls, but rather by being flattened at right angles to the long axis of the host needle. This resulted in the compression of the cell contents with the cell walls remaining rigid and clearly definable. This, in turn, formed large intercellular spaces in the affected mesophyll of the symptom area. In addition, the amount of damaged host tissue greatly exceeded that actually invaded by the pathogen. Other than where sporulation occurred, hyphae of *S. acicola* often were not observed or were present in very limited amounts in affected areas. Also, the separation of affected and unaffected tissue was usually distinct, and because *S. acicola* hyphae were not observed in the unaffected tissue and were rare in the adjoining affected tissue, the possibility exists that the effect on the host does not depend on the actual presence of hyphae of the pathogen.

This evidence strongly suggests that a toxin may be involved in the host-parasite relationship of *S. acicola* and longleaf pine and that much of the mesophyll damage in the symptom areas results from a toxin produced by *S. acicola*. This hypothesis is supported in part by the findings of Shain and Franich (10) in reports of *Dothistroma* blight symptom induction by a substance termed dothistromin.

The role of *Fumago* sp., *Lophodermium* sp., and *Pestalotia* sp.

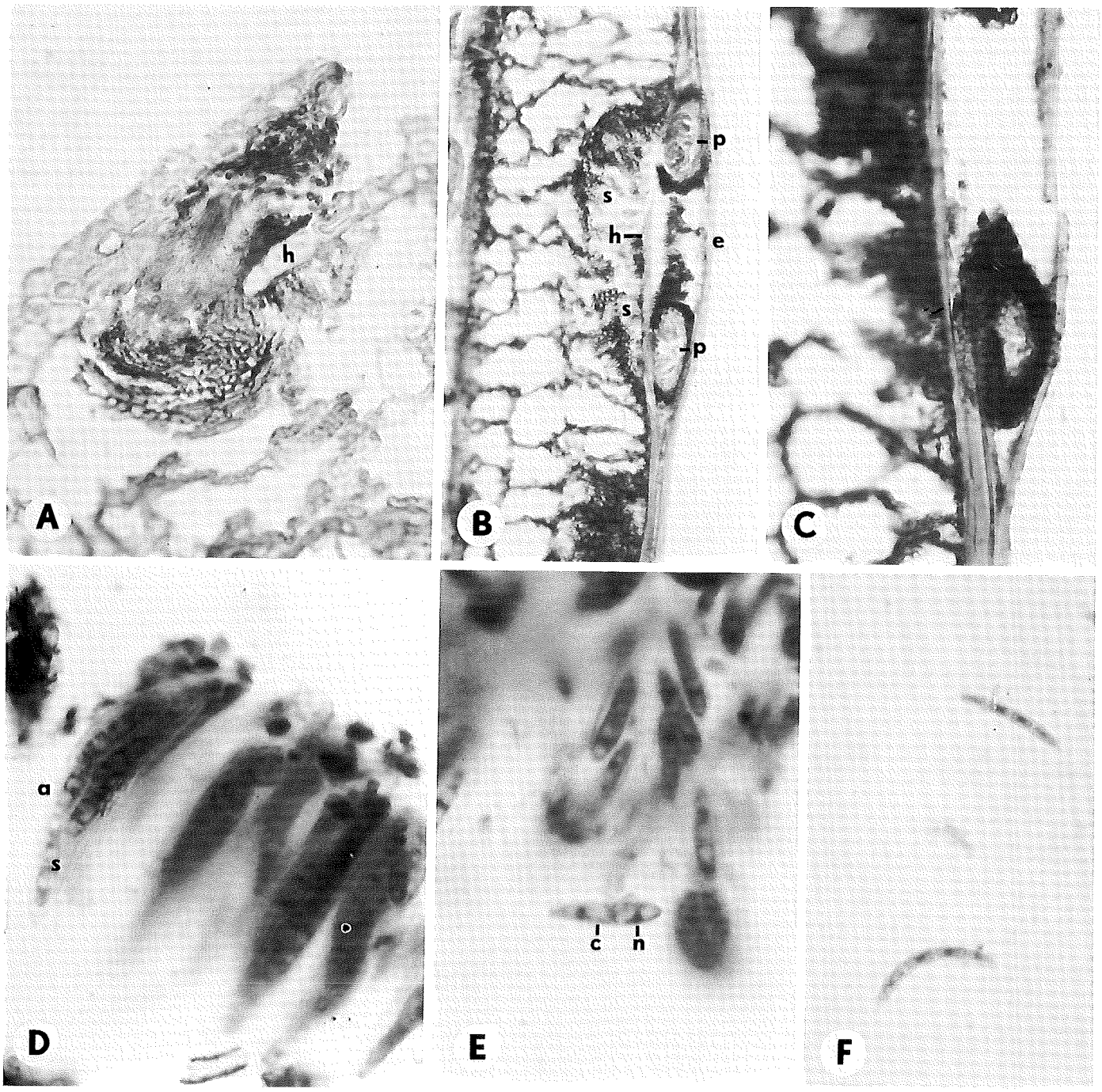


Fig. 4. Fruiting structures of *Scirrhia acicola*. **A,** Conidia being produced from stroma in outer mesophyll, splitting epidermis, and casting down hypodermal cells (h) (×320). **B,** Perithecial stroma (s) in outer mesophyll and locules (p) between hypodermis (h) and epidermis (e). Note the limited stroma hyphal area in the collapsed mesophyll (×150). **C,** Perithecial locule splitting epidermis (×320). Lactophenol-aniline blue preparations of **D,** asci (a) and ascospores (s) (×1,480); **E,** two-celled ascospores exhibiting a nucleus (n) in each cell and four normally hyaline areas (c), two per cell (×1,480); and **F,** four-celled conidia (×740).

in the relationship of *S. acicola* to longleaf pine is uncertain. These fungi were consistently found on the material studied, and their interaction with the brown spot disease deserves further investigation.

In general, the observations of the sexual and asexual phases of *S. acicola* conform to earlier taxonomic treatments of this fungus (1,2,12,13,15), although minor differences were noted. The mostly four-celled conidia were considered significant. Previously, *S. acicola* conidia were described as having one to three walls (two to four cells) (2,16) or with or without several indistinct walls (13). Rarely were mature conidia of less than four cells observed. The perithecial stromata, although initiated in the outer mesophyll, were mainly seated between the hypodermis and epidermis, and the perithecial locules were erumpent, but covered at maturity. The two-celled ascopsores were definitely hyaline, contained one nucleus per cell, and contained four hyaline areas (two per cell) (Fig. 4E), which may have been mistaken for oil globules previously (13,16). In addition, the brown spot fungus was placed in the genus *Systremma* of the Dothidiaceae due to reports that perithecial stromata were erumpent and not covered by host tissue at maturity and that ascospores were dilutely brown (16). My research on the brown spot fungus does not agree with these findings but instead supports the placement by Siggers (13) of the ascigerous stage of the brown spot fungus in the genus *Scirrhia* of the Phyllacoraceae.

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