Histopathology of Root Disease Incited by *Thielaviopsis basicola* in *Ilex crenata*

Robert L. Wick and Laurence D. Moore

Research associate and associate professor, respectively, Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg 24061.
Portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree, VPI & SU, Blacksburg.
Accepted for publication 17 November 1982.

ABSTRACT

Wick, R. L., and Moore, L. D. Histopathology of root disease incited by *Thielaviopsis basicola* in *Ilex crenata*. Phytopathology 73:561-564.

Black root disease caused by *Thielaviopsis basicola* in Japanese holly cultivar Helleri was examined histologically. All primary root tissues were colonized, but the hypodermis, endodermis, and vascular system were partially resistant. Lesions expanded by the intracellular growth of thin-walled, hyaline hyphae that were constricted behind the hyphal tip. Subsequent hyphae were thick-walled and contained many cross-walls and constrictions. *T. basicola* was compartmentalized in the cortex 9–14 days after inoculation.

A root disease caused in Japanese holly (*Ilex crenata* Thunb.) (cultivars Helleri, Rotundifolia, and Microphylla) by *Thielaviopsis basicola* (Berk. & Br.) Fr. was reported for the first time in 1976 by Lambe and Wills (5). Japanese holly cultivars Highlander, Compacta, Convexa, and Helleri were subsequently found to be susceptible (6). Lambe et al (7) compared seven species of holly (including six cultivars of Japanese holly) for resistance to *T. basicola* and found that *I. crenata* cultivar Helleri was highly susceptible. While young holly plants can be killed within weeks as the result of severe root destruction by *T. basicola*, it is the senior author's experience as a diagnostician at the plant disease clinic (Virginia Polytechnic Institute and State University [VPI & SU], Blacksburg) that plants with more mature roots decline more

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.

slowly. Similarly, Mathre et al (8) reported that young cotton seedlings are more susceptible to *T. basicola* than are mature plants.

Histopathological studies of root diseases incited by *T. basicola* have been reported for several hosts including tobacco (*Nicotiana* sp.) (3,4,10), bean (*Phaseolus vulgaris* L.) (2,9), citrus (*Citrus* sp.) (11), and cotton (*Gossypium hirsutum* L.) (8). A number of differences and similarities regarding colonization and response by hosts have been observed. Conant (3) determined that hyphae enter tobacco roots through wounds or by "mass action" and not by direct penetration, whereas Stover (10) described direct penetration of tobacco roots without mention of the necessity of wounds. In bean (2,9), citrus (11), and cotton (8), direct penetration of epidermal cells by *T. basicola* also was noted. Inter- and intracellular colonization by hyphae was noted in tobacco (10), bean (2,9), and cotton (8). The host cell nucleus of invaded tobacco (3), bean (2), and cotton (8) was described as normal in appearance following initial penetration, and in citrus (11) hyphae were reported within living cells.

Our objectives were to determine histologically the ability of *T. basicola* to colonize Japanese holly root tissues as well as the ability of the host to resist colonization.

MATERIALS AND METHODS

Japanese holly, cultivar Helleri, was obtained from established plantings in Blacksburg, VA. Cuttings were rooted in pasteurized Weblite® (Webster Brick Co., Roanoke, VA 24016) under mist and then transplanted into 10-cm-diameter pots containing a mixture of ground pine bark and Weblite (1:1, v/v). The transplants were fertilized every 2 wk with 100 ml of Peters'® water-soluble fertilizer (10-8.7-16.7, N-P-K; W. R. Grace & Co., Foglesville, PA 18051), which contained 300 μg of N per milliliter. Plants were maintained in a greenhouse at ambient temperatures and were 12 mo old and had well-established root systems when inoculated with *T. basicola*.

T. basicola (isolate 5) was obtained from Japanese holly by W. H. Wills, Department of Plant Pathology and Physiology, VPI & SU. It was maintained on V-8 juice agar, which consisted of 100 ml of V-8 juice (Campbell Soup Co., Camden, NJ 08101), 900 ml of distilled water, and 1.5% agar. Inoculum was obtained by seeding V-8 juice medium with a suspension of endoconidia and incubating at 28 C in the dark for 5–7 days. Endoconidia were harvested by flooding the surface of the medium with sterile distilled water. A hemacytometer was used to adjust the endoconidial concentration to 5×10^4 conidia per milliliter. Holly plants were inoculated by pouring 100 ml of the endoconidial suspension into each pot and the roots were harvested 1, 3, 5, and 7 days after inoculation. Five roots from each of two plants per date were examined. The experiment was repeated with plants harvested at intervals of 5, 7, 9, 11, 13, and 16 days after inoculation. Twenty roots from each of two plants per date were examined. In addition to feeder roots, 20 mature roots with lesions were examined on day 16.

Feeder roots 3–4 mm long with macroscopic lesions, as well as feeder roots from uninoculated plants, were fixed in formalin-acetic acid-alcohol (FAA) (1). The roots were dehydrated in a graded series of *t*-butanol and infiltrated with Paraplast® (Sherwood Medical Industries, St. Louis, MO 63100) and 12-μm sections were cut with a rotary microtome. Sections were affixed to glass slides with Haupt's solution and stained with a safranin-fast green combination (1). For scanning electron microscopy, roots were dehydrated, transferred to acetone, critical-point dried, and coated with gold.

RESULTS

Examination 24 hr after inoculation revealed germinated endoconidia and hyphae on the surface of roots (Fig. 1). Hyphae penetrated directly through the epidermis without forming appressoria. Examination of the whole root system 5 days after inoculation showed that lesions occurred primarily on feeder roots (Fig. 2). Most of the lesions developed initially at the zone of elongation and were limited to the cortex. Chlamydospores had formed within the cortex and both chlamydospores and endoconidiophores developed on the surface of the lesion. Hyphae and chlamydospores were occasionally found in the stele. Lesion expansion occurred by intracellular ramification of hyaline, thin-walled, 1–3 μm diameter hyphae. These hyphae had a characteristic lance shape due to a constriction just behind the hyphal tip (Fig. 3). Intercellular growth by *T. basicola* was not observed. Host cells were not killed in advance of penetration, and intact nuclei were common in newly invaded cells (Fig. 4). The hyphae either branched after breaching the cell wall or continued to penetrate the opposite wall. Branching often occurred in a digitate pattern from a swollen, darkly stained area near the hyphal tip. These newly formed hyphae were either long and lance-shaped or short and sickle-shaped. The hyphae eventually became thick-walled, constricted, and darkly pigmented (Fig. 3) and formed a pseudoparenchymatous stroma in the root cortex (Fig. 5). Stromatic tissue and hyphae also formed in the epidermis, including root hairs (Fig. 6), but only infrequently in the hypodermis. When stromatic tissue formed in the epidermis, the outer epidermal wall sloughed off and abundant chlamydospores, endoconidiophores, and endoconidia were produced.

Three morphological types of hyphae were recognized: hyaline, thin-walled (1–3 μm in diameter) hyphae with constrictions behind the hyphal tip; darkly pigmented, thick-walled, pseudoparenchymatous hyphae; and dichotomously branched, thick-walled, darkly pigmented hyphae (5–8 μm diameter), which gave rise to chlamydospores and endoconidiophores. Chlamydospores were also associated with the pseudoparenchmatous stroma that developed in the cortex and epidermis.

All primary tissues of the root were susceptible to colonization. Initial penetrations occurred from the root cap to the area of maturation, with the majority of lesions occurring in the zone of elongation. The hypodermis, endodermis, and vascular system were least frequently colonized. Phellem, produced by the pericycle, was never colonized and the proportion of susceptible tissue decreased as phellem was produced (Fig. 7).

First indication of a host wound-response was the presence of enlarged nuclei 1–5 cortical cells in advance of the thin-walled, hyaline hyphae (Fig. 8). Nuclear division and cell plate formation followed and resulted in the formation of a complete barrier of newly formed wound-response cells around the lesion (Fig. 9). When mature wound-response cells were examined with ultraviolet light microscopy, fluorescence of the walls occurred, which indicated that lignin or a ligninlike substance was present. Young wound-response cell walls did not fluoresce. The pathogen did not colonize or breach this barrier even after adjacent cells became filled with fungal tissue. In addition, there was no evidence that growth of the pathogen had ceased prior to the onset of cell division. Zones of metacutization, which resulted from episodic root growth, were also resistant to penetration (Fig. 10).

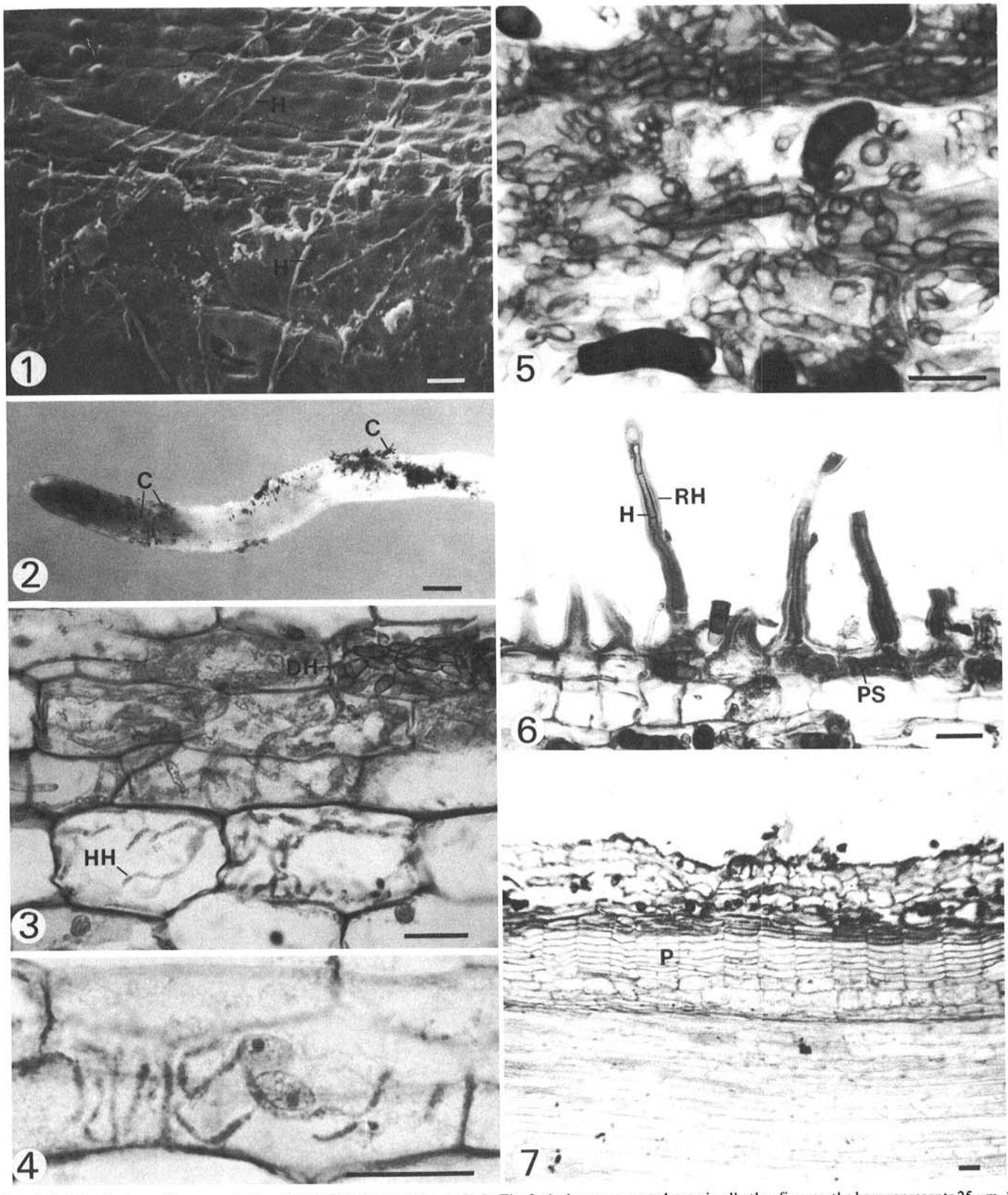
Cell wall swelling occurred in newly colonized cells and moribund host cells became thicker and stained red with safranin. The structural integrity of the root was maintained in the presence of *T. basicola* and rotted only when secondary organisms were present.

DISCUSSION

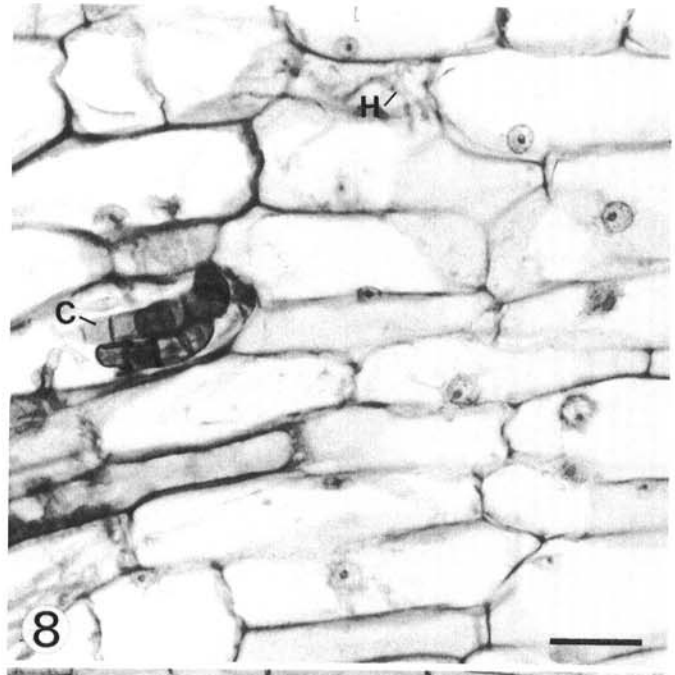
Direct penetration of holly roots by *T. basicola* occurred without the formation of appressoria and is consistant with other reports (2,9,11). Conant (3) reported that *T. basicola* entered tobacco roots chiefly through wounds, but this was not substantiated by Stover (10). No entry through wounds was observed in holly roots. Colonization of holly roots was exclusively intracellular, but in bean (2,9), tobacco (10), and citrus (11), intercellular colonization by *T. basicola* had been reported. Newly invaded holly root cells apparently remained live for a period of time as indicated by intact, normal staining host nuclei. Intact nuclei in colonized tobacco (10), bean (2,9), and citrus roots colonized by *T. basicola* have also been reported. Christou (2) suggested that a balanced parasitism was established in early stages of disease development in bean and

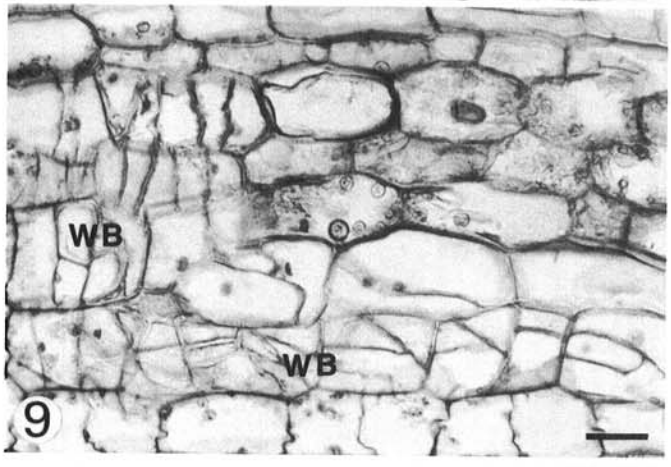
reported that digitate mycelia of *T. basicola* that were suggestive of haustoria had developed in roots. The fact that nuceli remain intact in newly colonized cells and that root tips of some plants are resistant to colonization by *T. basicola* is similar to a mycorrhizal condition.

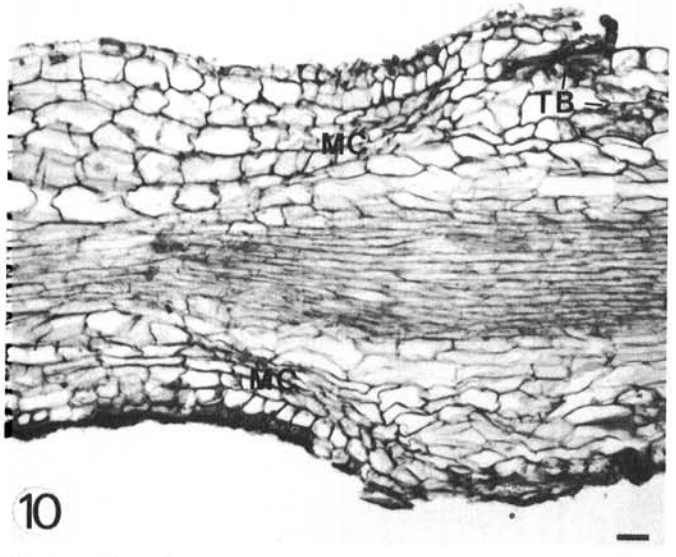
T. basicola did not rot Japanese holly roots. In the absence of secondary organisms, cell walls and middle lamellae were not macerated; thus, structural integrity of the root was retained. For this reason, we chose to refer to this disease as Thielaviopsis root disease, rather than the more commonly cited expression,



Figs. 1–7. Colonization of Japanese holly roots by *Thielaviopsis basicola*. In Fig. 2, the bar represents 1 mm; in all other figures, the bars represents 25 μm. **1,** Electron micrograph of hyphae (H) colonizing the surface of a feeder root. **2,** Lesions with chlamydospores (C) on the root surface. **3,** Hyaline, intracellular hyphae (HH) colonizing cortical cells in advance of darkly pigmented hyphae (DH). **4,** Hyphae in feeder root cells containing intact host nuclei. **5,** Pseudoparenchymatous stroma in cortex. **6,** Pseudoparenchymatous stroma (PS) in epidermal cells and hyphae (H) in root hairs (RH). **7,** Root with phellem (P) developed from the pericycle. *T. basicola* has colonized the cortex.







Figs. 8–10. Colonization of Japanese holly roots by *Thielaviopsis basicola*. Bar represents 25 μm. **8,** Host nuclei on the periphery of a root lesion. H = hyphae, C = chlamydospore. **9,** Wound-barrier (WB) formation resulting from colonization by *T. basicola*. **10,** Zone of metacutization (MC) in cortex of a feeder root. TB = *T. basicola*.

Thielaviopsis root rot.

Resistance of specific cell types to colonization by *T. basicola* has been reported for various hosts. The root cap and hypodermis of citrus (11), the epidermis, root tip, and zone of elongation of tobacco (3), and the epidermis of bean (2) were reported to be resistant. All cells of bean plants were colonized. The hypodermis, endodermis, and vascular system of Japanese holly roots were the least colonized. Conant (3) reported that more than 50% of the lesions in tobacco occurred at the base of branch roots. In Japanese holly, no lesions were found at such sites.

Conant (3) reported that periderm formation was the single most important factor in resistance of tobacco roots to *T. basicola*. However, Jewett (4), who studied some of the same tobacco cultivars, concluded that periderm formation did not occur in a significant number of cases. Cell division was initiated in endodermal cells of bean roots (9) and in hypodermal cells of citrus roots (11) when attacked by *T. basicola*. Mathre et al (8) reported that the cork cambium occasionally walled off the pathogen in colonized cotton roots. Pierre and Wilkinson (9) correlated cell division in bean roots with resistance to *T. basicola*, but felt that a chemical by-product was responsible for resistance and that cell division was a repair phenomenon.

The occurrence of thin-walled, hyaline hyphae in cells adjacent to dividing cortical cells indicated that wound-barrier formation in holly roots may be an important factor in resistance to colonization by *T. basicola*. Wound-barrier formation was initiated before cessation of growth by the pathogen. Subsequent lignification of these walls may not be important in containing the pathogen because lignification could not be detected until after a number of daughter cells had formed.

Wound-barriers, formed in response to colonization, and metacutization that occurred due to episodic root growth, did not prevent secondary disease development by *T. basicola* because endoconidia and chlamydospores formed on the root surface resulted in additional lesions. Rooted cuttings and young container-grown plants have a high proportion of primary root tissue that is susceptible to colonization. As the plants mature, pericycle activity results in resistance of the older root tissues. Container culture of holly plants tend to force roots into closer proximity to each other than that which occurs in field-grown plants. The high proportion of susceptible tissue and the crowding of the roots may explain the rapid destruction of young containerized holly plants by *T. basicola*.

LITERATURE CITED

1. Berlyn, G. P., and Miksche, J. P. 1976. Botanical Microtechnique and Cytochemistry. The Iowa State Univ. Press, Ames. 326 pp.
2. Christou, T. 1962. Penetration and host-parasite relationships of *Thielaviopsis basicola* in the bean plant. Phytopathology 52:194-198.
3. Conant, G. H. 1927. Histological studies of resistance in tobacco to *Thielaviopsis basicola*. Am. J. Bot. 14:457-480.
4. Jewett, F. L. 1938. Relation of soil temperature and nutrition to the resistance of tobacco to *Thielavia basicola*. Bot. Gaz. 100:276-297.
5. Lambe, R. C., and Wills, W. H. 1976. Thielaviopsis root-rot of Japanese holly. (Abstr.) Proc. Am. Phytopathol. Soc. 3:264.
6. Lambe, R. C., and Wills, W. H. 1977. Further incidence of black root rot of Japanese holly. (Abstr.) Proc. Am. Phytopathol. Soc. 4:217.
7. Lambe, R. C., Wills, W. H., and Bower, L. A. 1979. Susceptibility of some *Ilex* species to *Thielaviopsis basicola*. South. Nurserymen's Res. J. 6:8-13.
8. Mathre, D. E., Ravenscroft, A. V., and Garber, R. H. 1966. The role of *Thielaviopsis basicola* as a primary cause of yield reduction in cotton in California. Phytopathology 56:1213-1216.
9. Pierre, R. E., and Wilkinson, R. E. 1970. Histopathological relationship of *Fusarium* and *Thielaviopsis* with beans. Phytopathology 60:821-824.
10. Stover, R. H. 1950. The black root-rot disease of tobacco. I. Studies on the causal organism, *Thielaviopsis basicola*. Can. J. Res. (C) 28:445-470.
11. Tsao, P. H., and VanGundy, S. D. 1962. *Thielaviopsis basicola* as a citrus root pathogen. Phytopathology 52:781-786.