Pathogenesis of *Thielaviopsis basicola* on a Susceptible and a Resistant Cultivar of Burley Tobacco

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ABSTRACT

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Stages in pathogenesis were examined on cultivars of burley tobacco that are either susceptible (Burley 21 × Kentucky 10 [B21×10]) or completely resistant (Tennessee 90 [TN90]) to black root rot, caused by *Thielaviopsis basicola*. The initial interaction of *T. basicola* hyphae with host tissue was examined microscopically on roots grown in vitro and in soil under greenhouse conditions. *T. basicola* penetrated root hairs and epidermal cells of both cultivars within 24 h of inoculation. Epidermal cells were the most common sites of penetration, and infection of these cells often was characterized by extensive bell-shaped collars around the penetration hyphae. Collars also were observed in cortical cells during colonization. Amber discoloration of infected cells was apparent on both cultivars within 72 h of inoculation; however, the reaction of cortical cells of TN90 was very restricted compared to a more diffuse amber region on B21×10. Hyphae advanced from the necrotic regions into asymptomatic cells on B21×10, but hyphae were limited to discolored cells on TN90. Sporulation was prolific in and on roots of B21×10, but was rare on TN90. A computer-driven image analysis program was used to collect quantitative data on lesion development following inoculation of aeroponically grown roots with endoconidia of *T. basicola*. Lesions developed on both cultivars, but lesion number, lesion size, and secondary inoculum production were severely limited on TN90 compared to B21×10. Because of limited lesion expansion, absence of secondary infections, and continued root growth, TN90 root systems outgrew the effects of the initial inoculation; whereas, the root systems of B21×10 became severely diseased.

Additional keywords: complete resistance, histopathology, partial resistance.

Thielaviopsis basicola (Berk. and Broome) Ferraris (synanamorph *Chalara elegans* Nag Raj & Kendrick) is a soilborne, plant pathogenic fungus commonly found in cultivated and noncultivated soils (31). *T. basicola* parasitizes a wide range of hosts (16) and is reported to have significant saprophytic ability (6). Important agricultural hosts include cotton, beans, carrots, pansies, peanuts, and tobacco. On tobacco (*Nicotiana tabacum* L.), *T. basicola* causes black root rot and is found in all temperate growing regions of the world (22). Major symptoms of black root rot include dark cortical lesions that often result in root pruning, foliar stunting, and significant loss of yield (11).

Development of *T. basicola* during penetration and colonization of several hosts has been examined histologically. Susceptible tobacco genotypes are penetrated by threadlike hyphae followed by the development of spear-shaped swellings inside the cell (10). Growth of intracellular hyphae in tobacco occurs as characteristic crescent-shaped cells. Similar observations were made on bean (2,18) and citrus (28). Formation of appressoria was reported on cotton (13), carrots (20), peanuts (8), and chicory (19), whereas direct penetration without appressoria was observed on beans (2) and holly (29). Penetration of cotton and chicory occurs through root hairs and root epidermal tissue (8,12,19), whereas Conant (4) reported infection of tobacco roots primarily through wounds. The deposition of papillae onto the inner surface of host cell walls has been associated with *T. basicola* penetration of peanuts (8) and red clover (14). Ultrastructural examination of infections on red clover revealed that the host plasma membrane remained intact for more than 24 h following penetration (14). On all hosts, colonization of root cortical tissue and subsequent sporulation by *T. basicola* were reported.

Although chemical (6,11,25) and biological controls of black root rot (9,27) have been reported, genetic resistance is the primary means of disease management in burley tobacco production (3,25,26). Two types of genetic resistance are used for control of black root rot: low to moderate partial resistance from *N. tabacum* and single-gene resistance derived from *N. debneyi* Domin (3,30). *N. debneyi*-derived resistance was first implemented in burley tobacco production in the 1950's (3). The resistance of *N. debneyi* is considered complete (3) and appears to be durable because no isolate of *T. basicola* has been identified with the ability to cause disease on tobacco cultivars carrying this gene for resistance (24).

In work described herein, interactions of *T. basicola* with burley tobacco cultivars that are either susceptible or resistant to black root rot were examined using quantitative and histological methods. Significant differences were observed in stages of pathogenesis on these cultivars, and the role of these differences in disease development is discussed.

MATERIALS AND METHODS

Host cultivars, pathogen maintenance, and inoculum production. Two cultivars of burley tobacco, Burley 21 × Kentucky 10 (B21×10) and Tennessee 90 (TN90) (F. W. Rickard Seeds, Winchester, KY), were used in all experiments. Although B21×10 has a low level of partial resistance to black root rot (25,30), in current burley tobacco production B21×10 is among the most susceptible cultivars planted and suffers significant disease in *T. basicola*-infested fields (25). Therefore, B21×10 was considered a susceptible control in this study. In contrast, TN90 has complete, single-gene resistance to black root rot derived from *N. debneyi* (3,25,30).

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Seeds of each cultivar were germinated in flats of peat and vermiculite under continuous fluorescent lighting at 28°C. Seedlings were watered daily and were not fertilized prior to transfer from flats.

The isolate of *T. basicola* (Smith-Henson-2) used throughout this study was isolated from diseased roots of burley tobacco (23). The isolate was typical of wild-type isolates of *T. basicola*, and was similar to other isolates in pathogenicity tests conducted under greenhouse conditions. Cultures were maintained and inoculum was produced on 5% carrot agar (50 ml of canned carrot juice [Hollywood Carrot Juice; Pet, Inc., St. Louis] and 18 g of agar/liter of deionized water). Cultures were grown at 22 to 26°C in the dark for 2 to 3 weeks prior to harvesting endoconidia by vortexing colonies of *T. basicola* in deionized water for 30 s. The resulting suspension was filtered twice through four layers of cheesecloth to remove agar, hyphae, and chlamydospores. The concentration of endoconidia was determined with a hemacytometer and diluted with deionized water to 2×10^6 endoconidia/ml for the inoculation of roots.

Quantitative study of lesion development. Tobacco plants were grown in aeroponics systems prior to inoculation with *T. basicola*. The aeroponics systems consisted of mist chambers (15 by 25 by 25 cm) supplied with an atomized nutrient spray (5 s spray/3 min). The nutrient solution contained 300 μM potassium nitrate, 50 μM potassium phosphate (monobasic), 400 μM calcium sulfate, 200 μM magnesium sulfate, 1.7 μM iron sulfate, and Murashige and Skoog basal salt micronutrient solution (Sigma Chemical Co., St. Louis) at quarter-strength. The nutrient solution was adjusted to pH 5.0 with sodium hydroxide (1.0 N) and was not recirculated following delivery to the mist chamber.

Seedlings were removed from flats 1 month after seeding (two- to three-leaf stage), rinsed thoroughly in deionized water, and transferred to vials (30 ml) that contained deionized water to facilitate acclimation to the aeroponics systems. Stems were supported in sponge cylinders (15 by 15 mm) that were slit from edge to center. Seedlings were transferred to the aeroponics systems after 4 days in the vials. Seedlings were suspended in lids of the mist chambers by the sponge cylinders and grown at 24°C under continuous fluorescent lighting.

Three separate aeroponics systems were constructed and served as replications of treatments. Each system supplied two mist chambers with nutrient spray, and each mist chamber held six plants of a given genotype. Roots were inoculated 1 week after transfer to the aeroponics systems by atomizing 5 ml of the endoconidial suspension (total of 1×10^7 endoconidia) into each mist chamber with an artist's airbrush. Root systems were not sprayed with nutrient solution for 2 h following inoculation to prevent washing endoconidia from the roots. Two plants were removed from each mist chamber at 6, 9, and 12 days after inoculation. The root systems were fixed in 2% glutaraldehyde in 0.05 M potassium phosphate buffer (pH 7.1) and stored at 5°C. The test was run twice.

After fixation, the entire root system per plant was suspended in a petri dish of deionized water over a blue background. Root images were then captured through a stereo microscope via a high-resolution, color video camera (Sony CCD IRIS; Sony Corp. of America, Park Ridge, NJ), and data were collected with the BioScan OPTIMAS 4.10 (BioScan Inc., Edmonds, WA) image analysis program. Root images were converted to binary images, and threshold levels were set to delineate lesions from healthy root tissue. Calibration units were determined in square millimeters, areas representing lesions were measured, and data were exported to Excel (Microsoft Corp., Redmond, WA) for compilation prior to analysis in SAS (SAS Institute, Cary, NC). Entire roots systems were examined for the presence of lesions, and all positive measurements greater than 0.05 mm^2 were included in the statistical analysis of treatment effects. Total root tissue per root system was determined by wet weight.

All tests were performed in a split-plot design with cultivar as whole-plot, and data were analyzed by the GLM procedure of SAS (SAS Institute) in a two-way analysis of variance. Significant differences in the analysis of variance were determined by F tests with $P < 0.05$.

Histopathology. Twenty seedlings of each cultivar were transferred from flats to plastic petri dishes (100 by 15 mm) 21 days after seeding (two-leaf stage). Each seedling was placed in a separate dish and grown hydroponically in 10 ml of sterile Gamborg's B-5 basal medium with minimal organics at pH 5.7 (Sigma Chemical Co.). Prior to transfer, root systems were rinsed thoroughly with deionized water. Seedlings were placed on P5 filter paper discs (7.0-cm diameter) (Fisher Scientific Co., Pittsburgh), and the hypocotyls were supported in sponge discs (15 by 2 mm) that were slit from edge to center. Seedlings were grown in dishes at room temperature under continuous fluorescent lighting, and the nutrient solution in each dish was replaced every 48 h. Hydroponically grown plants were inoculated 8 to 10 days after transfer to petri dishes. Inoculum was applied following removal

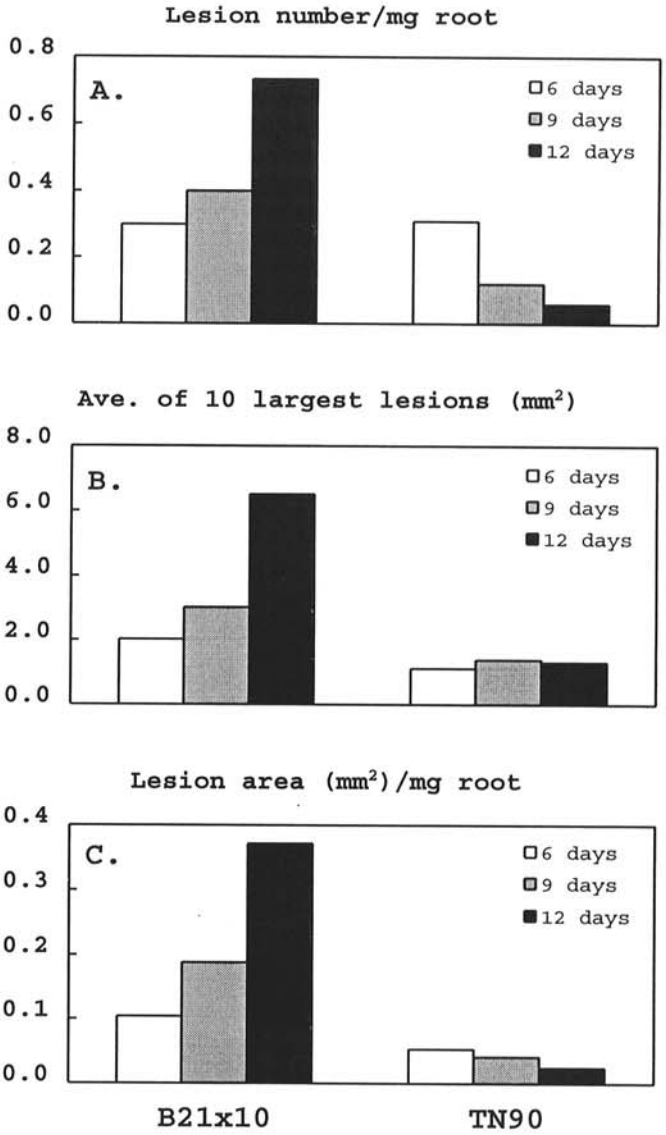

Fig. 1. Lesion development by *Thielaviopsis basicola* on susceptible (B21×10) and resistant (TN90) tobacco cultivars 6, 9, and 12 days after inoculation. Tobacco plants were grown in aeroponics systems and lesion data were collected with a computer-driven image analysis system. **A,** Number of lesions per milligram of root tissue. **B,** Estimate of lesion expansion from average of the 10 largest lesions (mm^2) per root system. **C,** Lesion area (mm^2) per milligram of root tissue.

of the nutrient solution by atomizing 0.3 ml of endoconidial suspension (6×10^5 endoconidia) onto each root system. Inoculum was sprayed with an artist's airbrush through a cylinder (7.5 by 22.5 cm) held over plates. Roots were observed 12, 24, 48, 72, and 96 h after inoculation.

An additional 20 seedlings of each cultivar were transferred 40 days after seeding (three-leaf stage) to pots (10-cm diameter) containing pasteurized sandy-loam soil. The plants were grown under greenhouse conditions for an additional 30 days prior to inoculation. Plants were fertilized weekly with 70 ml of Peter's 20-20-20 (Grace-Sierra Horticultural Products Co., Milpitas, CA) at 2 g/liter. Each plant was inoculated by pouring 10 ml of the endoconidial suspension onto the soil surface. Soil and roots were removed with a cork bore (1.8-cm diameter) 12, 24, 72, and 96 h after inoculation. Roots were washed free of soil with tap water prior to preparation for microscopic examination.

In an additional test, 20 plants of each cultivar were grown under sterile conditions on a nutrient medium. Seeds of each cultivar were surface sterilized for 5 min with constant agitation in a solution containing 40 ml of Clorox, 20 ml of 95% EtOH, 40 ml of deionized water, and 200 µl of Tween 80 (Fisher Scientific Co.), and then rinsed in three washes of sterile deionized water. For germination and detection of contamination, seeds were plated on 1% agar containing full-strength Gamborg's B-5 medium with sucrose (Gibco BRL, Life Technologies, Inc., Grand Island, NY). Two days after germination, sterile seedlings were transferred to plastic petri plates (100 by 15 mm) containing 10 ml of 1% agar with full-strength Gamborg's B-5 basal salt medium with minimal organics (Sigma Chemical Co.). Inoculum was placed within 5 mm of host roots by transferring a plug of carrot agar (4-mm diameter) containing hyphae of *T. basicola* to the sterile petri plates. Penetration of root hairs was examined 12 h after inoculation by inverting petri dishes on a microscope stage.

Microscopy. Inoculated in vitro and soil-grown roots were observed as nonstained whole mounts, stained whole mounts, and

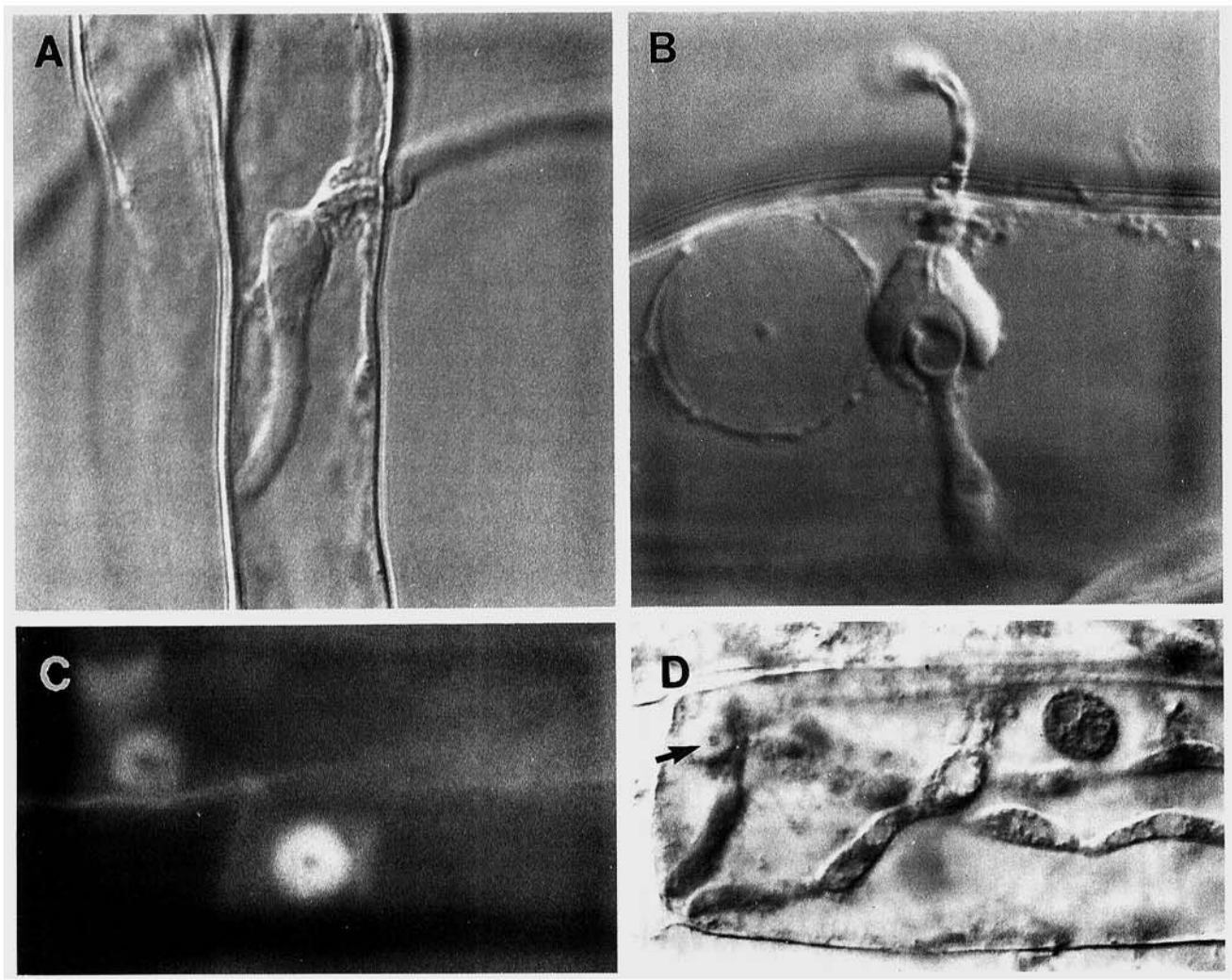

Fig. 2. Infection of burley tobacco root tissue by *Thielaviopsis basicola*. **A,** Penetration of root hair by hyphae and subsequent growth toward root cortex. Host specimen was grown under sterile conditions and photographed 12 h after inoculation. **B,** Penetration site of root epidermis showing distinct penetration morphology. The structure originated from the pathogen germ tube which showed a slight swelling at the site of wall penetration. Inside the host cell, the characteristics of the structure included an initial enlarged sphere, and central cytoplasmic column extending from the germ tube, a conspicuous bell-shaped deposit, and an extended hypha from which colonization continued. Host specimen was grown hydroponically and viewed 12 h after inoculation as nonstained whole mount under differential-interference-contrast microscopy. View of haustoriumlike structure is medial-longitudinal because of focal plane. **C,** Fluorescence visualization of the bell-shaped collar structure at the penetration site from the epidermal to the cortical cell, viewed in cross-section: central cytoplasmic column in the bell-shaped deposit. Excitation of cotton blue-stained specimens is indicative of callose composition. Host specimen was grown in soil and viewed as paradermal hand-section of cortical tissue, stained with lactophenol-cotton blue. **D,** Colonization of root cortical cell by *T. basicola* 24 h after inoculation. Intracellular hyphae showed characteristic sickle-shaped growth pattern. Bell-shaped collar structure at the penetration site from the epidermal to the cortical cell is viewed in cross-section: central cytoplasmic column (arrow) in the bell-shaped deposit. Host specimen was grown in soil and viewed as paradermal hand-section of cortical tissue stained with lactophenol-cotton blue.

stained sections. Nonstained roots were mounted in deionized water. Whole mounts were stained in 0.5% (wt/vol) toluidine blue O in 0.1% (wt/vol) Na_2CO_3 (pH 11.1) for 1 min at 40 to 60°C and destained in 95% EtOH for 12 h at room temperature. Stained roots were mounted in deionized water for observation. Paradermal hand-sections of root epidermis and cortex, obtained with the aid of a stereo microscope, were stained with lactophenol-cotton blue (Becton Dickinson Microbiology System, Cockeysville, MD) for 1 min at 40 to 60°C, and then transferred to lactophenol for mounting and observation. Microtome sections were prepared from root specimens fixed in 2% glutaraldehyde in 0.05 M potassium phosphate buffer at pH 7.1. Following fixation, specimens were washed (3 times) in 0.05 M potassium phosphate buffer (pH 7.1) and dehydrated through a graded series of alcohol concentrations at 4°C (12-h intervals in 30, 50, 70, 95, and 100% EtOH). Following dehydration, specimens were infiltrated and embedded at room temperature in JB-4 Plus (Polysciences, Inc., Warrington, PA) and sectioned longitudinally at 4 µm with a glass knife. JB-4 sections were stained in 0.5% (wt/vol) toluidine blue O in 0.1% (wt/vol) Na_2CO_3 (pH 11.1) for 15 s and rinsed in deionized water. All mounts were examined with a Zeiss axiophot microscope (Carl Zeiss, Inc., Thornwood, NY) equipped for fluorescence microscopy with an HBO 100-W mercury burner, G365-nm exciter, and LP 420-nm barrier fluorescence filters.

RESULTS

Lesion development was similar in both runs of the quantitative experiment, with cultivar, time after inoculation, and the cultivar × time interaction as significant factors in lesion development. Six days after inoculation, the number of lesions per milligram of root tissue did not significantly differ between the susceptible and resistant cultivars (Fig. 1A). However, between days 6 and 12, the number of lesions per milligram of root tissue increased from 0.30 to 0.73/mg of root tissue on B21×10, but decreased from 0.31 to 0.06/mg of root tissue on TN90. On day 6, the average size of the 10 largest lesions per plant was twice as great on B21×10 as on TN90 (Fig. 1B). Lesions on B21×10 continued to increase in size through day 12, from 2.02 to 6.51 mm^2, but lesions on TN90 remained unchanged at approximately 1.28 mm^2. Total lesion area per milligram of root tissue, a combination of lesion number and size, was twice as great on B21×10 as on TN90 at day 6. Total lesion area increased on B21×10 from 0.10 to 0.37 mm^2/mg of root between days 6 and 12. In contrast, on TN90, total lesion area decreased from 0.05 to 0.02 mm^2/mg of root between days 6 and 12 (Fig. 1C). Lesions on TN90 plants grown aeroponically were brown, while those on B21×10 were dark brown to black. No lesions were observed prior to inoculation of roots.

T. basicola penetrated the roots of both cultivars similarly within 12 h of inoculation without apparent inhibition of germination or germ tube growth on either hydroponically or soil-grown plants. Penetration of root hairs and epidermal cells was observed, but epidermal cells were the most commonly observed sites of infection. Penetration was characterized by one of two morphologies. Root hairs were penetrated by threadlike penetration hyphae followed by the formation of spear-shaped swellings, from which growth of the hyphae continued toward the root cor-

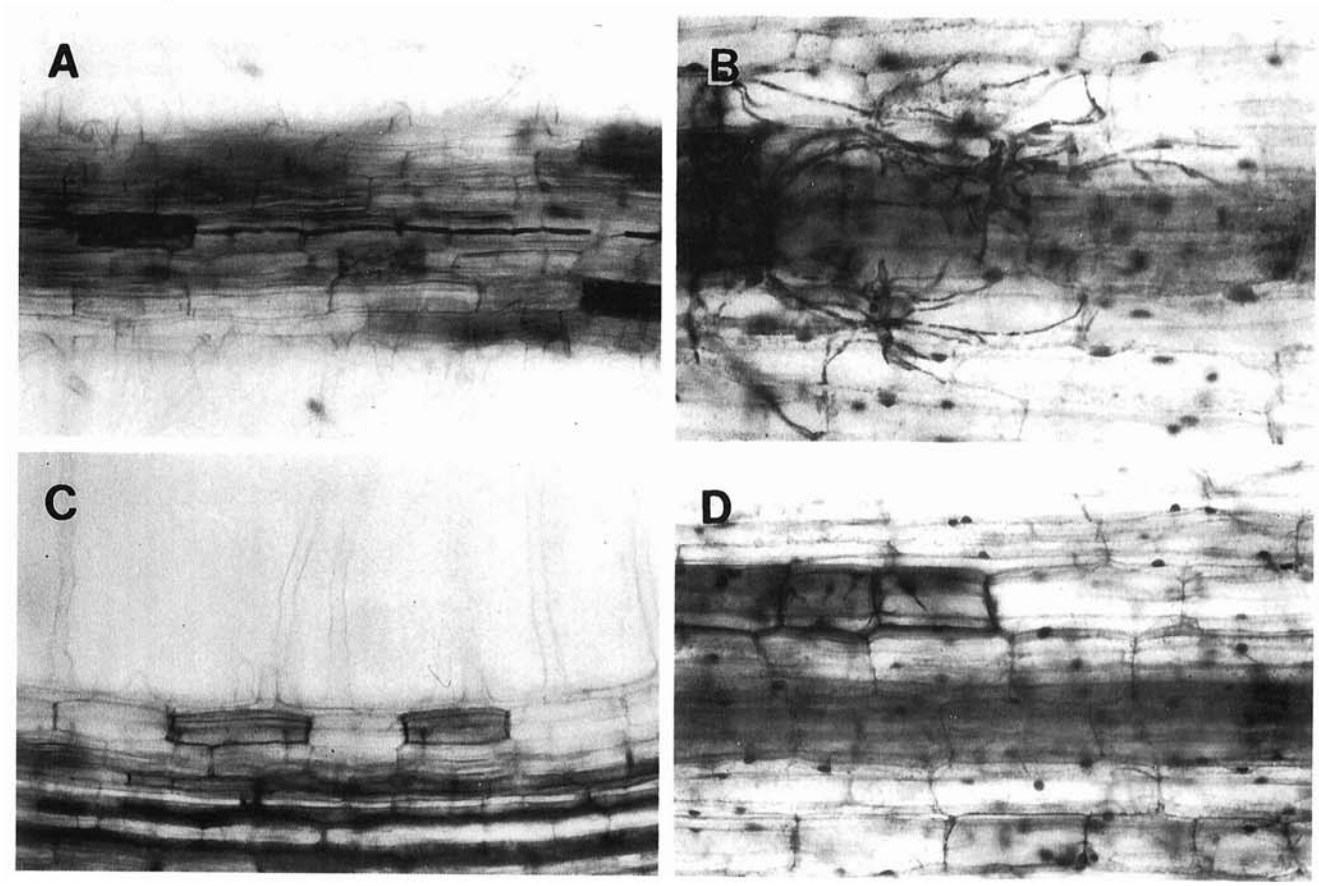

Fig. 3. Colonization of susceptible and resistant tobacco cultivars by *Thielaviopsis basicola* 72 h after inoculation. Host specimens grown hydroponically. **A,** Nonstained whole mount of susceptible root (B21×10). Amber cells and amber regions indicate sites of colonization. **B,** Stained whole mount of susceptible root showing extended colonization of cortical tissue. *T. basicola* hyphae advanced from discolored cells into asymptomatic tissue. **C,** Nonstained whole mount for resistant root (TN90) showing distinct discoloration of cortical cells. Discolored cells indicate sites of attempted colonization. **D,** Stained whole mount of resistant root showing no hyphae advancing from discolored cells.

tex (Fig. 2A). Most penetration structures in the epidermal cells were surrounded by a distinctive collar (Fig. 2B). These collars appeared bell shaped, encircling the neck region and the proximal end of the capitate swelling. Intracellular hyphae advanced from the swellings to continue colonization. Ultraviolet excitation of the cotton blue-stained collars showed yellow fluorescence indicative of callose (Fig. 2C) (5,7). A slight swelling of the germ tube was observed occasionally at the site of penetration. In some cases, epidermal infections resembled penetrations of root hairs, with only penetrating hyphae and swellings. Both types of penetration structures were observed upon cell-to-cell growth.

Penetration and colonization of cortical cells were observed on both cultivars 24 h after inoculation (Fig. 2D). The intracellular hyphae showed characteristic sickle-shaped morphology, and host nuclei were often observed in these cells more than 24 h after inoculation. Macroscopic lesions were apparent on both cultivars 72 h after inoculation, but significant differences in host response were observed between the cultivars. On B21×10, amber-colored regions indicated sites of cortical colonization (Fig. 3A), and *T. basicola* hyphae advanced from these amber-colored regions into neighboring cortical cells that were not yet discolored (Fig. 3B). Colonization was often extensive, with growth of inter- and intracellular hyphae. On TN90, amber cells were much more distinct than on B21×10, and only rarely were amber regions observed (Fig. 3C). *T. basicola* hyphae did not advance into neighboring cells from the amber cells (Fig. 3D). Endoconidia from the initial inoculation were consistently observed on the root surface of TN90 in close proximity to amber cells, and germ tubes and penetration sites in these cells often were visible. Occasionally two amber cells were adjacent in TN90, and each cell was either penetrated by a separate germ tube, or hyphae in one cortical cell advanced to the adjacent cell. However, no hyphae were observed in neighboring, asymptomatic cells at 72 h. Infections on TN90 occasionally showed discoloration only at the penetration site with no further growth by the fungus nor reaction by the host (Fig. 4).

Growth and sporulation by *T. basicola* were prolific on discolored and asymptomatic tissues of B21×10 (Fig. 5A); abundant phialides and characteristic chlamydospores with dark melanized segments were observed. Sporulation was observed in colonized cortical cells at 96 h after inoculation (Fig. 5B). Ectotrophic growth and sporulation were rarely observed on TN90, and spores that were produced often appeared immature and the segments were not melanized (Fig. 5C). Attempts to germinate these spores on growth media failed.

DISCUSSION

Spore germination, germ tube growth, and penetration of root tissue were similar on the two cultivars. Whether *T. basicola* hyphae produce appressoria prior to penetration of these cultivars is still unanswered. Production of hyphal swellings upon infection of burley tobacco was not common to all penetration sites. Reports of appressorium formation by *T. basicola* have varied with specific host interactions (2,8,13,19,20,29). Investigation into the presence or absence of mucilaginous substances at the hyphal-host cell interface would help clarify whether appressoria are necessary for infection.

Upon penetration of live root hairs, *T. basicola* formed the threadlike penetration hyphae and spear-shaped swellings observed previously (2,10,18,28). However, the production of distinct bell-shaped collars around the capitate penetration hyphae in epidermal cells has not been described in previous studies of *T. basicola*. The study of the collars under fluorescence microscopy indicated they were composed of callose, which is typical of haustorial collars and other host appositions (1). The observations of callose collars in this study and of host plasma membrane integrity during infection by *T. basicola* in a previous study (14) indicated that infection by *T. basicola* occurs by the formation of haustorialike structures that are not determinate but advance to colonize neighboring cells. Similar intracellular hyphal growth was reported in the foliar hemibiotroph *Colletotrichum lindemuthianum* (15). Relationships and similarities remain to be elucidated between the bell-shaped collars observed in tobacco and the papillae formed in the inner surface of host cell walls of red clover (14) and peanuts (8).

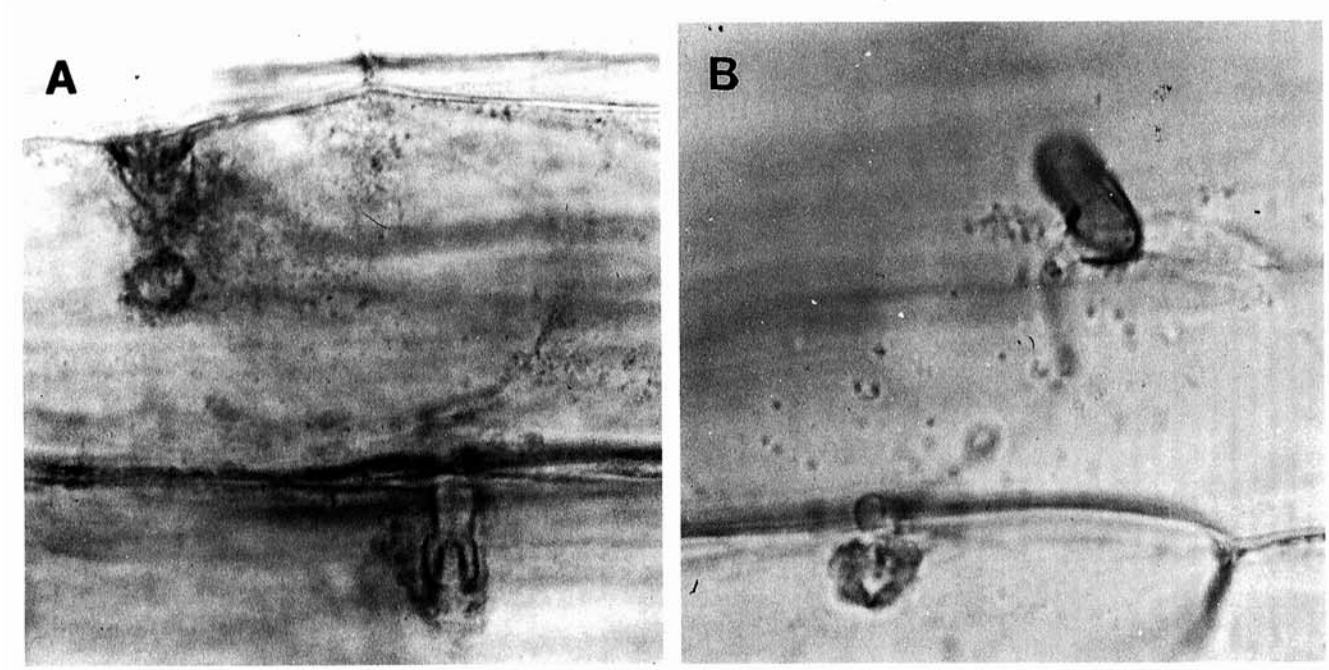

Fig. 4. Response of amber cells on the resistant tobacco cultivar (TN90) to penetration by *Thielaviopsis basicola*. Host plants were grown hydroponically and root tissues were observed 72 h after inoculation as nonstained whole mounts. **A,** Adjacent amber cells in resistant root cortex showing bell-shaped deposit formation upon cell-to-cell penetration. Both penetrated cells were discolored, and no hyphae were observed in neighboring asymptomatic cells. **B,** Attempted penetration of resistant host tissue showing discoloration only at penetration site. No further growth by the fungus nor response by the host was observed.

Hyphae advanced from the initially penetrated cells into neighboring cortical cells with the sickle-shaped growth pattern characteristic of *T. basicola* hyphae (2,10). Three days after inoculation, macroscopic symptoms were observed on roots of both cultivars. Cells of B21×10 that were near the site of initial penetration developed an amber discoloration, indicating cortical necrosis. Advance of *T. basicola* hyphae into neighboring, asymptomatic cells was often extensive on B21×10, and sporulation was prolific in and on the discolored and asymptomatic tissue. Sporulation among the necrotic cells of B21×10 indicated that host cell necrosis was not inhibitory to normal growth and reproduction of the fungus. This observation is consistent with descriptions of hemibiotrophic fungal pathogens.

In contrast to the development on B21×10, cell necrosis and growth of *T. basicola* hyphae were restricted to initially penetrated cells on TN90. In addition, no pathogen reproduction was observed on or near the necrotic cells. Because the macroscopic appearance of lesions on both hosts did not present an obvious temporal difference in the necrotic reaction, and because *T. basicola* hyphae had already advanced into neighboring cells of B21×10 before amber discoloration occurred, it appears that the resistance mechanism in TN90 is expressed following penetration and prior to cellular discoloration and necrosis.

Lesion expansion on TN90 also was greatly inhibited compared to B21×10. In general, the rate of lesion expansion relative to root growth is a major factor contributing to disease progress (24). In the quantitative study, TN90 rapidly outgrew the effects of the initial lesion development. Restricted lesion expansion and prevention of secondary inoculum production indicates that the resistance to black root rot expressed by TN90 can be considered complete resistance (17). This conclusion agrees with field observations of disease resistance in tobacco cultivars with this source of resistance (3,25).

Disease dynamics of polycyclic soilborne pathogens such as *T. basicola* are difficult to characterize. To a limited extent, secondary inoculum and hyphae of *T. basicola* are dispersed into the rhizosphere of susceptible cultivars, and the propagules may contribute to both auto- and alloinfections. This results in multiple cycles of inoculum production and secondary infections and may bring about a logarithmic increase in disease and in populations of the pathogen (25). In contrast, on TN90, the absence of secondary inoculum, combined with continued root growth, will result in disease progress typical of monomolecular pathosystems: new lesions form only as growing roots encounter primary inoculum.

An aeroponics system was used to investigate lesion development and secondary inoculum production in this study. Although the environment of an aeroponics system differs from naturally infested field soil, important benefits were provided by the use of this technique, and it has been used to investigate resistance to other root diseases (21). The amount of inoculum delivered to the root surface was easily controlled, as were nutrient concentrations, pH of nutrient solution, and temperature. Furthermore, host plants grown and inoculated without soil greatly facilitated visualization of root symptoms and allowed the use of an automated technique for quantitative measurement of disease. The computer-driven image analysis program not only provided efficient accumulation of data, it also provided an objective measure of lesion number and size.

This study provided information on *T. basicola* pathogenesis on tobacco and on the expression of resistance to black root rot in tobacco. However, further characterization of the resistance mechanism derived from *N. debneyi* will require a thorough understanding of the fungal differentiation and pathogenicity events that precede and occur concurrently with resistance expression. Current research on the cellular interactions of *T. basicola* and host tissue may provide a more accurate concept of *T. basicola* pathogenesis and ecology on susceptible and resistant genotypes.

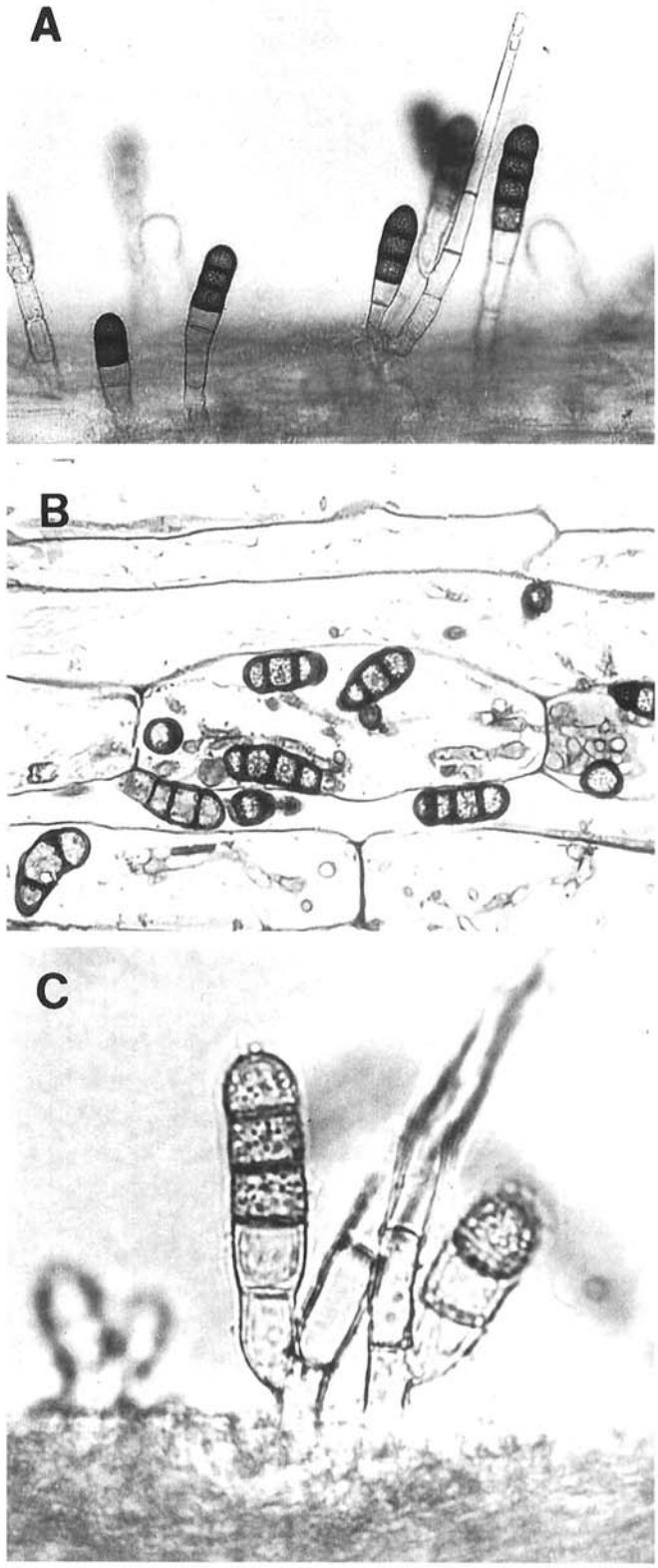

Fig. 5. Sporulation by *Thielaviopsis basicola* on a susceptible tobacco cultivar (B21×10). Host plants were grown hydroponically and root tissues were observed as nonstained whole mounts (**A** and **C**) and JB-4 Plus sections (**B**). **A,** Ectotrophic hyphae, phialides, and chlamydospore production on surface of susceptible roots 72 h after inoculation. Chlamydospores showed characteristic dark melanized segments. **B,** Sporulation of *T. basicola* in B21×10 cortical cells 96 h after inoculation. Section is longitudinal to root axis. **C,** Extremely rare *T. basicola* ectotrophic sporulation on resistant tobacco cultivar (TN90). Host plants were grown hydroponically and root tissue observed 72 h after inoculation as nonstained whole mounts. Chlamydospores appeared immature and nonmelanized.

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