Initial Cellular Interactions Between *Thielaviopsis basicola* and Tobacco Root Hairs

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ABSTRACT

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Cellular events that occur during the initial interactions between *Thielaviopsis basicola* and root hairs of tobacco (*Nicotiana tabacum*) were examined microscopically. Time-course documentation of the infection process indicated a dynamic interaction between *T. basicola* and the living host cell. Upon root hair contact and recognition, the vegetative apex of *T. basicola* rapidly differentiated to form infection structures, and the host cell responded cytologically. Penetration was achieved by threadlike hyphae that subsequently developed distal swellings, and intracellular hyphae of sickle-shaped morphology advanced from the distal swelling and colonized the cell. Streaming of the host cytoplasm became

aggregated near the infection site prior to penetration and accumulated around the infecting hyphae as long as the host cell was viable. Substantial callose deposition, in the form of a bell-shaped collar around infection structures, resulted from the cytological activity at the infection site. Penetration of dead root hairs was common, but did not lead to the development of infection structures or to a sustained association with the host tissue; *T. basicola* exited dead root hairs and resumed vegetative growth. The establishment of the parasitic relationship by *T. basicola* was characteristic of hemibiotrophic fungi in that, initially, biotrophic infection led to tissue colonization, and host cell survival was limited under parasitism.

Additional keywords: black root rot, *Chalara elegans*, histopathology.

Thielaviopsis basicola (Berk. & Broome) Ferraris (synanamorph *Chalara elegans* Nag Raj & Kendrick) is a soilborne, plant-pathogenic fungus commonly found in cultivated and non-cultivated soils (28,29). A wide range of plant species are parasitized by *T. basicola* including agricultural hosts such as cotton, bean, carrot, pansy, peanut, and tobacco (7,20). Infection of a susceptible host results in black root rot symptoms including dark cortical lesions, root pruning, and foliar stunting.

The initial interactions between plant-pathogenic fungi and their hosts involve physiological changes in both organisms that, in the fungus, reflect adaptations necessary for establishment of the parasitic relationship. Histological studies of *T. basicola* on several hosts have described events from penetration through sporulation (2,5,9,11,12,14,16–18,21–23,25–27). These descriptions have been consistent with regard to formation of threadlike, penetrating hyphae with distal swellings; intracellular colonization by sickle-shaped hyphae; and production of hyaline endoconidia and melanized aleuriospores. However, descriptions of other stages of infection are less consistent. For example, formation of appressoria upon contact with host tissue was reported on cotton (17), carrot (23), peanut (2,11), and chicory (22), whereas direct penetration without appressoria was observed on cotton (14), bean (21), red clover (18), and holly (27). Penetration by hyphae through host-deposited papillae was associated with epidermal infection of peanut (11) and red clover (18), whereas epidermal infection of tobacco was characterized by formation of substantial callose collars around the intracellular infection structures (9). In addition to epidermal penetration, root hair penetration (5,9,14,16,22) and infection through wounds (2,23,25) have been reported.

In the current study, events that occur during the initial cellular interactions between *T. basicola* and root hairs of tobacco (*Nico-*

tiana tabacum L.) were examined microscopically. Observations indicate a dynamic interaction between *T. basicola* and living host cells during establishment of the parasitic relationship.

MATERIALS AND METHODS

Host and pathogen maintenance. A black root rot-susceptible cultivar of burley tobacco, Burley 21 × Kentucky 10 (courtesy of F. W. Rickard Seed Inc., Winchester, KY) (9), was used as host material. In preliminary studies, additional tobacco cultivars also were examined (Judy's Pride [courtesy of R. C. Rufty, North Carolina State University, Raleigh] and Kentucky 15 [courtesy of F. W. Rickard Seed Inc.]), but did not differ appreciably from 'Burley 21 × Kentucky 10' in initial interactions with *T. basicola* as described here.

The isolate of *T. basicola* (Smith-Henson-2) used throughout this study was obtained from diseased roots of burley tobacco (24). It was morphologically typical of wild-type isolates of *T. basicola* and was similar to other isolates in pathogenicity tests conducted under greenhouse conditions. The isolate was periodically introduced to burley tobacco roots and reisolated following symptom development to avoid adverse effects of growth in culture on pathogen aggressiveness. Cultures were maintained and inoculum 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) under dark conditions at 22 to 26°C.

In vitro inoculation method. Petri plates were constructed such that infection events could be observed continuously on intact and undisturbed plants. Holes that were 3.5 cm in diameter were bored through the bottoms of plastic petri plates (100 by 15 mm); one hole was cut per plate. A thin ring of 100% silicone sealant (Dow Corning Corp., Midland, MI) was then applied to the outside surface of the petri plate 2 to 3 mm from the hole, a large cover slip (48 by 65 mm) (Fisher Scientific Co., Pittsburgh) was affixed over the hole to form a complete seal, and the silicone was allowed to cure overnight. Prior to use, the prepared, glass-

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bottom petri plates were soaked in 0.5% sodium hypochlorite for 30 min, rinsed under running tap water for 20 min, soaked in 95% ethyl alcohol for 2 min, and air-dried under sterile conditions. Lids also were constructed with corresponding holes and glass cover slips to avoid optical interference by the plastic.

Tobacco seeds were surface-sterilized for 5 min with constant agitation in an aqueous solution containing 2% sodium hypochlorite, 20% ethyl alcohol, and 0.2% 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 (Gibco BRL, Life Technologies, Inc., Grand Island, NY) amended to include 3% sucrose (pH 5.6). Two days after germination, sterile seedlings were transferred to glass-bottom petri plates containing 8 ml of 1% purified agar (Fisher Scientific Co.) with full-strength Gamborg's B-5 basal salt medium with minimal organics (Sigma Chemical Co., St. Louis) (pH 5.6); two or three seedlings were transferred to each plate. Radicles were positioned so that roots would grow at the agar-glass interface, and, after 7 to 10 days, one or two roots per plant were inoculated with endoconidia or hyphae of *T. basicola*.

Endoconidia were harvested for inoculation by removing 2- to 3-week-old colonies of *T. basicola* from culture plates and vortexing them in deionized water for 30 s. The resulting suspension was filtered twice through four layers of cheesecloth to remove agar, hyphae, and aleuriospores. The concentration of endoconidia was determined with a hemacytometer and diluted with sterile deionized water to 5×10^5 endoconidia/ml. Inoculation with endoconidia was performed by injecting 100 μ l of the endoconidia suspension under the agar in the proximity of the root using a 1-cm³ U-100 insulin syringe (Fisher Scientific Co.). Inoculation with hyphae was performed by transferring a plug of agar (1.5 mm in diameter) containing *T. basicola* hyphae to the plate containing the host plant. Plugs were obtained from the edge of a 3- to 5-day-old colony and transferred to a hole in the agar of the same size near the host roots, adjacent to the zone of maturation and 4 to 6 mm from the root hairs.

Observation of infection. Stages in infection were observed by inverting plates on the microscope stage following spore germination (about 8 h) or as hyphae grew through the agar to contact

root tissue (about 12 to 24 h). All specimens were observed with a Zeiss axiophot microscope (Carl Zeiss, Inc., Thornwood, NY) by brightfield, differential-interference-contrast, or fluorescence microscopy. The microscope was equipped for epifluorescence microscopy with an HBO 100-W/2 mercury burner (Carl Zeiss, Inc.) and G365-nm exciter:LP 420-nm barrier fluorescence filters (Carl Zeiss, Inc.). The fluorescent stain used was aniline blue, prepared as 0.05% aniline blue dye (color index #42755; Fisher Scientific Co.) in 0.067 M K₂HPO₄ (pH 9.0) (9,10) and applied by removing areas of agar near the inoculation site and flushing the plate with 50 ml of stain over 5 min.

Documentation of infection events was obtained by examination of roots in over 200 plates during a 1-year period, with multiple infection sites per root per plant. For quantitative assessment of fungal morphology at infection sites, plates were prepared as described above, one inoculation per plant and one plant per plate. Three plates were inoculated with hyphae and three plates were inoculated with endoconidia, as described above. The first five infections per plate to occur in the focal plane medial longitudinal to the root hair were documented with consideration to length, branching, and diameter of the infecting hyphae or germ tubes. The test was conducted twice. Images of infection sites were captured through the microscope via a high resolution, color video camera (Sony CCD IRIS; Sony Corp. of America, Park Ridge, NJ) following maturation of penetration structures. Measurements were determined with the Image-Pro Plus 1.3 (Media Cybernetics, Inc., Silver Spring, MD) image analysis program; a stage micrometer was used to calibrate the sizes of structures in the images.

Transmission electron microscopy. To generate specimens for transmission electron microscopy, tobacco seedlings were prepared and inoculated with hyphae as described above, except plates of layered agar were used instead of glass-bottom petri plates. Eight milliliters of 5% purified agar was allowed to solidify in the petri plates (100 by 15 mm) and then covered with an additional 8 ml of 1% purified agar with full-strength Gamborg's B-5 basal salt medium with minimal organics (pH 5.6). Roots grew at the interface between the 1 and 5% agar, and infection events were observed as described above. As infections progressed to specific stages, specimens were fixed by submerging

Fig. 1. Infection court establishment by *Thielaviopsis basicola* on root hairs of tobacco. **A**, Branch hyphae (small arrow) from which infections of root hairs (large arrow) were typically initiated among hyphal plug inoculations. Bar = 25 μ m. **B**, Noninductive contact. Growth of the hyphal apex continued, redirected by the physical interaction with the host root hair, but differentiation of the hyphal apex and cytological response with the host cell did not occur. Bar = 15 μ m.

the petri plate in 300 ml of 3% glutaraldehyde in 0.5 M potassium phosphate buffer (pH 6.2) at 4°C for 24 h. Following fixation, the petri plates were rinsed in three washes of the same buffer, and infection sites were excised with surrounding agar for support. Excess agar was trimmed away to give an agar block of 1 mm³ containing the specimen. Specimens were postfixed in 1% OsO₄ in 0.5 M potassium phosphate buffer (pH 6.2) for 3 h at 4°C, rinsed in three washes of the same buffer, and dehydrated in a graded series of ethyl alcohol (30, 50, 70, 95, and 100% ethyl alcohol at 12-h intervals). Specimens were flat-embedded in Spurr's media, sectioned at about 80 nm on a diamond knife, and stained with 4% uranyl acetate and Reynold's lead citrate. Sections were observed and documented using a JOEL 100S transmission electron microscope (JOEL, Ltd., Tokyo).

RESULTS

Infection sites initiated by germ tubes from endoconidia and by hyphae from agar were characterized ($n = 30$ per inoculation type). For clarity of presentation, apexes of vegetative hyphae of endoconidial origin will be referred to as germ tubes, regardless of length. Germ tubes initiating infection averaged 116 μm in length (standard error = 12.16). Infections by hyphae of hyphal plug origin generally were initiated by short and unbranched side branches of main hyphae (Fig. 1A). Branch hyphae attempting infection averaged 58 μm in length (standard error = 5.98). A small proportion of these short hyphae branched subsequent to infection (6.7%), but branching was not observed among the sampled germ tubes and was rarely observed during the course of the study. A slight increase in hyphal or germ tube diameter was commonly observed adjacent to root hairs upon establishment of an infection site (Figs. 2 and 3), but no distinct formation of an attachment organ (appressorium) was observed. Hyphal diameter was marginally greater than germ tube diameter; hyphal diameter was 3.19 μm (standard error = 0.07) and germ tube diameter was 2.27 μm (standard error = 0.06) 20 to 30 μm from the root hair surface at an infection site ($n = 30$). Hyphal diameter increased by

0.79 μm (standard error = 0.092) and germ tube diameter increased by 0.69 μm (standard error = 0.108) adjacent to the root hair surface at an infection site ($n = 30$).

Physical contact between *T. basicola* and root hair cells was not sufficient to cause *T. basicola* to initiate infection. In fact, host contact without differentiation of fungal structures (noninductive contact) was common with hyphae and germ tubes. In such cases, the vegetative apex of *T. basicola* continued to grow, redirected by the physical contact with the root hair, and no cytological reaction was discernible within the root hair (Fig. 1B). Noninductive contacts were observed with all approach angles of hyphae or germ tubes to root hairs. On occasion, branch hyphae and germ tubes infected root hairs subsequent to noninductive contacts, including subsequent contact with the same root hair.

Following an inductive contact (one that initiated infection), root hair infection proceeded rapidly, characterized by directed cytological activity in the root hair cell and differentiation of infection structures by the fungus (Fig. 3). Infections by hyphae and germ tubes were similar. No changes by the host or pathogen were observed prior to contact. Upon inductive contact, the apex of *T. basicola* discontinued vegetative growth. The hyphal apex increased slightly in diameter, and cytoplasmic streaming within the root hair became aggregated at the infection site 5 to 10 min after contact. Accumulation of cytoplasmic components intensified and persisted at the infection site as long as active streaming was observed within the host cell. Migration of the host nucleus in the direction of the infection site was commonly, but inconsistently, observed. Penetration of the root hair cell occurred 15 to 20 min after contact. The penetration site consisted of a relatively small diameter hole (Fig. 4A) through which a threadlike penetration hypha advanced into the root hair lumen. A brief cessation of growth of the penetration hypha was observed when the hypha had grown several micrometers into the root hair lumen (25 to 30 min after contact), but full extension of the threadlike penetration hyphae was completed 45 to 50 min after contact. Fifty to 55 min after contact, the distal end of the threadlike penetration hypha began to swell, and this swelling continued for about 15 min, resulting in a final diameter greater than 200% that of the vegetative hypha. Cessation of swelling and commencement of hyphal advance from the distal swelling occurred 65 to 70 min after contact. Formation of a callose collar around the threadlike penetration hypha and distal swelling was apparent 75 to 80 min after contact and became increasingly prominent at later stages of infection. Streaming of the host cytoplasm also resulted in aggregation of birefringent granules predominantly around the distal swelling during later stages of infection (Fig. 4B). The primary septation within the distal swelling was formed 110 to 115 min after contact, and, concurrently, advance of the parasitic apex (apex resulting in hypha directly involved in parasitism of host cells) gave rise to sickle-shaped growth morphology characteristic of *T. basicola*. Additional parasitic apexes originated from the distal swelling, the first being formed 115 to 150 min after contact. Survival of the root hair cells during parasitism varied from about 10 to 36 h, and advance of the parasitic apexes within the host cell often reached adjacent host cells in the root epidermis or cortex. *T. basicola* often exited the root hair cell when host necrosis occurred prior to hyphal advance into neighboring host cells. Cell-to-cell penetration by *T. basicola* proceeded via a similar series of stages.

Ultrastructural examination of the infection sites representing early stages of infection (about 60 min after contact) showed accumulation of cytoplasmic components, but no depositions around the distal swelling (Fig. 5B); however, in late stages of infection (about 800 min after contact), distinctly bell-shaped depositions around the threadlike penetration hyphae and proximal end of the distal swellings were observed (Fig. 5A). This deposition appeared outside the host plasma membrane, and evidence of its callose composition was provided by aniline blue-fluorescent staining of the structure at 600 min after contact (Fig. 3L). In ad-

Fig. 2. Inductive contact with endoconidial inoculum of *Thielaviopsis basicola* on root hair of tobacco. The germ tube apex increased slightly in diameter at the site of contact with the root hair surface. Bar = 10 μm .

Fig. 3. Infection of a tobacco root hair by *Thielaviopsis basicola*. Bar = 5 μ m. **A**, Two minutes prior to contact between the hyphal apex (small arrow) with the root hair surface (large arrow). **B**, Moment of contact. **C**, Ten minutes after contact. Cytoplasmic aggregation (arrow) was observed at the infection site. **D**, Twenty minutes after contact. *T. basicola* penetrated through the cell wall of the root hair. **E**, Thirty minutes after contact. The penetration peg (arrow) advanced into the host cell lumen. **F**, Forty-five minutes after contact. The penetration peg was fully extended. **G**, Sixty-five minutes after contact. The distal swelling (arrow) formed. **H**, Seventy-five minutes after contact. The distal swelling was mature. **I**, Ninety minutes after contact. The hyphal apex advanced from the distal swelling, and substantial host deposition (arrow) appeared around the penetration peg and proximal end of the distal swelling. **J**, One hundred and fifteen minutes after contact. A septation (arrow) was present in the distal swelling and sickle-shaped morphology of intracellular hyphae developed. **K**, One hundred and fifty minutes after contact. Additional hyphal apices arose from the distal swelling (arrow). **L**, Six hundred minutes after contact, viewed with fluorescence microscopy after aniline blue staining. Fluorescence was localized to regions of the penetration peg and the proximal end of the distal swelling, indicating callose deposition.

dition, ultrastructural examination of mature infection structures consistently showed a septation in the threadlike penetration hyphae (Fig. 5C); no neck ring or specialized plant-fungus interface was observed.

Formation of infection structures by *T. basicola* was dependent upon penetration of a living host root hair. Formation of infection structures occurred in most every case of inductive contacts with

root hairs in which the cytoplasm was actively streaming; very rarely, infection structures failed to form completely or formed abnormally. Initiation of infection of a root hair that was already infected by another hypha also was observed. In six of these cases, the primary infection of the root hair was far advanced (greater than 8 h) and cytoplasmic streaming within the root hair was evident but sparse. In such interactions, morphological and

Fig. 4. Additional morphological features of tobacco root hair infection by *Thielaviopsis basicola*. **A**, Narrow diameter of penetration site (arrow) relative to hyphal diameter. Bar = 2.5 μ m. **B**, Aggregation of birefringent granules (arrow) around the distal swelling during later stages of infection (105 minutes after contact of hyphal apex with root hair surface). Bar = 5 μ m.

Fig. 5. Transmission electron micrographs of tobacco root hair infection by *Thielaviopsis basicola*. **A**, Distinctly bell-shaped deposition around the penetration peg and proximal end of the distal swelling at later stages of infection (800 to 1,000 minutes after contact of hyphal apex with root hair surface). Deposition appeared outside host plasma membrane (arrow). Magnification = $\times 7,000$. **B**, Distal swelling of penetration structures at early stage of infection (60 to 70 minutes after contact). Bell-shaped collar was not present around penetration structures. Magnification = $\times 9,000$. **C**, Penetration site and septation (arrow) in penetration peg. Magnification = $\times 20,000$. Section represents alternative plane of specimen depicted in Figure 5A.

temporal development of infection structures by *T. basicola* proceeded normally, despite no appreciable accumulation of host cytoplasmic components at the infection site (Fig. 6A). Secondary infections that occurred in close temporal succession to initial infections elicited cytological reactions similar to singly infected root hairs.

Penetration of dead root hair cells by *T. basicola* was common. In such cases, a slight increase in hyphal or germ tube diameter was observed adjacent to the root hair. The penetration site was initially of small area relative to the hyphal or germ tube diameter, but penetration did not proceed via a threadlike hypha and none of the structures associated with parasitic infection were formed. Instead, the hypha resumed the diameter of the initial hypha or germ tube. Upon traversing the lumen and contacting the opposite cell wall, the hypha again swelled slightly and exited the cell via a hypha of normal diameter (Fig. 6B and C). Noninductive contacts also were observed with dead root hair cells.

DISCUSSION

Although the majority of histological studies concerning host infection by *T. basicola* has centered on colonization and lesion development within the root cortex, root hairs have been repeatedly noted as infection sites (5,9,14,16,22) and often may be the host tissue with which *T. basicola* makes initial contact. The in vitro inoculation method developed in this study allows for precise location of inoculum on host tissue, as well as effective visualization and documentation of cellular events during initial interactions. Host root hairs were particularly suited for this type of study, because they are hyaline and often could be observed in a single focal plane with little optical interference from other host tissues. In addition, fixation of specimens was possible at precise moments of interest for subsequent excision and examination by other histological methods such as transmission electron microscopy.

Observations of initial cellular interactions between *T. basicola* and root hairs of tobacco provided evidence of recognition by both organisms. *T. basicola* was similar to other plant-pathogenic fungi in that recognition of an exogenous signal (infection court) was required for the vegetative apex to initiate host penetration (3,8).

Substrate recognition was evident by morphological differentiation upon penetration (inductive contact). Physical contact alone was insufficient to cause hyphae and germ tubes to initiate penetration, and, because dead root hairs were often penetrated, the signal to induce penetration was not entirely a diffusible product of the host cytoplasm. It is possible that other factors that determine infection courts were not uniformly present on the host surface or that apexes of *T. basicola* differed in their ability to respond to the host substrate at instances of contact.

Host recognition of the pathogen was evident by directed streaming and accumulation of cytoplasmic components within minutes of inductive contact. This prepenetration response is well documented with other fungal pathogens (1). Production of papillae is a common result of such cytological activity; however, the rapidity of penetration by *T. basicola* apparently did not allow sufficient opportunity for papillae formation in root hairs of tobacco. Host synthesis and deposition of callose around infection structures indicated sustained cytological activity in response to infection by *T. basicola*, but, in general, the defense mechanisms were ineffective at preventing successful infection.

Previous reports described infection structures of *T. basicola* as threadlike hyphae with distal swellings that give rise to sickle-shaped intracellular hyphae (5,9,11,12,14,16). These structures also were observed in this study. However, previous histological studies of *T. basicola* examined samples destructively only from 8 h to days after inoculation, whereas the time-course observation of specimens in the current study more accurately documented the time required and the sequence of events during infection. The existing biotrophic interactions of *T. basicola* with roots of red clover were described at the cytological level by Nan et al. (18). They reported ramification of hyphae through the lumens of living host cells and the eventual loss of cytoplasmic integrity of the host. Several other studies of *T. basicola* also have reported hyphae within the host cells that appeared to be living (5,9,16,26). Results from the current study and from Nan et al. (18) on the interactions of *T. basicola* and host cells provide compelling evidence for the trophic classification of *T. basicola*.

Behavior of *T. basicola* during infection of root hairs was typical of a hemibiotrophic pathogen. Hemibiotrophs are character-

Fig. 6. Interactions of *Thielaviopsis basicola* with weakened and dead root hairs of tobacco. **A**, Penetration of weakened root hair. Bar = 5 μ m. Root hair cell did not direct cytoplasmic streaming to site of infection. Although no cytoplasmic aggregation and no accumulation of depositions were observed, the differentiation of infection structures (penetration peg and distal swelling) by *T. basicola* occurred normally. **B and C**, Penetration of dead root hair. Bar = 5 μ m. Infection structures associated with parasitism were not formed, and, after traversing the root hair lumen, the hypha exited the cell and resumed vegetative growth.

ized by host interactions that are initially biotrophic in nature, but in which cytoplasmic integrity of the host cell is ephemeral (15). Subsequent necrotrophic nutrient acquisition by hyphae in association with these necrotic cells is often suggested (15), and sporulation typically occurs within necrotic tissue. *Colletotrichum* has served as a model for the study of hemibiotrophism among foliar-phytopathogenic fungi (4,19), and root infection by *T. basicola* was analogous to foliar infection by *Colletotrichum* spp. in several ways. Similarities included the following: (i) the penetration site consisted of a relatively small hole through which the threadlike penetration hypha advanced into the cell lumen, (ii) the duration of biotrophic infection was on the order of days, (iii) the plant-fungus interface appeared unspecialized (i.e., no neck ring), and (iv) the advance of hyphae through the lumen of the host cell was achieved by invagination of the host plasma membrane and led to infection of neighboring cells (9). A low level of activity of cell wall-degrading enzymes that results in a small diameter penetration site is believed to limit disturbance of the host cell during biotrophic infection (13,19). Also, the lack of a specialized plant-fungus interface may contribute to the ephemeral nature of the biotrophic phase of parasitism by *Colletotrichum* (19). The same may also be true in *T. basicola*-host systems; however, it is possible that the rapid intracellular growth of *T. basicola* may contribute to host cell death by surpassing the limitations of plasma membrane invagination. Dissimilarities between infection by *T. basicola* and the model *Colletotrichum* include the following: (i) the differentiation of the vegetative apex of *T. basicola* does not result in the similar formation of a morphologically specialized appressorium, (ii) the threadlike penetration hyphae of *T. basicola* were markedly more pronounced than the analogous structure of *Colletotrichum* spp., (iii) the cytological activity of the host cell resulted in substantial callose deposition around infection structures of *T. basicola*, and (iv) the parasitic apex of *T. basicola* gave rise to sickle-shaped hyphae that were morphologically distinct from vegetative hyphae.

The differentiation of appressoria by *T. basicola* was examined histologically and quantitatively in the current study. Contradictions in the literature as to whether *T. basicola* forms appressoria upon infection have been repeatedly noted (2,9,11,17,18,22). However, it is evident, upon comparison of our observations and the numerous existing descriptions, that the morphology of *T. basicola* at the infection site is similar across host species (2,5,9,11,14,17,18,21–23,27). Discrepancies have been attributed to differences in experimental techniques (22), but it is more likely to have resulted from different uses of terminology. In the review by Emmett and Parbery (6), morphology and function were used as criteria for identification of appressoria. Identification of appressoria based upon morphology was suggested to be impossible or arbitrary when the structure is not delimited by a septum and consists of only a slight swelling. Such is the case with *T. basicola* on tobacco. The alternative concept defines appressoria as functioning in attachment to the host. No studies of *T. basicola* have been reported that specifically investigate this phenomena; although, the apex of prepenetration germ tubes commonly remain attached to the root surface through rigorous manipulations such as autoclaving and incubation in stain solutions (M. E. Hood, unpublished data). The swelling of hyphae or germ tubes of *T. basicola* at the host surface should not be referred to as an appressoria until the function of attachment has been confirmed.

Behavior of *T. basicola* upon penetration of weakened and dead root hairs demonstrated the importance of interactions with a living cell in the development of infection structures and establishment of the parasitic relationship. Penetration hyphae and associated structures were formed in the living cells whether or not the cell responded cytologically. Therefore, development of the distal swelling of penetration hyphae is independent of host accumulation of cytoplasmic components and the resulting structural depositions. This conclusion is inconsistent with that of Nan et al. (18),

who suggested that the morphology of infection structures (i.e., distal swellings) of *T. basicola* was influenced by expansion of the penetration peg following growth through existing papillae. In addition to differentiation of infection structures, inductive contact with a living host cell led to a sustained association of the parasite with host tissue. In contrast, *T. basicola* exited dead root hairs and resumed vegetative growth; threadlike penetration hyphae and distal swellings were not observed upon cell wall penetration of dead root hairs.

The distinctive intracellular morphology of *T. basicola* has been used as a diagnostic characteristic of this fungus for many decades (5,12,14,16,17,21,25,27). In the current study, important factors and events that lead to the formation of these structures and their role in sustaining a parasitic association with the host were examined. It was only upon contact and recognition with a living host cell that such differentiation occurred, and the infection was typical of hemibiotrophs in form and effect on the host cell. Root colonization resulted from infection of a living root hair, because *T. basicola* maintained a developmental association with the host and progressed to penetrate neighboring cells. Initial interactions with dead host cells failed to serve a similar function, and structures and development that are characteristic of parasitism by *T. basicola* did not occur. Further studies should address the importance of establishing the hemibiotrophic relationship to survival of *T. basicola* under natural conditions. Such information would allow more reliable evaluation of measures to control populations of *T. basicola* and agricultural losses to black root rot.

LITERATURE CITED

1. Aist, J. R. 1983. Structural responses as resistance mechanisms. Pages 33-70 in: *The Dynamics of Host Defense*. J. A. Bailey and B. J. Deverall, eds. Academic Press, New York.
2. Baard, S. W., and Laubsher, C. 1985. Histopathology of blackhull incited by *Thielaviopsis basicola* in groundnuts. *Phytophylactica* 17:85-88.
3. Bailey, J. A. 1983. Biological perspectives of host-pathogen interactions. Pages 1-32 in: *The Dynamics of Host Defense*. J. A. Bailey and B. J. Deverall, eds. Academic Press, New York.
4. Bailey, J. A., O'Connell, R. J., Pring, R. J., and Nash, C. 1992. Infection strategies of *Colletotrichum* species. Pages 88-120 in: *Colletotrichum: Biology, Pathology and Control*. J. A. Bailey and M. J. Jeger, eds. CAB International, Wallingford, United Kingdom.
5. Christou, T. 1962. Penetration and host-parasite relationships of *Thielaviopsis basicola* in the bean plant. *Phytopathology* 52:194-198.
6. Emmett, R. W., and Parbery, D. G. 1975. Appressoria. *Annu. Rev. Phytopathol.* 13:147-167.
7. Gayed, S. K. 1972. Host range and persistence of *Thielaviopsis basicola* in tobacco soil. *Can. J. Plant Sci.* 52:869-872.
8. Hoch, H. C., and Staples, R. C. 1991. Signaling for infection structure formation in fungi. Pages 25-46 in: *The Fungal Spore and Disease Initiation in Plants and Animals*. G. T. Col and H. C. Hoch, eds. Plenum Press, New York.
9. Hood, M. E., and Shew, H. D. 1996. Pathogenesis of *Thielaviopsis basicola* on a susceptible and a resistance cultivar of burley tobacco. *Phytopathology* 86:38-44.
10. Hood, M. E., and Shew, H. D. 1996. Applications of KOH-aniline blue fluorescence in the study of plant-fungal interactions. *Phytopathology* 86:704-708.
11. Jones, B. L. 1991. Penetration and development of *Chalara elegans* in peanut (*Arachis hypogaea*). *Phytophylactica* 23:81-84.
12. Koch, L. W. 1936. Recent investigations on tobacco root rot in Canada. *Can. J. Res. Sect. C Bot. Sci.* 13:584-589.
13. Lewis, D. H. 1973. Concepts in fungal nutrition and the origin of biotrophy. *Biol. Rev.* 48:261-278.
14. Linderman, R. G., and Toussoun, T. A. 1968. Pathogenesis of *Thielaviopsis basicola* in nonsterile soil. *Phytopathology* 58:1578-1583.
15. Luttrell, E. S. 1974. Parasitism of fungi on vascular plants. *Mycologia* 66:1-15.
16. 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.
17. Mauk, P. A., and Hine, R. B. 1988. Infection, colonization of *Gossypium hirsutum* and *G. barbadense*, and development of black root rot caused by *Thielaviopsis basicola*. *Phytopathology* 78:1662-1667.
18. Nan, Z. B., Long, P. G., Skipp, R. A., and Hopcroft, D. H. 1992. Micros-

- copy of invasion of red clover roots by *Trichocladium basicola*, and effects of benomyl and procloraz. *Plant Pathol.* 41:449-461.
19. O'Connell, R. J., and Bailey, J. A. 1991. Hemibiotrophism in *Colletotrichum lindemethianum*. Pages 211-222 in: *Electron Microscopy of Plant Pathogens*. K. Mendgen and D. E. Lesemann, eds. Springer-Verlag, New York.
 20. Otani, Y. 1962. Studies on the black root rot disease caused by *Thielaviopsis basicola* (Berk. & Br.) Ferris. *Bull. Okayama Tob. Exp. Stn.* 23:1-118.
 21. Pierre, R. E., and Wilkinson, R. E. 1970. Histopathological relationship of *Fusarium* and *Thielaviopsis* with beans. *Phytopathology* 60:821-824.
 22. Prinsloo, G. C., Baard, S. W., and Ferreira, J. F. 1992. A scanning electron microscope study of the infection and colonization of chicory root by *Thielaviopsis basicola*. *Phytophylactica* 24:293-296.
 23. Punja, Z. K., Chittaranjan, S., and Gaye, M. M. 1992. Development of black root rot caused by *Chalara elegans* on fresh market carrots. *Can. J. Plant Pathol.* 14:299-309.
 24. Shew, H. D., and Meyer, J. R. 1992. *Thielaviopsis*. Pages 171-174 in: *Methods for Research on Soilborne Phytopathogenic Fungi*. L. L. Singleton, J. D. Mihail, and C. M. Rush, eds. The American Phytopathological Society, St. Paul, MN.
 25. Stover, R. H. 1950. The black root rot disease of tobacco. I. Studies on the causal organism *Thielaviopsis basicola*. *Can. J. Res. Sect. C Bot. Sci.* 28:445-470.
 26. Tsao, P. H., and van Gundy, S. D. 1962. *Thielaviopsis basicola* as a citrus root pathogen. *Phytopathology* 52:781-786.
 27. Wick, R. L., and Moore, L. D. 1983. Histopathology of root disease incited by *Thielaviopsis basicola* in *Ilex crenata*. *Phytopathology* 73:561-564.
 28. Yarwood, C. E. 1974. Habitats of *Thielaviopsis* in California. *Plant Dis. Rep.* 58:54-56.
 29. Yarwood, C. E. 1981. The occurrence of *Chalara elegans*. *Mycologia* 73:524-530.