INOCULUM DENSITIES OF *THIELAVIOPSIS BASICOLA* IN TOBACCO FIELDS IN VIRGINIA, AND THE RELATIONSHIP OF INOCULUM DENSITY TO THE SEVERITY OF BLACK ROOT ROT AND GROWTH OF TOBACCO

by

Lawrence P. Specht

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APPROVED:

G. J. Griffin, Chairman	N. L. Powell
L. A. Link	P. J. Semtner
L. D. Moore	W. H. Wills

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(ABSTRACT)

A new selective medium (TB-CEN) was developed for isolating *Thielaviopsis basicola*, cause of black root rot of tobacco, from soil. TB-CEN medium contains etridiazol and nystatin to inhibit the growth of undesired fungi, and unautoclaved extract from carrot to selectively enhance for the growth of *T. basicola*. Inoculum and/or population densities of *T. basicola* in five burley tobacco fields were 74-166 propagules per g of soil, and 0-12 propagules per g of soil in three other burley fields. Inoculum and/or population densities of *T. basicola* in 12 flue-cured and 2 sun-cured tobacco fields were 0-26 propagules per g of soil, and 101 and 402 propagules per g of soil in two other flue-cured fields. Environmental factors apparently had a strong effect on black root rot development, since root rot and plant stunting were severe in two burley fields that had 148 and 158 propagules per g of soil, but were not severe in the two flue-cured fields that had 101 and 402 propagules per g of soil. All of the cultivars planted in the four fields were susceptible.

Black root rot was the major disease associated with the stunting of tobacco plants in the burley region of Virginia, but not in the flue- and sun-cured regions. No evidence was found to indicate that endomycorrhizae were involved in tobacco stunting in Virginia. *T. basicola* inoculum density-disease severity studies were conducted both in soil-temperature tanks and in the field. Tobacco seedlings were grown in temperature tanks (20-23 C) for 30-31 days in naturally infested field soil (pH 6.5). For all cultivars tested (Burley 21, NC95 and Va Gold), the mean percent of roots that were rotted increased significantly (P=0.001) as inoculum density increased (R^2 range for regressions=0.93-0.97). Severe levels of root rot occurred at inoculum densities of 50-200 propagules per g of soil. Significant (P=0.01) reductions in plant growth occurred at inoculum densities as low as 5-10 propagules per g of soil. In a study conducted on a commercial burley tobacco (cv. B21-Ky10) field, inoculum densities of 150 and 683 propagules per g of soil were associated with moderate and severe levels of black root rot, respectively. Differences between soil-temperature tank and field studies appeared to be due to variations in environmental- and host-related factors. In another burley field study, the fungicide imazalil, which completely inhibited the growth of *T. basicola* when amended into agar media at a concentration of 1.0 µg a.i./ml, failed to control black root rot when it was added to transplanting water (50 ml/plant) at concentrations as high as 1,500 µg a.i./ml.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

REVIEW OF LITERATURE

INTRODUCTION AND RESEARCH OBJECTIVES

Tobacco (*Nicotiana tabacum* L.) is Virginia's most important agronomic crop. The four types of tobacco grown in Virginia are burley, flue-cured, dark-fired and sun-cured. Burley tobacco is grown in the Appalachian region in southwestern Virginia. The flue-cured, dark-fired, and sun-cured types are grown in the Piedmont region in the central Virginia. In 1982, burley tobacco was produced on 14,300 acres, flue-cured on 42,000 acres, dark-fired on 4,800 acres, and sun-cured on 570 acres (41).

An important soilborne disease of tobacco is black root rot, caused by *Thielaviopsis basicola* (Berk. & Br.) Ferraris (=*Chalara elegans* Nag Raj & Kendrick). Tobacco plants affected with black root rot often exhibit poor growth and stunting (103). However, McLeod (115) found no correlation between the severity of black root rot and the growth of diseased flue-cured and burley tobacco plants. Black root rot is the most important fungal disease of burley tobacco in Virginia (L. A. Link, personal communication), but no extensive root-disease surveys have been conducted. Black root rot is typically a problem in those burley fields where crop rotation is not practiced and soil pH is 6.0 or higher (R. G. Henderson, personal communication). Stunting is most severe early in the season, particularly when the weather is cool and

wet after transplanting (R. G. Henderson, personal communication). In the burley-tobacco growing regions of the United States, yield reductions of 5-7% are currently estimated to occur as a result of damages caused by black root rot (90). However, no supporting data were presented in this publication.

The present importance of black root rot, and its role in tobacco stunting, in the Piedmont region of Virginia has not been fully examined; however, the warm weather in this region makes it less likely that the disease occurs to the same extent there that it apparently does in the cooler burley region. Black root rot has traditionally not been a serious problem in the flue-cured, dark-fired, and sun-cured tobacco types in Virginia (R. G. Henderson, personal communication). Clayton (24) did not consider black root rot to be important in flue-cured tobacco in the southeastern United States. Todd (172) reported that black root rot caused only an estimated 0.15% yield reduction in flue-cured tobacco in North Carolina. Losses due to black shank, caused by *Phytophthora parasitica var. nicotianae*, and brown root rot, caused by the lesion or meadow nematode (*Pratylenchus* spp.) accounted for much larger amounts, 0.76 and 0.86%, respectively, of the 3.14% total estimated loss caused by all diseases (172). Gayed & Watson (54) estimated that black root rot caused a 0.26% reduction in the yield of flue-cured tobacco in Ontario, Canada. Troutman (175) reported that black root rot in Virginia was frequently a severe problem in flue-cured tobacco seedbeds during cool, wet springs. In recent years, there has been an increasing

number of cases of black root rot in the Piedmont region of Virginia (D. A. Komm, personal communication).

The four objectives of this study were: 1. To more fully determine the incidence and severity of black root rot in Virginia tobacco fields, and also to determine the role of *T. basicola* in tobacco stunting. The possible role of pathogenic endomycorrhizal fungi, such as *Glomus* spp., in tobacco stunting was also studied. 2. To determine the relationship between the inoculum density of *T. basicola* and the incidence and/or severity of black root rot, both in soil-temperature tanks and in the field. Also, to determine the importance of *T. basicola*-inoculum clumping in the field to this relationship. 3. To evaluate the effectiveness of imazalil, when added to transplanting water, for controlling black root rot of tobacco. 4. To develop a new selective medium for the quantitative isolation of *T. basicola* from Virginia tobacco field soils. Presently reported selective media do not always inhibit undesired fungi satisfactorily enough to permit the full recovery of *T. basicola* on soil-dilution plates.

The information gained from this study will help to define more clearly the importance of black root rot in Virginia, especially in relation to tobacco stunting. Information obtained from this study will also be useful for establishing inoculum density versus disease incidence and/or severity relationships; such information is essential for the development of a black root rot disease prediction program. There are presently no

cost-effective chemical treatments for controlling black root rot in tobacco fields; the information obtained from this study also will help to evaluate the possible use of imazalil for this purpose.

THE ROLE OF ENDOMYCORRHIZAE IN TOBACCO STUNTING

The stunting of tobacco plants has been observed in all of the tobacco-growing regions in Virginia (D. A. Komm, personal communication). *T. basicola* probably plays a role in stunting in some fields, but other pathogenic organisms may also be involved. Hendrix & Csinos (68) reported on "tobacco stunt" disease in burley tobacco in Kentucky. Tobacco stunt was found to be controlled by fumigation with methyl bromide + chloropicrin. Burley cultivars considered resistant to black root rot, such as Ky 10, Ky 14, Ky 15 and Ky 17, were all susceptible to tobacco stunt. Roots of tobacco plants with this disease always appeared normal. The above-ground symptoms were similar to those associated with other factors (such as black root rot, manganese toxicity, and brown root rot caused by *Pratylenchus* spp.) that also cause stunting of burley tobacco. A pathogenic vesicular-arbuscular (endomycorrhizal) fungus, *Glomus macrocarpum* (Tul. & Tul.) Gerd. & Trappe was isolated from several fields with tobacco stunt (69, 119, 120). The fungus, which severely inhibited the growth of tobacco seedlings in greenhouse trials, sporulated heavily on roots without inducing any visible necrosis (69, 119). Wendt & Griffin (unpublished data, personal communication) also found an association between the stunting of burley tobacco and the presence of *Glomus*-like spores in tobacco plant root systems. However, some of the stunted plants had either root and/or stem damage caused by cutworms (*Agrotis*

ypsilon) and wireworms (*Conoderus vespertinus*).

Data collected by Fox & Spasoff (48) indicated that *Endogone mosseae* may be pathogenic on some tobacco cultivars. The growth of 'BVA 523', a burley breeding line, was decreased (compared to plants grown in noninfested soil) when plants were grown in soil infested with *E. mosseae*. Csinos (34) reported that tobacco (cv. NC 2326) inoculated with azygospores of *Gigaspora margarita* only showed a positive growth response at the lowest of three soil fertility levels tested; no differences were found between inoculated and uninoculated plants at two higher soil fertility levels. In a survey of the endomycorrhizal flora in 30 tobacco fields in northern Florida, Rich & Schenck (145) found that *G. margarita* and *Acaulospora trappei* predominated. The number of different species of endomycorrhizal fungi on tobacco was considered low in comparison to other agronomic crops in the area.

Crush (33) reported that several species of grasses and legumes grew better when they were colonized by endomycorrhizae, but only when soil phosphate levels were low. Crush (33) suggested that mycorrhizal fungi exist in relationships with their hosts that vary from mutualism to mild parasitism, depending upon how much phosphorus the host can take up from the soil unassisted. Mycorrhizae are usually considered beneficial to plant growth, however. Menge (116) even concluded that the stunting of some plants that occurs following fumigation results from the elimination of beneficial mycorrhizae.

Mycorrhizal plants are generally less susceptible to disease (37, 151). Tobacco plants colonized by either *E. mosseae* (12) or *Glomus mosseae* (152) were more resistant to *T. basicola* than were nonmycorrhizal plants. Colonization of tobacco roots by *E. mosseae* reduced infection by *Meloidogyne incognita* (158). Cotton seedlings inoculated with *G. mosseae* had as much root rot caused by *T. basicola* as did uninoculated plants; however, reductions in shoot growth were less when the plants were also mycorrhizal (153). The incidence of infection of soybean by *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Fusarium solani* was the same whether plants were mycorrhizal or not, but plants colonized by *G. mosseae* tolerated infection by these pathogens better than nonmycorrhizal plants (196).

Mycorrhizal and nonmycorrhizal plants are sometimes equally susceptible to disease. Colonization of sweet orange seedling roots by *G. fasciculatus* did not influence the severity of root rot caused by *T. basicola* (35). Wick & Moore (189) reported that the presence of *G. mosseae*, and unidentified *Glomus* and *Gigaspora* species, did not influence the infection of Japanese holly feeder roots by *T. basicola*. The reproduction of *Pratylenchus brachyrus* on cotton roots was not altered by colonization with *Gigaspora margarita* (76).

Mycorrhizal plants are sometimes more susceptible to disease. Phytophthora root rot of soybeans, caused by *Phytophthora megasperma* var. *sojae*, was more severe when plants were colonized by an *Endogone* sp. (148). Similarly, Verticillium

wilt of cotton, caused by *Verticillium dahliae*, was more severe when plants were colonized by *G. fasciculatus* (36).

Nematodes, especially lesion or meadow nematodes (*Pratylenchus* spp.), root knot nematodes (*Meloidogyne* spp.), and the tobacco cyst nematode (*Globodera solanacearum*) also may cause stunting of tobacco in Virginia (56, 57, 77, 84). Powell & Nusbaum (143) reported that a black shank (*Phytophthora parasitica* var. *nicotianae*)-root-knot (*Meloidogyne* spp.) complex causes a severe stunting of flue-cured tobacco in North Carolina. Olthof (125) reported that the colonization of tobacco (cv. Burley 49) by *T. basicola* favored the development of one strain of *Pratylenchus penetrans* within tobacco roots; however, the presence of the nematode did not influence the severity of black root rot. Green et al. (58) reported that colonization of pea roots by *T. basicola* inhibited invasion by *Pratylenchus thornei*, but encouraged invasion by *Pratylenchus crenatus*.

BLACK ROOT ROT OF TOBACCO CAUSED BY *THIELAVIOPSIS BASICOLA*

Taxonomic classification. *Thielaviopsis basicola* (Berk. & Br.) Ferraris is a Deuteromycete of the order Moniliales and class Dematiaceae (14). *T. basicola* has both phialidic (endoconidiophores) and aleuriosporic (chlamydospores) states. *T. basicola* does not have a known teleomorphic state. A nonpathogenic Ascomycete, *Thielavia basicola*, is sometimes found in association with *T. basicola* in decayed root tissues (101). Early researchers, such as Johnson (79), mistakenly assumed that *Thielavia basicola* was the teleomorph of *T. basicola* and referred to the black root rot pathogen as *Thielavia basicola*. However, McCormick (113) showed that these two fungi were different organisms.

Nag Raj & Kendrick (121) proposed that the latin binomial for *T. basicola* be changed to *Chalara elegans*. They combined the genera *Thielaviopsis* and *Chalaropsis* with the closely related genus *Chalara*. The genus *Chalara*, as defined by Nag Raj & Kendrick (121), exhibits pleomorphism, which refers to the production of more than one conidial state. Under Nag Raj and Kendrick's system, the chlamydosporic states of these fungi are only used for species determination. Delon & Kiffer (38) list *T. basicola* as *Chalara elegans*, and *Chalaropsis thielavioides* as *Chalara thielavioides*. The distinction is that the former produces chlamydospores in chains while the latter usually forms them singly.

Host plants. *T. basicola* attacks many genera of plants within at least 33 families (103). Above-ground symptoms of black root rot are typical of those associated with decreased root function, such as stunting, chlorosis, and loss of foliage. Young plants may damp-off when attacked as seedlings, but stunting can still occur if older plants are attacked. *T. basicola* is particularly damaging to members of the Fabaceae (formerly Leguminosae), Malvaceae, Solanaceae and Cucurbitaceae families (79, 103, 195).

Besides tobacco, some other economically important plants susceptible to black root rot are soybean (5, 99), snap and pinto beans (53, 93, 106), peanut (107), cowpea (194), pea (18, 96), lentil and chickpea (19), cotton (49), sesame and Japanese holly (85), poinsettia (82), sweet orange and other citrus species (181, 182), cherry (140), plum (155), and catnip (Mark Vizvary, personal communication). *T. basicola* does not attack members of the Poaceae (formerly Gramineae) and related families (195).

Pathogenesis. Garrett (50) classified *T. basicola* as an unspecialized parasite that has a high degree of competitive saprobic ability. *T. basicola* colonizes and sporulates on green, decomposing residues of such crops as tobacco, legumes, and even cereals (53). As a parasite, *T. basicola* penetrates epidermal cells directly without forming an appressorium (23). Hyphae then invade, both inter- and intracellularly, all surrounding tissues. Chlamydospores are eventually produced in colonized tissues, including the tracheary elements (23). Endoconidia and

chlamydospores are formed abundantly at the surface of diseased roots (23). Bateman (16) found greatly increased populations of *T. basicola* in the rhizosphere of susceptible hosts, and concluded that this was a reflection of pathogen sporulation on roots.

Hildebrand & Koch (70) reported that *T. basicola* was capable of definite intracellular penetration of strawberry (a nonhost) roots; however, invaded cells were not killed and infection was halted. On tobacco, the development of black root rot involves extensive maceration of host root tissue (183). Thornberry (170) reported that culture filtrates of *T. basicola* contained pectase (pectin methylesterase), and that the degree of virulence was correlated with the production of this enzyme. Unbehaun & Moore (183) reported that the principal difference between a black root rot-susceptible (Vesta 5) versus a resistant (Virginia Gold) cultivar was that pectic enzyme activity increased more rapidly and to the highest level after inoculation of the former. Endopolygalacturonate-trans-elimanase activity coincided closely with symptom development, and appeared to be largely responsible for the maceration of diseased host tissues. Two other pectic enzymes, pectin methylesterase and endopolygalacturonase, were more important in penetration and early stages of root tissue colonization. Unbehaun & Moore (184) reported that nearly 30% less pectic substances were extracted from diseased than from healthy roots of Vesta 5. Phosphatidase enzymes also apparently play a role in pathogenesis, since the concentration of these

enzymes in diseased tissue paralleled the development of symptoms of black root rot on bean (104).

Survival. Little is known about how *T. basicola* survives in soil, but the chlamydospores appear to play an important role (26, 73, 111, 139, 179). The direct microscopic observation of germinated propagules on soil-dilution plates showed that chlamydospores were the origin of all colonies (179). Otani (128) reported that chlamydospores survived up to 3 yr in soil. Endoconidia have been reported to persist as long as 7 mo (91). The survival of *T. basicola* is reduced in microbially active, moist soil (132). Chlamydospore germinability is temporarily lowered when soil is air-dried, but rewetting dry soil greatly increases germinability (91).

Because the chlamydospores of *T. basicola* survive in soil for long periods of time, crop rotation will not completely eliminate this pathogen from infested fields. However, it will reduce infestation levels. Small grains, especially oats, barley and corn, are not susceptible to black root rot and make effective rotation crops (103). However, *T. basicola* still survives as a parasite on many weeds (53). Legumes, such as soybean, red clover, sweet clover, and alfalfa, should not be used in a rotation with tobacco because they are susceptible to black root rot, which increases pathogen infestation levels (103). Any crop grown prior to tobacco should be turned under well in advance to allow for complete decomposition. Decomposing

crop residues often produce phytotoxic compounds that injure tobacco roots, which increases susceptibility to black root rot (137). Similarly, decomposing barley residues increase the severity of black root rot on cotton (92).

Influence of temperature. The optimum soil temperature range for the development of black root rot on tobacco is 17-23 C, with no disease occurring at 30-32 C (80). In contrast, the optimum temperature for the growth of *T. basicola* in pure culture is 26-30 C (80, 102). This apparent discrepancy is discussed by Garrett (50). Garrett considers black root rot of tobacco to be an example of a disease where the optimum temperature for the growth of the host is more important than the optimum temperature for the growth of the pathogen. Tobacco is of tropical origin and grows more vigorously at high (eg. 30 C) than at low (eg. 20 C) soil temperatures. *T. basicola*, an unspecialized parasite, most readily attacks young, immature tissue, while mature tissue is relatively resistant. The rapid maturation of tobacco roots at high soil temperatures therefore enables them to resist infection. Conant (32) reported that wound periderm formation in tobacco was favored by high soil temperatures and was closely associated with resistance to black root rot.

Lloyd & Lockwood (98) reported that black root rot on pea was most severe at a high soil temperature (28 C). The optimum temperature for the growth of pea is low (13-17 C). Black root rots on poinsetttia (82) and citrus (181), which both grow best

at high temperatures, are most severe at low temperatures. These results also support Garrett's hypothesis.

Influence of soil pH. The optimum pH value for the in vitro growth of *T. basicola* is markedly lower than the optimum value for the development of black root rot. The optimum pH range for the in vitro growth of *T. basicola* is reported to be 4.4-6.4 (102) and 4.7-5.5 (15). In contrast, the optimum soil pH value for black root rot development is 6.0 or greater (103). Doran (42) reported that at soil temperatures of 15, 18, 21-24, and 27 C that marked black root rot injury did not begin until soil pH was increased (from below 5.7) to 5.7, 5.7-5.8, 5.8, and 5.8-5.9, respectively. No black root rot occurred at 30 C or above, regardless of soil pH. The avaliability of calcium may also influence the development of black root rot, since excess calcium stimulates the growth of *T. basicola* over a range of pH values (102). The manner in which calcium does this is uncertain, but it may stimulate the activity of phosphatidase enzymes, which function in the utilization and breakdown of lipids (104). Soils high in pH are usually high in calcium, since lime is often supplied to increase soil pH.

Wicox (190) and Papavizas (131) have suggested that there is possibly a microbial pre- or post-host invasion inhibition of *T. basicola* in acid soil. Microorganisms present in the rhizosphere of plants growing in acid soil may be more inhibitory to *T. basicola* than those in the rhizosphere of plants growing in

neutral or alkaline soil. Data presented by Bateman (15) supports this hypothesis. Bateman reported that bean plants were equally susceptible to black root rot over a range of soil pH values (4.7-8.0), but only when the normal soil microbiota was eliminated and aseptic conditions were maintained.

Influence of soil texture. Soil texture influences the development of some diseases caused by *T. basicola.* For instance, fine-textured soils with poor drainage increased the severity of peanut blackhull in New Mexico (73). Severe black root rot of soybeans was prevalent in the fine-textured, clay soils of southeastern Michigan, but not in the coarse-textured soils of southwestern Michigan (99). High soil moisture directly favors the development of black root rot and other diseases of tobacco (103). Sidebottom & Shew (157) reported that the development of black shank was correlated with sand content of soil. The fine-textured soils were often waterlogged, which inhibited root development and probably contributed to the increased severity of root infection that was observed.

High soil moisture may increase the severity of black root rot indirectly by lowering soil temperature. The effects of soil texture, moisture and temperature on the development of black root rot are interrelated. For instance, fine-textured soils hold more water and remain cooler than light-textured soils (71). Lucas (103) concluded that black root rot on tobacco is generally more severe in cold, wet, clay soils.

Resistance. Resistance to black root rot within the species *N. tabacum* is multigenic, and is influenced by environmental conditions (103). One type of resistance within *N. tabacum* is a moderate level of resistance controlled by a group of recessive genes. This resistance was originally obtained from the cultivar Harrow Velvet (25). The best quality burley cultivar developed using the Harrow Velvet-type resistance is Burley 21; however, this cultivar is only moderately resistant (25). The burley cultivar Ky 14 has a relatively high level of resistance. The first cross in its development was Warner X Burley 21 (94). Ky 14 yields slightly better than Burley 21, but has a lower leaf quality (27). MS B21-Ky10 is a first generation hybrid that was made by crossing Ky 10 onto male-sterile Burley 21. This cultivar has a low to medium black root rot resistance (29). Virginia 509 is a burley cultivar that originated from the cross Burley 37 X Burley 21 (66, 67). It has moderate black root rot resistance, in addition to black shank and wildfire resistance. Virginia 509 has excellent leaf quality and yields as well as Burley 21. L8 burley germplasm confers moderate resistance (28). The burley cultivar Ky 12 has relatively high *N. tabacum*-type resistance (95).

An extremely high level of resistance, or near "immunity", to black root rot, conferred by a single dominant gene, has been obtained from the distantly related species *N. debneyi*. (25). The first commercial burley cultivar containing the *N. debneyi*-type resistance was Burley 49 (72). The burley cultivars Burley

64 (62), Ky 15 (30), and Ky 17 (31) also contain this type of resistance. Two dark-fired cultivars with *N. debneyi*-type resistance are Ky-170 (1) and DF-911 (88). Tobacco cultivars with *N. debneyi*-type resistance do not yield as well as other cultivars under disease-free conditions (90). Legg et al. (90) concluded that the linkage of undesirable genetic material with *N. debneyi*-type resistance was a possible explanation for the differences in yield and chemical composition between resistant and susceptible cultivars. A recently developed burley cultivar with *N. debneyi*-type resistance is TN-86. This cultivar is also reported to be high yielding (P. Hunter, personal communication).

Todd (171) reported that black root rot caused severe losses in burley tobacco in North Carolina prior to the development of resistant cultivars. However, this disease continues to cause some damage in North Carolina, even though moderately resistant cultivars are now grown (172). Smiley et al. (160) reported that Burley 49 was subject to yield reductions of about 500 lb/acre when planted on infested land.

Va 3160 was the first flue-cured cultivar with resistance to black root rot (87), but it has reduced yields and lower leaf quality (87). White & Povilaitus (188) reported on the development of Dehli 34, a flue-cured cultivar with "tolerance" to black root rot (i.e. susceptible to *T. basicola*, but still yields well when diseased). Va Gold is a flue-cured cultivar considered to have high black root rot resistance, but it may also be partly tolerant (R. G. Henderson, personal communi-

cation).

Clayton and others (25) suggested that the genes in tobacco which provide resistance to black root rot (and other diseases) could be involved in general metabolism as well, and are therefore pleiotropic. The metabolic action(s) associated with the expression of black root rot resistance has not been determined. As stated earlier, Conant (32) found that resistance (*N. tabacum* type) was correlated with wound periderm formation. Jewett (78) could not confirm Conant's findings and had to conclude that resistance was due primarily to chemical factors. Evidence presented by Gayed (52) suggests that resistance is phenolic in nature. The *N. debneyi*-type resistance was mainly attributed to this chemical factor, while the *N. tabacum*-type was partly attributed to this and partly to other unknown factors (52). Mathre et al. (112) reported that young tissues of cotton seedlings were very susceptible to infection by *T. basicola*, while older tissues with a well developed phellogen were fairly resistant.

Physiological specialization. Johnson (79) reported that isolates of *T. basicola* from tobacco infected nearly 100 different species of plants and concluded that no specialized races appeared to exist. No host specificity was found among isolates of *T. basicola* collected from and tested for pathogenecity on pea, lentil and chickpea (19). Isolates of *T. basicola* tested on cotton, bean, pea, peanut and soybean were not

host specific (168).

There are many reports indicating that physiological specialization in *T. basicola* does occur (4, 82, 85, 93, 98, 124, 165 166, 191). Stover (165, 166) reported that *T. basicola* occurs as two distinct types, which he called the gray and brown wild biotypes. The latter, which was predominant in nature, was more pathogenic and aggressive in culture. Studies by Huang & Patrick (75) showed that these biotypes were unstable and that each could be derived from the other in culture. Linderman & Toussoun (93) reported that isolates of *T. basicola* taken from tobacco, soybean, garbonzo and pinto beans, cherry, and citrus existed as clones ranging from nearly avirulent to highly virulent on these hosts. Keller & Shanks (82) reported that isolates of *T. basicola* taken from tobacco and poinsettia varied in pathogenecity; isolates from one host never injured the other. Lambe & Wills (85) reported that isolates of *T. basicola* taken from Japanese holly varied in pathogenicity from mild to severe. Ohashi & Murai (124) reported on the occurrence of isolates of *T. basicola* with low, intermediate and high virulence on tobacco. Wright & Biss (191) reported that tobacco cultivars susceptible to black root rot in North America (eg. NC95 and Hick's Broadleaf) were resistant to strains of *T. basicola* present in New Zealand. Virginia Gold is reported highly resistant to strains of *T. basicola* present in Japan (124), but only moderately resistant to strains present in New Zealand (191). Clayton (25) concluded that there are many different races of *T.*

basicola that vary widely in their virulence and their ability to attack different host genotypes. A tobacco cultivar might appear resistant in one state, but highly susceptible in another. For instance, Ky 16 has shown distinct evidence of resistance to strains of *T. basicola* present in North Carolina, Virginia, Tennessee and Kentucky, but not in Wisconsin (25). Studies on the physiologic race problem indicate that only the *N. debneyi*-type resistance appears to be equally effective against all races of *T. basicola* (25).

Chemical control. Fumigation with methyl bromide + chloropicrin, methylisothiocyanate, or metham are recommended practices for controlling black root rot in tobacco seedbeds in Virginia (D. A. Komm, personal communication). Methylisothiocyanate (138) and metham (89) will also control black root rot in tobacco fields. Papavizas & Lewis (133) reported that these compounds reduced the inoculum density of *T. basicola*. However, the fumigation of tobacco fields in Virginia is expensive and is not practiced often (D. A. Komm, personal communication).

Several nonfumigant fungicides that control black root rot of tobacco under greenhouse conditions and in seedbeds are benomyl, thiabendazole, thiophanate-methyl, captan and maneb (51, 64, 133, 136, 150). Some studies indicate that soil incorporation of benomyl reduces the inoculum density of *T. basicola* (74, 109, 150). Other studies show that benomyl can control black root rot of bean and tobacco without reducing

pathogen inoculum density (133, 136). For instance, the incorporation of benomyl into soil at the rate of 6 µg/g soil controlled black root rot without appreciably lowering the inoculum density of *T. basicola* (136). Gayed (51) reported that a 24-hr-root-dip treatment with benomyl (5 µg/ml) prior to transplanting 4-wk-old tobacco seedlings controlled black root rot in growth chambers, but failed to protect older, field-sized transplants placed into infested field soil. Other crops where benomyl is effective for controlling black root rot include cotton (49, 118), bean (133, 134, 135, 136), peanut (74), citrus (3), and poinsettia (109, 144). However, even if found effective, the use of benomyl for controlling black root rot in tobacco fields is not economically advantageous (64).

A study conducted by S. K. Gayed (unpublished data, personal communication) indicated that the addition of the systemic, imidazole fungicide imazalil to transplanting water (2,000 µg/ml, 50 ml/plant) reduced the severity of black root rot in a flue-cured tobacco field in Canada. However, a phytotoxic effect of imazalil may have been responsible for the lack of any associated increase in plant growth.

Methods of isolation. Some general methods of assaying for soilborne plant pathogens include direct microscopic observation, dilution end-point or most probable number (MPN) techniques, baiting, and selective media (117). The direct microscopic observation of chlamydospores of *T. basicola* is accomplished by

spreading a soil suspension over an agar surface, staining, and applying a cover glass over the area to be viewed (130). This method can only be used when the inoculum density of *T. basicola* is high. One type of MPN technique involves the preparation of a series of soil dilutions, which are then used for inoculating susceptible host plants to test for the presence of the pathogen (108).

Yarwood (193) reported on a method for the qualitative isolation of *T. basicola* from soil by means of carrot disks. Tsao & Canetta (180) modified Yarwood's procedure and developed a MPN technique using carrot disks. Tabachnik et al. (168) developed an assay procedure that involves placing soil on carrot disks, by means of a modified Anderson soil sampler. Rittenhouse & Griffin (147) used a modified carrot-bait procedure for the quantitative isolation of *T. basicola* from soil. In the latter study, the multiple colonization of carrot disks was accounted for by correcting the percent colonization data with Gregory's multiple-infection equation (59). The carrot-disk procedures are very sensitive, but they also are time consuming, do not enumerate propagules of *T. basicola* as discrete colonies, and require a large number of replicates for statistical accuracy. Lloyd & Lockwood (97) cautioned users of the carrot-disk methods against the use of carrots contaminated with *Chalaropsis thielavioides* Peyr.

The most desirable method for enumerating propagules of *T. basicola* is the dilution-plate technique. Several media reported

selective for this fungus are RB-M2 medium (177), VDYA-PCNB medium (129), and TBM-C and TBM-V8 media (105). However, none of these media satisfactorily inhibit undesired fungi when low soil dilutions are required (Griffin & Rittenhouse, unpublished data, 169). Personal experience (L. P. Specht, unpublished data) indicates that when Virginia tobacco field soils are used, none of these media can be employed successfully with 10^{-1} and 10^{-2} soil dilutions. Plates are quickly overrun with undesired fungi at these dilutions, which prevents the recovery of *T. basicola*.

Two general principles employed in the development of selective media are selective inhibition of undesired organisms and selective enhancement of the desired organism (178). Vaartaja (185) reported that two antifungal compounds, nystatin and pentachloronitrobenzene (PCNB), were relatively noninhibitory to the growth of *T. basicola*. Most of the above media depend upon these compounds for the selective inhibition of soil fungi. However, Vaartaja (185) also reported that nystatin and PCNB were relatively noninhibitory to the growth of a *Fusarium* sp. Indeed, the development of interfering soil fusaria on dilution plates prepared with the present selective media is a problem.

Extract from carrot tissue appears to be useful for the selective enhancement of *T. basicola* on agar media. Tsao & Van Gundy (182) reported that carrot agar (water agar containing propylene-oxide sterilized, macerated, fresh carrot roots) was useful for the selective enhancement of *T. basicola*. The mechanism(s) which operates in carrot tissue and extract for the

selective isolation of *T. basicola* are not known; however, it also occurs to a lesser extent in other species of Umbelliferae (114).

A study by Mathre & Ravenscroft (111) showed that *T. basicola* grows poorly on synthetic media. The germination of endoconidia and chlamydospores of *T. basicola* after 24 hr in media containing root extracts, simple sugars, or minerals + complex carbohydrates was 100%, 65-85%, and near 0%, respectively. The addition of calcium to culture media enhances the growth of *T. basicola* over a range of pH values (102). Steinberg (164) and Yamaguchi (192) also reported that calcium stimulates the growth of *T. basicola*.

Interestingly, a root-dip inoculation of carrot seedlings with endoconidia of *T. basicola* resulted in increased plant growth (194). No root lesions associated with *T. basicola* were seen on carrot, which suggests that the *T. basicola* component of the soil microflora may be symbiotic with carrots.

INFLUENCE OF INOCULUM DENSITY ON SOILBORNE DISEASE

Black root rot/greenhouse studies. The stunting of plants with black root rot has been reported for most susceptible crops, including tobacco (25, 42, 146, 153, 191, 175, and R. G. Henderson, personal communication), soybeans (99), peas (96), cotton (112), Japanese holly (86), poinsettia (82), citrus (182), and cherry (140). In greenhouse studies, Clayton (25) found a positive relationship between a black root rot index versus decreases in both tobacco plant height and weight.

Only a few studies have attempted to determine quantitative relationships among the inoculum density of *T. basicola*, black root rot incidence and/or severity, and plant growth. In addition, most of these studies have been conducted under greenhouse conditions, in artificially infested, sterilized soil. This makes it difficult to relate the results obtained to what occurs under field conditions (13). In a greenhouse study (112) that utilized an artificially infested, sterilized soil mixture, low levels of black root rot on cotton seedlings occurred at 10^2 propagules per g of soil. The pathogen could not be isolated from roots at 10^1 propagules per g of soil. The degree of hypocotyl and root discoloration was directly proportional to inoculum density over the range 10^1-10^4 propagules per g of soil. Slight and moderate stunting occurred at 10^3 and 10^4 propagules per g of soil, respectively.

The inoculum density threshold for *T. basicola* to cause any

root rot on bean plants, in artificially infested, nonsterile soil, was reported to be 500 propagules (70-80% chlamydospores and 20-30% endoconidia) per g of soil (131). In that study, a black root rot disease-severity index increased from 0 to near maximum as inoculum density was increased from 300 to 40,000 propagules per g of soil.

Bowden et al. (19) found a positive linear relationship between the log of the inoculum density of *T. basicola*, in an artificially infested, sterilized soil mixture, and the percentage of the root surfaces of pea and lentil plants with visible necrosis. The inoculum densities tested were 10^2, 10^3 and 10^4 endoconidia per cm^3 of soil. There was a negative linear relationship between log inoculum density and pea dry weight. Lentil plants were apparently tolerant to disease, since increasing levels of root rot were not associated with reduced plant growth.

Blume & Harmon (18) found that reduced black root rot ratings on pea seedlings were generally associated with increases in both root and total plant weight. Maduewesi et al. (105) reported that an inoculum density of 10 propagules per g of soil was sufficient to cause black root rot on soybean in artificially infested soil. It was not reported whether or not the soil had been sterilized in that study. In a study by Gayed (53), tobacco seedlings grown in sterilized soil artificially infested with 0, 2,000, 10,000 and 50,000 endoconidia per g of soil had black root rot ratings (0-5 scale) of 0, 1.0, 1.7 and 2.5, respectively.

Similar results were obtained with cowpea and bean.

The above studies indicate an exponential type of relationship between inoculum density and disease severity, primarily determined using disease indexes. Rittenhouse (146), using naturally infested field soil, found no relationship between the inoculum density of *T. basicola* and percent visible root necrosis on tobacco plants, at least over an inoculum density range of 100-3,000 propagules per g of soil. Disease severity ratings on susceptible cultivars were high even at 100 propagules per g of soil.

Tabachnik et al. (168) found a direct proportional relationship between the inoculum density of *T. basicola*, in artificially infested, sterilized soil, and a disease severity index on cotton seedlings. One, 5-10, 25, and 50-100 endoconidia per g of soil resulted in up to 25%, up to 50%, 26-75%, and up to 100%, respectively, of cotton seedling taproot surfaces becoming blackened or rotted. Maier (106) reported that the severity of black root rot on pinto beans, growing in nonsterile soil artificially infested with chlamydospores of *T. basicola*, increased each week over a 4-wk period. This result emphasizes the importance of length of incubation period when attempting to determine quantitative relationships between inoculum density and disease.

Black root rot/field studies. The initial inoculum of *T. basicola* in field soil apparently consists primarily of

chlamydospores. Mathre & Ravenscroft (111) considered endoconidia to possibly function as secondary inoculum, by increasing pathogen populations within growing seasons. As stated earlier, endoconidia and chlamydospores have been reported to survive in soil as long as 7 mo and 3 yr, respectively (91, 128).

Very little information is presently available concerning *T. basicola* and inoculum density-disease incidence and/or severity relationships in tobacco fields. Rittenhouse & Griffin (147) reported that the inoculum density of *T. basicola* in soil cores taken from two burley tobacco fields in southwestern Virginia were typically less than 10^3 propagules per g of soil, as determined using a carrot-disk method. However, no field studies were attempted to determine how inoculum density related to disease development. In a field study, Klarner et al. (83) found a correlation between the severity of black root rot and the height of individual dark-fired cultivars of tobacco. However, McLeod (115) reported that there was no correlation between the severity of black root rot and plant vigor, since the growth of some of the most severly diseased flue-cured and burley cultivars was superior to uninfected material of the same line.

A number of black root rot field studies for other crops have been conducted. For instance, Mathre et al. (112) reported that 38 of 49 cotton fields in California had 1 or fewer propagules of *T. basicola* per g of soil, as determined using a carrot-disk assay technique. Seven, two, and two fields

contained 1-100, 200-1,000, and over 1000 propagules per g of soil, respectively. The frequency of isolation of *T. basicola* from cotton seedling roots was positively correlated with pathogen population density in each field. Garber et al. (49) reported that an inoculum density of 200 propagules per g of soil (carrot-disk technique) was sufficient to cause black root rot in cotton fields. Tabachnik et al. (168) reported that the inoculum density of *T. basicola* in eight cotton fields with black root rot seedling problems was 10 or fewer propagules per g of soil, as determined using a carrot-disk procedure.

The inoculum densities of *T. basicola* in 16 peanut fields in New Mexico were 189-504 propagules per g of soil, as determined using the dilution-plate technique with a modified RB-M2 medium (73). Peanut fields that had high and low previous levels of blackhull contained averages of 410 and 208 propagules per g of soil, respectively (73). Bowden et al. (19) reported that in fields where *T. basicola* was detected (by the direct microscopic observation of decayed roots for chlamydospores) that chickpea plants averaged 24% visible root necrosis, whereas in fields where the pathogen was not detected, plants had only 5% visible root necrosis.

A survey of soybean fields in southwestern Ontario indicated that population densities of *T. basicola*, present in soil collected from within rows in mid season, were 0-248.5 propagules per g of soil, as determined using both a carrot-disk and a most probable number method (5). Most of the fields had fewer than 10

propagules per g of soil, and black root rot was not evident unless roots were examined carefully. No stunting of soybean was observed, even on a highly susceptible cultivar that had extensive root necrosis. However, the percentage of root segments colonized by *T. basicola* was greatest in fields with the highest population densities. Pathogen population densities were probably overestimated, since a study at one location showed that populations were higher in soil taken from within rows (around soybean plant root systems) than from between rows (248.5 versus 126.8 propagules per g of soil, respectively). This result was probably due to pathogen reproduction on plant roots.

Other soilborne diseases. Inoculum density versus disease incidence and/or severity studies have been conducted for other root diseases of tobacco besides black root rot. In a growth chamber study, Kannwischer & Mitchell (81) found a positive relationship between the inoculum density of *Phytophthora parasitica* var. *nicotianae* and the percentage of diseased tobacco roots; the relationship was linear at the lower inoculum densities. Flowers & Hendrix (47) reported that the development of black shank was slower in a field that had a low rather than a high inoculum density of *P. parasitica* var. *nicotianae*. Interestingly, final pathogen populations were higher when black shank-resistant rather than susceptible cultivars were grown. Propagules of the pathogen were produced rapidly directly beneath a susceptible cultivar, but this cultivar was quickly killed,

while the resistant cultivars remained alive. New roots were probably produced on the latter after initial infection. This would have provided new substrate for the fungus, so total pathogen reproduction may have been higher on the resistant cultivars when pathogenesis was extensive (47). A related conclusion was made by Hanounik et al. (63) in a greenhouse study on the population dynamics of *Meloidogyne incognita* on tobacco. High initial nematode populations caused severe initial root damage, which resulted in less substrate for subsequent nematode reproduction.

In a greenhouse study, Chen & Echandi (22) found that a bacterial wilt severity index of tobacco was correlated with the log of the inoculum dosage (root-dip technique) of *Pseudomonas solanacearum* over the range 2 X 10^3 to 2 X 10^9 cfu/ml. Lownsbery & Peters (100) reported that, in a outdoor pot experiment, the final weight of tobacco plants was inversely proportional to the logarithm of the initial inoculum density of *Globodera solanacearum*.

Barker & Olthof (13) have reviewed the fundamental quantitative relationships between the population density of plant parasitic nematodes and plant growth. The relationship is usually linear when plant growth is plotted against the logarithm of the nematode population density. Seinhorst (154) had previously concluded that an exponential type of model, that generates a sigmoidal curve, was more suitable. Seinhorst's model was based partly on the assumption that no decrease in

plant growth occurs until the nematode population density reaches a "threshold level". However, Oostenbrink (126, 127) has questioned Seinhorst's model.

Inoculum density versus disease incidence and/or severity relationships have been studied for other pathogen-host systems. For instance, the relationship between the inoculum density of *Cylindrocladium crotalariae* and the number of infections on peanut roots was linear in arithmetic plots (60, 169, 174). The microsclerotial inoculum density of *C. crotalariae* in a field study was better correlated with disease incidence when a susceptible rather than a resistant cultivar was tested (40). In a greenhouse study, Phipps & Beute (141) found a positive relationship between the log of the inoculum density of *C. crotalariae* versus both a root rot index and decreases in root and total plant weight. Higher inoculum densities were required to cause root rot on a resistant than on a susceptible cultivar in that study. In controlled soil-temperature tank studies, the number of infections (per plant and per m root) by *C. crotalarie* on peanut was greatest when a long incubation period was used (174).

In greenhouse experiments, Zambolim et al. (197) found an approximate linear relationship when the percentage of diseased soybean seedlings (arithmetic scale) was plotted against the inoculum density (geometric progression scale) of *Rhizoctonia solani*. In greenhouse studies, Duczek et al. (43) found a similar relationship for both the incidence and severity of

common root rot on two barley cultivars versus the inoculum density (0-256 conidia/cm^3) of *Cochliobolus sativus*.

In a field experiment conducted by Ashworth et al. (6), the incidence of tomato plants infected with *Verticillium dahliae* increased as inoculum density increased from 0.1 to 27 microsclerotia per g of soil. In a greenhouse experiment, the inoculum density of *Fusarium oxysporum* f. sp. *lycopersici* was positively correlated with a wilt severity index on tomato plants (2). The severity of wilt symptoms on potato was related to the inoculum density of *Verticillium albo-atrum* in soil (173). In greenhouse studies, Trujillo & Hine (176) found a positive correlation between the inoculum densities of both *Pythium aphanidermatum* and *Phytophthora parasitica* and the severity of root rot on pineapple.

In greenhouse studies, Warren (187) found an approximate linear relationship between the inoculum density of *R. solani* versus a disease index on hypocotyls of lima bean. With a resistant cultivar, the relationship was linear over the entire inoculum density range tested, but with the susceptible cultivar, the relationship was only linear at lower inoculum densities.

Sippell & Hall (159) did not find any relationship between the severity of root rots caused by *P. ultimum* or *F. solani* f. sp. *phaseoli* versus the growth of bean plants. In their greenhouse studies, they found that plant weight was influenced primarily by temperature and that root rot severity was determined mainly by pathogen inoculum density.

Theoretical relationships. Baker & colleagues (7, 8, 9, 10) developed a set of models for studying quantitative relationships between inoculum density and disease incidence. The models are supposed to be appropriate for determining whether a rhizoplane or a rhizosphere host-pathogen interaction exists. They propose that this question can be answered by examining the exponent of a power-law equation in the general form of the allometric equation: $S=k(ID)^b$, where S equals the number of infections, ID equals inoculum density, and k and b are constants estimated from the data. For instance, for nonmotile inoculum situated around a fixed infection court, a rhizosphere interaction is supposedly indicated by a b value equal to 1.0. A value of b equal to 1.0 also indicates a linear or directly proportional relationship between inoculum density and disease incidence. For instance, Tomimatsu & Griffin (174) found that values of b for a *C. crotalariae*-peanut system were close to 1.0, indicating direct proportionality. A rhizoplane effect supposedly exists if the value of b equals 0.67. Baker & colleagues propose that the value of b be determined by calculating the slope of a linear equation regressed on a $\log_{10}$-$\log_{10}$ transformation of inoculum density versus disease incidence [corrected by Gregory's multiple infection transformation (59)], but Ferriss (46) calculated the value of b directly using an iterative stastical method. Baker & Drury (9) emphasize that the models are only valid for data points taken from the logarithmic portion of inoculum density-disease incidence curves, and that the analysis of

transitional or plateau portions will result in a underestimation of b. They also emphasize that the models are strictly for infections, not necrosis. Few researchers have actually attempted to measure "infections" per se, as Griffin & Tomimatsu (60), and Tomimatsu and Griffin (174) have done. Rather, infections are usually estimated from $\log_{10}$-$\log_{10}$ transformations of inoculum density versus disease incidence corrected by Gregory's multiple infection transformation.

Grogan et al. (61) and Vanderplank (186) question the validity of Baker's models and also the existence of a strict rhizoplane effect. Vanderplank (186) also points out that the transformation of arithmetic inoculum density-disease incidence data obscures relationships that are most easily visualized from straight arithmetic plots, and that at lower inoculum densities, the number of infections should always be directly proportional to inoculum density. Vanderplank (186) proposed that disease incidence be modeled as a function of inoculum density by the Poisson equation: $Y=n(1-e^{-aID})$, where y equals number of infections, e equals base e of natural logarithm, ID equals inoculum density, and n and a are constants estimated from the data. The parameter n is a measure of site susceptibility. Black & Beute (17) used this equation to model the severity of *C. crotalariae* root rot on peanut. However, this (or any other) model does not have a theoretical or biological basis for measuring disease severity, unless certain assumptions, which will be considered shortly, are made.

Relatively little is known about the efficiency of inoculum (present within the host rhizosphere) for infection (8, 174). If "inoculum efficiency", or the percent of germinating propagules that successfully infect roots, is high, then the overall slope values of arithmetic inoculum density-infection plots will also be high (174). A low inoculum efficiency may be due to low endogenous reserves, inadequate exogenous nutrients (exudates), microbial antagonism in the rhizosphere, or host-defense mechanisms (174). Once infections have occurred, the "infection efficiency", or the percent of infections that lead to necrosis, will determine how many lesions actually develop. All or only a portion of infections may eventually lead to necrosis (174). For instance, for a *C. crotalariae*-peanut system, Tomimatsu & Griffin (174) reported that estimates of the efficiency of inoculum for observed infections were near 100%, but that estimates of the efficiency of observed infections for necrosis were low (0.27 to 0.28%). In another study, Griffin & Tomimatsu (60) calculated infection efficiency estimates indicating that from 0.02 to 1.03% of the observed *C. crotalariae*-root infections resulted in visual shoot symptoms on peanut plants.

The relationship between infection and necrosis would not be proportional unless lesions do not overlap and their development are completely independent of each other (11). Since these assumptions cannot be made, there are presently no models for describing theoretical or biological relationships between inoculum density and disease severity.

Influence of inoculum pattern. The inoculum patterns of many soilborne plant pathogens are nonrandom or clumped. Some of the pathogens reported to have clumped inoculum patterns include *T. basicola* in tobacco fields (146, 147), *Phytophthora parasitica* var. *nicotianae* in tobacco fields (44), *Sclerotium rolfsii* in peanut fields (20, 156), *Cylindrocladium crotalariae* in peanut fields (65, 169), *Cylindrocladium* spp. in a black walnut nursery (149), *Sclerotinia minor* in lettuce fields (39), *Verticillium dahliae* in potato fields (161), *Rhizoctonia* spp. in tall fescue turf (110), and nematodes (55, 123). The inoculum pattern of *Fusarium solani* f. sp. *phaseoli* in a bean field was considered to be uniform by Nash & Snyder (122); however, calculations on their data by Taylor et al. (169) indicated high variance to mean ratios, which indicates clumped inoculum patterns.

Patterns of disease in fields also occur in a nonrandom fashion, such as the pattern of blackrot lesions on cabbage caused by *Xanthomonas campestris* (167), hypocotyl rot disease of snapbeans caused by *Rhizoctonia solani* (21), and the frequencies of observed infections by *C. crotalariae* on peanut roots (60). All but a couple of these inoculum and disease patterns were fit well by the negative binomial distribution, which is the most flexible of several clumped or aggregated distributions (162). The negative binomial is described by two parameters: the mean and the dispersion parameter (k). The distribution approaches a random pattern as k values get progressively larger than 2, and a logarithmic series (indicating extreme clustering) as k values

become smaller than 2.

One possible mechanism for the clustering of inoculum is that portions of soil contain diseased plants and/or infested plant debris upon which the pathogen reproduces itself. A clumped inoculum pattern would result if the mechanical actions of plowing and disking failed to completely breakup and disperse inoculum present in soil (65, 149). Clumped inoculum patterns may also be the result of heterogenous soil type and nonuniform cultural practices (156). Martin et al. (110) point out the importance of both the abiotic and biotic site microenvironments in influencing pathogen inoculum patterns. For instance, soil texture influences nematode population patterns (55, 123). However, for *T. basicola*, there was no correlation between inoculum density and soil type (112).

Extreme clumping of inoculum can complicate inoculum density-disease incidence relationships (45, 60, 146, 169). Taylor et al. (169) suggest that a measurement of the degree of inoculum clumping may be useful in refining such relationships. In a *C. crotalariae*-peanut system studied by Griffin & Tomimatsu (60), the degree of clumping of root infections, as measured by Lloyd's index of patchiness (142), was negatively correlated with the number of observed infections on peanut roots. When the observed root infection densities were corrected with Lloyd's index of patchiness, the R^2 value, for the relationship between the number of observed root infections and disease incidence, increased from 0.88 to 0.93. A greenhouse study by Tomimatsu &

Griffin (174) indicated that the number of observed root infections was directly proportional to inoculum density. Therefore, in that particular system, the degree of clumping of root infections may be directly related to the degree of inoculum clumping, if one inoculum unit results in one root infection.

Studies conducted by Stanghellini et al. (163), on the population dynamics of *P. aphanidermatum* in sugar beet fields, indicate that temporal fluctuations in pathogen population densities should also be considered when attempting to establish quantitative relationships between pathogen population density and disease.

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Chapter 2

A NEW SELECTIVE MEDIUM FOR ENUMERATING LOW POPULATIONS OF *THIELAVIOPSIS BASICOLA* IN NATURALLY INFESTED TOBACCO FIELD SOILS

INTRODUCTION

Thielaviopsis basicola (Berk & Br.) Ferraris (=*Chalara elegans* Nag Raj & Kendrick) is a widespread soilborne plant pathogen that causes a black root rot on a variety of economically important plants. The carrot-bait technique reported by Yarwood (13) in 1946 is a highly sensitive, qualitative method of assaying for *T. basicola*. Various modifications (7, 11) of the carrot-bait technique can provide quantitative data on population densities of *T. basicola* in soil. However, these procedures are time consuming and require a large number of replicates for statistical accuracy. The most desirable method for enumerating propagules of *T. basicola* in soil is the dilution-plate technique. This technique is simple and also provides highly quantitative data. Several media reported selective for *T. basicola* are TBM-C and TBM-V8 (2), VDYA-PCNB (5) and RB-M2 (9). Population densities of *T. basicola* in tobacco fields in Virginia are typically between 10^1 and 10^3 propagules per g of soil (L. P. Specht and G. J. Griffin, unpublished data). Low soil dilutions (10^{-1} and 10^{-2}) are required to assay for propagules of *T. basicola* at these populations. However, present media do not satisfactorily

inhibit undesired fungi on soil-dilution plates at low dilutions (L. P. Specht and G. J. Griffin, unpublished data). Tabachnik et al. (8) reported that the carrot-disk technique was superior to TBM-C (the best of the present selective media) for isolating *T. basicola* from soil.

The objective of this study was to develop a selective medium that could be used for enumerating low populations of *T. basicola* in naturally infested tobacco field soils. The new medium that was developed contains etridiazol and nystatin to inhibit undesired fungi, and raw (unautoclaved) extract from carrot (*Daucus carota* L. cv. sativa) for selective enhancement. Etridiazol has a wide spectrum of antifungal activity and is especially effective against Oomycetes. Etridiazol also inhibits the growth of many soil fusaria that are not adequately controlled on other selective media.

MATERIALS AND METHODS

The medium. The new selective medium, called *T. basicola*-carrot, etridiazol, nystatin (TB-CEN) medium, was developed by testing the in vitro activity of 30 different fungicides against undesired fungi that developed on soil-dilution plates of TBM-V8 medium. The most effective fungicides were tested further, using either V-8 juice agar or carrot-extract agar as basal media.

The per-L contents of TB-CEN medium were 80 ml of 50% raw (unautoclaved) carrot extract, 400 mg etridiazol (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole, added as 1.14 g Terrazole™ 35WP, Uniroyal Corp.), 250,000 units nystatin, 500 mg streptomycin sulfate, 30 mg chlortetracycline hydrochloride, 1 g $CaCO_3$, and 15 g agar. The 50% carrot extract solution was prepared by comminuting 100 g of peeled, raw carrot tissue in 100 ml of distilled water in a Waring blendor at high speed for 2 min. The carrots were fresh and were obtained from local commercial sources. The resulting slurry was strained through several layers of cheesecloth. Eighty ml of this extract was used per L of medium. The nystatin solution was prepared by dissolving 250,000 units nystatin in 45 ml of distilled water. A mixed streptomycin and chlortetracycline solution was prepared by dissolving 500 mg streptomycin sulfate and 30 mg chlortetracyline hydrochloride in 25 ml of distilled water. To prepare 1 L of medium, the following were added to 850 ml of molten, 1.8% water agar cooled to 48 C: 80 ml 50% carrot extract, 45 ml nystatin

solution, 25 ml mixed streptomycin and chlortetracycline solution, 1.14 g Terrazole™ 35WP, and 1 g $CaCO_3$. The liquid components were warmed to 48 C in a water bath just prior to use. The pH of the medium was adjusted to 5.3 with 1.0 N H_2SO_4. The medium was poured immediately into 9-cm glass petri plates, while also swirling the flask to keep the fungicide uniformly suspended. For purposes of comparison, TBM-C, TBM-V8 and VDYA-PCNB media were prepared in a similar manner.

Soil dilution procedures. The following procedure was used to assay for propagules of *T. basicola* in naturally and artificially infested soils. Initial 10^{-1} soil dilutions (w/v) were prepared by adding 11 g quantities of moist soil to 95-ml-distilled-water blanks, and shaking on a wrist-action shaker for 15 min. Separate samples were oven dried (24 hr at 105 C) for soil dry weight determinations. The selective media were tested using both pour- and spread-plate techniques. For the pour-plate technique, 1-ml aliquots of soil suspensions were pipetted into empty glass petri plates. Twenty-seven to 30 ml of molten medium was poured into each plate. The agar was agitated to distribute the soil particles thoroughly. A rubber policeman, with most of the rubber tip removed, was used to mix the agar-soil suspension. The medium was prepared in 500-ml or smaller quantities to facilitate more accurate pouring. Aseptic technique was not practiced, so it was possible to prepare a single, large flask of molten water agar, and then pour the proper volume of agar into a

beaker that was used for combining the components of the medium. Ten plates per soil sample were prepared unless stated otherwise. For the spread-plate technique, 1-ml aliquots were pipetted onto and distributed over the surface of hardened agar media. Petri lids were temporarily removed while excess surface water was allowed to evaporate under a forced-air hood. Except for an incubation-temperature study, all plates were kept at room temperature (20-22 C) on a laboratory bench. The plates were incubated for 6 (TBM-V8, TBM-C and VDYA-PCNB) or 14 (TB-CEN) days prior to counting colonies. Population densities of *T. basicola* were calculated as propagules per g of oven-dry soil. The carrot-disk technique was tested by using a modification (7) of Yarwood's carrot-bait method.

Soil samples. The soils used were taken from burley and flue-cured tobacco fields in Virginia. The samples were passed through a 4.8-mm-opening sieve, mixed thoroughly, placed in plastic bags with pinholes to allow for gas exchange, and stored at room temperature prior to use. Eleven isolates of *T. basicola* were used to test TB-CEN medium for: 1. percent recovery of endoconidia from artificially infested soils, 2. percent germination of endoconidia and chlamydospores, and 3. colony growth. The isolates of *T. basicola* were from burley and flue-cured tobacco, Japanese holly, bean, soybean (provided by J. L. Lockwood) and cotton (provided by J. E. DeVay). For the artificial infestation experiment, endoconidia were obtained from

2-wk-old cultures grown on V8-juice agar. Spore concentrations were determined using a haemacytometer and adjusted accordingly. Chlamydospores were collected by comminuting mycelium of 6-wk-old cultures in distilled water in a Waring blendor. The resulting suspension was passed through a 44-um-opening sieve that was nested on top of a 25-um-opening sieve. Most of the chlamydospores were retained on the bottom sieve. This procedure did not eliminate all mycelial fragments, but was sufficient for chlamydospore germination studies. A chlamydospore chain (usually 4-6 cells) was counted as one unit, and was considered to have germinated if at least one cell produced a germ tube. Percent germination of endoconidia and chlamydospores on TB-CEN medium were determined after 24 and 72 hr, respectively, at 20-22 C.

RESULTS

The recovery of *T. basicola* from 10 naturally infested soils was significantly greater on TB-CEN (pour-plate technique and 10^{-1} dilution) than on TBM-C, TBM-V8 or VDYA-PCNB media (Table 2.1). *T. basicola* was not recovered on any plates of the latter three media when soils contained 30 or fewer propagules per g. Fig. 2.1 shows the appearance of *T. basicola* colonies and other fungi that developed on dilution plates. The mean diameter of *T. basicola* colonies on TB-CEN medium was 10.5 mm. The average no. of undesired fungi (ave. diam.=5.1 mm) per plate was 40. On TBM-C, the second best medium in these studies, the mean diameter of *T. basicola* colonies was 4.1 mm. The average no. of undesired fungi (ave. diam.=4.0 mm) per plate was 110.

TB-CEN medium and a carrot-disk technique gave similar results (Table 2.2). The recovery of *T. basicola* from four of nine naturally infested soils was greater with TB-CEN medium than with carrot disks. The two methods were not compared statistically because data collected by the carrot-disk technique was corrected for multiple colonization (12), and thus did not lend itself to direct comparison with the discrete colony-count data obtained with TB-CEN medium.

The recovery of *T. basicola*, using various assay techniques (pour- versus spread-plates and glass versus plastic petri plates), from a naturally infested soil containing approximately 10^3 propagules per g is shown in Table 2.3. The recovery of *T.*

Table 2.1. Relative recovery of *Thielaviopsis basicola*, obtained with four selective media, from naturally infested tobacco field soils using 10^{-1} soil dilutions[a]

	PROPAGULES PER G OF SOIL			
SOIL CODE	*TB-CEN*	*TBM-C*	*TBM-V8*	*VDYA-PCNB*
1	13 A[b]	0 B	0 B	0 B
2	20 A	0 B	0 B	0 B
3	30 A	0 B	0 B	0 B
4	74 A	21 B	0 C	0 C
5	92 A	40 B	13 C	0 C
6	94 A	50 B	0 C	0 C
7	120 A	7 B	8 B	2 B
8	167 A	27 B	2 C	0 C
9	214 A	132 B	13 C	0 C
10	422 A	340 B	73 C	0 D

[a]Selective media were used with pour-plate technique and glass petri plates. Ten plates of each medium were prepared per soil sample. TB-CEN plates were incubated for 14 days and the other media for 6 days.

[b]Values followed by the same letter are not significantly different (P=0.05) according to the Duncan's Multiple Range Test. Statistical comparisons were made horizontally among media, and not vertically among soil samples.

Figure 2.1. Soil-dilution plates prepared from a tobacco field soil naturally infested with *Thielaviopsis basicola*. The pour-plate technique was used in combination with 10^{-1} soil dilutions for A) TB-CEN, B) TBM-C, C) TBM-V8 and D) VDYA-PCNB media. Arrows indicate typical colonies of *T. basicola*. No colonies of *T. basicola* were recovered on VDYA-PCNB medium.

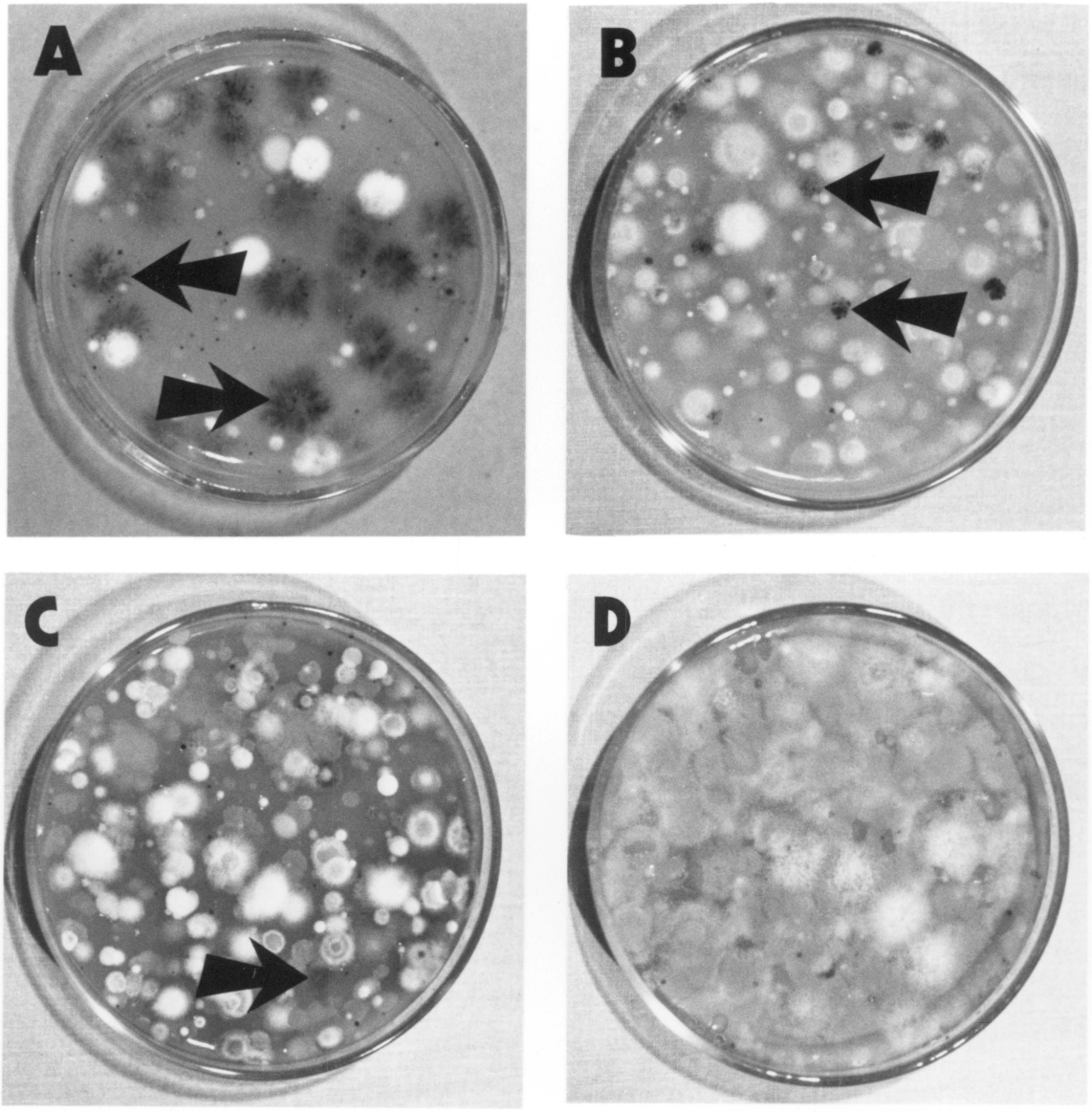
A
B
C
D

Table 2.2. Relative recovery of *Thielaviopsis basicola* from naturally infested tobacco field soils using TB-CEN medium and a carrot-disk technique.

	PROPAGULES PER G OF SOIL	
SOIL CODE	*TB-CEN*[a b]	*CARROT DISK*[c]
11	0	8.9
12	0.5 ± 1[d]	0
13	4.3 ± 2	0
14	8.4 ± 3	9.1
15	12 ± 5	0
16	26 ± 5	42
17	76 ± 14	97
18	101 ± 18	82
19	166 ± 17	226

[a] The pour-plate technique and glass petri plates were used with 10^{-1} soil dilutions.
[b] Twenty plates per soil sample were prepared.
[c] Twenty carrot disks per soil sample, and data were corrected for multiple colonization (12).
[d] 95% confidence interval.

Table 2.3. Relative recovery of *Thielaviopsis basicola* from a naturally infested tobacco field soil (containing about 10^3 propagules per g) using 10 assay techniques[a]

	PROPAGULES PER G OF SOIL	
MEDIUM[b]	*POUR-PLATE TECHNIQUE*	*SPREAD-PLATE TECHNIQUE*
TB-CEN (G)	2110 A[c]	1900 A
TB-CEN (P)*[d]	1410 B	830 C
TBM-C (G)	1500 B	1460 B
TBM-V8 (G)*	1260 B	840 C
VDYA-PCNB (G)*	410 C	30 D

[a]Soil sample assayed, using a 10^{-2} dilution, was collected from around the root systems of tobacco plants colonized by *T. basicola*.

[b]Glass (G) or plastic (P) petri plates tested.

[c]Values followed by the same letter within a column are not significantly (P=0.05) different according to the Duncan's Multiple Range Test.

[d]Asterisk indicates that the pour- and spread plate techniques are significantly (P=0.05) different according to an unpaired t-test.

basicola was significantly greater on TB-CEN (glass petri plates) than on the other media. The recovery of *T. basicola* on TB-CEN medium was significantly lower with plastic than with glass petri plates (Table 2.3). Undesired fungi were a problem on all of the media except TB-CEN in glass petri plates. Plastic petri plates containing TB-CEN medium were opaque instead of transparent. The opaqueness apparently was caused by adsorbtion of etridiazol to the plastic, since the addition of the fungicide to the medium caused plastic petri plates to become opaque. This would explain the reduced effectiveness of TB-CEN medium in plastic petri plates. With TB-CEN medium in glass petri plates, the spread- and pour-plate techniques were not significantly different when a 10^{-2} dilution was assayed (Table 2.3). However, the spread-plate technique (using TB-CEN in glass. petri plates) gave a low recovery of *T. basicola* when a 10^{-1} dilution was assayed (data not shown).

The recovery rates of 11 isolates of *T. basicola* on TB-CEN medium, from soils artificially infested with 100 endoconidia per g and assayed using 10^{-1} dilutions, were 50-95% and averaged 81%. The recovery rates of three tobacco isolates (T1, T2 and T6) on TB-CEN medium were below 70%. However, the other selective media did not recover any colonies of *T. basicola*. Numerous colonies of undesired fungi covered the surfaces of these plates. The experiment was repeated with similar results. Percent germination of endoconidia on TB-CEN medium were 90-97% and averaged 95%. Percent germination of chlamydospores were 92-100%

and averaged 97%. Percent germination of endoconidia and chlamydospores on V8-juice agar averaged 100 and 99%, respectively. TB-CEN medium in glass, but not in plastic, petri plates inhibited the growth of *T. basicola* significantly more than any of the other media (Table 2.4). The possible adsorbtion of etridiazol by plastic would reduce the concentration of the fungicide in the medium, thus making the medium less inhibitory.

An experiment was conducted to determine if the performance of TB-CEN medium varied with carrot source. Different lots of carrots were obtained from local commercial sources. There were no significant (P=0.05) differences in the recovery of *T. basicola* among seven lots; however, one lot that had a bitter taste (determined by subjective evaluation) gave the lowest recovery. A qualitative difference noted was that colonies of *T. basicola* were less pigmented and somewhat difficult to count with three of the lots.

Incubation temperature had a significant effect on the recovery of *T. basicola* on TB-CEN medium in glass petri plates (Table 2.5). Recovery was lower at 27 C than at 20-22 or 16 C. Colonies of undesired fungi covered the plates at 27 C. Recovery rates at 20-22 C and 16 C were equal, but fewer undesired fungi and better sporulation of *T. basicola* occurred at 16 C. A disadvantage of the lower temperature was the long incubation period (21 days) that was required for complete colony development. Other tests showed that the recovery of *T. basicola* was the same whether plates of TB-CEN medium were incubated under

Table 2.4. Colony diameter (mm) of 11 isolates of *Thielaviopsis basicola* grown on five media for 10 days at 20-22 C

ISOLATE	*VDYA*	*VDYA-PCNB*	*TBM-V8*	*TB-CEN(P)*[a]	*TB-CEN(G)*[a]
Tobacco (T7)	35	20	11	14	8
Tobacco (T1)	44	22	13	15	9
Tobacco (T2)	44	22	13	14	9
Tobacco (T4)	44	23	16	16	9
Tobacco (T6)	43	22	10	17	10
Tobacco (T3)	46	23	17	18	11
Tobacco (T5)	45	23	18	17	11
J. holly (J1)	45	22	16	15	10
Bean (B1)	55	30	17	17	11
Soybean	37	25	16	10	6
Cotton (F374)	44	22	14	15	10
AVERAGE[b]	43.8 A[c]	23.1 B	14.6 C	15.3 C	9.5 D
% INHIBITION[d]	---	47%	67%	65%	78%

[a]Plastic (P) or glass (G) petri plates tested.
[b]Average colony diameter for all 11 isolates.
[c]Values followed by the same letter are not significantly (P=0.05) different according to the Duncan's Multiple Range Test. Isolates were used as replications for the analysis.
[d]Percent inhibition in growth (average for all isolates) compared to VDYA (V8-juice, dextrose and yeast-extract agar) control plates.

Table 2.5. Effect of incubation temperature on the recovery of *Thielaviopsis basicola* from a naturally infested tobacco field soil using TB-CEN medium[a]

INCUBATION TEMPERATURE	*INCUBATION PERIOD*[b] *(days)*	*PROPAGULES PER G OF SOIL*
16 C	21	594 A[c]
20-22 C	14	583 A
27 C	12	300 B

[a] The pour-plate technique and glass petri plates were used with a 10^{-1} soil dilution.

[b] Number of days soil-dilution plates were incubated until colonies of *T. basicola* were completely developed.

[c] Values followed by the same letter are not significantly (P=0.05) different according to the Duncan's Multiple Range Test.

dark or light conditions. TB-CEN medium was also used to isolate *T. basicola* from colonized plant roots; the best results were obtained when a thin layer of TB-CEN was poured over root segments after they had been placed on the surface of the medium.

DISCUSSION

TB-CEN medium was as effective as carrot disks for isolating *T. basicola* from naturally infested soils. TB-CEN medium was also better than TBM-C, TBM-V8 and VDYA-PCNB media. The pour-plate technique had to be used when 10^{-1} soil dilutions were required in assays. Some soil fungi (probably mostly *Fusarium* spp.) were unable to develop fully when embedded in TB-CEN agar medium, since large surface colonies of undesired fungi occurred more often on spread- than pour-plates. Recovery of 8 of 11 isolates of *T. basicola* on TB-CEN medium, from soil artificially infested with endoconidia, was greater than 80% when 10^{-1} dilutions were used. The recovery of the other three isolates was below 70%. The reason for the occasional low recovery of *T. basicola* was not determined, but it was apparently not caused by reduced spore germination, since endoconidia of all isolates germinated at high percentages (range=90-97%) on TB-CEN medium.

Tsao & Bricker (10) reported that chlamydospores are the major survival propagule of *T. basicola*. They wisely suggest that the artificial infestation of soil be restricted to chlamydospores, since they found that colonies of *T. basicola* on

soil-dilution plates never originated as endoconidia or mycelium when naturally infested soils were assayed. The recovery of chlamydospores from artificially infested soil was not tested here because of difficulties associated with obtaining individual chlamydspores free of mycelium. However, the germination of chlamydospores on TB-CEN medium was always high (range=92-100%).

Glass petri plates were not sterilized for use with TB-CEN, nor was aseptic technique maintained during medium preparation, since few airborne contaminants grew on the medium. Carrots are a natural product of variable composition, so the performance of TB-CEN medium could vary depending upon carrot source, but this was not a problem here. In preliminary tests, the stimulatory property of carrot extract was partially destroyed by autoclaving. The mechanism(s) which operate in carrot tissue and extract for the selective enhancement of *T. basicola* is not known, but the selective property apparently occurs to a lesser extent in other species of Umbelliferae (4). The stimulatory factor(s) is not present in synthetic media (3).

The use of unautoclaved carrot extract in TB-CEN medium would be a problem if carrots were contaminated with *T. basicola* and/or related fungi (1, 6). Surface colonization by *T. basicola* and other contaminating fungi can be eliminated by peeling carrots or by surface sterilization. Control plates that contain no soil should always be prepared to check for possible contamination of carrots by *T. basicola*.

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Chapter 3

INOCULUM DENSITIES OF *THIELAVIOPSIS BASICOLA* IN TOBACCO FIELDS AND THE ROLE OF BLACK ROOT ROT IN TOBACCO STUNTING IN VIRGINIA

INTRODUCTION

Black root rot, caused by *Thielaviopsis basicola* (Berk. & Br.) Ferraris (=*Chalara elegans* Nag Raj & Kendrick), is an important soilborne disease of tobacco (*Nicotiana tabacum* L.). Tobacco fields with black root rot often contain plants with varying degrees of stunting (8); however, no extensive black root rot disease or yield loss surveys have been conducted to our knowledge. Legg et al. (6) reported that black root rot causes an estimated 5-7% reduction in the yield of burley tobacco in the United States, but no supporting data on disease occurrence or yield loss were presented. The present status of black root rot in Virginia (where flue-cured and burley are the two major types of tobacco grown) is uncertain, but plant stunting is commonly observed in tobacco fields. Endomycorrhizae may be involved in the stunting of tobacco in Virginia, since a pathogenic endomycorrhizal fungus, *Glomus macrocarpum* (Tul. & Tul.) Gerd. & Trappe, causes tobacco stunt disease of burley tobacco in Kentucky (4, 5, 9). Also, there has been an increasing number of cases of root rot in the Virginia flue-cured region in recent years (unpublished).

This paper is a report on the results of a statewide black

root rot survey that was conducted to evaluate the role of *T. basicola* in tobacco stunting in Virginia. In the survey, the inoculum densities of *T. basicola* in tobacco fields were examined in relation to the incidence and/or severity of black root rot and associated plant stunting. Little information is available concerning inoculum densities of *T. basicola* in tobacco fields.

MATERIALS AND METHODS

Black root rot survey. Commercial burley, flue-cured, and sun-cured tobacco fields considered possibly to have black root rot and/or plant stunting problems were located with the assistance of county agricultural extension agents. Most of the fields were visited 1-2 wk prior to transplanting in May or early June. Fifty to 75 soil cores (2.5 cm diam. x 15-20 cm deep) were systematically taken from a 150-200 m^2 area marked off at each location. The cores were bulked to produce one composite sample per location. The samples were passed through a 4.8-mm-opening sieve, mixed thoroughly, and assayed for inoculum densities of *T. basicola* using TB-CEN medium (Chapter 2). Twenty soil-dilution plates were prepared per soil sample. The fields, which were maintained by growers according to standard production practices, were visited again in mid July. At this time, stunted and normal-sized plants were carefully dug up, washed free of soil, and immediately rated for the mean percent of roots with black root rot (0-100% basis). For the purpose of sampling, a

tobacco plant was "stunted" if its height was less than half the height of "normal-sized" or representative larger plants in the field. Additional soil samples were collected from around root systems of stunted and normal-sized plants. A minimum of five stunted plants, and three to five normal-sized plants, were evaluated at each location. Samples taken from around root systems of stunted and normal-sized plants were analyzed for soil pH, soil fertility and plant-parasitic nematodes. Soil from two of the fields were also analyzed for soil texture. Soil pH was measured in 0.01 M $CaCl_2$ (11), and the other analyses were conducted in the clinical laboratories at VPI&SU.

Isolation of endomycorrhizae. Four of the fields sampled for black root rot were selected for this study. Tobacco stunting was not associated with black root rot in two of the fields (burley field No. 17 and sun-cured field No. 2), but was in the other two (burley field No. 19 and flue-cured field No. 11). Endomycorrhizal-like spores were isolated, by a wet sieving and sugar-floatation-centrifugation technique (9), from soils previously taken from around root systems of stunted and normal-sized plants. Individual spores were transferred with a pasteur pipette into the root zones of 7-to-8-wk-old tobacco seedlings growing in pots containing a steam-pasteurized, 50/50 mixture of loamy soil and sand. Ten spores (1/pot) were selected at random from each soil sample. All of the spores used were filled with protoplasm. The cultivars grown were Burley 21 and

NC95, depending upon whether soils were from burley or flue/sun-cured fields, respectively. The seedlings were grown in the greenhouse for 14 wk, at which time plant root systems were carefully washed free of soil, cleared in KOH, stained in trypan blue-lactophenol (12), and observed for endomycorrhizal colonization. Final plant height measurements were also taken. Isolates of endomycorrhizae were identified by N. C. Schenck, Univ. of Florida.

RESULTS

Black root rot survey. Fig. 3.1 shows the location of Virginia counties included in the 1984-85 survey. Nineteen commercial tobacco fields were visited the first year (Table 3.1). Estimated inoculum densities of *T. basicola* in three (Nos. 12-14) of eight burley fields were 0-12 propagules per g of soil. Tobacco stunting in these fields was infrequent, and mean percent root rot ratings on all plants were 0%. Estimated inoculum densities of *T. basicola* in two other burley fields (No. 15 and No. 16) were 74 and 76 propagules per g of soil. Five percent or fewer of the plants in these fields were stunted, with mean percent root rot ratings on stunted versus normal-sized plants averaging 8.0 and 3.6%, respectively (for field No. 15), and 15 and 1.0%, respectively (for field No. 16). Another burley field (No. 17) had an estimated inoculum density of 166 propagules per g of soil. Two percent of the plants in this field were stunted;

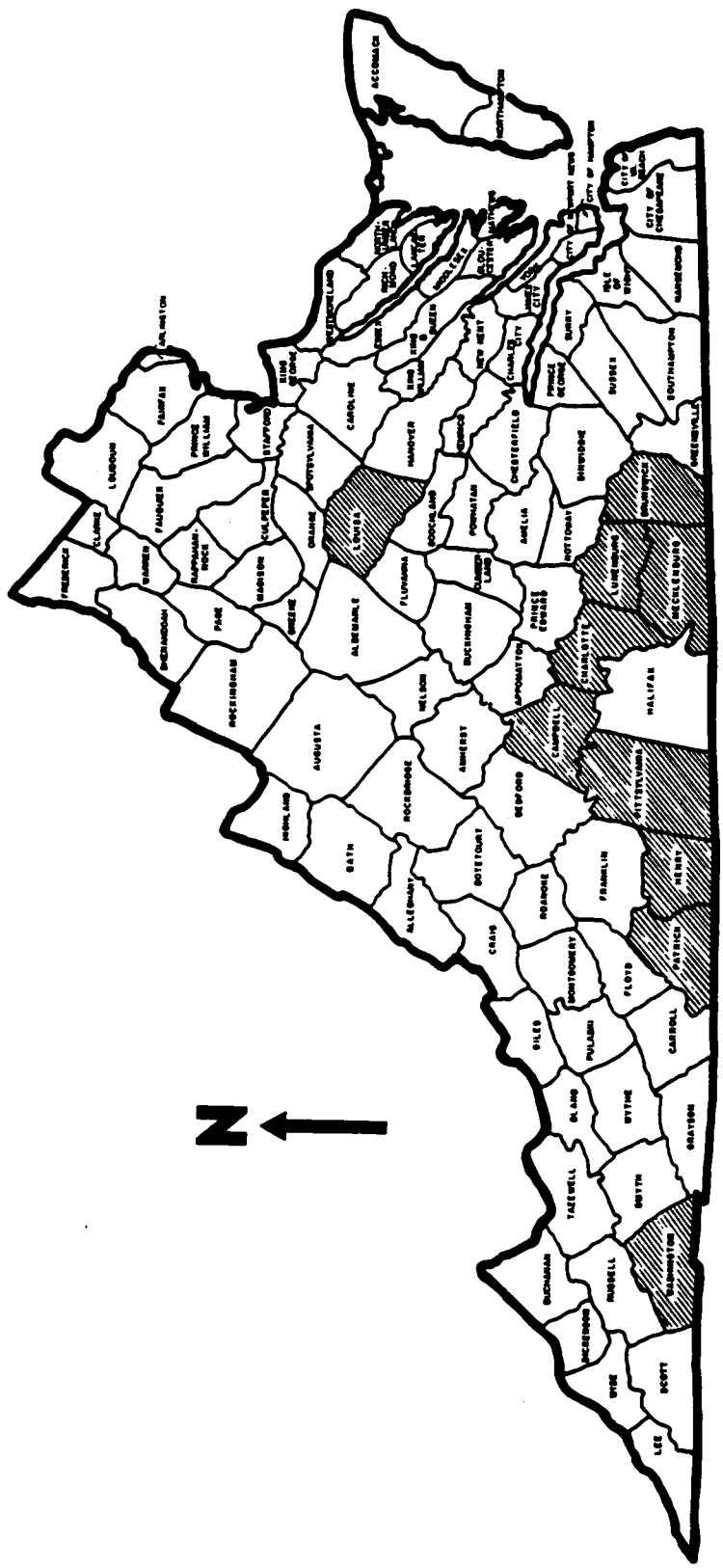

Figure 3.1. Location of counties included in the 1984-85 Virginia black root rot/tobacco stunting survey. A total of eight burley fields (all in Washington County in southwestern Virginia), 16 flue-cured fields (in Patrick, Henry, Pittsylvania, Campbell, Charlotte, Mecklenburg, Lunenburg and Brunswick counties in southcentral Virginia), and two sun-cured fields (both in Louisa County in central Virginia) were sampled.

Table 3.1. Population densities of *Thielaviopsis basicola*, and levels of black root rot associated with stunted and normal-sized tobacco plants in Virginia fields in 1984

FIELD NO.	CULTIVAR	T. BASICOLA-INOCULUM DENSITY[d]	%STUNTED[g]	STUNTED PLANTS[h] MRRR[i]	STUNTED PLANTS[h] T. BASICOLA-POPULATION DENSITY[j]	STUNTED PLANTS[h] pH[k]	NORMAL-SIZED PLANTS[h] MRRR[i]	NORMAL-SIZED PLANTS[h] T. BASICOLA-POPULATION DENSITY[j]	NORMAL-SIZED PLANTS[h] pH[k]
1	sun-cured[a]	0		0	0	6.8	0	0	6.8
2	sun-cured[a]	5		0	11	5.0	0	16	5.2
3	SP G-28[b]	0		0	0	5.7	0	0	5.5
4	McNair 944[b]	0		0	0	5.4	0	0	5.2
5	McNair 944[b]	0.5		0	0	5.6	0	0	5.7
6	K 326[b]	NT[e]		0	1	5.8	0	0	5.8
7	Coker 319[b]	NT[e]		0	3	5.2	0	2	5.5
8	K 326[b]	8		0.4	3*	5.8	0.7	9	5.9
9	Coker 319[b]	12		1.2	11*	5.9	1.0	20	5.2
10	NC82[b]	26		1.0	29	6.1	0.7	28	6.3
11	Coker 319[b]	101	5 (10)	20*	139*	6.3	5.0	265	6.4
12	Ky 14[c]	0		0	0	6.1	0	0	6.1
13	B21-Ky10[c]	2		0	1	4.5	0	1	4.6
14	B21-Ky10[c]	12		NT[l]	NT[l]	NT[l]	0	6	5.5
15	Ky 14[c]	74	5 (10)	8.0*	102*	6.2	3.6	285	6.0
16	Ky 14[c]	76	1 (13)	15*	61*	6.2	1.0	266	5.7
17	Ky14-L8[c]	166	2 (7)	1.8	186	6.4	1.0	195	6.3
18	B21-Ky10[c]	148 vs 10[f]	40 (3)	32*	2040*	6.5	1.0	234	6.5
19	B21-Ky10[c]	158 vs 31[f]	40 (4)	30*	1051*	5.4	1.0	204	5.2

[a] Official test field.
[b] Flue-cured field.
[c] Burley field.
[d] Propagules of *T. basicola* per g of soil in a composite sample collected at transplanting time in May or early June.
[e] Not tested.
[f] Field not sampled prior to transplanting, but population densities of *T. basicola* (present in areas that had either mostly stunted versus mostly normal-sized plants, respectively) were estimated by assaying soils taken from between rows in early August.
[g] Percent of plants in the test area that were stunted. A minimum of 100 plants were counted. A plant was considered to be stunted if its height was less than half the height of normal-sized or representative larger plants in the field. Data are only presented for medium to high inoculum density fields. Values in parentheses are the average fresh shoot weight of stunted plants expressed as a percent of the average fresh shoot weight of normal-sized plants.
[h] Data on stunted versus normal-sized plants. Plant and soil samples were taken in mid July (field Nos. 1-17) or early August (fields No. 18 and No. 19). An asterisk indicates that there was a significant difference (as determined by an unpaired t-test) between the value given for the stunted versus the normal-sized plants. Soil pH data were not analyzed statistically.
[i] Mean percent of roots with black root rot (0-100%).
[j] Propagules of *T. basicola* per g of soil in a sample taken from aound root systems.
[k] Soil pH was measured in 2 parts 0.01 M $CaCl_2$:1 part soil (v/w).
[l] Not tested because of resets in the field.

however, *T. basicola* was not associated with the stunting, since mean percent root rot ratings on both stunted and normal-sized plants were low (1.8 and 1.0%, respectively). The last two burley fields sampled (No. 18 and No. 19) were the only ones in the survey with significant stunting problems associated with black root rot. Forty percent of the plants in both of these fields were stunted. No soil samples were taken at these locations prior to transplanting by the grower, but population densities of *T. basicola* in these fields were estimated by assaying soil taken from between rows in early August. These two fields were unusual because there were distinct areas that had either mostly stunted or mostly normal-sized plants. When these areas were sampled separately, the population densities of *T. basicola* were 148 (for field No. 18) and 158 (for field No. 19) propagules per g of soil in areas containing mostly stunted plants, and 10 (for field No. 18) and 31 (for field No. 19) propagules per g of soil in areas containing mostly normal-sized plants. Fig. 3.2 shows the appearance of stunted burley tobacco plants present in field No. 19 in 1985.

Estimated inoculum densities of *T. basicola* in two of two sun-cured fields (No. 1 and No. 2) and eight of nine flue-cured fields (Nos. 3-10) were 0-26 propagules per g of soil. Tobacco stunting occurred to some extent in all of these fields; however, *T. basicola* was not associated with the stunting, since mean percent root rot ratings on both stunted and normal-sized plants were low (range=0-1.2%). One flue-cured field (No. 11) had an

Figure 3.2. The appearance of stunted burley tobacco (cv. B21-Kyl0) plants in field No. 19 in 1985. Tobacco stunting in this field was associated with black root rot.

inoculum density of 101 propagules per g of soil. Five percent of the plants in this field were stunted, with mean percent root rot ratings on stunted versus normal-sized plants averaging 20 and 5%, respectively.

For five of the fields (Nos. 8, 9, 11, 15 and 16), population densities of *T. basicola* in July were significantly higher in soils taken from around root systems of normal-sized than of stunted plants (Table 3.1). This occurred even in three fields (Nos. 11,15 and 16) where black root rot was more severe on stunted plants. However, in the two burley fields that had the severest levels of black root rot and plant stunting (No. 18 and No. 19), significantly higher population densities of *T. basicola* were found in soils taken from around root systems of stunted plants.

At the request of county agricultural extension agents, five additional flue-cured fields were visited in 1985. Population densities of *T. basicola* were estimated at these locations by assaying soil taken from between rows in late June to mid July. Population densities in four of the fields (Nos. 20-23) were 0-6 propagules per g of soil. The other field (No. 24) had a population density of 402 propagules per g of soil. The cultivar planted in field No. 24 was K-326, which has low to medium black root rot resistance. Seven percent of the plants in this field were stunted, with mean percent root rot ratings on stunted versus normal-sized plants averaging 32 and 1.3% (significantly different at P=0.05), respectively. Population densities of *T.*

basicola in soils taken from around root systems of stunted versus normal-sized plants were 2,150 and 825 propagules per g (significantly different at P=0.05), respectively. The pH values of soil associated with stunted versus normal-sized plants were 5.2 and 5.5, respectively.

Direct comparisons among fields concerning the sizes of stunted and normal-sized tobacco plants were not possible because of differences in factors such as weather and soil conditions, time of planting and sampling, cultural practices, and cultivar planted. However, in the medium to high inoculum density fields (Nos. 11, 15-19, 24), which contained 74 propagules per g of soil or greater, fresh shoot weights were 29-106 g for stunted plants, and 443-1649 g for normal-sized plants. Within fields, there were no appreciable differences in percent organic matter, P, K, Ca, Mg, Zn, and Mn levels in soil between samples associated with stunted versus normal-sized plants. Nematodes damaging to tobacco were found occasionally. *Pratylenchus* spp. were present in field No. 5, while both *Pratylenchus* spp. and *Tylenchorhynchus* spp. were present in field No. 13; however, their population densities were well below levels considered by the VPI&SU Nematode Assay Clinic to be damaging. A complicating factor in field No. 24 was the presence of damaging levels of *Globodera solanacearum* (tobacco cyst nematode); population densities in soil taken from around root systems of stunted versus normal-sized plants were 280 and 540 larvae per one-half L, respectively.

Soil pH values were generally 6.0 or greater in the medium to high inoculum density fields. The only notable exceptions were the low soil pH values (range=5.2-5.5) in fields No. 19 and No. 24. Soil texture analyses were carried out for the two fields that appeared to have the extremes in soil type. The soil in flue-cured field No. 11 (which had 101 propagules per g of soil, and exhibited a moderate incidence of stunted plants) was 59.9% sand, 31.0% silt and 9.1% clay, and was classified as a sandy loam. On the other hand, the soil in burley field No. 18 (which had 148 propagules per g of soil in an area that had mostly stunted plants) was 9.7% sand, 60.0% silt and 30.3% clay, and was classified as a silty-clay loam. Soil calcium levels, which may influence the severity of black root rot (7), varied widely among fields, but not between samples taken within fields. Soil calcium levels (µg per g) in the medium to high inoculum density fields averaged 564 (No. 11), 1002 (No. 15), 864 (No. 16), 1200 (No. 17), 1152 (No. 18), 1200 (No. 19), and 372 (No. 24). Soil calcium levels in the other fields ranged from 216 µg per g in No. 13 up to 1200 µg per g in No. 1. *T. basicola* was consistently isolated from tobacco plant root segments that had representative lesions.

Isolation of endomycorrhizae. Endomycorrhizal-like spores were found in all soils assayed; however, endomycorrhizae successfully colonized root systems of only 5 of 40 burley and 0 of 40 flue-cured tobacco plants grown in the greenhouse. The

endomycorrhizal fungus *Glomus clarus* Nicolson & Schenck (10) was isolated from soils taken from around root systems of both stunted and normal-sized plants in field No. 19. *G. clarus* was isolated also from soil taken from around root systems of stunted plants in field No. 17. Two different *Acaulospora* spp. were isolated from soil taken from around root systems of normal-sized plants in field No. 17. No endomycorrhizae were isolated from either of the flue-cured fields (No. 2 and No. 11). The height of greenhouse-grown tobacco plants not colonized by endomycorrhizae were 56-89 cm, while the height of plants colonized by endomycorrhizae were 56-81 cm.

DISCUSSION

Black root rot appears to be a major cause of tobacco stunting in the burley region of Virginia, but not in the other Virginia regions. To our knowledge, the data presented here are the first to document the relative importance of black root rot in tobacco stunting. We found no evidence to indicate that endomycorrhizae or other factors were associated with burley stunting, as found in Kentucky (4, 5, 9). *G. macrocarpum* was not isolated from any fields; however, *G.clarus* and two different *Acaulospora* spp. were. The results of the endomycorrhizal survey are preliminary, and further research is required to document the unfavorable or favorable effects of endomycorrhizae on burley and flue-cured tobacco in Virginia.

T. basicola generally occurred more frequently and was present at higher inoculum densities in burley than in flue- and sun-cured tobacco fields. Most of the flue- and sun-cured cultivars have low or low-medium levels of resistance, so other factors are probably responsible for the low incidence and severity of black root rot in these types. For instance, black root rot is most severe when soil temperatures are 23 C or lower (8). The flue- and sun-cured cultivars are grown in the low elevation, Piedmont region in central Virginia, while the burley cultivars are grown in the higher elevation, Appalachian region in southwestern Virginia. Air temperatures at the Virginia Tech Southern Piedmont Agricultural Research Center, located at Blackstone, averaged 18.4, 23.8, 23.2 and 23.7 C during the months May, June, July and August of 1984, respectively. Air temperatures at the Abingdon weather station, located in southwestern Virginia, during these months averaged 15.6, 22.2, 21.7 and 22.3 C, respectively. Soil temperatures (10 cm depth) at Blackstone during these months averaged 20.5, 26.9, 25.9 and 26.9 C, respectively. Since the flue- and sun-cured cultivars were transplanted in early-mid May, soil temperatures in tobacco fields in central Virginia were apparently only favorable for black root rot development for the first few weeks after transplanting. No corresponding soil temperature data were available for southwestern Virginia, but soil temperatures in the burley tobacco fields, which were transplanted late May-early June, were very likely several degrees C lower throughout most of

the growing season. Therefore, soil temperatures in the burley fields were probably close to the upper limit of the temperature range (17-23 C) that is considered most favorable for black root rot development. Other environmental factors also may have contributed to the greater severity of black root rot in the burley than in the flue- and sun-cured tobacco fields. In Virginia, soils in burley tobacco fields tend to be finer-textured and slightly higher in pH than soils in flue- and sun-cured tobacco fields. Fine soil texture and high soil pH (generally 6.0 or greater), in addition to low soil temperature, both favor black root rot development (8).

The cultivars B21-Kyl0, Ky 14, and Kyl4-L8 have low-medium, high, and high levels of black root rot resistance, respectively (1, 2, 3). Low host resistance, coupled with relatively high inoculum densities of *T. basicola* (148 and 158 propagules per g of soil in areas containing mostly stunted plants) are apparently the primary reasons for the severe black root rot and tobacco stunting problems that occurred in fields No. 18 and No. 19. On the other hand, high host resistance of Ky 14 and Kyl4-L8, even in the presence of medium to high inoculum densities of *T. basicola* (74-166 propagules per g of soil), is apparently the major reason why black root rot and tobacco stunting were not significant problems in burley field Nos. 15-17. Kyl4-L8, which is the most popular burley cultivar in Virginia (Leo A. Link, personal communication), probably has an even higher level of black root rot resistance than Ky 14, since mean percent root rot

ratings on Ky14-L8 (field No. 17, with 166 propagules per g of soil) were lower than on Ky 14 (fields No. 15 and No. 16, with 74 and 76 propagules per g of soil, respectively). The burley cultivars Ky 15 and Ky 17 are practically immune to black root rot, but are not widely grown because they do not yield as well as other cultivars under disease-free conditions (6). Field No. 12 was the only burley location where *T. basicola* was not found. This was a new field that had been planted to tobacco for only a couple of years and the pathogen was apparently not yet established.

The population density of *T. basicola* in one of five flue-cured fields (No. 24) sampled in 1985 was very high (402 propagules per g of soil). Additional studies conducted in 1985 (unpublished data) showed that the inoculum densities of *T. basicola* in fields No. 18 and No. 19 had increased greatly compared to the 1984 values (as high as 649 propagules per g of soil, from 148 propagules per g of soil, in field No. 18). The generally higher inoculum densities of *T. basicola* observed in 1985 were probably due to a large buildup of inoculum on diseased root systems in 1984. Root systems of normal-sized plants were always much larger than those of stunted plants. By virtue of their larger size, root systems of normal-sized plants were apparently in some instances capable of supporting greater levels of reproduction by *T. basicola* than those of stunted plants, since significantly higher populations were found in soils taken from around root systems of normal-sized plants for five of the

fields. However, in the fields that had the largest differences in mean percent root rot ratings between stunted and normal-sized plants, and also the greatest levels of root rot on stunted plants (Nos. 18, 19 and 24), significantly higher populations of *T. basicola* were found in soil taken from around root systems of stunted plants. Both the size of plant root systems and the severity of black root rot appear to be factors related to *T. basicola* reproduction. These two factors were also related to each other, since root systems of tobacco plants with severe black root rot were always smaller than root systems of tobacco plants with low levels of black root rot.

In addition to host resistance, factors such as soil temperature, pH and texture should be considered when relating the inoculum density of *T. basicola* to black root rot development. An inoculum density of 150 propagules per g of soil, coupled with low to medium host resistance, caused severe levels of black root rot and plant stunting in two burley tobacco fields in this study. A high soil pH was not an absolute requirement for black root rot, as evidenced by the low soil pH values in one of these two fields. The influence of soil texture on black root rot occurrence was not examined directly in this study; however, black root rot was very severe in field No. 19, which had a silty-clay-loam soil type. The information presented here, though not complete, should be useful in developing *T. basicola*-inoculum density versus disease incidence and/or severity relationships in commercial tobacco fields.

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Chapter 4

RELATION OF INOCULUM DENSITY OF *THIELAVIOPSIS BASICOLA* TO ROOT ROT SEVERITY AND GROWTH OF TOBACCO IN NATURALLY INFESTED SOIL

INTRODUCTION

Thielaviopsis basicola (Berk. & Br.) Ferraris (=*Chalara elegans* Nag Raj & Kendrick) is a widespread, soilborne plant pathogen that causes a black root rot on tobacco (*Nicotiana tabacum* L.). Black root rot is the principal disease associated with tobacco stunting in the burley region of Virginia (Chapter 3). Black root rot also causes minor losses in the flue-cured region of Virginia (Chapter 3). Rittenhouse & Griffin (9) reported that population densities of *T. basicola* in two burley tobacco fields were typically less than 10^3 propagules per g of soil. However, very little information is available concerning *T. basicola*-inoculum density versus disease incidence and/or severity relationships for tobacco in naturally infested soils. The objective of this study was to examine these relationships, both in soil-temperature tanks and in the field.

MATERIALS AND METHODS

Soil-temperature tank studies. Soils were collected from two burley tobacco fields in southwestern Virginia. One of the fields was heavily infested with *T. basicola*. Soil at this location was taken soon after harvest from around remains of tobacco plant root systems. Both infested and uninfested soils were passed through a 6.0-mm-opening screen. The infested soil was also passed through a 2.0-mm-opening sieve. Soils were assayed for propagules of *T. basicola* using TB-CEN medium (Chapter 2). The uninfested and infested soils contained 0 and 2,440±160 (95% confidence interval) propagules per g of oven-dry (24 hr at 105 C) soil, respectively. The uninfested soil contained 25 µg NO_3-N/g, 45 µg P/g, 137 µg K/g, 492 µg Ca/g, 120 µg Mg/g, 1.5 µg Zn/g, 15.7 µg Mn/g, 3.3% organic matter, 9.7% sand, 60.0% silt and 30.3% clay. The infested soil contained 37 µg NO_3-N, 45 µg P/g, 157 µg K/g, 1200 µg Ca/g, 120 µg Mg/g, 2.1 µg Zn/g, 16.1 µg Mn/g, 2.6% organic matter, 23.7% sand, 62.5% silt and 13.8% clay. Varying amounts of these soils were combined to produce a range of inoculum densities of *T. basicola*. Both soils were adjusted to a pH of 6.5 by adding 2,500 µg CaOH per g of soil. Fifty, 44, and 83 µg of N (added as NH_4NO_3), P (added as P_2O_5), and K (added as K_2O), respectively, were also added per g of soil. These nutrients were supplied as granular fertilizers. The soils were mixed thoroughly in a cement mixer. One-kg quantities of soil (dry weight basis) were placed in 11-cm

diam. plastic containers. Seven or eight-week-old flue-cured (cv. NC95 and Va Gold) or burley (cv. Burley 21) tobacco seedlings were transplanted into containers, one per pot. Resistance to black root rot in these cultivars range from low to high. In accordance with standard production practices, a higher N fertilization rate (100 µg/g of soil) was used for Burley 21. Three separate experiments were conducted, one for each cultivar. For each of the experiments, five to ten extra tobacco seedlings, of the same size as those that were transplanted, were used to determine an average initial (i.e. at-transplanting-time) value for oven-dry (70 C) shoot weight, shoot height, and oven-dry root weight. The inoculum densities prepared (15-20 replications) were 0, 10, 25, 50 and 100 propagules per g of soil (for NC95), and 0, 5, 20, 60 and 200 propagules per g of soil (for Va Gold and Burley 21). The pots were placed at random into soil-temperature tank floating devices with manifolds for water drainage. Burley 21, NC95, and Va Gold seedlings were grown at soil temperatures of 20-22, 21-23, and 21-23 C, respectively. Air temperatures in the greenhouse fluctuated from 20-32 C. Flourescent lights were used to supplement natural sunlight. Pots were watered daily or as needed to keep the soil moist. At the end of 30-31 day plant-growth periods, plant root systems were carefully washed free of soil, and rated immediately for mean percent of roots with black root rot. The rating categories used were 0, 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 95%. Roots with representative lesions were plated onto TB-CEN medium

(Chapter 2). For each inoculum density, the average (mean of 15-20 plants) increase in shoot dry weight, shoot height, and root dry weight was determined by subtracting the at-transplanting-time value from the final measured value.

The inoculum density-mean percent root rot data were modeled [SAS, Nonlinear Regression (11)] using a power-law equation of the form $Y=aX^b$, where Y equaled mean percent root rot, X equaled propagules of *T. basicola* per g of soil, and a and b were constants estimated from the data (1). Regressions involving mean percent root rot data were weighted to correct for unequal variances among inoculum densities (11, 12). Mean percent root rot ratings for plants grown in uninfested soil were not included in the analyses, since the observed values (0% in all cases) were not subject to variation. The plant growth data were modeled (SAS, Nonlinear Regression) after a power-law equation of the form $Y=Y_o-aX^b$, where Y equaled predicted plant growth, Y_o equaled plant growth in uninfested soil, X equaled propagules of *T. basicola* per g of soil, and a and b were constants estimated from the data.

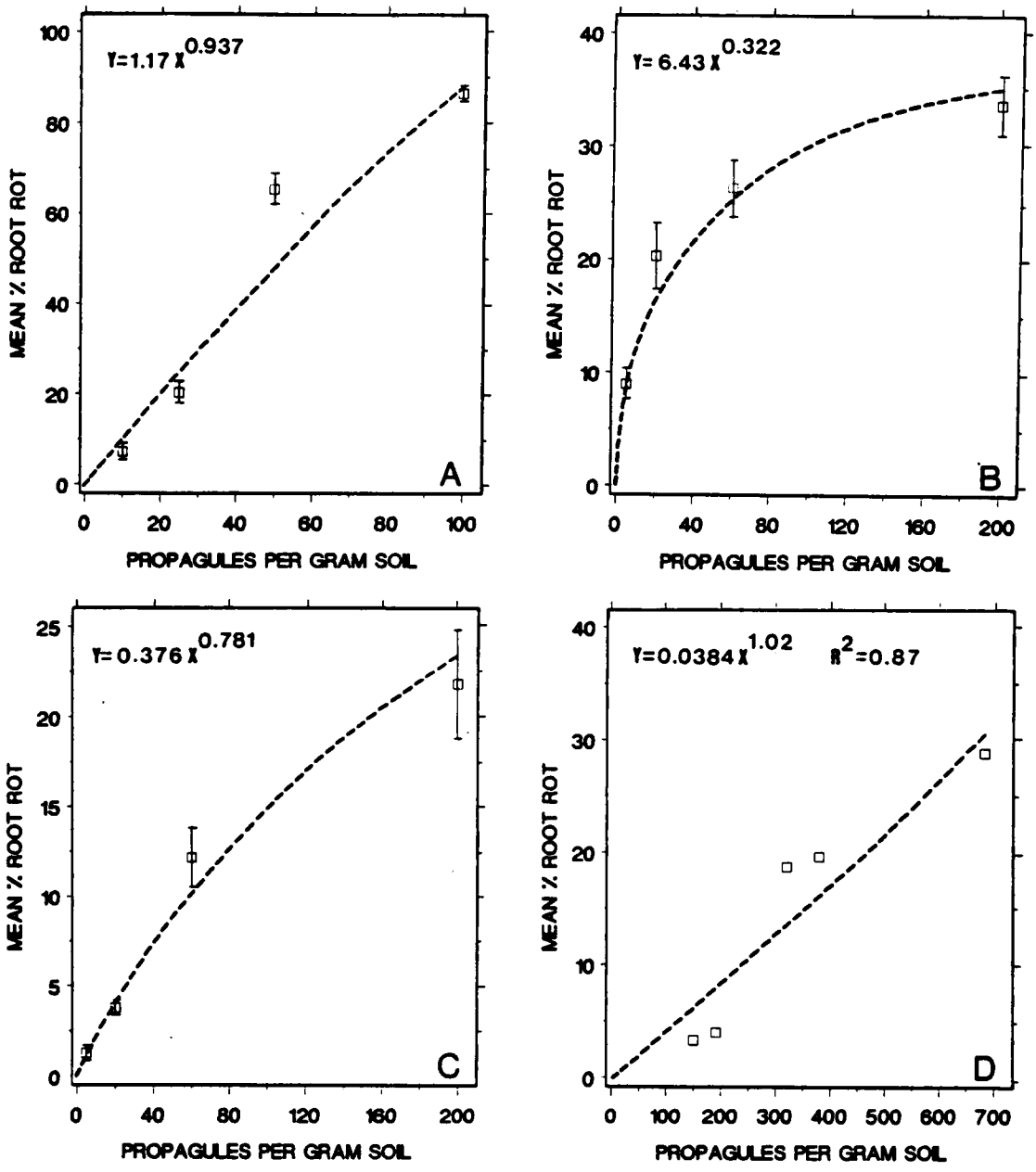
Field-plot study. A study was conducted in 1985 on a 0.45 ha commercial burley tobacco (cv. B21-Ky10) field infested with *T. basicola*. A preliminary population density survey was completed 1 mo prior to transplanting on 21 May. Eight 5.7 x 3.6 m plots were set up several days after transplanting. Each plot contained four rows and approximately 50 plants. Fifty soil

cores (2.5 cm diam. x 15-20 cm deep) were systematically taken from each plot by sampling between the transplants. A composite sample for each plot was made by bulking the cores. The samples were passed through a 4.8-mm-opening sieve, mixed thoroughly, and assayed on TB-CEN medium. The samples were also analyzed for soil pH, water contents at -0.01, -0.033 and -1.5 MPa, soil texture, percent organic matter, soil fertility, and plant-parasitic nematodes. Soil pH was measured in 0.01 M $CaCl_2$ (7). Pressure plates were used for determining soil water content (8). The other soil analyses were conducted in the clinical laboratories at VPI&SU. The field plots were maintained by the grower according to standard production practices. Pesticides applied were metalaxyl, napropamide and carbofuran. Tobacco plants were sampled from the plots on 9 July. Every other plant was carefully dug up, washed free of soil, and immediately rated for mean percent black root rot. Roots with representative lesions were plated onto TB-CEN medium. Oven-dry shoot weight, shoot height, and oven-dry root weight were determined for each plot (average of 24-26 plants per plot).

RESULTS

Soil-temperature tank studies. For all cultivars, the incidence of plants with black root rot was 100% when inoculum densities of *T. basicola* were greater than 0 propagules per g of soil. *T. basicola* was isolated from all root segments that had representative lesions. No root rot associated with *T. basicola* was found on plants grown in uninfested soil. Mean percent root rot ratings on NC95 (Fig. 4.1A) were 7.2 and 86.5% at 10 and 100 propagules per g of soil, respectively. A 95% confidence interval for the exponent or b value obtained by regression included 1.0, which indicated that the relationship between inoculum density and mean percent root rot was not significantly (P=0.05) different from a directly proportional or linear one (1). Mean percent root rot ratings on Va Gold (Fig. 4.1B) were 1.3 and 21.8% at 5 and 200 propagules per g of soil, respectively. Mean percent root rot ratings on Burley 21 (Fig. 4.1C) were 9.0 and 33.7% at the same inoculum densities. With these two cultivars, the exponent or b values obtained by regression were significantly (P=0.05) less than 1.0, which indicated curvilinear relationships between inoculum density and mean percent root rot, with the most rapid increases (per unit increase in inoculum) in mean percent occurring at the lower inoculum densities. All of the root rot data for the individual plants were included in the analyses used to obtain the best fitting lines. R^2 values of 0.93-0.97 were obtained when the

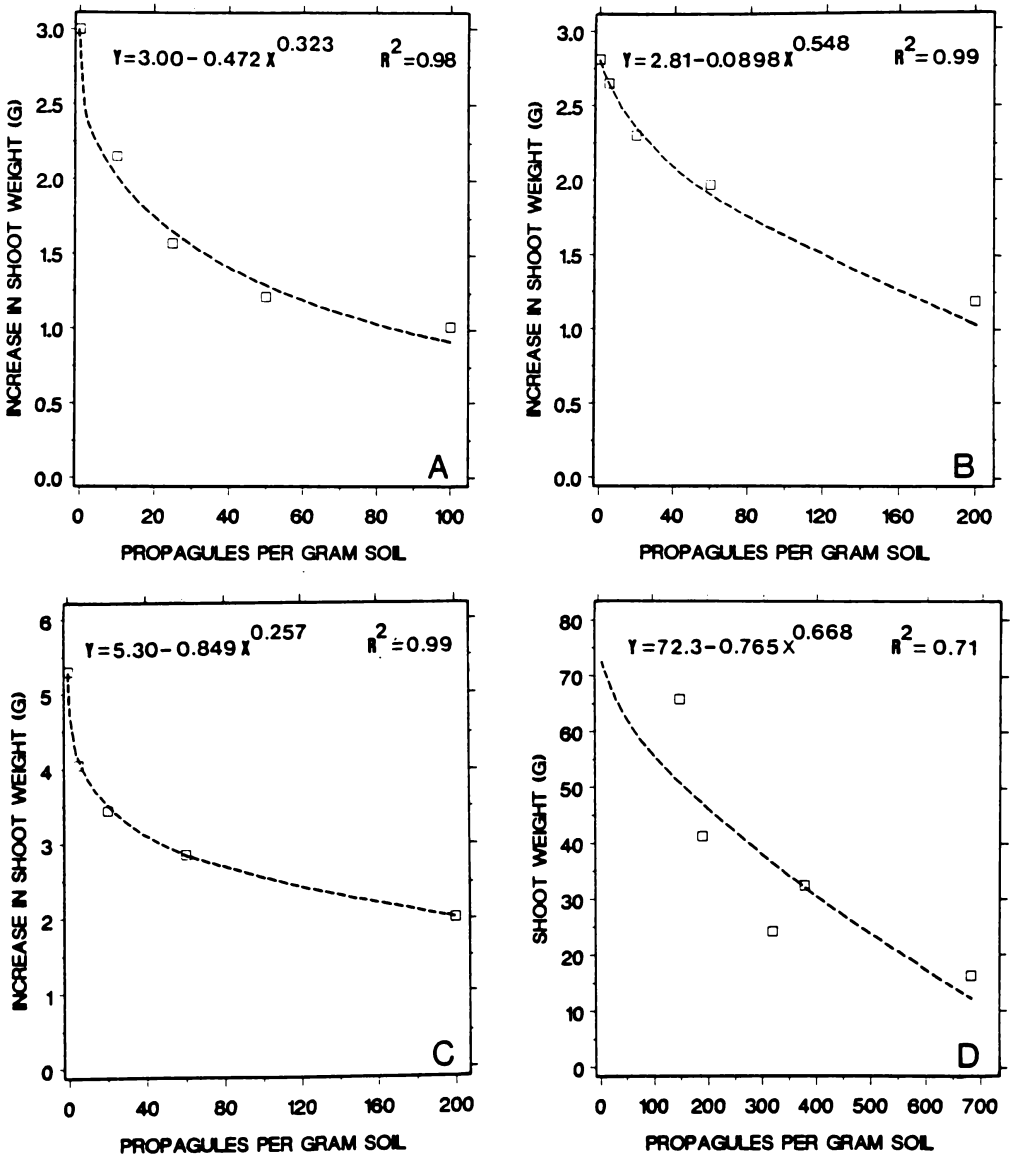
Figure 4.1. Graphs showing the observed relationships between inoculum density of *Thielaviopsis basicola*, expressed as propagules per g of soil (X), and mean percent root rot (Y). **A–C.** Soil-temperature tank studies with: **A,** cv. NC95. **B,** cv. Va Gold. **C,** cv. Burley 21. Vertical bars represent 95% confidence intervals for the mean of 15-20 replications per inoculum density. Regressions were weighted to correct for unequal variances among inoculum densities. Mean percent root rot ratings for plants grown in uninfested soil were 0%, but were not included in the analyses because they were not subject to variation. R^2 values of 0.93, 0.97 and 0.96, respectively, were obtained when the mean values were fitted to the calculated regression lines. Ninety five percent asymptotic confidence intervals for the exponents were 0.937 ± 0.118, 0.322 ± 0.067 and 0.781 ± 0.129, respectively. Regressions were significant at the P=0.001 level. **D,** Field-plot study with cv. B21-Ky10. Regression was carried out on plot means and was significant at the P=0.01 level.


Y=1.17 X^0.937
MEAN % ROOT ROT
PROPAGULES PER GRAM SOIL
A
Y= 6.43 X^0.322
B
Y= 0.376 X^0.781
C
Y=0.0384 X^1.02 R^2=0.87
D

mean values (15-20 replications per inoculum density) were fitted to the calculated regression lines (Fig. 4.1A-C). No root rot or discoloration not associated with *T. basicola* was found on NC95. However, up to 50% of the roots of Va Gold and Burley 21 plants grown in uninfested soil were slightly discolored or mildly rotted. The cause of the discoloration was not determined, but it was not associated with *T. basicola*. Only extensively necrotic roots, which were associated with *T. basicola*, on these two cultivars were rated as positive for root rot.

Significant (P=0.01) reductions in shoot dry weight occurred at inoculum densities as low as 5-10 propagules per g of soil. The reductions were greatest with NC95 (Fig. 4.2A) and Burley 21 (Fig. 4.2C). However, all of the cultivars showed the largest reductions in shoot weight at inoculum densities of 50-200 propagules per g of soil. With NC 95, shoot dry weight was reduced from 3.00 to 2.15 to 1.01 g as inoculum densities increased from 0 to 10 to 100 propagules per g of soil, respectively. With Burley 21, shoot dry weight was reduced from 5.30 to 4.05 to 2.00 g as inoculum densities increased from 0 to 5 to 200 propagules per g of soil, respectively. With Va Gold, shoot dry weight was reduced from 2.81 to 2.65 to 1.18 g as inoculum densities increased from 0 to 5 to 200 propagules per g of soil, respectively. Exponent or b values obtained by regression (R^2 range=0.98-0.99) were all much less than 1.0, which indicated strong curvilinear relationships between inoculum density and shoot dry weight, with the most rapid decreases (per

Figure 4.2. Graphs showing the observed relationships between inoculum density of *Thielaviopsis basicola*, expressed as propagules per g of soil (X), and increases in shoot dry weight in soil-temperature ranks, or shoot dry weight in the field (Y). **A–C.** Soil-temperature tank studies with: **A**, cv. NC95. **B**, cv. Va Gold. **C**, cv. Burley 21. Regressions were carried out on the means (15–20 plants per inoculum density) and were significant at the P=0.01 level. **D**, Field-plot study with cv. B21-Ky10. Regression was carried out on plot means and was significant at the P=0.10 level.


Y=3.00-0.472X^0.323
R^2=0.98
INCREASE IN SHOOT WEIGHT (G)
PROPAGULES PER GRAM SOIL
A
Y=2.81-0.0898X^0.548
R^2=0.99
INCREASE IN SHOOT WEIGHT (G)
PROPAGULES PER GRAM SOIL
B
Y=5.30-0.849X^0.257
R^2=0.99
INCREASE IN SHOOT WEIGHT (G)
PROPAGULES PER GRAM SOIL
C
Y=72.3-0.765X^0.668
R^2=0.71
SHOOT WEIGHT (G)
PROPAGULES PER GRAM SOIL
D

unit increase in inoculum) occurring at the lower inoculum densities. Similar results were obtained with the shoot height and root dry weight data (Table 4.1).

Field-plot study: The inoculum densities of *T. basicola* in five plots were 150, 191, 320, 380 and 683 propagules per g of soil. The incidence of tobacco (B21-Ky10) plants with black root rot was 100% in all plots. Mean percent root rot ratings were 3.3 and 28.8% at 150 and 683 propagules per g of soil, respectively (Fig. 4.1D). The exponent or b value obtained by regression (R^2=0.86) was close to 1.0, which indicated a linear relationship between inoculum density and mean percent root rot. *T. basicola* was isolated from all root segments that had representative lesions. Shoot dry weight generally decreased with increasing inoculum density (Fig. 4.2D), but there was an appreciable amount of deviation from the regression line (residual=0.29). The shoot dry weight of plants grown in uninfested soil was estimated, since no plots not containing *T. basicola* were located. A simple linear regression of shoot height on inoculum density indicated that the best-fitting straight line was Y=61.3-0.735X. However, the straight line (R^2=0.66) did not fit the data as well as the curved line (R^2=0.71). Similar results were obtained with the shoot height and root dry weight data (Table 4.1).

Table 4.2 gives some of the chemical and physical characteristics of the soil in the field plots. There were usually no large differences among the plots. Soil pH values

were 5.2-5.4. All soils were classified either as silt-loams or as loam/silt-loams. However, soil in plot 5 did have the lowest phosphorus and also the highest calcium levels, 17 and 960 μg/g of soil, respectively. Figs. 4.4-4.8 show representative tobacco plants and root systems observed in the soil-temperature tank and field-plot studies.

Table 4.1. Regression equations describing observed relationships between the inoculum density of *Thielaviopsis basicola*, expressed as propagules per g of soil (X), and increases in shoot height or root dry weight (Y)

CULTIVAR	*INCREASE IN SHOOT HT (cm)*	*INCREASE IN ROOT DRY WT (g)*
NC95[a]	$Y=6.25-0.895X^{0.325}$ ($R^2=0.99$, $P>F=0.01$)	$Y=0.672-0.207X^{0.214}$ ($R^2=0.96$, $P>F=0.01$)
Va Gold[a]	$Y=7.16-0.244X^{0.559}$ ($R^2=0.97$, $P>F=0.01$)	$Y=0.368-0.110X^{0.167}$ ($R^2=0.98$, $P>F=0.01$)
Burley 21[a]	$Y=12.8-0.415X^{0.510}$ ($R^2=0.97$, $P>F=0.01$)	$Y=0.821-0.116X^{0.274}$ ($R^2=0.99$, $P>F=0.01$)
B21-Ky10[b]	$Y^c=49.6-0.470X^{0.674}$ ($R^2=0.72$, $P>F=0.07$)	$Y^c=12.8-0.0786X^{0.747}$ ($R^2=0.77$, $P>F=0.06$)

[a] Soil-temperature tank study.
[b] Field-plot study.
[c] Actual shoot height or root dry weight (i.e. at-transplanting-time value was not subtracted off).

Table 4.2. Inoculum densities of *Thielaviopsis basicola* with 95% confidence intervals, and chemical-physical characteristics of soils taken from the five field plots

	PLOT #				
	1	*2*	*3*	*4*	*5*
T. basicola (propagules per g soil)	150±18	191±22	320±26	380±24	683±32
pH[a]	5.2	5.3	5.3	5.4	5.3
Phosphorus[b]	37	34	29	32	17
Potassium[b]	152	157	145	157	157
Calcium[b]	540	600	528	576	960
Magnesium[b]	69	78	83	83	81
NO_3-Nitrogen[b]	43	50	45	70	58
Zinc[b]	1.1	1.2	1.0	1.0	1.2
Manganese[b]	9.4	12.0	7.5	8.1	8.9
% Organic Matter	2.4	2.7	2.0	2.1	2.9
Water Content[c] (%) at:					
-1.5 MPa	8.8	7.3	7.2	7.8	10.4
-0.033 MPa	19.1	17.6	19.8	18.0	20.4
-0.01 MPa	22.6	21.1	23.9	22.1	23.8
% Sand	26.0	33.2	31.4	29.4	25.2
% Silt	55.2	48.7	52.4	53.6	56.2
% Clay	18.8	18.1	16.2	17.0	18.6
Textural Class[d]	sil	l-sil	sil	sil	sil

[a]Measured in 2 parts 0.01 M $CaCl_2$:1 part soil (v/w).
[b]µg per g of soil.
[c]Dry weight basis. Analyses conducted on samples that were passed through a 2.0-mm-opening sieve and packed to bulk densities (after wetting) of 1.2 g per cm^3.
[d]silt-loam or loam/silt-loam.

Figure 4.3. Soil-temperature tank study. Representative tobacco plants of cultivar NC95 grown in soil naturally infested with 100 (left) and 0 (right) propagules of *Thielaviopsis basicola* per g of soil.

Figure 4.4. Soil-temperature tank study. Representative tobacco plants of cultivar Burley 21 grown in soil naturally infested with (from left to right) 0, 5, 20, 60, and 200 propagules of *Thielaviopsis basicola* per g of soil.

Figure 4.5. Soil-temperature tank study. Representative tobacco plants of cultivar Va Gold grown in soil naturally infested with (from left to right) 200, 60, 20, 5, and 0 propagules of *Thielaviopsis basicola* per g of soil.

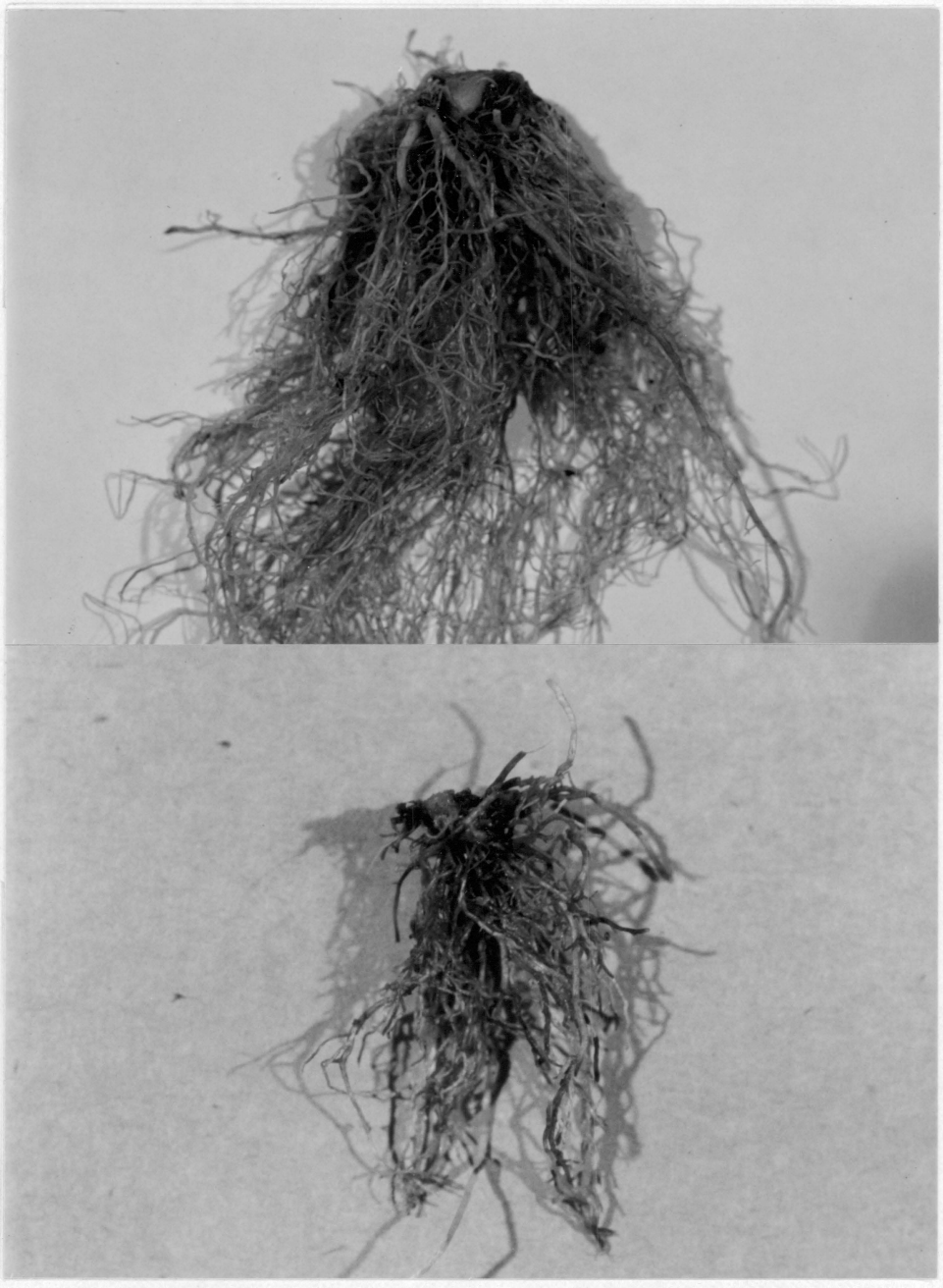


Figure 4.6. Soil-temperature tank study. Close-up of root systems of representative tobacco plants (cv. Va Gold) grown in soil naturally infested with either 0 (top) or 200 (bottom) propagules of *Thielaviopsis basicola* per g of soil.

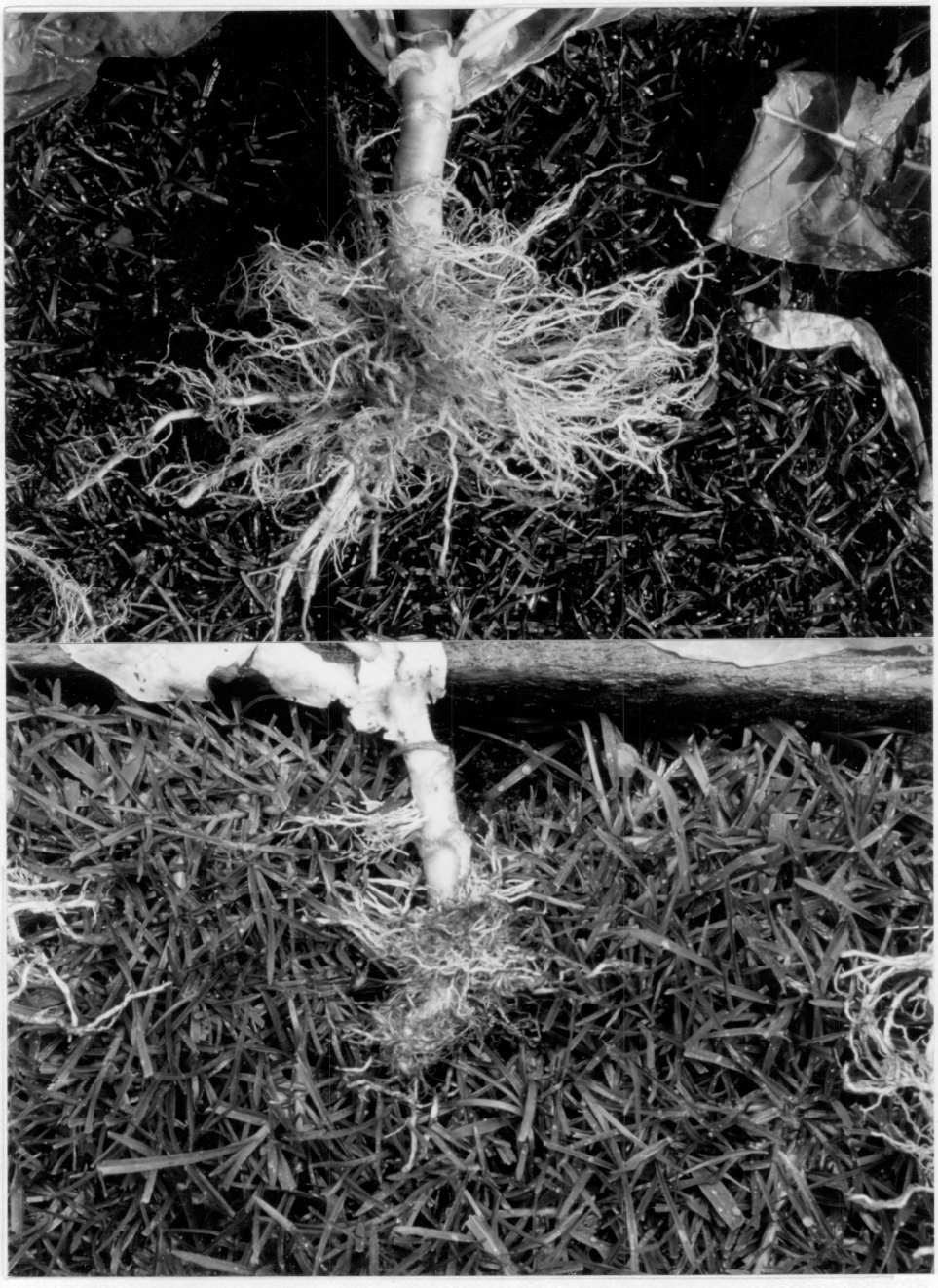


Figure 4.7. Field-plot study. Root systems of representative tobacco plants (cv. B21-Ky10) grown in field plots containing an average of 150 (top) or 683 (bottom) propagules of *Thielaviopsis basicola* per g of soil.

DISCUSSION

Fig. 4.8 shows the types of curves that are generated by the power-law equations that were used to analyze the mean percent root rot and plant growth data. When the variables X and Y are related through a power-law function (such as $Y=aX^b$), a linear regression of log Y on log X will produce a straight line of the form log Y=log a + b(log X). The values of b and a can be estimated directly using iterative procedures, or by calculating the slope (for the value of b), and the antilog of the Y intercept (for the value of a). However, log-log transformations obscure relationships that are best visualized by arithmetic data plots. Hence, and iterative method (SAS, Nonlinear Regression) appeared to be more desirable and was used here.

Linear relationships between inoculum density and mean percent root rot were found for cultivars NC95 and B21-Ky10. Curvilinear relationships were found for cultivars Va Gold and Burley 21. However, the accurate judgement of *T. basicola*-associated root rot on the latter two cultivars was hampered by a partial root discoloration that was not associated with the pathogen. The power-law relationship was used to fit inoculum density units (propagules per g of soil) to disease severity units (%), but no theoretical or biological bases about the number of infections or lesions are implied. Each plant root system had numerous lesions, many of which coalesced, particularly at the higher inoculum densities. Mean percent root

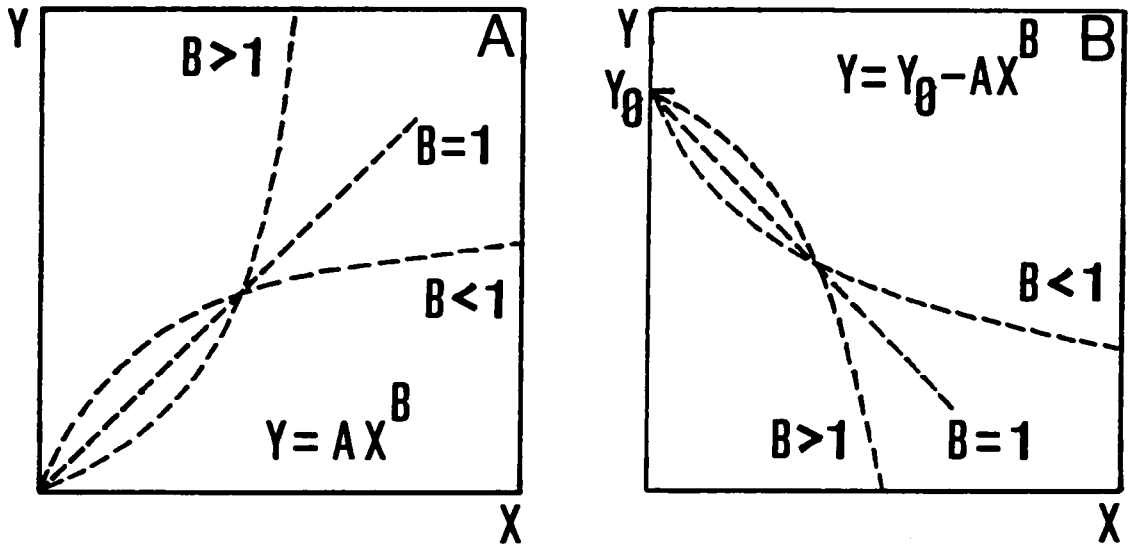


Figure 4.8. Types of curves generated by power-law equations used for analyzing: A, Mean percent root rot data. B, Plant growth data. X equals inoculum density, Y equals predicted value, Yo equals plant growth in uninfested soil, and a & b are constants estimated from the data. Fiqure A modified from Arya & Lardner (1).

rot is not a complete representation of total root damage, since the size of plant root systems invariably decreased as mean percent root rot increased. In addition, some rotted roots probably remained in the soil during harvesting. Even so, the percentage of roots that were rotted was apparently not affected greatly by the size of the remaining root system, at least for NC95 and B21-Ky10, where direct proportional relationships between inoculum density and mean percent root rot were found.

In the field-plot study, inoculum densities of 150 and 683 propagules per g of soil were associated with moderate and severe levels of black root rot, respectively. In the soil-temperature tank studies, severe levels of root rot occurred at inoculum densities of 50-200 propagules per g of soil. Direct evaluations between the studies are not possible, since environmental- and host-related (such as cultivar and age of transplants) factors varied or could not be controlled. Two environmental factors that favor black root rot are a soil pH of 6.0 or greater and soil temperatures of 17-23 C (5). Three soil factors (pH 6.5, 20-23 C temperature, and high moisture) and a host factor (the planting of young seedlings) were optimal for black root rot occurrence in soil-temperature tanks. In contrast, soil pH was low (range=5.2-5.4), and much larger field-sized transplants were used in the field-plot study. Older tobacco plants are more resistant than seedlings to black root rot (5). Less conducive environmental- and host-related factors are probably the reason why higher inoculum densities of *T. basicola* were required to

cause root rot in the field-plot study. Even though environmental factors in the field could not be controlled per se, attempts were made to select plots that appeared to have similar soil conditions. These initial decisions (in early spring) were based primarily upon soil color and apparent texture. Eight plots were originally set up, but only five were evaluated for root rot and plant growth because the other three were later found to have higher soil pH values (range=6.5-6.8). However, differences in environmental factors among the plots may possibly explain the lower R^2 values (0.71-0.77, Fig. 4.2D & Table 4.1) obtained in regressions with the plant growth data. For instance, plot 1 had a slightly lower soil pH, and also the lowest level of root rot and the largest tobacco plants. Plot 5 had the highest level of soil calcium, and also the highest level of root rot and the smallest tobacco plants. High soil calcium levels may promote black root rot independent of any effect on soil pH (4).

The inoculum densities of *T. basicola* in the field plots were observations measured with error. Standard regression procedures are not ordinarily appropriate when the "independent" variable is an observation (3). However, 95% confidence intervals for the inoculum densities were small relative to the means (Table 4.2). Also, only plots that provided a wide range of inoculum densities (based upon the preliminary survey) were selected. Standard regression procedures are generally accepted when the latter condition is met (3). The inoculum density of *T.*

basicola in the infested soil that was used for the temperature tank studies also was an observation, but all inoculum densities were prepared from the same soil, and environmental conditions were uniform.

Taylor et al. (14) point out that extreme inoculum clumping or aggregation can complicate inoculum density-disease incidence relationships in the field. A clumped inoculum pattern may result if the mechanical actions of plowing and disking fail to break up and disperse infested plant debris or other forms of inoculum (2, 10). Rittenhouse & Griffin (9) reported that the inoculum pattern of *T. basicola* in two burley tobacco fields was clumped, but the degree of clumping was not high (Lloyd's index of patchiness values were 1.5-5.7), and it was concluded that it may be possible to develop satisfactory relationships between inoculum density and black root rot development without correcting for the degree of inoculum clumping. Coefficients of variation of the mean percent root rot data are given in Table 4.3. The variability of the field-plot data (B21-Ky10) was no greater than the soil-temperature tank data (NC95, Va Gold and Burley 21). Random or near random inoculum patterns in soil-temperature tanks were probably assured by thorough soil mixing. At comparable levels of root rot, such as 20.3% (NC95), 21.8% (Va Gold), 20.3% (Burley 21) and 19.6% (B21-Ky10), coefficients of variation were similar (63.8, 65.0, 43.9 and 55.6%, respectively). Inoculum clumping apparently did not greatly influence mean percent root rot ratings in the field, at

Table 4.3. Coefficients of variation of the mean percent root rot data, and shoot/root dry weight ratios for four tobacco cultivars

CULTIVAR	*PROPAGULES PER G SOIL*	*MEAN PERCENT ROOT ROT*	*COEFFICIENT OF VARIATION*	*SHOOT/ROOT RATIO*
NC95[a]	0	0	----	4.46
	10	7.2	84.6	5.61
	25	20.3	63.8	7.48
	50	65.3	25.8	7.25
	100	86.5	8.8	6.69
Va Gold[a]	0	0	----	7.6
	5	1.3	92.9	11.3
	20	3.8	43.5	13.8
	60	12.1	61.9	12.1
	200	21.8	65.0	11.8
Burley 21[a]	0	0	----	6.46
	5	9.0	60.6	6.59
	20	20.3	43.9	6.07
	60	26.3	34.0	5.85
	200	33.7	26.6	6.45
B21-Ky10[b]	150	3.3	84.8	5.65
	191	4.0	56.5	4.66
	320	18.7	60.3	5.28
	380	19.6	55.6	5.40
	683	28.8	30.0	5.11

[a] Soil-temperature tank study. Soil was thoroughly mixed to assure a random or near-random inoculum pattern.

[b] Field-plot study. Propagules per g of soil represents the plot average.

least on a *per plant root system basis*. However, there were "root-rot areas" on root systems of some field-grown tobacco plants. The clusters of inoculum of *T. basicola* observed by Rittenhouse & Griffin (9) may occupy volumes of soil that are relatively small compared to the size of tobacco plant root systems. Evidence supporting this hypothesis was found by Rittenhouse & Griffin (9). They reported that population densities of *T. basicola* in soil cores varied as much within a 3.66-m interval as they did within a 21.8-m interval.

The cultivars NC95, Burley 21, B21-Ky10 and Va Gold are generally considered to have low, low-medium, low-medium and high levels of black root rot resistance, respectively. However, they were all susceptible in these studies, with significant reductions in plant growth occurring at inoculum densities as low as 5-10 propagules per g of soil for those cultivars tested in soil-temperature tanks. Shoot/root weight ratios increased greatly when Va Gold was grown in soil infested with *T. basicola* (Table 4.3). An increase in shoot/root weight ratio may be an indication of tolerance to black root rot, since shoot growth did not decrease in proportion to the reduction in root system size.

Naturally infested field soil was used in the soil-temperature tank studies to simulate the condition of natural inoculum as much as possible. The nature of the propagules of *T.basicola* in the naturally infested soil was not determined, but chlamydospores are reported to be the major survival structure (15). The chlamydospores, which are formed in chains, eventually

break up in soil (6). Therefore, propagule counts (or counts of colony forming units) of *T. basicola* on soil-dilution plates (Chapter 2), and on carrot-disks (13, 16) would probably tend to be underestimated if soils were assayed prior to chlamydospore separation. However, soil assays would still provide useful information on relative population differences among soils.

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Chapter 5

THE EFFECTIVENESS OF IMAZALIL FOR CONTROLLING BLACK ROOT ROT IN BURLEY TOBACCO

INTRODUCTION

Black root rot, caused by *Thielaviopsis basicola* (Berk. & Br.) Ferraris (=*Chalara elegans* Nag Raj & Kendrick), is an important soilborne disease of burley tobacco in the United States (8). Black root rot can be controlled by soil fumigation with volatile fungicides such as methylisothiocyanate (12) and metham (7); Papavizas & Lewis (10) reported that these compounds reduced the inoculum density of *T. basicola*. However, the fumigation of burley tobacco fields in Virginia is expensive and not often practiced, especially since other soilborne pathogens of tobacco, including *Globodera solanacearum* (tobacco cyst nematode) and *Phytophthora parasitica* var. *nicotianae* (black shank), are not a problem.

The application of several nonvolatile fungicides to soil have shown promise for controlling black root rot of tobacco under greenhouse conditions and in seedbeds; these fungicides include benomyl, thiabendazole, thiophanate-methyl, captan and maneb (4,6,10,11,14). Of these, benomyl is one of the most effective, but even it has failed to protect tobacco under field conditions (4).

Imazalil (1-[2-(2,4-dichlorophenyl)-2-(2 propenyloxy)ethyl]-

1H-imidazole) is a nonvolatile, systemic fungicide that is active against a range of plant-pathogenic fungi, especially Deuteromycetes and Ascomycetes. Imazalil controls several post-harvest rots of fruits and vegetables, including Penicillium rot of pome and citrus fruits (3, 5), Alternaria rot of tomato (15), and stem-end rot of pineapple caused by *Thielaviopsis paradoxa* (1). Imazalil also controls common root rot of wheat and barley caused by *Cochliobolus sativus* (16, 17). The addition of imazalil to transplanting water has shown some promise for controlling black root rot of flue-cured tobacco in Canada (S. K. Gayed, personal communication). The purpose of this study was to evaluate the effectiveness of imazalil for controlling black root rot of burley tobacco in Virginia. A laboratory study was also conducted to compare the effectiveness of imazalil (versus benomyl) for inhibiting the in vitro growth of *T. basicola*.

MATERIALS AND METHODS

Field Study. A field experiment was conducted in 1985 over a 0.1 ha area of a commercial burley tobacco field in Washington County, Virginia. Observations made in 1984 indicated that black root rot was associated with a severe plant stunting problem in the field. For the experiment, the grower transplanted tobacco (cv. B21-Kyl0, low to medium black root rot resistance) and maintained the field according to standard production practices. Transplants were set out on 27 May, and on 28 May the soil around the base of each plant was drenched with 50 ml of a solution containing 0, 250, 750 or 1,500 µg a.i. imazalil/ml. Imazalil was added as Fungaflor™ 75WSP (100% sulfate salt formulation, Janssen Pharmaceutica Corp.). The experimental design was a randomized complete block with six replications (i.e. rows) per treatment. Row length was 33 m, with 70-75 plants depending upon grower spacing. Row width was 1.2 m. A guard row was present on each side of the field. Pesticides applied by the grower were metalaxyl, pendimethalin, diazinon and acephate. A composite soil sample (50 cores each 2.5 cm diam. x 15-20 cm deep) of the test area was taken on the same day that imazalil was applied. The inoculum density of *T. basicola* was determined by assaying the composite soil sample on TB-CEN medium (Chapter 2). Soil pH was measured in 0.01 M $CaCl_2$ (13). Additional assays for soil texture, percent organic matter and plant-parasitic nematodes were made in the clinical laboratories at VPI&SU.

All treatments were evaluated on 31 July. The height of every 10th plant in each row was measured at that time (plants had not yet been topped). The and 40th plant in each row was dug up, washed free of soil, and immediately rated for the mean percent of the root surface that was rotted (0-100%). The root rot and plant height data were analyzed by ANOVA (with imazalil concentration as the class variable) and trend analysis (9). The significance of both linear and quadratic relationships between imazalil concentration and plant height, and also between imazalil concentration and mean percent root rot, were tested.

Laboratory Study. Imazalil and benomyl were incorporated into a basal agar medium (pH 6.0) that contained 100 ml V8 juice, 1 g $CaCO_3$ and 18 g agar per L. Solutions containing imazalil (added as Fungaflor™ 75WSP) and benomyl (tech. grade, 98% a.i.) were prepared in water and a 1:1 (v/v) acetone-methanol mixture, respectively. One-hundred-ml quantities of basal molten agar medium were amended with the fungicides immediately prior to pouring plates. The fungicide concentrations tested were 0.001, 0.00316, 0.01, 0.0316, 0.1, 0.316, 1.0, 3.16, and 10.0 µg a.i./ml. The concentrations were chosen so that they would be equally spaced when plotted on a logarithmic scale. Control plates containing no fungicide were also prepared.

Five-mm^2 agar blocks were removed from the margins of actively growing colonies of *T. basicola* and placed on fungicide-amended medium. Five isolates of *T. basicola*, all from

burley or flue-cured tobacco, were used. Plates were incubated for 10 days at 20 C prior to measuring radial colony growth. The data were analyzed by ANOVA (with fungicide concentration and fungus isolate as class variables) and trend analysis. The significance of linear relationships between $\log_{10}$ fungicide concentration and radial colony growth were tested. For each fungicide concentration/fungus isolate combination, a direct comparison between imazalil and benomyl was made by an unpaired t-test.

RESULTS

Field study. All concentrations of imazalil failed to reduce the severity of black root rot compared to the control treatment, since there were no significant (P=0.05) linear or quadratic relationships between imazalil concentration and either plant height or mean percent root rot (Table 5.1). *T. basicola* was consistently isolated from tobacco plant root segments that had representative lesions. The test area had an average of 649±27 (95% confidence interval) propagules of *T. basicola* per g of soil. The soil was classified as a silty-clay loam, with 2.9% organic matter and a pH of 5.7.

Laboratory study. For both imazalil and benomyl, there were significant (P=0.01) negative linear relationships between $\log_{10}$ fungicide concentration and radial colony growth, over the range

Table 5.1. Effect of imazalil concentration on shoot height and mean percent root rot of burley tobacco[a b]

CONCENTRATION (µg a.i./ml)	*SHOOT HT* (cm)	*M%RR*
0	54.5[c]	7.3[c]
250	47.5	6.4
750	55.1	4.8
1500	46.5	6.1

[a] Tobacco plants (cv. B21-Kyl0, low-medium black root rot resistance) were transplanted 27 May, 1985. On 28 May, the soil around the base of each plant was drenched with 50 ml of a solution containing imazalil at the specified concentration.

[b] Shoot height and mean percent root rot (M%RR) were recorded on 31 July, 1985. M%RR represents the mean percent of root surfaces covered with black root rot lesions.

[c] There were no significant (P=0.05) linear or quadratic relationships between imazalil concentration and either shoot height or mean percent root rot.

of fungicide concentrations that were included in the analyses, which are shown in Fig. 5.1. All five isolates of *T. basicola* reacted similarly. For all concentrations less than 0.1 µg a.i./ml, imazalil inhibited the radial colony growth of each isolate significantly (P=0.01) more than benomyl did. Imazalil concentrations of 0.001 and 0.1 µg a.i./ml agar medium reduced radial colony growth by 17 and 95%, respectively, when growth was averaged across isolates. None of the isolates grew when the concentration of imazalil was greater than 0.1 µg a.i./ml. These results indicate that imazalil has a high degree of in vitro activity against *T. basicola*.

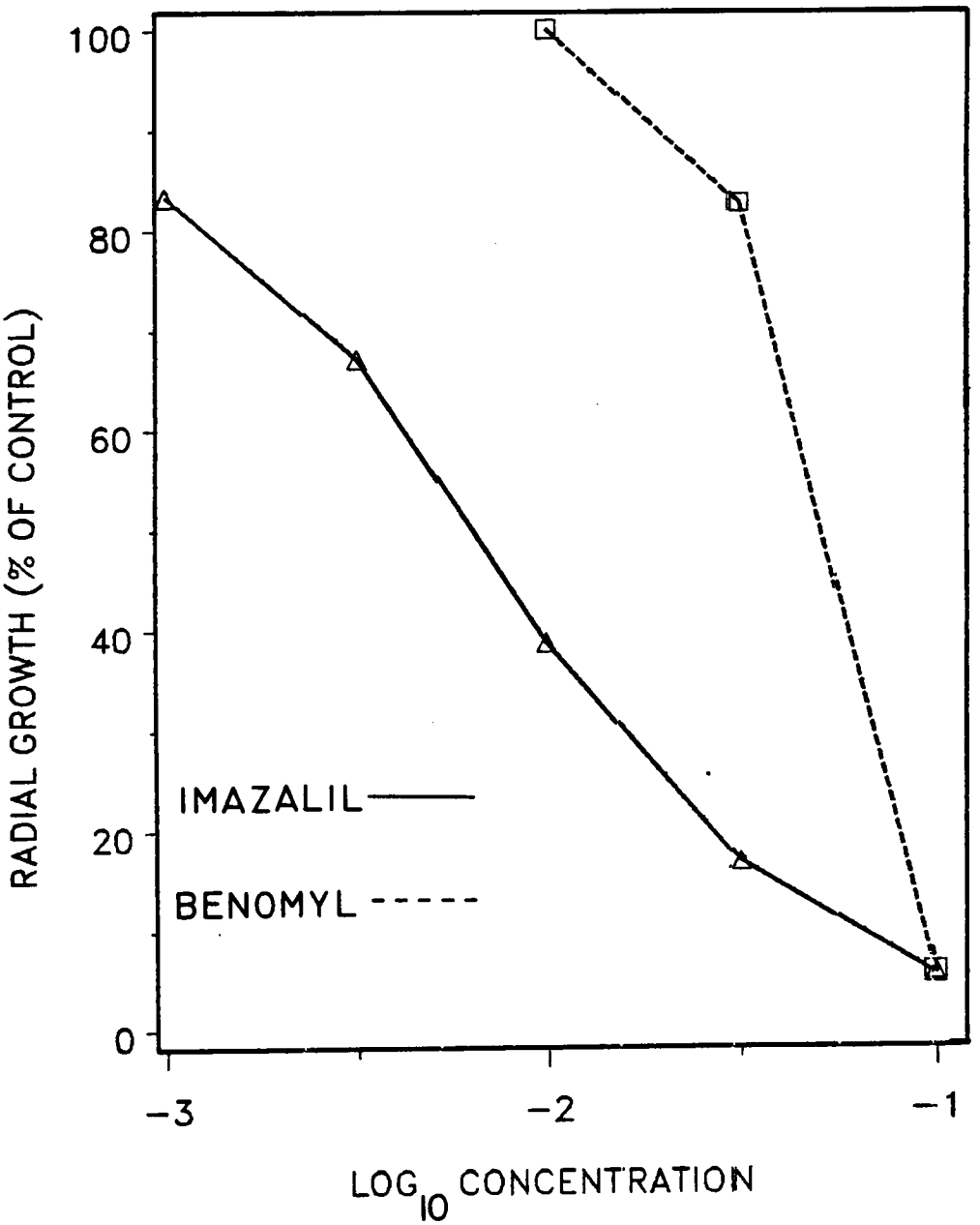

Figure 5.1. Average growth of five isolates of *Thielaviopsis basicola* on agar media amended with either imazalil or benomyl. Log_{10} values of -3.0, -2.5, -2.0, -1.5 and -1.0 correspond to concentrations of 0.001, 0.00316, 0.01, 0.0316, and 0.1 µg a.i./ml, respectively. No growth occurred on media containing concentrations of benomyl or imazalil greater than 0.1 µg a.i./ml. Benomyl did not inhibit the growth of *T. basicola* at concentrations of 0.01 µg a.i./ml or lower.

DISCUSSION

Imazalil was very effective for inhibiting the growth of *T. basicola* when amended into agar media, but did not control black root rot in a tobacco field. The latter result conflicts with data obtained by S. K. Gayed (personal communication, unpublished data). In Gayed's study, which was conducted in Canada on a sandy loam soil (2% organic matter and pH 7.2), the severity of black root rot in a flue-cured tobacco field was reduced by 50% when imazalil was supplied in the planting water at a concentration of 2,000 µg a.i./ml. However, no increased plant growth was associated with the reduction in black root rot in that study. The lack of a positive growth response may have been due to a phytotoxic effect. The possible phytotoxic effect of imazalil was the reason why lower concentrations (250-1500 µg a.i./ml) were tested in this study. J. J. Reilly (personal communication, unpublished data) found that imazalil at an aqueous concentration of 25 µg a.i./ml was phytotoxic to tobacco seedlings grown under hydroponic-like conditions in the greenhouse.

The failure of imazalil to control black root rot in this study may have been due to a partial inactivation of the chemical in soil, especially since the soil type was a silty-clay loam. This contrasts with the sandy loam soil type in the field used by Gayed. A fine-textured soil has a greater surface area and a subsequently greater ability to adsorb ions and molecules than

does a coarse-textured soil (2). Two soil fractions involved in the chemical adsorbtion of ions and molecules are clay and humus colloids. These colloids are predominantly negatively charged, so they attract positively charged ions and molecules more than negatively charged ions and molecules. Physical adsorbtion, which is due to ion-dipole or dipole-dipole interactions (also called van der Waals forces) also plays an important role in the adsorbtion of ions and molecules to soil colloids. No information was obtained from the manufacturer concerning the chemical and physical retention of imazalil in soil; however, imazalil was apparently applied in this study as a cation (100% sulfate salt formulation, very high solubility in water), so the chemical may have been inactivated by adsorbtion to either soil clay and/or humus fractions. There is no evidence directly supporting this hypothesis, but a similar conclusion was made by Hartill & Campbell (6) to explain why benomyl failed to control black root rot in a tobacco field. They reported that benomyl appeared to be rapidly bound in finer-textured soils.

Further studies would be needed to determine if higher concentrations of imazalil, or a different method of application, would be effective for controlling black root rot in burley tobacco fields in Virginia. However, phytoxicity may still be a problem.

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Chapter 6

SUMMARY

A new selective medium (TB-CEN) useful for isolating *T. basicola* from soil was developed. The medium contains etridiazol and nystatin to inhibit undesired fungi, and raw (unautoclaved) extract from carrot (*Daucus carota* L. cv. sativa) to enhance for *T. basicola* isolation. TB-CEN medium, in combination with the dilution-plate technique, was equally effective as a carrot disk technique for isolating *T. basicola* from naturally infested tobacco field soils. The advantage of the selective medium over the carrot disk technique was that propagules of *T. basicola* developed into discrete colonies on soil-dilution plates, but not on carrot disks. TB-CEN medium also was better than three other selective media when 10^{-1} and 10^{-2} soil dilutions were required in assays. The recovery of *T. basicola* from soils artificially infested with endoconidia was greater than 80% for 8 of 11 isolates tested. The percent germination of chlamydospores on TB-CEN medium were 92-100%, while the percent germination of endoconidia were 90-97%.

In a black root rot/tobacco stunting survey, estimated inoculum and population densities of *T. basicola* were 0-402 propagules per g of soil when samples, taken either at transplanting time or from between rows in June-August, were assayed on TB-CEN medium. Black root rot was the major disease associated with tobacco stunting in the burley region of

Virginia, but not in the flue- and sun-cured regions. *T. basicola* was absent or was a minor problem in most of the fields sampled in the latter two regions. No evidence was found to indicate that endomycorrhizae were involved in tobacco stunting in Virginia; however, further research is needed to document the favorable or unfavorable effects of endomycorrhizae on tobacco in Virginia.

Environmental conditions apparently had a strong effect on black root rot development, since black root rot and plant stunting were severe in two burley fields that contained 148 and 158 propagules per g of soil, but not in two flue-cured fields that contained 101 and 402 propagules per g of soil. All of the cultivars planted in the four fields were susceptible. An inoculum density of 150 propagules of *T. basicola* per g of soil, in combination with low to medium levels of host resistance and disease-favorable environmental conditions (such as low soil temperature, high soil pH, fine soil texture and high soil moisture), is apparently capable of causing severe black root rot and plant stunting in commercial tobacco fields.

The results of *T. basicola*-inoculum density versus disease incidence and/or severity studies (Chapter 4) indicated that there were strong relationships in regressions between inoculum density and mean percent black root rot on tobacco plants (R^2 range=0.86-0.97), and also between inoculum density and plant growth (R^2 range=0.71-0.99). For one of three cultivars tested (cv. NC95) in soil-temperature tanks, and also for cultivar

B21-Kyl0 tested in the field-plot study, the relationship between inoculum density and mean percent root rot was linear. *T. basicola*-inoculum clumping probably did not play a significant role in influencing the relationship between inoculum density and mean percent root rot in the field-plot study, since coefficients of variation of the mean percent root rot data were no greater in the field-plot study than in the soil-temperature tank studies, and also because a high R^2 value (0.86) was obtained in regression between the two variables. Curvilinear relationships were found for the other two cultivars (Burley 21 and Va Gold) tested in soil-temperature tanks; however, accurate judgement of *T. basicola*-associated root rot on these cultivars was hampered because of a partial root discoloration, which was not associated with *T. basicola*, on plants grown in uninfested soil. For all three cultivars tested in soil-temperature tanks, there were very strong curvilinear relationships in regressions between inoculum density and plant growth (R^2 range=0.96-0.99). The largest decreases in shoot weight, shoot height and root weight (per unit increase in inoculum) occurred at the lower inoculum densities. With NC95 and Burley 21, relatively large decreases in shoot growth occurred at inoculum densities as low as 5-10 propagules per g of soil. In the field-plot study conducted with B21-Kyl0, the relationship between inoculum density and shoot weight was described slightly better by a curved line (R^2 for regression =0.71) than by a straight line (R^2 for regression=0.66). Much higher inoculum densities were required to cause moderate to

severe levels of black root rot in the field-plot study than in soil-temperature tanks. One field plot that contained an average of 150 propagules of *T. basicola* per g of soil had tobacco plants with a mean percent root rot rating of 3.3%, while in soil-temperature tanks, mean percent root rot ratings of 1.3-9.0% occurred at inoculum densities as low as 5-10 propagules per g of soil. However, it is not possible to make direct evaluations between the results of the the soil-temperature tank and field-plot studies, since environmental and host-related factors varied or could not be controlled. Attempts were made to make soil-environmental conditions favorable for black root rot development in the soil-temperature tank studies, but this was not possible in the field studies.

The results of these investigations, though not complete, should provide useful information for developing practical relationships between the inoculum density of *T. basicola* and the development of black root rot in commercial tobacco fields. However, environmental and host-related factors also will have to be considered, since these studies show that the damaging effects of black root rot are difficult to predict when based upon inoculum density alone. There are still no economically feasible chemical means for controlling black root rot in tobacco fields in Virginia, since imazalil failed to provide control in the field study that was conducted. Resistant cultivars, such as Ky14-L8 and Ky 14, are presently the most practical way of insuring minimal yield losses when burley tobacco is planted on

infested land.

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