INOCULUM PATTERN AND RELATIONSHIP BETWEEN INCIDENCE OF BLACK ROOT ROT OF TOBACCO AND INOCULUM DENSITY OF *THIELAVIOPSIS BASICOLA* IN FIELD SOIL

by

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I. INTRODUCTION

Thielaviopsis basicola (Berk. and Br.) is a soil-borne pathogenic fungus. It is of major importance because of its extensive host range on economic crops. Its ability to persist in the soil in a dormant state during unfavorable conditions enables the fungus to survive throughout the world for extended periods in the absence of a host. In Virginia, T. basicola produces a root rot of the major money crop, tobacco (Nicotiana tabacum L.).

A large amount of research has been done on the growth requirements (26,41), survival mechanisms (37,50), and control of T. basicola (12,38). Research has shown equivalent inoculum densities of endoconidia and chlamydospores of T. basicola produce varying degrees of symptom expression on cotton, peanut, bean, soybean, pea, and sunflower (44).

In order to develop a disease prediction program for black root rot, the inoculum density-disease incidence relationship that occurs in the field must be determined. The inoculum pattern of T. basicola in the field, based on the inoculum density of T. basicola in the soil, must also be determined for use in the inoculum density-disease incidence studies. A third requirement for an effective predictive system is a quick, accurate method for determining the quantity of propagative units of T. basicola in the soil.

This research had three objectives. The first was to develop an improved procedure for estimating the population of T. basicola in naturally infested soil. Previous work had shown that recommended selective media were not satisfactory in quantifying T. basicola in the soil.

The second objective of this research was to determine the horizontal inoculum pattern of T. basicola in tobacco field soil. The effect of systematic and random sampling techniques on the calculating of the pattern was investigated. The spatial pattern of T. basicola, whether random or nonrandom, will influence the third objective: to determine the relationship between the inoculum density of T. basicola in field soil and black root rot incidence and severity on tobacco.

It may be possible to develop a mathematical relationship between inoculum density of T. basicola and the incidence of black root rot in the greenhouse and/or in the field, as has been suggested by other researchers (3,45). This could then be incorporated into the foundation of an effective disease prediction program.

II. LITERATURE REVIEW

2.1 The Fungus

Theilaviopsis basicola (Berk. and Br.) Ferr., was originally described in 1850 by Berkeley and Broom who observed chlamydospores on Pisum sativum and Nemophila auriculata. They gave the fungus the name Torula basicola. Since that time the fungus has undergone several name changes. Zopf, in 1876, described the endoconidia and chlamydospores of the fungus on Senecio elegans. He assumed the ascosporic stage of Thielavia basicola to be the perfect stage of the fungus and included it in his description, calling the fungus Thielavia basicola. Ferraris renamed the fungus Thielaviopsis basicola. In 1925, McCormick was able to demonstrate that Thielavia basicola was a separate species which only grows in the presence of T. basicola. Stover later referred to the relationship as a commensalistic one (41). Recently, another name change, Trichocladium basicola (Berk. and Br.) Carmichael comb. nov., has been suggested by Carmichael et al (8).

Thielaviopsis basicola is a Deuteromycete in the Order Moniliales, and the Family Dematiaceae. The fungus produces septate hyphae which are hyaline when young but darken with age. The fungus produces two types of conidia. The endoconidia, or microconidia, are produced within endoconidiophores. Each endoconidiophore consists of one

to several basal cells upon which is borne a long, narrow, tapering cell with a swollen base. The rectangular, hyaline spores are pushed out of the apex of the conidiophore by spores being produced behind them. As many as 160 spores may be produced in one conidiophore (9). The endoconidia are produced first and in larger numbers than the chlamydospores. The chlamydospores, or macroconidia, are produced on short lateral stems which can arise from any point on the mycelia. The conidiophores may arise singly or in groups. Dark, thick-walled chlamydospores are produced above one or two thin-walled basal cells. Chains of chlamydospores may contain from one to eight spores which separate at maturity. Each spore is capable of germinating (9,30,43).

It had been assumed by most researchers that chlamydospores were the most resistant of the two propagules and that endoconidia were relatively short lived (39). Observations by Stover (41) showed no variation between the survival and resistance of the two spore types, in vitro, after drying, heating, and extended dormant periods. He reported that a "small percentage" of endoconidia germinated after being stored at room temperature in various types of soil. He felt that the survival mechanism could not necessarily be attributed to the characteristics of the spore wall, but might be attributed to the inherent characteristics of the protoplasm present in all spore forms. Tsao and Bricker

(50) concluded from their germination studies, in which 95.5% of the colonies produced on dilution plates of naturally infested soil originated from chlamydospores, the fungus probably exists and survives in the soil as chlamydospores. Other research has reported a small percentage of viable endoconidia after storage at room temperature in soil for seven to ten months (25,36).

Mathre and Ravenscroft (33) found germination and germ tube elongation of both spore forms were maximal from 20-33 C, with a minimum temperature of 9 C. Germ tube elongation was suppressed above 30 C. Isolates examined by Lucas (29) grew optimally from 22-28 C with a minimum temperature of 8 C and a maximum temperature of 35 C. He noted that optimum temperature ranges varied with the isolate used.

Lucas (29) reported the optimum pH for the growth of T. basicola was between 3.9 and 6.2 but noted that this would vary with the substrate used. However, he reported no growth above pH 8.0 and below 3.0. Mathre and Ravenscroft (33) concluded that both spore forms germinated between 4.0 and 8.5. They also found that both spore forms reacted similarly to various sugars; glucose, considered to be a common component of root exudates of many plants, was one of the best stimulants of germination. In the same experiment they also found that germination of endoconidia in pure culture was highly stimulated by fractions of cotton and carrot root extracts. Linderman and Toussoun (25)

reported germination of chlamydospores increased after air-dried soil was moistened above saturation for five days prior to planting.

Thielaviopsis basicola requires potassium, phosphorous, magnesium, sulfur, calcium, iron, zinc, copper, manganese, and molybdenum for normal growth. Excess of calcium enhances the growth of the fungus at all pH levels (29). The fungus requires thiamine for normal growth (42).

Thielaviopsis basicola is found worldwide but most commonly in temperate regions. In one survey (59), the fungus was found in noncultivated areas as frequently as cultivated areas. Plants of the Leguminosae, including clover, pea, alfalfa, and bean are the most frequent hosts. Other hosts are found in the Cucurbitaceae and Solanaceae families (30). The fungus causes severe damage on a wide variety of cultivated plants. Johnson (21) compiled a list of over 100 species which were susceptible to attack by T. basicola. Susceptible ornamentals include pansy, peony, violet, pelargonium, poinsettia, and begonia (56). Other economic crops, which may serve as hosts, include cherry, cotton, citrus, peanut, and tobacco. The fungus is known to be involved in pea and bean root rot complexes (6,28), causes the "little leaf" disease of oil palms (41), and has been reported by Yarwood (60) to increase the growth of carrot, cucumber, and cat's ear, in inoculation experiments.

Johnson (21) concluded from his research there was no evidence of specialized races of the fungus since he was able to produce infection from one tobacco isolate on over 100 species of plants. Results from work by Keller and Shanks (23) showed that isolates from host plants exhibited some degree of host specificity. Lloyd and Lockwood (26) demonstrated that the tobacco strain of T. basicola was nonpathogenic on bean, and the strains isolated from pea, orange, and poinsettia were nonpathogenic on tobacco. Lambe and Wills (24) reported isolates from tobacco, sesame, and bean caused some black root rot on a cultivar of Japanese holly.

Stover (41) further subdivided the tobacco strains into two types, brown and grey. The brown type, a more pathogenic type to tobacco, frequently mutates to the grey type. The grey type is less pathogenic to tobacco, less resistant during periods of exogenous dormancy, slower growing, and more thiamine deficient than the brown type. All isolations by Stover from soils obtained from southwestern Virginia produced only the grey type.

Yarwood (58), in an attempt to isolate Sclerotinia sclerotiorum, discovered a quick, simple method for isolating T. basicola from soil with the use of carrot discs. Tsao (49,51) modified this method for quantitative studies, and Stover (41), using the same technique and potato discs, isolated the fungus from diseased tobacco root tissue.

Friedman et al (15) reported a high incidence of T. basicola occurring on carrots grown in California. Work by Lloyd and Lockwood (27) revealed that the fungus Chalaropsis thielaviodes Peyr., was frequently isolated from carrots and could be easily mistaken for T. basicola if only endoconidia were observed.

A survey conducted by McIlveen and Edgington (34) of the effectiveness of other umbelliferous tissue showed carrots to be the best for isolating T. basicola, although several others were capable of isolating the fungus. Papavizas (35) modified the selective VDYA selective medium for isolating T. basicola and this was further modified by Maduewesi et al (31) in 1975. Many researchers, however, still find the carrot disc method a more sensitive one for isolating T. basicola (44). Low populations of the fungus are difficult to recover with the Papavizas (35) and Maduewesi et al (31) selective media.

2.2 The Host

Nicotiana tabacum L., has been cultivated in the United States since 1612. In 1980, 2.0 billion pounds of tobacco were grown in the United States (47). It is the number one money crop in Virginia with the estimated product value of the entire manufacturing industry approximated at 3.2 billion dollars (14,54). Virginia is presently the second largest manufacturer or tobacco products in the U.S. The state

produces 30% of the cigarettes and 33% of the pipe tobacco in the U.S. (14). Flue cured tobacco, which is primarily used for cigarettes, is grown in what is known as the Old and Middle Belt which encompasses the eastern section of North Carolina and the Piedmont region of Virginia. Other types of tobacco that are grown in Virginia include burley, sun cured, and fire cured. These are primarily used for chewing and pipe tobacco and cigarettes. Several cultivars are grown as ornamentals.

In Virginia, a large amount of time and money are invested in a tobacco crop. Growing tobacco requires approximately 250 man hours and 1700 dollars to produce one acre of flue cured tobacco (7). Seeds are sown as early as February-March, seedlings are transplanted in April-May, and crop harvesting may continue through October.

2.3 Black Root Rot

Black root rot of tobacco is thought to have been first described in 1884 in Pennsylvania by Killebrew; however, he gave no identification to the fungus (30). It was definitely described in 1897 by Peglion in Italy who observed that the fungus was pathogenic to tobacco (30). It was first described in the U.S. by Selby in 1904 (30). The name black root rot, because it best describes the symptoms produced, is the most commonly used name for the disease. It is also referred to as root rot, Thielavia root rot, and

maricume radicale (57). The disease has been reported in all the regions of the world where tobacco is grown commercially, including Canada, Holland, Japan, Russia, New Zealand, United States, and areas of South America.

In 1919, Johnson and Hartman (22) referred to black root rot as the most serious disease that tobacco farmers had to contend with, and Anderson and his coworkers, in 1926, felt that black root rot probably caused more losses to tobacco growers of the U.S. than any other disease. Black root rot is considered a major disease of burley, eastern dark-fire, western dark-fire, dark air cured, shade cigar wrapper, and cigar filling tobaccos (46). In 1981 black root rot produced the highest estimated loss of flue-cured tobacco by a soil-borne fungus in Canada (46). In Virginia, T. basicola caused the highest estimated loss of burley tobacco by a soil-borne fungus, costing growers 337,811 dollars in 1981.

Christou (10) has shown that the fungus is capable of penetrating the epidermis directly. T. basicola rapidly invades the epidermal, endodermal, and cortical cells but the fungus is capable of penetrating all the tissues, including the xylem. The result is the death of cells and the blockage of conductive tissue. Flow of material in the tissues is interrupted and roots are unable to absorb water efficiently. Under high soil moisture conditions, the hyphae will grow towards the exterior of the root. Once at

the surface, the fungus readily produces endoconidia and chlamydospores (10). Small lesions coalesce to form large blackened areas. Pectic enzymes have been shown to play a role in the maceration of the roots (52).

Invasions of seedlings causes them to damp off and their roots are usually rotted completely through (30). Infection of mature plants is evident by large black decayed areas produced on the root system. Destruction of roots stimulates the formation of adventitious roots which can also be attacked by the fungus (11). Roots of affected plants are severely reduced and stems are easily pulled away from them. Plants wilt and become stunted; foliage turns pale green or yellow. Plants not severely infected may exhibit little or no above ground symptoms. Infested areas in a field will have plants which flower prematurely and are uneven in development (9,30).

Wolf (57) indicates both Peglion, in 1897, and Gilbert, in 1909, noticed that the disease caused by T. basicola varied depending on environmental conditions which, according to Gilbert, included the degree of infestation of the fungus in the soil of both the seed bed and the field, the amount of humus in the soil, the amount of watering and ventilation of the seedbed. Many researchers have attempted to name one factor as responsible for promoting disease, although it appears now that it is the interaction of several factors that allow for infection by the fungus.

In 1919, Johnson and Hartman (22) felt that soil temperature determined the degree of infection of tobacco by T. basicola. Others have reported a relationship between temperature and soil acidity in helping promote disease (13). The temperature most favorable for root rot of tobacco is between 17-23 C, with no root rot disease development occurring above 31 C (30). Thielaviopsis basicola produces little disease below pH 5.6, except in cold soils. A pH above 5.6, which is slightly acid to alkaline, is most favorable for disease development (9,30, 43). Black root rot is also severe in moist soils which are high in humus. Tobacco grown in light, sandy soil may have a moderate yield of tobacco even if the soil is infested with the fungus (9,56,57).

Thielaviopsis basicola is capable of surviving in the absence of hosts in a saprophytic state, according to some researchers (30,43). However, Bateman's work (5) disputes this showing that the fungus is incapable of growth or reproduction without the presence of a host. Nevertheless, its tremendous host range and ability to survive prolonged periods without a host, allows the fungus to maintain or increase its population. Spread of the fungus occurs primarily by transplanting of infected seedlings (30). Spread may also occur by the transportation of infested water and soil (9,30).

Black root rot of tobacco has been most successfully controlled through the use of resistant cultivars. There are numerous varieties of tobacco and each has been developed with various resistance qualities. Breeding for resistance for black root rot of tobacco began in 1912 (12). The old type of tobacco, obtained from areas where tobacco originated in Mexico and Central and South America, had little resistance to T. basicola. It was discovered that the North American types and several from the Near East had moderate to high levels of resistance (20,55). Attempts to incorporate the genes responsible for this resistance into tobacco with acceptable leaf qualities were unsuccessful. Therefore, a screening was done in 1934 of the Nicotiana species, and many were found to be immune or resistant. These were used for breeding and a wide range of resistance was achieved. Burley 49 was the first successful black root rot resistant variety to incorporate resistance from N. debneyi and was released in 1965 (12). Resistance from N. debneyi is believed to originate from the plant's ability to withstand the effects of phytotoxins in decomposing plant material. These materials produce increased leakage of root exudates which stimulate chlamydospore germination and decrease the hypersensitive response of the tobacco plant which allows establishment of T. basicola. The plant's ability to overcome exoenzymes which interact with polyphenols secreted by the roots and which are fungitoxic to

T. basicola also is involved in the resistance mechanism (16). Resistance from N. tabacum involves several factors, including the chemical ones previously mentioned, and the influence of the microbial populations existing in the rhizosphere of the tobacco plant. These populations vary with the species of tobacco and have been shown to be lower in soils where resistant varieties of N. tabacum were grown. The microorganisms secrete thiamine which results in higher populations of thiamine-deficient T. basicola (16). Some of the early black root rot resistant varieties were Harrow Velvet, Briarvet, Haranova, Little Dutch, Virginia Gold, and White Burley (11). In some cases, resistance varied with the location because of the type of T. basicola present in the soil (30,57).

Another recommended control for black root rot of tobacco is the use of crop rotation. Research by Bateman (5) in 1963 indicated that growth or reproduction of T. basicola is dependent on its parasitic activities. In the absence of a host, the fungus does not reproduce. Therefore, he recommended rotation with nonhost plants to help control disease. Recent work by Hsi (19) with sesame plants confirmed this. Cropping sequences using host of the fungus, especially legumes, such as soybean, red clover, sweet clover, and alfalfa are discouraged (9,30).

Severla fungicides have proved to be effective against T. basicola. Benomyl (methyl 1-(butylcarbamoyl)-2-

benzimidazolecarbamate) and DMT (tetra hydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) are both able to control black root rot when incorporated into the soil prior to planting. PCNB (pentachloronitrobenzene) and DCNA (2,6-dichloro-4-nitroaniline) were ineffective in controlling disease (17,38). Multipurpose fumigants containing chloropicrin are also available for control of black root rot.

Much work has been done on the use soil amendments to reduce the population of T. basicola (1,38). Incorporation of alfalfa hay, corn stover, and cabbage tissue in soil all lowered disease incidence. This method was found to be effective over a wide range of soil temperatures. If soil moisture and pH are lowered, control is increased (1). At extremely low temperatures, control is reduced. The amendments presumably work in two ways. First, they promote germination and subsequent lysis of the spores and second they increase the fungistatic influence of the rhizosphere so that it is greater than the germination stimulation produced by the host's roots' exudates. Maier (32) suggested that a delay in planting, which would allow the soil temperature to increase, would help prevent disease development.

III. MATERIALS AND METHODS

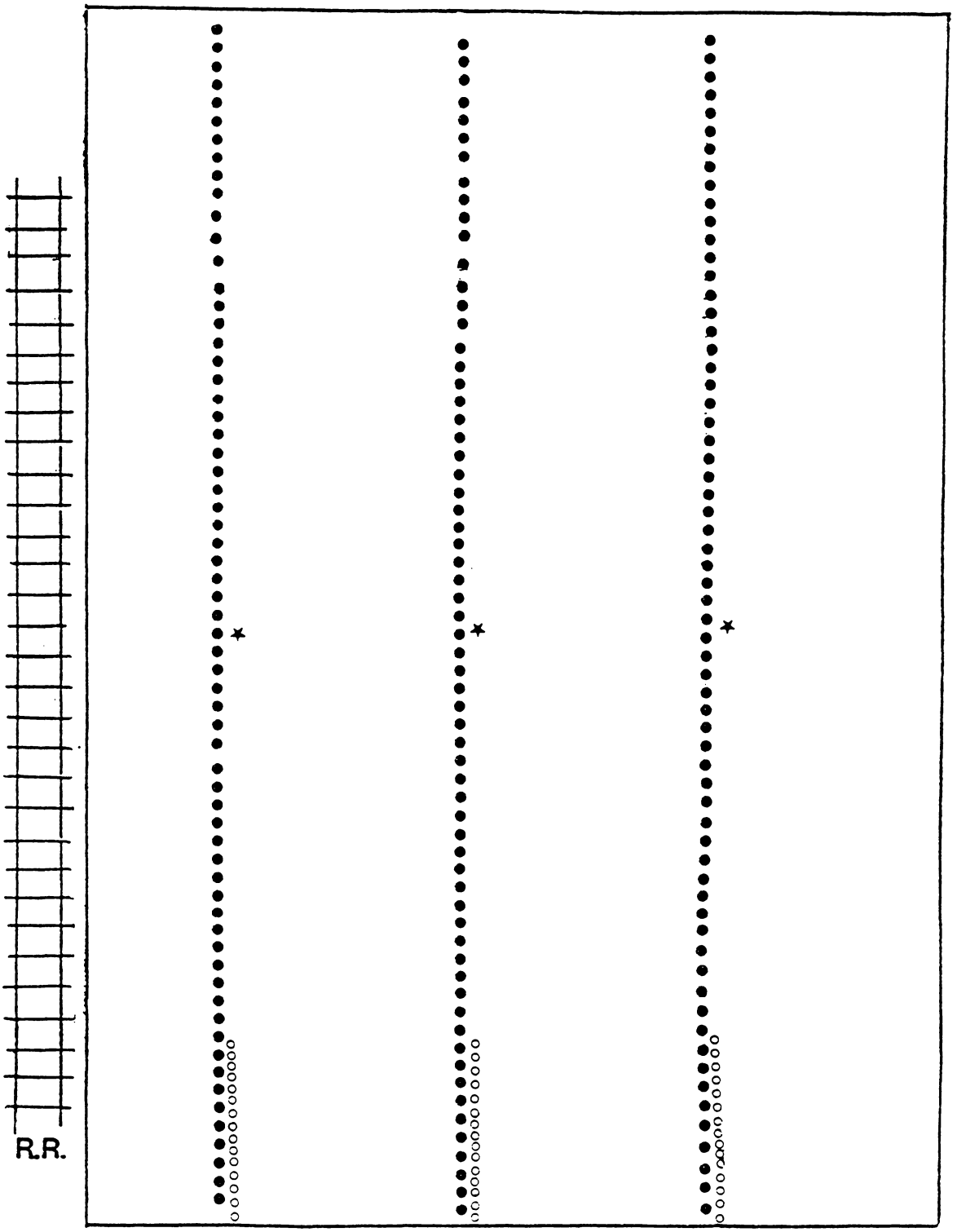
3.1 Establishment of Field Plots

Experimental field plots were located in Washington Co., Va. Field plot A was located in a field at Glade Springs Research Station, Glade Springs, Va. and has a history of black root rot. The field was planted with burley tobacco in 1969, 1972, and 1975-80, and with orchardgrass in 1970-71 and 1973-74. The field was plowed and disked once a year and was treated with potassium sulfate (K_2SO_4) and ammonium nitrate (NH_4NO_3). The field was also limed ($CaCO_3$) at 1,721.6 kg/ha in the fall of 1974 and 7,6208.4 kg/ha in the fall of 1976. The pH of the soil was 7.2 in 1980. No insecticides or herbicides were applied to the field until 1980. Ridomil(N-(2,6-Dimethylphenyl)-N-(methoxyacetyl-alanine methyl ester)) was applied at 1.1 kg ai/ha and Paarlan (4-Isopropyl-2,6-dinitro-N,N-dipropylaniline) at 1.7 kg ai/ha. Sevin (1-napthyl n-methyl carbamate) was also sprayed on the field for the control of flea beetles (L. Link, 1981, personal communication).

On April 25, 1980, the tobacco cultivar, Burley 21, which is slightly resistant to black root rot, was transplanted into three rows of the field, with plants spaced approximately 46 cm apart (Fig. 3.1).

Field plot B was located in a private grower's field adjacent to the Glade Springs Research Station. This field

Fig. 3.1 Vertical (★), horizontal (o), and individual plant (•) sampling locations in tobacco Field A. Spacing between tobacco plants is approximately 45.7 cm.


R.R.

also had a history of black root rot and was planted to tobacco in 1980 and 1981.

3.2 Soil Sampling

In 1980, tobacco Field A was sampled systematically to determine the relationship between populations of T. basicola and root rot of tobacco. Soil sampling was done on May 2, approximately one week after transplanting. A core sample 2.5 cm (diam) by 30 cm (deep), was used to collect the soil. Samples were obtained adjacent to each plant. Soil from each sixteen consecutive samples was pooled to produce four samples per row. For determining the vertical population distributions, soil samples were also taken at the midpoint of each of the three sampling areas of the rows. The soil cores were then divided by soil depth into 0.7.62 cm, 7.62-15.24 cm, 15.24-22.86 cm, and 22.86 cm-30.48 cm subsamples.

In August, another set of samples were taken in order to determine the horizontal population distribution. Fifteen samples, each a foot (30.48 cm) apart were taken at the head of each row of Burley 21 tobacco in the inter-row area.

All soil samples were stored in plastic bags with pinholes to allow gas exchange. The samples were transported to the laboratory in a cooler and stored at room temperature (25-27 C) until processing.

In May 1981, soil sampling was done to compare the effect of random and systematic sampling on population pattern calculations. A smaller plot was established in Field A that was sampled randomly and systematically (Figs. 3.2 and 3.3).

To determine the random locations the field plot (2537.5 cm by 2537.5 cm) was divided into 998,001 squares each 2.54 cm by 2.54 cm. Sampling sites were picked from a table of random numbers and located in the field with the use of a surveyor's transit. To systematically sample, the same plot was divided into 49 subplots, each 363.2 cm by 363.2 cm. A soil sample was taken at the center of each of the subplots. The soil sampling was done with a soil-core sampler as indicated above. Soil was transported and incubated as earlier samples. The same methods were used to establish and randomly and systematically sample in tobacco Field B.

3.3 Disease Ratings

In late July, the Burley 21 tobacco plants in Field A were dug up with a shovel, the roots washed with tapwater, and inspected for rot. Root rot was rated as none, trace, slight, moderate, and severe. Plant height was recorded in the field and fresh plant weight was measured after transport to the laboratory in an insulated container.

Fig. 3.2. Systematic (★) and random (o) locations in tobacco Field A. Each square is 363.2 cm by 363.2 cm

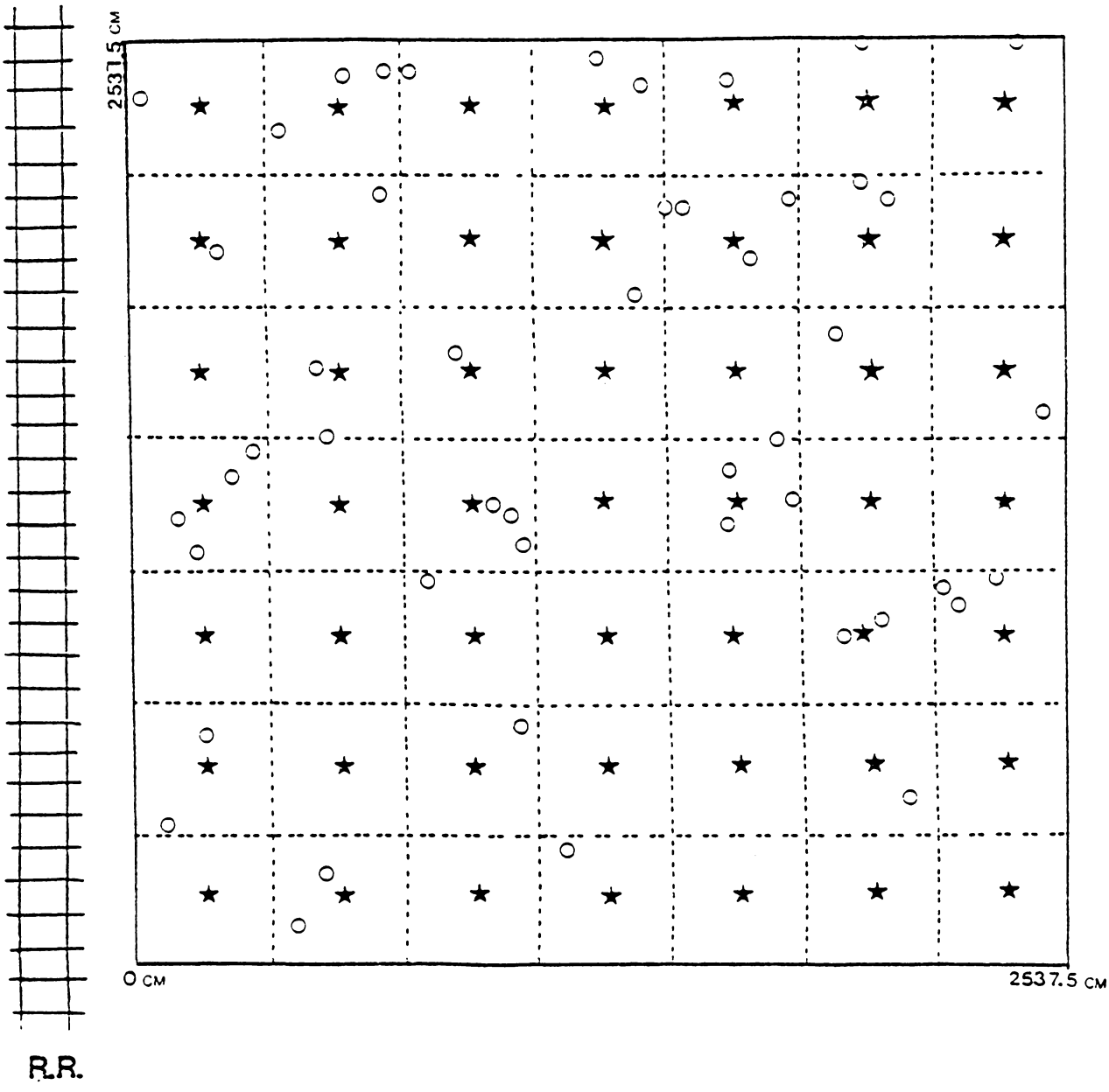
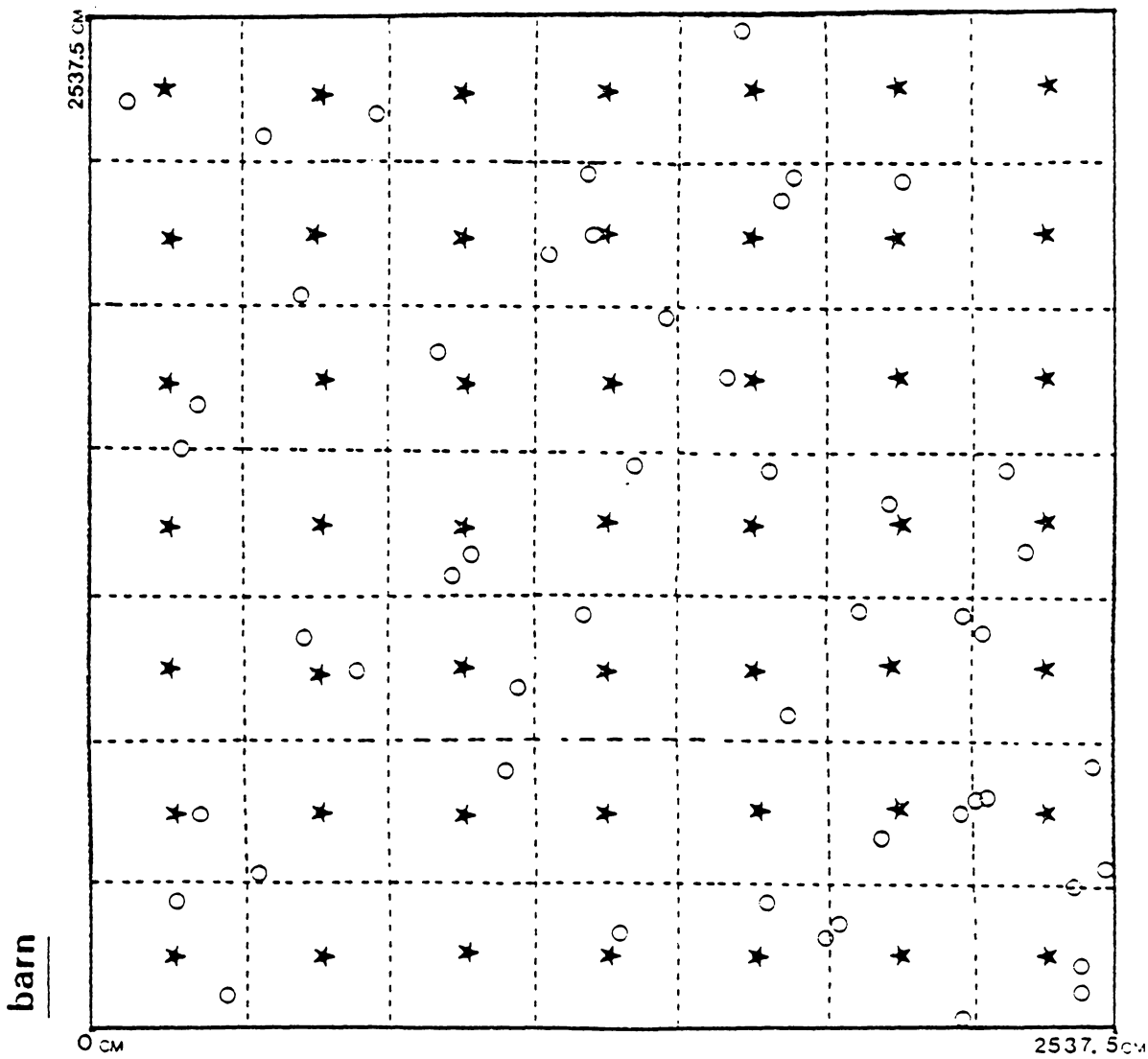

2531.5 CM
0 CM
2537.5 CM
R.R.

Fig. 3.3. Systematic (★) and random (o) sample locations in tobacco Field B . Each square is 363.2 cm by 363.2 cm


2537.5 CM
0 CM
2537.5 CM
barn

Four of the stunted tobacco plants in each group of sixteen were examined for the presence of chlamydospores and endoconidia of T. basicola. A representative number of root segments, 1 cm in length, were crushed on microscope slides and observed under a bright field microscope for spores. Other 1-cm root segments, from the same tobacco plants, were surface sterilized in 0.5% NaClO. They were then placed on carrot discs which had also been surface sterilized. Five carrot discs were placed in autoclaved petri plates lined with moistened filter paper. The plates were put into a moist chamber and incubated at room temperature (25-27 C) for 5-8 days. The carrots were then examined for growth of T. basicola.

3.4 Population Assays

Populations of T. basicola in the soil were determined using a modification of the method developed by Yarwood (46) and Tabachnik et al (44). Soil samples were thoroughly mixed in a plastic bag by hand and all clumps of soil were broken up. Between 0.5 and 10.0 g of soil was weighed and placed into autoclaved 250-ml erlemeyer flasks containing distilled water to produce a total volume of 100 ml. A magnetic stirring bar and 50 mg of streptomycin sulfate (US Biochemical Corp.), used to control bacteria, were also added to the flasks. The flasks were placed on a stirring plate and stirred continuously. Carrot discs, sliced 3-5 mm

thick, were surface sterilized in 0.5% NaClO for 5 min (27). They were then rinsed well in tap water. Five carrot slices were placed on a piece of filter paper in an autoclaved petri plate. Five petri plates were used for each soil dilution. A 0.1 ml aliquot of soil suspension was pipetted onto each carrot disc and a second carrot disc was placed on top. A second piece of filter paper was placed over the carrots and moistened. The petri plates were placed in a moist chamber and incubated at room temperature (25-27 C) for 5-8 days. At that time, the plates were examined for evidence of T. basicola; slide mounts were prepared and examined under a bright-field microscope for endoconidia and chlamydospores. The dry weight of each soil sample was determined by drying at 105 C for 24 hr and was used in calculating the original amount of soil put into each flask. The number of colonized carrot discs was used to calculate the propagules per gram of soil of T. basicola. Gregory's multiple colonization correction factor (53) was used to adjust the population figure.

The soil samples collected in tobacco Field A in 1980 were analyzed by the Virginia Tech Soil Testing and Plant Analysis Laboratory for pH, phosphorous, potassium, calcium, and magnesium.

The 1981 soil samples from tobacco Field A were all prepared with approximately 1 g dry weight of soil per dilution flask. Field B, however, having a lower population,

was diluted using approximately 2 g dry weight of soil per flask. To obtain discrete, comparable populations of T. basicola, equivalent concentrations were used for every sample of soil from each field. Populations were calculated with and without Gregory's multiple colonization correction factor in order to evaluate its influence in determining the population pattern of the fungus in the soil.

3.5 Greenhouse Experiments

Temperature-tank experiments were performed using soil, naturally infested with T. basicola, that was collected from the Glade Springs Research Station. The populations of T. basicola in the soils were determined by the method indicated above and adjusted, if necessary, to five inoculum densities (127, 179, 229, 425, 2799 propagules per gram of soil). Pots, 12-cm diam, were planted with 6-week-old tobacco seedlings. The cultivar Judy's Pride was used because it is rated as being black root rot susceptible (R. Henderson, 1981, personal communication). Five seedlings were transplanted into each pot and eight pots were used for each T. basicola inoculum density. Pots were placed into the tanks which were maintained at 28 C. Plants which died within the first week after transplanting were replaced, and the pots were watered when necessary.

The tobacco plants were removed from the pots 6 weeks after transplanting. Shoot height, fresh shoot weight, and

root fresh weight were measured. Roots were examined for root rot and a visual estimate of the percentage, to the nearest 5%, of the roots that were rotted was made. Shoots and roots were dried at 77 C for 48 hr and reweighed. Several root segments of representative plants were examined microscopically for evidence of endoconidia and chlamydospores.

A second temperature-tank experiment was performed using the tobacco cultivar Burley 21, which is considered moderately resistant to black root rot (D. Komm, 1981, personal communication). The soil was obtained from Glade Springs Research Station and was naturally infested with T. basicola. Populations of T. basicola in the soil were determined and adjusted (318, 541, 546, 597, 1271 propagules per gram of soil) and five-week-old seedlings were transplanted into 12-cm pots. Seven pots per soil sample were used with five plants per pot. Temperature-tanks were maintained at 28 C throughout the experiment. Seedlings which died within the first week after transplanting were replaced. Seven weeks after planting the tobacco plants were removed from the pots and examined. Shoot height, fresh shoot weight, and fresh root weight were measured. Several root segments from representative plants were examined microscopically for evidence of endoconidia and chlamydospores. Roots were inspected for root rot and a visual estimate of the percentage of root rot to the nearest

5% was made. Shoots and roots were dried at 77 C for 48 hr and dry weights were recorded.

3.6 Determining the Cultural Type of T. basicola

Stover (41) reported the existence of two cultural types of T. basicola and listed criteria for differentiating between the two. To determine the cultural type of T. basicola in the soil used in the present study, the fungus was isolated using the modified Yarwood's (58) carrot disc method from soil obtained from Glade Springs Research Station. The isolate was then transferred to potato dextrose agar and colony growth pattern and color were noted after five days at room temperature (25-27 C). These observations were compared to the characteristics presented in Stover's report.

IV. RESULTS

4.1 Field Experiments

Populations of T. basicola in the soil collected from Field A in May 1980 are listed in Table 4.1 along with the mean plant height, mean fresh root weight, and root rot ratings for each subplot. Populations of T. basicola ranged from 42.6 to 4,012.2 propagules per gram of soil. All of the roots of the plants examined had some evidence of rot; however, no severe root rot was observed on any of the roots. Moderate root rot of Burley 21 plants was seen in subplots with 471.9 and 4,012.2 propagules per gram of soil. The shortest tobacco plant was seen in subplot 3D which had one of the lowest mean populations (69.1 propagules per gram of soil). Ninety percent of the representative root segments of the stunted plants which were examined had lesions and 70% of the representative root segments from these plants, which were plated on carrot discs, yielded colonies of T. basicola.

A regression analysis of the two dependent variables, mean shoot height, and mean fresh weight, indicated that there was no relationship between either of them and the independent variable, inoculum density of T. basicola, with $R^2 = 0.0005$ ($P = 0.94$) and $R^2 = 0.006$ ($P = 0.81$), respectively. A regression of mean percent rot of roots per tobacco plant versus inoculum density showed no

Table 4.1. Populations of Thielaviopsis basicola in soil of tobacco Field A, ratings of root rot of tobacco, mean shoot height, and mean fresh root weight in 1980

Location	T. basicola population (propagules/g soil)[a]	Root rot[b] index	Mean shoot height (cm)[c]	Mean fresh root weight (g)[d]
Row 1				
subplot a	471.9	3.0	52.0	42.8
subplot b	4012.2	3.0	42.8	37.3
subplot c	3114.3	2.7	46.8	41.3
subplot d	1102.3	2.7	49.0	44.1
Row 2				
subplot a	892.9	1.8	45.6	38.4
subplot b	2654.8	2.0	37.5	28.5
subplot c	2761.9	2.0	40.9	30.5
subplot d	883.3	2.8	39.1	27.5
Row 3				
subplot a	460.1	2.0	46.8	30.7
subplot b	42.6	2.0	37.3	29.1
subplot c	3916.7	2.0	37.9	22.5
subplot d	69.1	2.0	30.6	15.5

a - Based on sixteen pooled soil samples, calculated with Gregory's multiple colonization correction factor
b - Mean root rot index of sixteen plants, 0 - none, 1 - trace, 2 - slight, 3 - moderate, 4 - severe
c - Based on sixteen plants
d - Based on sixteen plants

relationship between them with $R^2 = 0.024$ ($P = 0.80$).

Populations of the vertical soil samples collected in May 1980 are listed in Table 4.2. The highest mean population, 801.7 propagules per gram of soil, was in the top-layer sample. A gradual decline was observed with increased depth, and the lowest population, 93.8 propagules per gram of soil, was found in the bottom-layer sample. A R^2 value of 0.89 ($P = 0.06$) indicates a direct dependence of the population of T. basicola on soil depth.

Table 4.3 shows the results of the Virginia Tech Soil Testing and Plant Analysis Laboratory analyses of the soil samples collected in tobacco Field A in 1980. All of the soil samples had a pH greater than 6.4. Measurements for P, K, Ca, and Mg did not vary among the soil samples.

The systematic horizontal sampling of tobacco Field A done in August of 1980 showed populations ranging from 118.1 propagules per gram of soil to 3,803.8 propagules per gram of soil, which is approximately the same range of the populations of the samples taken earlier in the season, although the area sampled was considerably smaller (Tables 4.4 and 4.5).

Evaluation of the populations revealed that the pattern of T. basicola in the field was not random. The variance to mean ratios ($s^2/\bar{Y}$) of the populations calculated with and without Gregory's multiple colonization correction factor were 6.76 and 2.47, respectively. Lloyd's index of

Table 4.2. Mean population of *Thielaviopsis basicola* at various soil depths in tobacco Field A in 1980

Depth (cm)	*T. basicola* population[a] (propagules/gram of soil)
0.0- 7.6	801.7[b]
7.6-15.2	611.7
15.2-22.9	113.7
22.9-30.5	93.8

a - Population was based on 3 pooled soil samples and colonization of 25 carrot discs. Population was calculated with Gregory's multiple colonization correction factor.

b - $R^2 = 0.89$ ($P = 0.06$) indicates a strong dependence of *T. basicola* populations on soil depth.

Table 4.3. Soil pH, Ca, Mg, P, and K levels in tobacco Field A used to study Thielaviopsis basicola populations and black root rot incidence in 1980

Sample[a]	pH	P[b]	K[b]	Ca[b]	Mg[b]
1A	6.6	60	157	1200	120
1B	6.4	60	157	1200	120
1C	6.6	60	157	1200	120
1D	7.1	60	157	1200	120
2A	6.6	60	157	1200	120
2B	6.6	60	157	1200	120
2C	6.7	60	157	1200	120
2D	6.8	60	157	1200	120
3A	6.4	60	157	1200	120
3B	6.9	60	157	1200	120
3C	7.3	60	157	1200	120
3D	6.9	60	157	1200	120

a - Based on sixteen pooled soil samples

b - Expressed in µg/g

Table 4.4. Populations of Thielaviopsis basicola obtained in the 1980 horizontal sampling of tobacco Field A and calculated with Gregory's multiple colonization correction factor.

Row-location[a] (cm)	T. basicola[b] population (propagules/ g soil)	Row-location (cm)	T. basicola population (propagules/ g soil)	Row-location (cm)	T. basicola population (propagules/ g soil)
1 - 30.5	932.6	2 - 30.5	1222.5	3 - 30.5	674.5
1 - 60.9	762.8	2 - 60.9	2512.0	3 - 60.9	1199.5
1 - 91.4	2151.2	2 - 91.4	844.0	3 - 91.4	820.7
1 - 121.9	1202.6	2 - 121.9	3238.3	3 - 121.9	382.6
1 - 152.4	1640.2	2 - 152.4	1619.2	3 - 152.4	684.1
1 - 182.9	1010.8	2 - 182.9	3497.8	3 - 182.9	1213.8
1 - 213.4	247.5	2 - 213.4	2488.2	3 - 213.4	530.9
1 - 243.8	383.1	2 - 243.8	3808.8	3 - 243.8	523.5
1 - 274.3	519.5	2 - 274.3	2846.3	3 - 274.3	1393.9
1 - 304.8	2157.3	2 - 304.8	2430.6	3 - 304.8	381.7
1 - 335.3	247.3	2 - 335.3	3200.9	3 - 335.3	382.6
1 - 365.8	832.2	2 - 365.8	2839.6	3 - 365.8	118.1
1 - 396.2	246.1	2 - 396.2	2196.6	3 - 396.2	525.9
1 - 426.7	1402.8	2 - 426.7	1403.8	3 - 426.7	120.3
1 - 457.4	1406.4	2 - 457.4	1893.4	3 - 457.4	118.3

a - Location of systematic sampling sites along Burley 21 tobacco plants

b - Population was based on colonization of 25 carrot discs per soil sample

Table 4.5. Populations of Thielaviopsis basicola obtained in horizontal sampling of tobacco Field A in 1980 and calculated without Gregory's correction factor

Row-location[a] (cm)	T. basicola[b] population (propagules/ g soil)	Row-location (cm)	T. basicola population (propagules/ g soil)	Row-location (cm)	T. basicola population (propagules/ g soil)
1 - 30.5	769.4	2 - 30.5	956.9	3 - 30.5	585.5
1 - 60.9	559.8	2 - 60.9	1555.0	3 - 60.9	938.9
1 - 91.4	1409.1	2 - 91.4	709.2	3 - 91.4	689.7
1 - 121.9	941.4	2 - 121.9	1752.3	3 - 121.9	352.1
1 - 152.4	1183.4	2 - 152.4	1168.2	3 - 152.4	593.8
1 - 182.9	820.0	2 - 182.9	1923.1	3 - 182.9	950.1
1 - 213.4	235.7	2 - 213.4	1540.3	3 - 213.4	476.2
1 - 243.8	352.5	2 - 243.8	1891.3	3 - 243.8	469.5
1 - 274.3	465.9	2 - 274.3	1654.9	3 - 274.3	1048.9
1 - 304.8	1413.1	2 - 304.8	1547.6	3 - 304.8	351.3
1 - 335.3	232.3	2 - 335.3	1732.1	3 - 335.3	352.1
1 - 365.8	699.3	2 - 365.8	1650.9	3 - 365.8	115.7
1 - 396.2	234.4	2 - 396.2	1438.9	3 - 396.2	470.6
1 - 426.7	1055.6	2 - 426.7	1056.3	3 - 426.7	117.9
1 - 457.4	1058.3	2 - 457.4	1303.2	3 - 457.4	116.0

a - Location of systematic sampling sites along Burley 21 tobacco plants

b - Population was based on colonization of 25 carrot discs per soil sample

mean crowding ($\overset{*}{Y} = \overline{Y} + (s^2/\overline{Y} - 1)$), and Lloyd's index of patchiness ($\overset{*}{Y}/\overline{Y}$) were 9.31 and 1.19 for the populations calculated without Gregory's multiple colonization correction factor, and 17.16 and 1.51 for the populations calculated with the correction factor.

The Chi-square test for a goodness of fit to a Poisson distribution for the populations calculated with and without Gregory's multiple colonization correction factor showed no fit (P = 0.00). A good fit to the negative binomial (Poisson-logarithmic) distribution, indicating a contagious distribution, was obtained with the populations calculated without Gregory's multiple colonization correction factor ($x^2 = 4.69$, P = 0.25, $\hat{N} = 5.33$). However, a higher probability was obtained with the corrected populations ($x^2 = 4.19$, P = 0.38, $\hat{N} = 1.98$). For both forms of the population there was no fit to the Neyman Type A (Poisson-Poisson) distribution (P = 0.00), which also indicates a contagious distribution.

The location and assay results of the systematic samples taken in 1981 in Field A are given in Table 4.6. The analysis of the populations again indicated that T. basicola was distributed in the field in a nonrandom fashion. The variance to mean ratios for the populations with and without Gregory's multiple colonization correction factor was 8.90 and 4.13, respectively. Lloyd's index of mean crowding was 9.75 for the uncorrected populations and 16.90

Table 4.6. Proportion of carrot discs colonized by Thielaviopsis basicola in soil systematically collected from tobacco Field A in 1981

Sample # and location	Discs colonized[a] by T. basicola	Proportion of discs colonized[b]	Corrected value[c]
1 181.6 cm x 181.6 cm	5.0	0.20	0.22
2 544.8 cm x 181.6 cm	1.0	0.04	0.04
3 908.0 cm x 181.6 cm	5.0	0.20	0.22
4 1271.2 cm x 181.6 cm	1.0	0.04	0.04
5 1634.4 cm x 181.6 cm	10.0	0.40	0.51
6 1997.6 cm x 181.6 cm	4.0	0.16	0.17
7 2360.8 cm x 181.6 cm	4.0	0.16	0.17
8 181.6 cm x 544.8 cm	18.0	0.72	1.27
9 544.8 cm x 544.8 cm	10.0	0.40	0.51
10 908.0 cm x 544.8 cm	5.0	0.20	0.22
11 1271.2 cm x 544.8 cm	9.0	0.36	0.45
12 1634.4 cm x 544.8 cm	18.0	0.72	1.27
13 1997.6 cm x 544.8 cm	2.0	0.08	0.08
14 2360.8 cm x 544.8 cm	11.0	0.44	0.58
15 181.6 cm x 908.0 cm	9.0	0.36	0.45
16 544.8 cm x 908.0 cm	0.0	0.00	0.00
17 908.0 cm x 908.0 cm	1.0	0.04	0.04
18 1271.2 cm x 908.0 cm	13.0	0.52	0.73
19 1634.4 cm x 908.0 cm	2.0	0.08	0.08
20 1997.6 cm x 908.0 cm	8.0	0.32	0.39
21 2360.8 cm x 908.0 cm	5.0	0.20	0.22
22 181.6 cm x 1271.2 cm	15.0	0.60	0.92
23 544.8 cm x 1271.2 cm	-	-	-

a - Based on 0.001 g soil/disc
b - Based on 25 carrot discs
c - Corrected according to Gregory's multiple colonization correction factor

Table 4.6. cont. Proportion of carrot discs colonized by T. basicola in soil systematically collected from tobacco Field A in 1981

Sample # and location	Discs colonized[a] by T. basicola	Proportion of discs colonized[b]	Corrected value[c]
24 908.0 cm x 1271.2 cm	1.0	0.04	0.04
25 1271.2 cm x 1271.2 cm	3.0	0.12	0.13
26 1634.4 cm x 1271.2 cm	3.0	0.12	0.13
27 1997.6 cm x 1271.2 cm	0.0	0.00	0.00
28 2360.8 cm x 1271.2 cm	1.0	0.04	0.04
29 181.6 cm x 1634.4 cm	1.0	0.04	0.04
30 544.8 cm x 1634.4 cm	4.0	0.16	0.17
31 908.0 cm x 1634.4 cm	15.0	0.60	0.92
32 1271.2 cm x 1634.4 cm	8.0	0.32	0.39
33 1634.4 cm x 1634.4 cm	7.0	0.28	0.33
34 1997.6 cm x 1634.4 cm	5.0	0.20	0.22
35 2360.8 cm x 1634.4 cm	9.0	0.36	0.45
36 181.6 cm x 1997.6 cm	5.0	0.20	0.22
37 544.8 cm x 1997.6 cm	8.0	0.32	0.39
38 908.0 cm x 1997.6 cm	8.0	0.32	0.39
39 1271.2 cm x 1997.6 cm	0.0	0.00	0.00
40 1634.4 cm x 1997.6 cm	18.0	0.72	1.27
41 1997.6 cm x 1997.6 cm	6.0	0.24	0.28
42 2360.8 cm x 1997.6 cm	13.0	0.52	0.73
43 181.6 cm x 2360.8 cm	7.0	0.28	0.33
44 544.8 cm x 2360.8 cm	3.0	0.12	0.13
45 908.0 cm x 2360.8 cm	18.0	0.72	1.27
46 1271.2 cm x 2360.8 cm	1.0	0.04	0.04

a - Based on 0.001 g soil/disc
b - Based on 25 carrot discs
c - Corrected according to Gregory's multiple colonization correction factor

Table 4.6. cont. Proportion of carrot discs colonized by T. basicola in soil systematically collected from tobacco field A in 1981

Sample # and location	Discs colonized[a] by T. basicola	Proportion of discs colonized[b]	Corrected value[c]
47 1634.4 cm x 2360.8 cm	8.0	0.32	0.39
48 1997.6 cm x 2360.8 cm	8.0	0.32	0.39
49 2360.8 cm x 2360.8 cm	5.0	0.20	0.22

a - Based on 0.001 g soil/disc
b - Based on 25 carrot discs
c - Corrected according to Gregory's multiple colonization correction factor

for the corrected populations. Lloyd's index of patchiness was 1.47 for the uncorrected populations and 1.86 for the corrected populations.

There was no fit to the Poisson distribution (P = 0.00), using a Chi-square test for either the uncorrected or corrected populations. There was a good fit to a negative binomial distribution (x^2 = 2.32, P = 0.64, $\hat{N}$ = 2.1) for the uncorrected pouplations and the corrected populations (x^2 = 1.44, P = 0.90, $\hat{N}$ = 1.15), suggesting that the fungus is clumped or aggregated in the field. There was not fit of either of the populations to the Neyman Type A distribution.

The locations and results of the carrot disc assays of the random samples taken in 1981 in Field A are given in Tables 4.7 and 4.8. The variance to mean ratios for the populations calculated without and with Gregory's multiple colonization correction factor were 2.01 and 2.92, respectively. Lloyd's index of mean crowding was 5.43 for the uncorrected population and 7.08 for the corrected population. Lloyd's index of patchiness was 1.23 for the uncorrected population and 1.37 for the corrected population. A fit to the Poisson distribution was not possible with the uncorrected populations (P = 0.00) or the corrected populations (P = 0.00). A fit to the negative binomial distribution was achieved with the uncorrected pouplation (x^2 = 2.02, P = 0.70, $\hat{N}$ = 4.36) and the corrected population (x^2 = 2.22, P = 0.40, $\hat{N}$ = 2.69). There was no fit to the

Table 4.7. Locations of random soil-sampling sites in tobacco Field A for 1981

Sample #	Location	Sample #	Location
1	4'10" x 13'6" (147.3 cm x 411.5 cm)	14	35'6" x 5'11" (1082.1 cm x 180.4 cm)
2	7'5" x 16'8" (226.0 cm x 508.0 cm)	15	35'8" x 33'9" (1087.1 cm x 1028.7 cm)
3	9'11" x 39'7" (302.2 cm x 1206.5 cm)	16	36'6" x 50'7" (1109.9 cm x 1541.8 cm)
4	12'11" x 1' (393.7 cm x 30.5 cm)	17	36'8" x 31'8" (1117.6 cm x 965.2 cm)
5	13'9" x 63'6" (419.1 cm x 1934.8 cm)	18	37'9" x 4'2" (1150.6 cm x 127.0 cm)
6	19' x 4'10" (579.1 cm x 147.3 cm)	19	38'8" x 30'5" (1178.5 cm x 927.1 cm)
7	23'7" x 34'3" (718.8 cm x 1044.5 cm)	20	39'6" x 57'1" (1203.9 cm x 1752.7 cm)
8	28'4" x 61'3" (863.6 cm x 1866.9 cm)	21	40'5" x 7'8" (1231.9 cm x 233.7 cm)
9	28'10" x 64'7" (878.9 cm x 1968.5 cm)	22	41' x 50'6" (1249.7 cm x 1539.3 cm)
10	30'2" x 70'11" (919.5 cm x 2161.6 cm)	23	43'4" x 11'3" (1320.7 cm x 340.9 cm)
11	33'4" x 25'3" (1016.0 cm x 769.6 cm)	24	45'8" x 17'3" (1391.7 cm x 525.8 cm)
12	34' x 69'5" (1044.0 cm x 2115.8 cm)	25	45'8" x 55'5" (1391.7 cm x 1689.1 cm)
13	35' x 75'4" (1066.8 cm x 2296.1 cm)		

Table 4.7. cont. Locations of random soil-sampling sites in tobacco Field A for 1981

Sample #	Location	Sample #	Location
26	45'8" x 55'5" (1391.7 cm x 1689.1 cm)	39	65'8" x 22'1" (2001.3 cm x 673.1 cm)
27	46'7" x 79'7" (1419.3 cm x 2425.1 cm)	40	66'2" x 61'10" (2016.7 cm x 1884.6 cm)
28	49'9" x 17' (1516.4 cm x 518.2 cm)	41	71'3" x 13'3" (2171.7 cm x 403.9 cm)
29	52'1" x 29'4" (1587.5 cm x 893.9 cm)	42	74'9" x 1'1" (2278.4 cm x 30.7 cm)
30	53'2" x 62'2" (1620.5 cm x 1894.8 cm)	43	74'9" x 44'7" (2278.4 cm x 1358.8 cm)
31	54'9" x 46'8" (1668.8 cm x 1422.2 cm)	44	75'6" x 63'10" (2301.2 cm x 1945.5 cm)
32	57'2" x 43'6" (1742.4 cm x 1325.9 cm)	45	77'6" x 18'2" (2362.2 cm x 553.7 cm)
33	59'10" x 53'4" (1823.6 cm x 1625.5 cm)	46	77'9" x 21'8" (2369.8 cm x 660.2 cm)
34	60'2" x 7'1" (1833.9 cm x 215.9 cm)	47	77'9" x 24'7" (2369.8 cm x 749.2 cm)
35	63'9" x 46'10" (1943.1 cm x 1427.4 cm)	48	78'5" x 41' (2390.2 cm x 1249.7 cm)
36	63'9" x 48' (1943.1 cm x 1463.0 cm)	49	79'4" x 78'4" (2417.9 cm x 2387.5 cm)
37	65'3" x 57'10" (1988.8 cm x 1762.7 cm)	50	81'3" x 62'10" (2476.5 cm x 1915.1 cm)
38	65'3" x 70'8" (1988.8 cm x 2153.7 cm)		

Table 4.8. Proportion of carrot discs colonized by *Thielaviopsis basicola* in soil randomly collected from tobacco Field A in 1981

Sample #	Discs colonized[a] by *T. basicola*	Proportion of discs colonized[b]	Corrected value[c]
1	5.0	0.20	0.22
2	8.0	0.32	0.39
3	0.0	0.00	0.00
4	3.0	0.12	0.13
5	3.0	0.12	0.13
6	6.0	0.24	0.27
7	8.0	0.32	0.39
8	14.0	0.56	0.82
9	3.0	0.12	0.13
10	5.0	0.20	0.22
11	6.0	0.24	0.27
12	4.0	0.16	0.17
13	1.0	0.04	0.04
14	8.0	0.32	0.39
15	5.0	0.20	0.22
16	4.0	0.16	0.17
17	1.0	0.04	0.04
18	3.0	0.12	0.13
19	4.0	0.16	0.17
20	9.0	0.36	0.45
21	6.0	0.24	0.27
22	3.0	0.12	0.13
23	3.0	0.12	0.13

a - Based on 0.001 g soil/disc
b - Based on 25 carrot discs
c - Corrected according to Gregory's multiple colonization correction factor

Table 4.8. cont. Proportion of carrot discs colonized by T. basicola in soil randomly collected in tobacco Field A in 1981

Sample #	Discs colonized[a] by T. basicola	Proportion of discs colonized[b]	Corrected value[c]
24	3.0	0.12	0.13
25	9.0	0.36	0.45
26	3.0	0.12	0.13
27	5.0	0.20	0.22
28	0.0	0.00	0.00
29	4.0	0.16	0.17
30	5.0	0.20	0.22
31	2.0	0.08	0.08
32	2.0	0.08	0.08
33	4.0	0.16	0.17
34	1.0	0.04	0.04
35	2.0	0.08	0.08
36	2.0	0.08	0.08
37	3.0	0.12	0.13
38	6.0	0.24	0.27
39	3.0	0.12	0.13
40	5.0	0.20	0.22
41	9.0	0.36	0.45
42	0.0	0.00	0.00
43	5.0	0.20	0.22
44	6.0	0.24	0.27
45	4.0	0.16	0.17
46	2.0	0.08	0.08

a - Based on 0.001 g soil/disc
b - Based on 25 carrot discs
c - Corrected according to Gregory's multiple colonization correction factor

Table 4.8. cont. Proportion of carrot discs colonized by T. basicola in soil randomly collected in tobacco Field A in 1981

Sample #	Discs colonized[a] by T. basicola	Proportion of discs colonized[b]	Corrected value[c]
47	4.0	0.16	0.17
48	0.0	0.00	0.00
49	9.0	0.36	0.45
50	9.0	0.36	0.45

a - Based on 0.001 g soil/disc
b - Based on 25 carrot discs
c - Corrected according to Gregory's multiple colonization correction factor

Neyman Type A distribution for either of the populations.

Tobacco Field B had a much lower population of T. basicola than Field A. Populations from systematically sampled soil (Table 4.9) and calculated without Gregory's multiple colonization correction factor had a variance to mean ratio of 2.29. The corrected populations had a variance to mean ratio of 5.31. Lloyd's index of mean crowding for the uncorrected populations and corrected populations were 7.45 and 12.8, respectively. Lloyd's index of patchiness for the uncorrected and corrected populations were 1.21 and 1.51, respectively. There was no fit of either of the populations to the Poisson distribution ($P = 0.00$) or the Neyman Type A distribution ($P = 0.00$). There was a fit of the uncorrected population to the negative binomial distribution ($x^2 = 6.13$, $P = 0.40$, $\hat{N} = 4.78$) and the corrected populations to the negative binomial ($x^2 = 1.07$, $P = 0.80$, $\hat{N} = 1.97$).

Populations from randomly collected soil samples from Field B (Tables 4.10 and 4.11) and calculated without Gregory's multiple colonization correction factor, had a variance to mean ratio of 11.32. The same populations calculated with Gregory's correction factor had a variance to mean ratio of 72.15. Lloyd's index of mean crowding for the uncorrected populations and the corrected populations were 15.06 and 86.36, respectively. Lloyd's index of patchiness for the uncorrected and corrected populations

Table 4.9. Proportion of carrot discs colonized by Thielaviopsis basicola in soil systematically collected from tobacco Field B

Sample # and location	Discs colonized[a] by T. basicola	Proportion of discs colonized[b]	Corrected value[c]
1 181.6 cm x 181.6 cm	10.0	0.40	0.51
2 544.8 cm x 181.6 cm	19.0	0.76	1.43
3 908.0 cm x 181.6 cm	2.0	0.08	0.08
4 1271.2 cm x 181.6 cm	6.0	0.24	0.27
5 1634.4 cm x 181.6 cm	15.0	0.60	0.92
6 1997.6 cm x 181.6 cm	7.0	0.28	0.33
7 2360.8 cm x 181.6 cm	8.0	0.32	0.39
8 181.6 cm x 544.8 cm	1.0	0.04	0.04
9 544.8 cm x 544.8 cm	15.0	0.60	0.92
10 908.0 cm x 544.8 cm	3.0	0.12	0.13
11 1271.2 cm x 544.8 cm	7.0	0.28	0.33
12 1634.4 cm x 544.8 cm	3.0	0.12	0.13
13 1997.6 cm x 544.8 cm	8.0	0.32	0.39
14 2360.8 cm x 544.8 cm	6.0	0.24	0.28
15 181.6 cm x 908.0 cm	7.0	0.28	0.33
16 544.8 cm x 908.0 cm	10.0	0.40	0.51
17 908.0 cm x 908.0 cm	4.0	0.16	0.17
18 1271.2 cm x 908.0 cm	8.0	0.32	0.39
19 1634.4 cm x 908.0 cm	3.0	0.12	0.13
20 1997.6 cm x 908.0 cm	8.0	0.32	0.39
21 2360.8 cm x 908.0 cm	11.0	0.44	0.58
22 181.6 cm x 1271.2 cm	5.0	0.20	0.22
23 544.8 cm x 1271.2 cm	4.0	0.16	0.17

a - Based on 0.002 g soil/disc
b - Based on 25 carrot discs
c - Corrected according to Gregory's multiple colonization correction factor

Table 4.9. cont. Proportion of carrot discs colonized by T. basicola in soil systematically collected from tobacco field B

Sample # and location	Discs colonized[a] by T. basicola	Proportion of discs colonized[b]	Corrected value[c]
24 908.0 cm x 1271.2 cm	4.0	0.16	0.17
25 1271.2 cm x 1271.2 cm	8.0	0.32	0.39
26 1634.4 cm x 1271.2 cm	2.0	0.08	0.08
27 1997.6 cm x 1271.2 cm	6.0	0.24	0.27
28 2360.8 cm x 1271.2 cm	4.0	0.16	0.17
29 181.6 cm x 1634.4 cm	7.0	0.28	0.33
30 544.8 cm x 1634.4 cm	3.0	0.12	0.13
31 908.0 cm x 1634.4 cm	2.0	0.08	0.08
32 1271.2 cm x 1634.4 cm	6.0	0.24	0.27
33 1634.4 cm x 1634.4 cm	5.0	0.20	0.22
34 1997.6 cm x 1634.4 cm	8.0	0.32	0.39
35 2360.8 cm x 1634.4 cm	9.0	0.36	0.45
36 181.6 cm x 1997.6 cm	9.0	0.36	0.45
37 544.8 cm x 1997.6 cm	13.0	0.52	0.73
38 908.0 cm x 1997.6 cm	8.0	0.32	0.39
39 1271.2 cm x 1997.6 cm	9.0	0.36	0.45
40 1634.4 cm x 1997.6 cm	2.0	0.08	0.08
41 1997.6 cm x 1997.6 cm	6.0	0.24	0.27
42 2360.8 cm x 1997.6 cm	14.0	0.56	0.82
43 181.6 cm x 2360.8 cm	2.0	0.08	0.08
44 544.8 cm x 2360.8 cm	5.0	0.20	0.22
45 908.0 cm x 2360.8 cm	7.0	0.28	0.33
46 1271.2 cm x 2360.8 cm	10.0	0.40	0.51

a - Based on 0.002 g soil/disc
b - Based on 25 carrot discs
c - Corrected according to Gregory's multiple colonization correction factor

Table 4.9. cont. Proportion of carrot discs colonized by T. basicola in soil systematically collected from tobacco Field B

Sample # and location	Discs colonized[a] by T. basicola	Proportion of discs colonized[b]	Corrected value[c]
47 1634.4 cm x 2360.8 cm	4.0	0.16	0.17
48 1997.6 cm x 2360.8 cm	9.0	0.36	0.45
49 2360.8 cm x 2360.8 cm	2.0	0.08	0.08

a - Based on 0.002 g soil/disc
b - Based on 25 carrot discs
c - Corrected according to Gregory's multiple colonization correction factor

Table 4.10. Locations of random sampling sites in tobacco Field B for 1981

Sample #	Location	Sample #	Location
1	2'8" x 71'4" (81.1 cm x 2174.2 cm)	14	27'11" x 35'5" (850.9 cm x 1079.5 cm)
2	5'4" x 8'5" (162.5 cm x 256.6 cm)	15	29'4" x 36'11" (893.9 cm x 1125.2 cm)
3	6'7" x 45'1" (200.6 cm x 1374.1 cm)	16	31'1" x 19'3" (947.4 cm x 586.7 cm)
4	7'5" x 16'8" (226.1 cm x 507.8 cm)	17	33'4" x 25'4" (1015.9 cm x 772.1 cm)
5	8'6" x 48'2" (258.6 cm x 1468.1 cm)	18	35'3" x 66'4" (1074.4 cm x 2021.7 cm)
6	9'5" x 2'8" (287.0 cm x 81.1 cm)	19	38'9" x 32' (1181.1 cm x 975.4 cm)
7	11'10" x 12' (360.6 cm x 365.8 cm)	20	39'2" x 61'7" (1193.8 cm x 1876.9 cm)
8	12'9" x 69'3" (388.6 cm x 2110.7 cm)	21	39'3" x 66'11" (1196.3 cm x 2039.6 cm)
9	16'8" x 30'10" (507.8 cm x 939.7 cm)	22	39'8" x 6' (1208.8 cm x 182.9 cm)
10	17'1" x 56'10" (520.7 cm x 1732.2 cm)	23	42'3" x 43'7" (1287.8 cm x 1328.3 cm)
11	20'6" x 28'2" (624.8 cm x 858.5 cm)	24	45'8" x 55'5" (1391.7 cm x 1689.1 cm)
12	23'1" x 71' (703.6 cm x 2164.1 cm)	25	49'11" x 50'11" (1521.5 cm x 1551.9 cm)
13	26'4" x 52'11" (802.5 cm x 1612.9 cm)		

Table 4.10. cont. Locations of random sampling sites in tobacco Field B for 1981

Sample #	Location	Sample #	Location
26	50'8" x 77'4" (1544.1 cm x 2356.1 cm)	39	67'2" x 17'1" (2047.2 cm x 520.7 cm)
27	51'2" x 9'11" (1559.5 cm x 302.3 cm)	40	67'5" x 33'8" (2054.9 cm x 1025.9 cm)
28	53'2" x 43'11" (1620.5 cm x 1338.6 cm)	41	68'1" x 15'11" (2075.2 cm x 485.2 cm)
29	53'5" x 24'2" (1628.2 cm x 736.6 cm)	42	68'2" x 15'11" (2077.7 cm x 485.2 cm)
30	54'11" x 65' (1673.9 cm x 1981.2 cm)	43	68'2" x 30' (2077.7 cm x 914.4 cm)
31	55'2" x 67'9" (1681.5 cm x 2065.0 cm)	44	69'7" x 45'4" (2120.8 cm x 1381.7 cm)
32	57'3" x 7'4" (1744.9 cm x 223.4 cm)	45	72'11" x 38'6" (2222.5 cm x 1173.5 cm)
33	58'5" x 6'11" (1780.6 cm x 210.8 cm)	46	78'4" x 4'7" (2387.5 cm x 139.6 cm)
34	60'10" x 32'11" (1854.1 cm x 1003.3 cm)	47	78'9" x 2'3" (2400.3 cm x 68.6 cm)
35	61'4" x 14'2" (1869.3 cm x 431.8 cm)	48	79' x 11'5" (2407.9 cm x 347.9 cm)
36	62'6" x 41'3" (1894.8 cm x 1257.3 cm)	49	82'1" x 20'9" (2501.9 cm x 632.5 cm)
37	63' x 65'8" (1920.2 cm x 2001.3 cm)	50	82'11" x 11'10" (2527.3 cm x 360.5 cm)
38	67'2" x 1' (2047.2 cm x 30.5 cm)		

Table 4.11. Proportion of carrot discs colonized by *Thielaviopsis basicola* from soil randomly collected from tobacco Field B in 1981

Sample #	Discs colonized[a] by *T. basicola*	Proportion of discs colonized[b]	Corrected value[c]
1	2.0	0.08	0.08
2	0.0	0.00	0.00
3	6.0	0.24	0.27
4	1.0	0.04	0.04
5	12.0	0.48	0.65
6	2.0	0.08	0.08
7	1.0	0.04	0.04
8	1.0	0.04	0.04
9	6.0	0.24	0.27
10	4.0	0.16	0.17
11	3.0	0.12	0.13
12	12.0	0.48	0.65
13	0.0	0.00	0.00
14	2.0	0.08	0.08
15	1.0	0.04	0.04
16	0.0	0.00	0.00
17	15.0	0.60	0.91
18	0.0	0.00	0.00
19	0.0	0.00	0.00
20	13.0	0.52	0.73
21	2.0	0.08	0.08
22	1.0	0.04	0.04
23	2.0	0.08	0.08

a - Based on 0.002 g soil/disc
b - Based on 25 carrot discs
c - Corrected according to Gregory's multiple colonization correction factor

Table 4.11. cont. Proportion of carrot discs colonized by T. basicola from soil randomly collected from tobacco Field B in 1981

Sample #	Discs colonized[a] by T. basicola	Proportion of discs colonized[b]	Corrected value[c]
24	0.0	0.00	0.00
25	24.0	0.96	3.22
26	0.0	0.00	0.00
27	0.0	0.00	0.00
28	2.0	0.08	0.08
29	1.0	0.04	0.04
30	6.0	0.24	0.27
31	2.0	0.08	0.08
32	0.0	0.00	0.00
33	5.0	0.20	0.20
34	6.0	0.24	0.27
35	1.0	0.04	0.04
36	2.0	0.08	0.08
37	1.0	0.04	0.04
38	25.0	1.00	4.61
39	25.0	1.00	4.61
40	2.0	0.08	0.08
41	2.0	0.08	0.08
42	1.0	0.08	0.08
43	4.0	0.16	0.17
44	25.0	1.00	4.61
45	2.0	0.08	0.08
46	23.0	0.92	2.53

a - Based on 0.002 g soil/disc
b - Based on 25 carrot discs
c - Corrected according to Gregory's multiple colonization correction factor

Table 4.11. cont. Proportion of carrot discs colonized by T. basicola from soil randomly collected from tobacco Field B in 1981

Sample #	Discs colonized[a] by T. basicola	Proportion of discs colonized[b]	Corrected value[c]
47	25.0	1.00	4.61
48	0.0	0.00	0.00
49	4.0	0.16	0.17
50	1.0	0.04	0.04

a - Based on 0.002 g soil/disc
b - Based on 25 carrot discs
c - Corrected according to Gregory's multiple colonization correction factor

were 2.88 and 5.68, respectively.

There was no fit of either of the populations to the Poisson distribution (P = 0.00) or the Neyman Type A distribution (P = 0.00). The uncorrected populations had a good fit to the negative binomial distribution (x^2 = 1.80, P = 0.40, $\hat{N}$ = 0.53). The corrected populations did not fit the negative binomial distribution (x^2 = 22.70, P = 0.00, $\hat{N}$ = 0.21). A summary of all the statistics on T. basicola population patterns is given in Table 4.12.

4.2 Greenhouse Experiments

Inoculum densities in greenhouse experiment 1 ranged from 127 to 2,799 propagules per gram of soil. Incidence of root rot was 100% at all inoculum densities. Percentages of rot of the roots of the Judy's Pride tobacco seedlings ranged from 5 to 70% after 6 weeks at 25 C. A regression analysis of mean percent root rot versus inoculum density indicated that there was no relationship between the two variables (R^2 = 0.16; P = 0.48). There also was no indication of a relationship between inoculum density and the calculated variable CR (% root rot x dry root weight) (R^2 = 0.12; P = 0.60), inoculum density and dry root weight (R^2 = 0.37; P = 0.30), inoculum density and dry shoot weight (R^2 = 0.54; P = 0.15), and inoculum density and shoot weight (R^2 = 0.05; P = 0.75). Regression analyses of shoot height versus inoculum density and root dry weight

Table 4.12. Summary of variance to mean ratios, $\hat{N}$, indexes of mean crowding, indexes of patchiness, and Chi-square results for populations of Thielaviopsis basicola in Field A and B, and calculated with and without Gregory's correction factor

		Uncorrected Populations[a]					Corrected Populations[b]				
		s^2/Y	$\hat{N}$[c]	IMC[d]	LIP[e]	Dist.[f]	s^2/Y	$\hat{N}$	IMC	LIP	Dist.
Field A											
1980 -	S[g]	2.47[h]	5.33	9.31	1.19	NB	6.76	1.98	17.16	1.51	NB
Field A											
1981 -	S	4.13	2.10	9.75	1.47	NB	8.90	1.15	16.90	1.86	NB
	R	2.01	4.36	5.43	1.23	NB	2.92	2.69	7.08	1.37	NB
Field B											
1981 -	S	2.29	4.78	7.45	1.21	NB	5.31	1.97	12.80	1.51	NB
	R	11.32	0.53	15.80	2.88	NB	72.15	0.21	86.36	5.68	

a - Calculated without Gregory's multiple colonization correction factor

b - Calculated with Gregory's multiple colonization correction factor

c - Parameter for negative binomial

d - Lloyd's index of mean crowding ($\overset{*}{Y} = Y + (s^2/Y - 1)$)

e - Lloyd's index of patchiness ($\overset{*}{Y}/Y$)

f - Distribution derived from chi-square goodness of fit test, N.B. = negative binomial, P = Poisson, N.T. = Neyman Type A

g - Sampling method, S = systematic, R = random

h - All variance to mean ratios were significantly greater than 1.0 (P = 0.05) according to a t-test.

versus inoculum density showed no relationship between them (R^2 = 0.05; P > 0.99, R^2 = 0.46; P = 0.22). There was a slight relationship between shoot dry weight and inoculum density (R^2 = 0.79; P = 0.04) (Table 4.14).

Greenhouse experiment 2 was performed using soils with populations ranging from 330 to 1300 propagules per gram of soil. Root rot incidence was 96-100% at all inoculum densities. Rot of the roots of the Burley 21 seedlings ranged from 0-60% after seven weeks at 25 C. Regression analyses indicated no relationship between inoculum density and shoot height (R^2 = 0.00; P > 0.99), inoculum density and shoot dry weight (R^2 = 0.03; P = 0.80), inoculum density and root dry weight (R^2 = 0.32; R^2 = P > 0.99), inoculum density and root rot (R^2 = 0.07; P = 0.75), and inoculum density and the calculated variable CR (R^2 = 0.55, P = 0.15). A summary of these data is given in Table 4.13.

A regression analysis of mean percent roots rotted per tobacco plant versus shoot height indicated a strong relationship (R^2 = 0.91, P = 0.02). A relationship was also seen between shoot dry weight and inoculum density (R^2 = 0.60; P = 0.06). No relationship was seen between root dry weight and inoculum density (R^2 = 0.23, P = 0.41) (Table 4.14, Fig. 4.1).

The assay for nematodes indicated that only one soil sample contained nematodes. This was the sample with the highest population of T. basicola. The genus

Table 4.13. R^2, slope, and y-intercept values of regression analyses of plant measurements vs. inoculum density taken of tobacco seedlings grown in soil naturally infested with Thielaviopsis basicola at 25 C

	cv. Judy's Pride[a]			cv. Burley 21[b]		
	R^2	slope	y-intercept	R^2	slope	y-intercept
shoot height vs. ID[c]	0.05 (P=0.75)	3.12	2.99	0.00 (P>0.99)	0.00	6.56
shoot dry weight vs. ID[c]	0.54 (P=0.15)	0.00	0.10	0.03 (P=0.80)	0.00	0.60
root dry weight vs. ID[c]	0.37 (P=0.30)	0.00	0.03	0.32 (P>0.99)	0.00	0.27
root rot[d] vs. ID[c]	0.16 (P=0.48)	0.00	20.45	0.07 (P=0.75)	-0.01	23.21
CR[e] vs. ID[c]	0.12 (P=0.60)	0.00	0.56	0.55 (P=0.15)	0.00	5.50

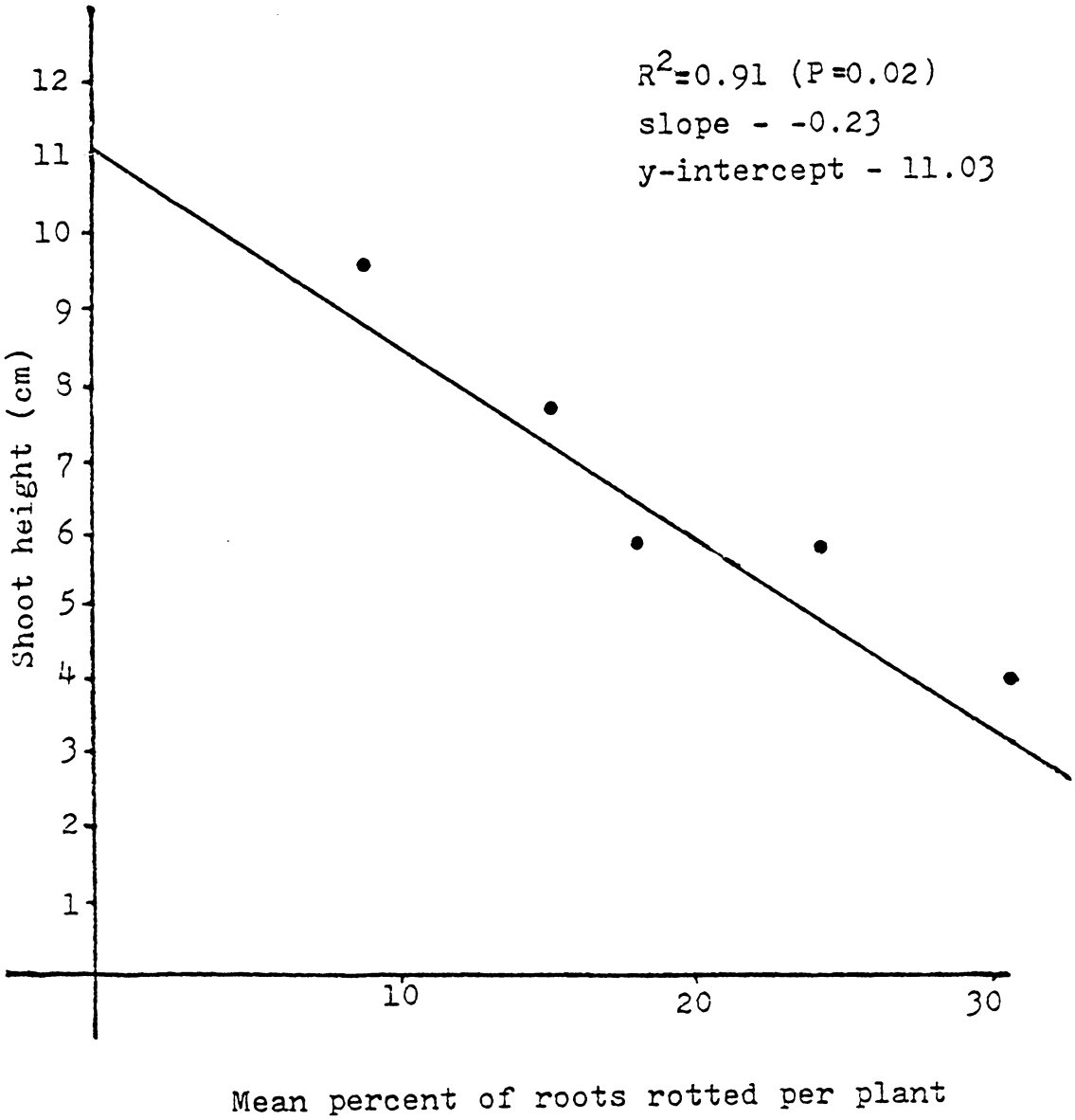
a - A susceptible black root rot cultivar
b - A slightly resistant black root rot cultivar
c - Inoculum density
d - Indicates the mean percent of roots rotted per tobacco plant
e - Indicates percent of roots rotted per tobacco plant x root dry weight

Table 4.14. R^2, slope, and y-intercept values of regression analyses of plant measurements versus mean percent roots rotted per tobacco plant grown in soil naturally infested with Thielaviopsis basicola at 25 C

	cv. Judy's Pride[a]			cv. Burley 21[b]		
	R^2	slope	y-intercept	R^2	slope	y-intercept
shoot height vs. RR[c]	0.05 (P 0.99)	-0.01	3.19	0.91 (P=0.02)	-0.23	11.03
shoot dry weight vs. RR[c]	0.79 (P=0.04)	-0.01	0.23	0.60 (P=0.06)	-0.02	0.88
root dry weight vs. RR[c]	0.46 (P=0.44)	0.00	0.06	0.23 (P=0.41)	-0.01	0.28

a - A susceptible black root rot cultivar
b - A slightly resistant black root rot cultivar
c - Root rot, indicates the mean percent of roots rotted per tobacco plant

Fig. 4.1. Relationship between mean percent of roots rotted per plant and shoot height of Burley 21 tobacco seedlings after seven weeks at 28 C.


R^2=0.91 (P=0.02)
slope - -0.23
y-intercept - 11.03
Shoot height (cm)
12
11
10
9
8
7
6
5
4
3
2
1
10
20
30
Mean percent of roots rotted per plant

Tylenchorhynchus had a population of 10 larvae per 500 cc of soil in this sample.

4.3 Isolate Type

Isolations from the field produced only the grey-black type of T. basicola; no olivaceous or brown colonies were observed. The colonies on PDA were at least 25 mm in diameter, and some were as large as 38 mm after 8 days at 25-27 C. The perimeter of each colony was growing slightly below the surface of the agar.

V. DISCUSSION

The stunting of the Burley 21 tobacco plants in many sections of tobacco Field A in 1980 (Fig. 5.1) was severe and root rot incidence in the field was 100%. Stunting is considered a major effect of infection of tobacco plants by T. basicola. The inoculum density of the fungus in many parts of the field was extremely high and sufficient to produce disease. T. basicola is a fungus which apparently requires a minimum of 100, but more often, 1,000, propagules per gram of soil to produce disease (2). Maduewesi et al (31), however, reported that 10 propagules per gram of soil is sufficient to produce root rot on soybeans in soil artificially infested with T. basicola. In the present study root rot was observed on both Burley 21 and Judy's Pride tobacco plants which were planted in soil having 120 to 2800 propagules of T. basicola per gram of soil. Nevertheless, data collected in 1980 showed no statistical relationship between the degree of stunting and the inoculum density of T. basicola in the field.

Baker (3) has attempted to more accurately define the relationship between inoculum density of soil-borne plant pathogens and disease incidence. At low inoculum levels, disease incidence increases with an increase in pathogen populations. At higher populations, multiple infections occur but the relationship can be reestablished by

5.1. Severly stunted 4-month-old Burley 21 tobacco plants in tobacco Field A in 1980. Note large tobacco plant to the right of the stunted plant.

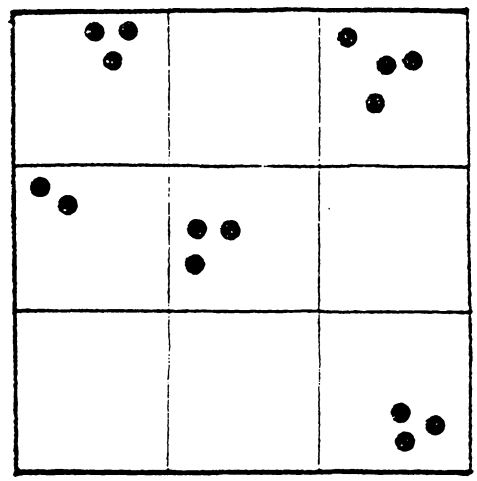
transforming the population of diseased plants with the use of Gregory's multiple colonization correction factor. At extremely high inoculum densities, competition occurs and disease does not increase with increasing pathogen populations. Baker suggested that this relationship would vary depending on the condition of the host-parasite interaction resulting in what he termed the rhizosphere effect and the rhizoplane effect. He proposed mathematical models to illustrate this. Baker cautioned that his models were only applicable if an idealized situation were present; where propagules were randomly arranged in the field and capacity factors (environmental influences) are relatively constant. Observations from tobacco Field A and B indicate that T. basicola is not randomly or uniformly distributed in either of the fields, but, instead, is arranged in clumps or aggregations. Both the systematic and the random sampling suggested a clumped pattern of the propagules of T. basicola in both fields. A fit of all the populations was obtained to the negative binomial distribution which indicates a clumped pattern of the propagules in the fields. None of the calculated populations, whether uncorrected or corrected with Gregory's multiple colonization correction factor, fit the Poisson or the Neyman Type A distribution.

Taylor et al (45) reported that the clumped pattern of Cylindrocladium crotalariae in the field, resulting in high variances, may have contributed to the low slope value of

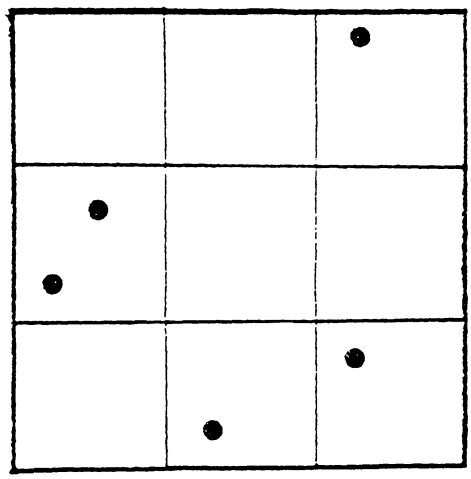
the regression curve relating inoculum density and disease incidence that they obtained in their study. A clumped pattern of T. basicola in the tobacco fields may have been a factor in the results obtained in this study.

The variance to mean ratio of the T. basicola population density, a measurement of aggregation, also supported the idea of a clumped pattern of T. basicola in the field soil. A ratio less than one indicates a uniform pattern of propagules. A ratio equal to one suggests a random pattern, and a ratio greater than one occurs when propagules are clumped (Fig. 5.2). All the variance to mean ratios calculated from the populations of T. basicola in Field A and B were significantly greater than one. Lloyd's index of mean crowding, defined as the number per individual of other co-occupants in a unit, increases with increased crowding. Lloyd's index of patchiness is the ratio of mean crowding to mean density, and is an indicator of a clumped spatial pattern which disregards the organism's total density (40). The indexes of patchiness in this study ranged from 1.19 to 2.88 for the uncorrected populations and 1.37 to 5.68 for the corrected populations. This is comparable to the index of patchiness, 2.0, obtained in the peanut field soil naturally infested with C. crotalariae. An index as high as 10.2 was obtained for Cylindrocladium in nursery field soil (45). In the C. crotalariae study, a higher correlation was found between disease incidence

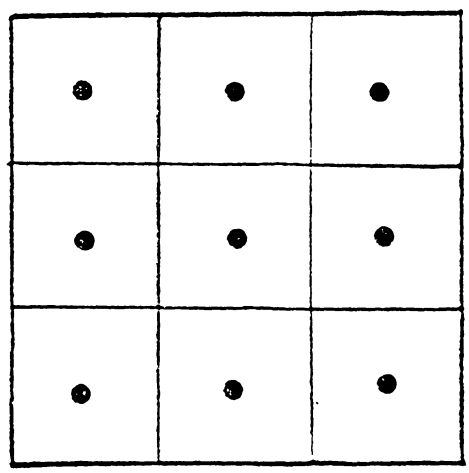
Fig. 5.2. The relationship between population patterns and variance to mean ratios. (a) $s^2/\bar{Y}<1$, (b) $s^2/\bar{Y}=1$, (c) $s^2/\bar{Y}>1$.



c



b



a

and pathogen population than was found in the present study.

The low patchiness value obtained here may indicate that the clumped pattern of T. basicola in the soil may not have been the dominant factor in the weak inoculum density-disease incidence relationship seen and that other environmental or capacity factors may have been more important.

The use of Gregory's multiple colonization correction factor, a logarithmic transformation, altered the data and the degree to which a goodness of fit was achieved to the distributions. However, the use of a correction factor may have resulted in a more reliable estimate of the population of T. basicola in the soil. Random samples had a reduced probability of fit when corrected, while the corrected populations of the systematically sampled soil had a higher probability of fit to the negative binomial than the uncorrected population. The variance to mean ratios and the indexes of mean crowding increased for the corrected populations overall, whereas, the indexes of patchiness were not altered (Table 5.1).

Both the systematic and random sampling generally indicated a clumped distribution, although the degree of the probability varied. A possible explanation for the discrepencies may have been the locations of the random sampling sites. Many of the sites were extremely close to one another leaving large portions of the field unsampled. This produced inadequate coverage of the field and would result

Table 5.1. Ranges of variance to mean ratios, indexes of mean crowding, and indexes of patchiness derived from populations of T. basicola in tobacco field soil calculated with and without Gregory's multiple colonization correction factor

	Variance to mean ratio	Index of mean crowding	Index of patchiness
Uncorrected	2.01 - 9.29	5.43 - 15.80	1.19 - 2.88
Corrected	2.92 - 72.15	7.08 - 86.36	1.37 - 5.68

in a poor representation of the spatial pattern of T. basicola in the field.

Both greenhouse experiments had a high incidence, 96-100%, of disease. The experiments showed no relationship between the dependent variables (shoot height, shoot weight, and root rot) and the independent variable, inoculum density. These results are similar to the results obtained in the field test. There was no evidence of a relationship between inoculum density and disease incidence although the soil was mixed thoroughly prior to planting of the tobacco seedlings. This would have prevented the clumping or aggregation of the propagules of T. basicola which occurred in both Fields A and B. Mixing could have produced a random distribution of the propagules in the soil used in the greenhouse experiments. This would allow the conditions that are optimal for linear regression analysis. Research by Tomimatsu and Griffin (48) found that mixing of soil reduced the variability of observed infections of peanut roots by C. crotalariae in greenhouse experiments. This fungus is known to occur in a nonrandom pattern in field soil (45). A stronger relationship between disease incidence (infections) and inoculum density, than found in the field, was observed in their study, which may have been due to the probable random pattern or low degree of patchiness of the pathogen in the soil of the greenhouse experiments. The high disease incidence in these experiments

may have prevented the development of a mathematical model between disease incidence and inoculum density. Regression analysis did show a good relationship between root rot and shoot height and shoot weight of the Burley 21 plants.

The soil analysis results indicated that there were no consistent variations among the soil samples which could have produced the stunting which was observed. The pH values were all above 6.4, which is considerably higher than the pH of 5.2, at which liming is recommended (Leo Link, personal communication).

Several other factors have been shown to produce stunting of tobacco but were not investigated in this research. The moisture level of the soil was not monitored throughout any of the experiments. Leo Link (personal communication) reported "very poor growth" at the end of June in 1980, which he attributed to the extremely dry weather.

Another explanation proposed recently by Hendrix and Modjo (18) is that a large percentage of the stunting of tobacco may be caused by isolates of the endomycorrhizal fungus Glomus macrocarpus. Results obtained by Wendt (1981, unpublished) in southwestern Virginia supported this hypothesis, in part. However, work done by Baltruschat and Schönbeck (5) suggests that mycorrhizae may inhibit spore formation by T. basicola, and thus, mycorrhizal tobacco plants may be less damaged by T. basicola than nonmycorrhizal plants.

Another possible explanation for the stunting suggested by Wendt (1981, unpublished) is damage caused by cutworms, Agrostis ypsilon, and wireworms, Conoderus vespertinus. He reported that 29% of the stunted plants which he examined in 1981 tobacco fields near Glade Springs, Va. had above-ground stem damage which may have contributed to decreased growth.

These and other factors (viruses and virulences in clones of T. basicola) could have interacted with T. basicola to produce the overall reduction of the height of the plants in the fields studied. Furthermore, other symptoms, such as number of leaves and leaf area, should possibly have been measured and incorporated into the disease category along with stunting. The use of only stunting to quantify disease could be much too simplistic a view of the actual situation.

VI. SUMMARY

Analysis of the populations in the soil of T. basicola in two tobacco fields showed a good fit of the observed frequencies to the expected frequencies of the negative binomial distribution, using either systematic or random sampling. This indicates that T. basicola is distributed in a clumped or aggregated pattern in the fields. The variance to mean ratios, all significantly greater than one, also support the idea of a clumped pattern of T. basicola in the field.

A clumped or nonrandom pattern in the field may have contributed to the weak statistical relationship seen in regression analyses between inoculum density and shoot height (R^2 = 0.0005; P = 0.94) and root weight (R^2 = 0.006; P = 0.81). However, the nonrandom pattern may not be the only reason that the R^2 values were low. The Lloyd's index of patchiness values, all somewhat low (range = 1.19 - 2.88 for populations not corrected with Gregory's multiple colonization correction factor, and 1.37 - 5.68 for populations corrected with Gregory's multiple colonization correction factor), may indicate that the clumped pattern of T. basicola was not the major influence, and that some other factor(s) may have contributed to the low R^2 values.

In the greenhouse experiments, thorough mixing of the soil most likely prevented a clumped distribution of

propagules of T. basicola and may have resulted in a random distribution of propagules. No relationship was seen between any of the dependent variables and the independent variable, inoculum density. Disease incidence was high in both greenhouse experiments and this may have prevented development of a mathematical relationship between disease incidence and inoculum density. A good relationship (R^2 = 0.91; P = 0.02) was seen between shoot height and mean percent rot of roots per tobacco plant; and shoot weight (R^2 = 0.60; P = 0.06) and mean percent rot of roots per tobacco plant of Burley 21 plants. These values may possibly indicate that some factor, other than inoculum pattern, may have affected the mathematical relationship between plant growth or disease incidence and population density of T. basicola.

Major variables which may alter the relationship between inoculum density and disease incidence are biological, physical and chemical environmental (or capacity) factors. The influence of these on the mathematical relationship between inoculum density and disease incidence was not investigated in this research. This may be necessary before a disease prediction program for T. basicola in tobacco fields can be developed.

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INOCULUM PATTERN AND RELATIONSHIP BETWEEN INCIDENCE OF BLACK ROOT ROT OF TOBACCO AND INOCULUM DENSITY OF *THIELAVIOPSIS BASICOLA* IN FIELD SOIL

by

Christine M. Rittenhouse

(ABSTRACT)

Thielaviopsis basicola (Berk. and Br.) is a widespread, soil-borne, pathogenic fungus which causes a root rot on tobacco (*Nicotiana tabacum* L.). Presently, control is achieved primarily through the use of resistant cultivars of tobacco or chemicals. In order to develop a disease prediction program, disease-inoculum density relationships must be determined. It was the purpose of this study to develop an improved procedure for estimating the populations of *T. basicola* in naturally infested soil. A second objective was to determine the horizontal inoculum pattern of *T. basicola* in tobacco field soil, and the third objective was to determine the relationship between black root rot of tobacco and inoculum density of *T. basicola* in naturally infested soil.

Two fields in southwestern Virginia were randomly and systematically sampled to assay the population density of *T. basicola* in the soil. Populations of *T. basicola* were isolated using a modified carrot-disc baiting technique

and calculated using a colonization correction factor. Frequencies of calculated population densities of T. basicola in soil samples were compared, using a Chi-square test, to the expected frequencies indicated by the Poisson, Neyman Type A, and negative binomial distributions. Temperature-tank studies were conducted to investigate inoculum density - disease relationships. Two cultivars of tobacco were grown in soil naturally infested with T. basicola. The soil was thoroughly mixed before use to preclude inoculum clumping.

The results obtained with either systematic or random sampling indicated that propagules of T. basicola are clumped or aggregated in tobacco field soil, as there was a good fit of the data to the negative binomial distribution. Also, the variance to mean ratios were significantly ($P = 0.05$) greater than 1.0 which is indicative of a clumped pattern. Other indexes of aggregation indicated a low to moderate degree of inoculum clumping in soil.

For soil temperature-tank tests, root rot incidence was 96-100% at all natural population densities for both slightly resistant cv. Burley 21 and susceptible cv. Judy's Pride plants, and T. basicola was consistently associated with rotted roots. Low R^2 values were obtained in regression analyses of T. basicola inoculum density versus mean

percent root rot per Burley 21 plant and per Judy's Pride plant.