Inoculum Potential of *Cylindrocladium crotalariae*: Infection Rates and Microsclerotial Density-Root Infection Relationships on Peanut

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ABSTRACT

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Numerous infections (1 to >1,000 per plant) caused by *Cylindrocladium crotalariae* were observed on asymptomatic taproots, lateral, and fine roots of peanut plants grown in naturally infested soils in the field or greenhouse. The majority of observed infections did not appear to be restricted to surface tissues, based on tests involving surface sterilization of roots with 0.1 and 0.25% NaClO. In a time-course experiment (25 C), the infection rate, I_r°, was 0.120, 0.162, and 0.199 observed infections per meter of root per day per microsclerotium per gram of soil for the first, second, and third 21-day periods, respectively. The infection rate, R_e, for estimated infections ($\log_e [1/1-y]$, in which y is the proportion of plants with necroses) was 0.0017, 0.0038, and 0.0084 infections per plant per day per microsclerotium per gram of soil for the first, second, and third 21-day periods, respectively. Based on the infection-rate curve, each plant had about 300 observed root infections when 50% of the plant population had root necroses. Regression line slopes of 0.98 ($R^2 = 0.94$) and 0.99 ($R^2 = 0.94$) were obtained for $\log_{10}$–$\log_{10}$ plots of microsclerotial inoculum density vs the number of observed root infections per plant and per unit root length, respectively. Slopes of 21.4 ($R^2 = 0.95$) and 2.3 ($R^2 = 0.95$), respectively, were obtained for first-order regression lines in arithmetic plots of the same variables. Efficiency of inoculum for observed infection (percent of germinating microsclerotia that infect roots) estimates were high (near 100%), while efficiency of observed infection for necrosis (percent of infections that develop necroses, calculated from $\log_e [1/1-y]$) estimates were low (0.27 to 0.28%).

Additional key words: inoculum efficiency, infection efficiency, multiple-infection correction.

Cylindrocladium black rot (CBR) of peanut (*Arachis hypogaea* L.), caused by *Cylindrocladium crotalariae* (Loos) Bell & Sobers, is a destructive root rot that in some years can kill nearly all peanut plants in infested areas of a field (22). Some progress has been made on the study of inoculum potential relationships of this pathogen, especially in regard to inoculum density-disease incidence relationships (13,21,24) and in regard to the influence of physical environmental factors on microsclerotium germinability (11,23). Nothing is known, however, about the quantitative relationship between microsclerotium germination in the rhizosphere and observed root infections. As is indicated in a review by Baker (2), a similar situation exists for other root-infecting fungi. Also, except for analyses of Vanderplank (26,27) and the work of Kannwischer

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and Mitchell (16), with black shank of tobacco, little is known about infection rates of soilborne pathogens.

Krigsvold et al (17,18) demonstrated that a high percentage (39.8%) of the microsclerotia of *C. crotalariae* germinated in a 1-mm-wide volume of rhizosphere soil collected from defined regions of peanut root tips. If inoculum efficiency (percent of germinating propagules that infect roots) is high, this germination in the rhizosphere could lead to development of many root infections on each peanut plant at microsclerotial inoculum densities that occur in nature. Infection rates (infections per plant per unit time) and overall slope values of arithmetic inoculum density-infection plots would be high also. All or a portion of the infections could lead to development of lesions and CBR symptoms. If inoculum efficiency is low, due to low endogenous reserves, inadequate exogenous nutrients (exudates), or antagonism in the peanut rhizosphere or host-defense mechanisms, few or no infections would occur on an individual peanut plant. As pointed out by Baker (2), little is presently known about inoculum efficiency and, to our knowledge, no estimates based on propagule germination data have been made for root-infecting fungi.

This paper reports the occurrence of numerous observed infections of *C. crotalariae* on asymptomatic root systems of field-grown and greenhouse-grown peanut plants (determined by plating methods) infection rates for observed root infections, and the relationship between microsclerotial inoculum density and the number of observed infections. In addition, estimates are made for efficiency of inoculum for observed infection, and efficiency of observed infection for necrosis (percent of infections that develop into necroses). Portions of the information reported here have been presented in a preliminary report (25).

MATERIALS AND METHODS

Field study. In September 1979, in Southampton County, VA, a plot measuring 4.6 m long by 3.9 m (four rows) wide was established in an area of a peanut field in which the plants were showing CBR symptoms. The field was known to be infested with *C. crotalariae* since 1974. All plants in the plot were excavated, the roots were washed, and the plants were rated for shoot symptoms, root rot, and discoloration. A composite soil sample was made from soil cores (2 cm diameter × 20–25 cm deep) taken every 30.5 cm, in each of the three interrow areas. After transport to the laboratory and thorough mixing, the soil sample was assayed for microsclerotia of *C. crotalariae* by the method of Griffin (8). Asymptomatic root systems of 10 plants were washed for an additional 25 min in running tap water and the entire root systems were cut into portions as extensive (long) as possible for plating on 9-cm-diameter petri plates containing sucrose-QT medium (8). For each plant the total length of plated roots (fine, lateral, and taproots) was measured with a ruler. Colonies of *C. crotalariae* growing from roots were counted after 5–7 days of incubation at 25 C. Extreme care was taken to prevent duplicating the recording of colonies that originated from both ends of a cut root or that were closely associated but were not clearly discrete units. The number of discrete colonies of *C. crotalariae* growing from asymptomatic roots was used as a measure of the number of observed apparent infections (hereafter referred to as observed infections).

TABLE 1. Number of observed infections caused by *Cylindrocladium crotalariae* on asymptomatic taproots, lateral, and fine roots of 10 field-grown cultivar Florigiant peanut plants after 15 wk

Plant code	Root length (m)	Observed infections:			
			Per meter of root		
		Per plant	Fine	Lateral + taproots	All roots
A	4.4	52	5.0	24.0	11.8
B	8.2	11	0.5	2.6	1.3
C	2.7	0	0.0	0.0	0.0
D	4.4	1	0.0	0.4	0.2
E	5.9	114	14.5	25.0	19.3
F	7.8	0	0.0	0.0	0.0
G	4.3	0	0.0	0.0	0.0
H	4.1	27	1.2	1.4	6.6
I	5.4	0	0.0	0.0	0.0
J	4.1	13	4.3	1.3	3.2

Infection rate and relationship between microsclerotial inoculum density and the number of observed infections. Generally, in the greenhouse studies, *C. crotalariae*-free peanut-field soil (sandy loam, pH = 5.2) was mixed with naturally infested soil containing high populations of microsclerotia of *C. crotalariae* to obtain the desired inoculum densities. Initial microsclerotial populations were determined by the method of Griffin (8). Soil with high microsclerotial populations was obtained from the field by excavating the root zones of plants with symptomatic shoots, removing intact roots, and mixing the soil thoroughly before using it as a source of inoculum. Thiram-treated peanut seeds of CBR-susceptible cultivars (Florigiant or VA-72-R) were planted four per 1-L plastic container (11-cm diameter). These pots were placed in a temperature tank at 25 C on a floating apparatus with a manifold underneath to allow for water drainage from the pots. Plants were watered daily or as needed to maintain the moisture level near field capacity. Following the plant-growth period, plants were removed gently and washed free of remaining soil in the same manner as used in field studies. Shoot symptoms, as well as percentage root necrosis and discoloration, were noted for each plant. In the infection-rate study, observed infections per plant were determined on 10, 10, and four asymptomatic root systems for the 21-, 42-, and 63-day periods, respectively. For each period, plants were removed from pots until 10 asymptomatic root systems were obtained. Estimated infections of Vanderplank (26,27), determined from $\log_e (1/1-y)$ in which y is root rot incidence, were based on 14, 29, and 58 plants for the same three periods. In the inoculum-density experiment, entire root systems of three plants with asymptomatic roots were plated for each of the microsclerotial densities after 21 days. Observed infections were determined from plated entire asymptomatic root systems, as described previously. Root lengths (fine, lateral, and taproots) were measured for each plant with a ruler.

Lesion development by *C. crotalariae* isolates from observed infections. Isolates of *C. crotalariae* obtained from asymptomatic roots and necrotic portions of roots were grown on plates of Hunter's medium (15) in agar for at least 6 wk at 25 C. Strips of agar medium (1 × 3 cm), containing mature microsclerotia, were washed for 8 hr in running tap water to remove exogenous nutrients and enzymes before they were macerated in a blender and wet-sieved on a 25-μm sieve for 10 min. Microsclerotia were suspended in enough distilled water to give a concentration of 150 microsclerotia per 0.5 ml for each isolate. A 0.5-ml aliquot was pipetted onto and spread over washed 0.5 × 1.0-cm strips of water agar. The strips of microsclerotial inoculum were placed on a glass plate (root-slide) designed by Krigsvold (17). The agar strip was covered with a plastic screen and held in place with rubber bands. A glass rod, bent to form a "V," was used for guiding the peanut taproot toward the agar strip of microsclerotia. The root-slide was placed at an acute angle in a 1-L plastic pot, having a window on one side (hereafter called a windowed pot) to monitor the growth of the taproot, root necrosis and root discoloration. Unsterile peanut-field soil free of *C. crotalariae* was placed on top of the root-slide in the windowed pot. A pregerminated peanut seed (radicle length, 1.5 cm) was placed in each pot. Plants were subirrigated twice daily and incubated at 25 C in a growth chamber with a 14-hr photoperiod at 4,500 lux. After 2–3 wk of incubation, depending on taproot growth rate, each plant was carefully removed, washed free of soil, and lengths of each necrotic and discolored area of the taproot were measured. Symptomatic tissue was plated on sucrose-QT medium (8) for isolation of the pathogen.

RESULTS

Infection of field-grown peanut plants by *C. crotalariae*. Of the 80 15-wk-old peanut plants excavated from the field plot in September 1979, 41% had root necroses, while only 25% of the plants had CBR shoot symptoms (chlorosis, wilting, or necrosis).

Surface discoloration of fine roots, commonly observed in field- or greenhouse-grown peanut plants in the absence of *C. crotalariae,* was not considered a CBR symptom. Six of 10 asymptomatic root systems assayed were colonized by *C. crotalariae* and had a mean of 36.3 observed root infections per infected plant (range, 1 to 114); observed infections were found on fine roots as well as on the taproots and main lateral roots (Table 1). Four of the 10 asymptomatic root systems were not colonized by *C. crotalariae.* The inoculum density of the composite sample of soil cores from the plot was 0.2 microsclerotia per gram of soil.

Infection rate and disease progress. The disease-progress curves (Fig. 1) showed an increase in the number of observed infections and estimated infections ($\log_e [1/1-y]$, in which y is equal to the proportion of peanut plants with rot) over time. Root length increase between 21 and 63 days is indicated also in Fig. 1. The average number of observed infections per plant were 161 at 21 days, 470 at 42 days, and 1,080 at 63 days. The number of estimated infections per plant were 0.33 at 21 days, 1.05 at 42 days, and 2.66 at 63 days. The incidence of root rot (not shown in Fig. 1) was 28.3% at 21 days and 93.0% at 63 days. Based on interpolation of the infection-rate curves, each plant had about 300 observed root infections when 50% of the plants had root necroses (equals estimated infection value of 0.693). The infection rate for observed infections on a unit-root-length basis, I_r^o, increased slightly for each of the three 21-day periods. Calculated values of 0.120, 0.162, and 0.199 observed infections per meter of root per day per microsclerotium per gram of soil were obtained for the first, second, and third 21-day periods, respectively. On a per-plant basis, the two infection rates, R_o, for observed infections, and R_e, for estimated infections (26,27), increased greatly over the three 21-day periods. Values for R_o were 0.84, 1.62, and 3.19 observed infections per plant per day per microsclerotium per gram of soil for the first, second, and third 21-day periods, respectively. Values for R_e (based on $\log_e [1/1-y]$ in which y is the proportion of the plant population with root necrosis) were 0.0017, 0.0038, and 0.0084 estimated infections per plant per day per microsclerotium per gram of soil, for the first, second, and third 21-day periods, respectively. In these and other greenhouse tests, observed infections were distributed over all portions of the root system and many were located near the root tips. No perithecia of *Calonectria crotalariae* (Loos) Bell & Sobers were visible on any plants in the course of the experiments.

Surface-sterilization tests. To determine if observed infections were restricted to the outer cortical tissues of peanut roots (peanut root has no epidermis), washed roots were treated with two concentrations of NaClO. Generally, there was a reduction in the number of observed infections per meter of root length for roots treated with the two concentrations of NaClO (0.1 and 0.25%), compared to the washed controls (Table 2). Overall, treatment of five entire root systems with 0.1% NaClO gave 24.3% reduction of observed infections, compared to the washed controls, and treatment with 0.25% NaClO resulted in a 43.7% reduction. Despite this reduction in observed infections per meter of root for NaClO-treated root systems, the average number of observed infections per meter of root was not significantly different from that of the washed controls ($P = 0.05$). Thus, most observed (apparent) infections were probably not limited to surface cortical cells.

Inoculum density experiments. One or more observed root infections were found on all except one of the plants in the

TABLE 2. Effects of two concentrations of NaClO on the number of observed *Cylindrocladium crotalariae* infections on asymptomatic root systems of peanut

	Observed infections per meter of root[a]			
Root type	Washed[b]	0.1% NaClO[c]	Washed[b]	0.25% NaClO[c]
Fine	11.6	8.0	23.1	13.1
Lateral	31.2	22.0	53.1	32.2
Taproots	21.0	24.0	50.3	20.3
All roots	23.0	17.4[d] (−24.3%)	37.8	21.3[d] (−43.7%)

[a] Five root systems were used for each treatment.
[b] Washed in running tap water for 25 min.
[c] Washed in running tap water for 25 min plus treatment with NaClO for 30 sec.
[d] Data are not significantly different from washed root systems, according to the F-test ($P = 0.05$). The percentage reduction in number of observed infections per meter of root due to treatment with NaClO is indicated in parentheses.

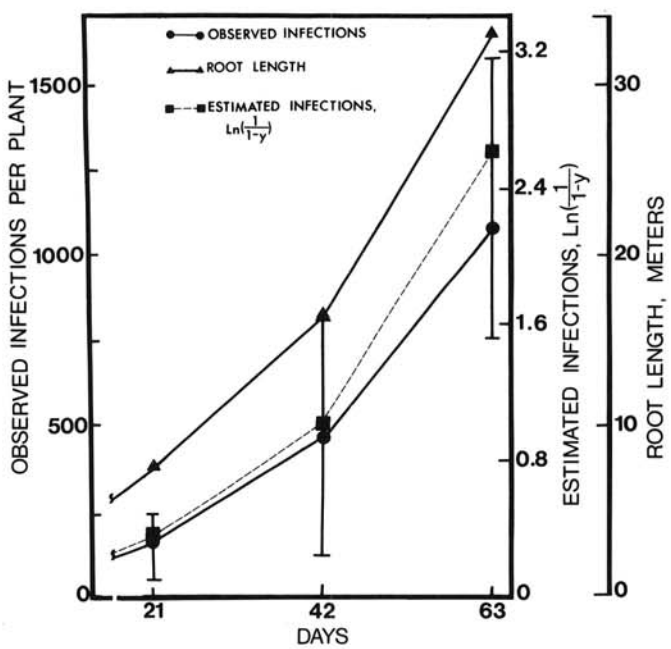

Fig. 1. Number of observed root infections caused by *Cylindrocladium crotalariae* per cultivar Florigiant peanut plant, estimated root infections per plant (calculated from $\log_e [1/1-y]$, y is based on proportion of root systems with necrosis), and root length per plant after 21, 42, and 63 days at 25 C. Vertical bars for each of the three periods represent the range of observed infections per plant. The microsclerotial density was 9.1 microsclerotia per gram of soil.

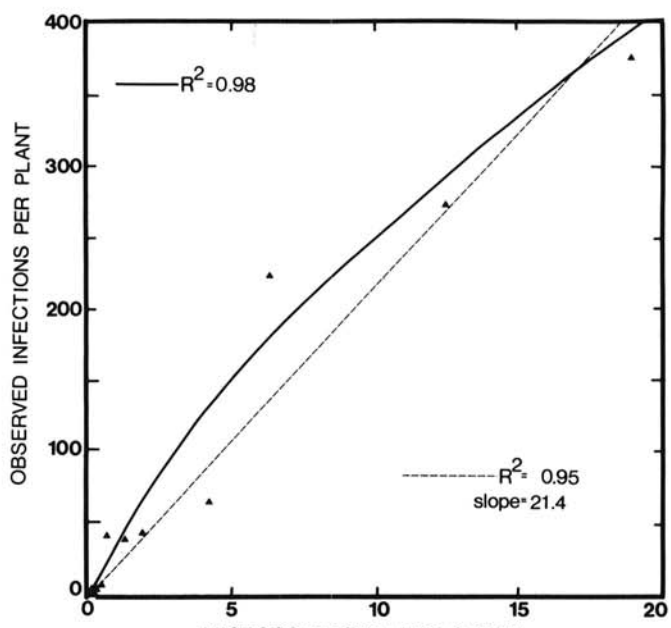

Fig. 2. Arithmetic plot of first-order (----) and second-order (——) linear regression curves for the relationship between numbers of observed infections caused by *Cylindrocladium crotalariae* per cultivar Florigiant peanut plant and numbers of microsclerotia per gram of soil. Values are the means of three replicates. Curves were forced through the origin. The first- and second-order equations were $y = 21.4X$ and $y = 29.6X - 0.52X^2$, respectively.

inoculum density experiment after 21 days. For the arithmetic plot, first-order regression equation analyses indicated the average number of observed infections per plant increased in direct proportion to the microsclerotial density (Fig. 2). The slope value of this straight line is 21.4 with an R^2 value of 0.95. A similar relationship was found also in an arithmetic plot (Fig. 3) of observed infections per meter of root vs the inoculum density (slope = 2.3, $R^2 = 0.95$). As there appeared to be some apparent curvature in the data of both plots, second-order regression (quadratic) equations also were examined (3). For the second-order regression equations, there was more curvature in the regression line in the upper region of the curve for the infections-per-meter-of-root plot than in the infections-per-plant plot. Table 3 shows the results of statistical analyses designed to test the fitness of the first-order and second-order regression equations. The robust form (14) of both equations deemphasized the five highest inoculum density data points (upper region of the curve) most removed from the regression lines, as these points strongly influenced regression analysis. Based upon parameter estimates and on error and R^2 values, both the robust form and the least-square form of the second-order regression equations gave a better fit than the first-order equation. However, when the highest inoculum density data point was omitted from the data set, the coefficient for the X^2 term in the second-order regression equation was not significant ($P = 0.05$). Thus, the first-order regression equation appeared to be the best fit. At 3 wk, approximately the same number of observed infections per plant (195) are predicted by the first-order equation for 9.1 microsclerotia per gram of soil, as was found in the time-course experiment that utilized this inoculum density (Fig. 1).

Log_{10}-log_{10} plots of microsclerotial inoculum density vs observed infections per plant and per 10 m of root are shown in Figs. 4 and 5,

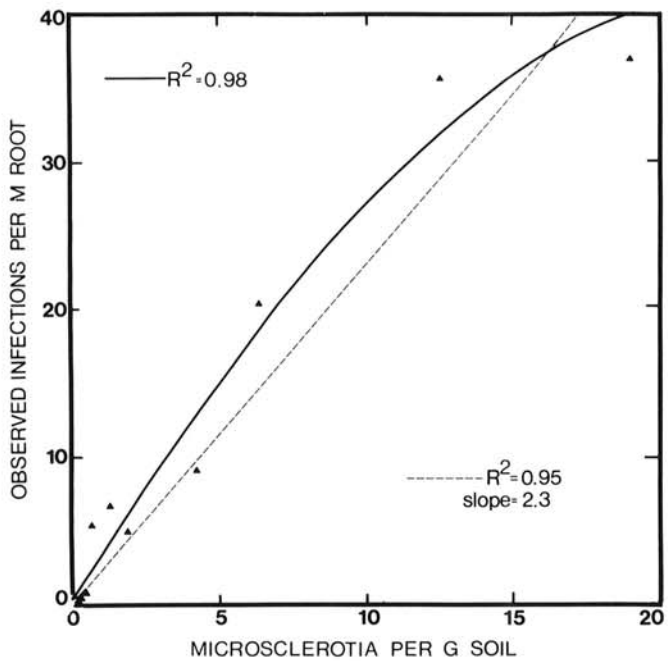

Fig. 3. Arithmetic plot of first-order (----) and second-order (——) linear regression curves for the relationship between numbers of observed infections caused by *Cylindrocladium crotalariae* per meter of root and microsclerotia per gram of soil. Values are the means of three replicates. Curves were forced through the origin. The first- and second-order equations were $y = 2.3X$ and $y = 3.7X - 0.09X^2$, respectively.

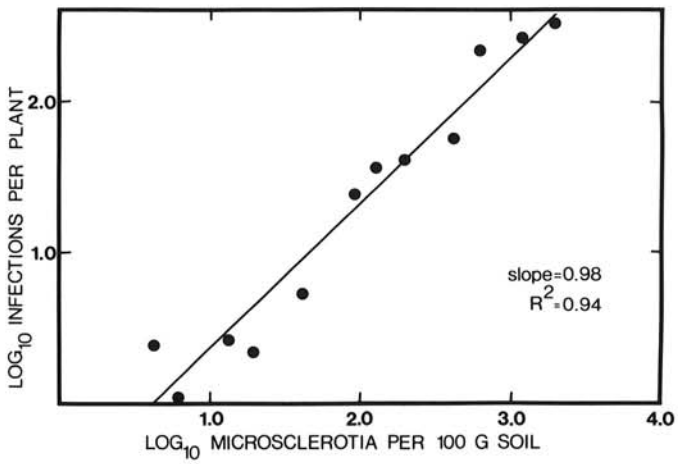

Fig. 4. Relationship between log_{10} numbers of observed infections caused by *Cylindrocladium crotalariae* per cultivar Florigiant peanut plant and log_{10} of the number of microsclerotia per 100 g of soil. Values are the means of three replicates.

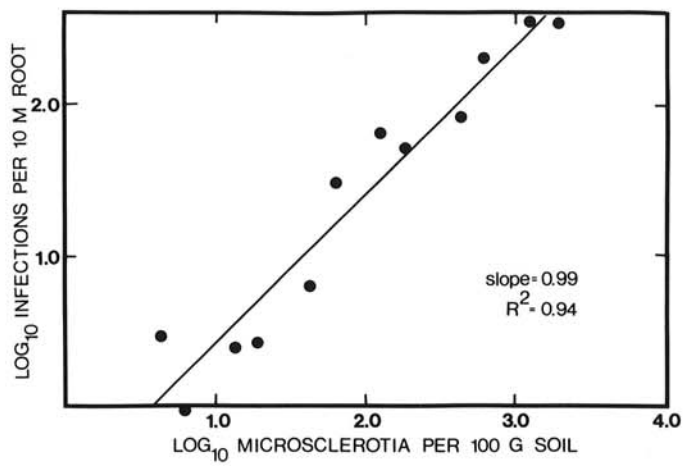

Fig. 5. Relationship between log_{10} numbers of observed infections caused by *Cylindrocladium crotalariae* per 10 m of root and log_{10} of the number of microsclerotia per 100 g of soil. Values are the means of three replicates.

TABLE 3. Statistics of first- and second-order regression analyses of the relationship between the number of *Cylindrocladium crotalariae* observed infections per meter of root and microsclerotial density

Regression equation		Sum of absolute error	R^2	Variable	Estimate
$y = bX + E$	Least-square form	28.96	0.95	X	2.30
	Robust form	28.91		X	2.26
	Least-square form[a]	13.90	0.98	X	2.89
$y = b_1X + b_2X^2 + E$	Least-square form	18.85	0.98	X	3.72
				X^2	−0.09
	Robust form	17.97		X	3.87
				X^2	−0.10
	Least-square form[a]	13.70	0.98	X	2.99
				X^2	−0.009[b]

[a] Includes all data points except highest inoculum density.
[b] Parameter estimate is not significant ($P = 0.05$).

respectively. Both plots had slope values near 1.0, again indicating that the number of observed infections increased in direct proportion to inoculum density. An R^2 value of 0.94 was obtained for each of the data sets of Figs. 4 and 5. Based on the models of Baker et al (1,4), a rhizosphere host-pathogen interaction is indicated by the slope values obtained.

Lesion development on taproots. The results in Table 4 show the average lengths and average rates of lesion development for four isolates of *C. crotalariae,* each obtained from observed infections or from lesions. Lesion isolates produced higher lesion growth rates and larger lesions than observed-infection isolates, but the differences in both cases were not significantly different ($P = 0.05$).

Efficiency of inoculum and efficiency of infection. The estimate of inoculum efficiency for observed infection of temperature-tank-grown (25 C) VA-72-R peanut plants by germinating microsclerotia of *C. crotalariae* after 3 wk was high (103%). This estimate was based on the data that Krigsvold (17) obtained for percent microsclerotium germination (39.8%) in a 1-mm-wide zone of rhizosphere soil collected from defined regions of VA-72-R peanut root tips at 25 C, and on data obtained in the present study, which included root length per VA-72-R plant, and the number of observed root infections per VA-72-R plant. The infection rate, I_r°, for VA-72-R plants (0.091 to 0.108 observed infections per meter of root per day per microsclerotium per gram of soil) was somewhat lower than that obtained for Florigiant plants. The volume of rhizosphere soil that root tips pass through, as they grow, was calculated from the mean plant root length, the radius of the root tip plus rhizosphere soil (1.13 mm) and the radius of the root tip alone (0.13 mm). The weight of rhizosphere soil was calculated from the bulk density (1.4 g/cm^3) of the soil and the volume of the rhizosphere soil. The number of microsclerotia germinating per plant was calculated from the percent germination data of Krigsvold (17), the inoculum density (9.1 microsclerotia per gram of soil), and the weight of rhizosphere soil per plant. Efficiency of inoculum was calculated as follows:

Efficiency of inoculum for observed infection $= (O_{t_i}/G_{t_i}) \times 100$ in which O_{t_i} represents the number of observed infections per plant at time, t_i; and G_{t_i} represents the estimated number of microsclerotia germinating in a 1-mm zone of rhizosphere soil per plant at time, t_i. The time between microsclerotium germination and infection (infection time) is estimated to be short and should not critically influence these calculations. That the inoculum efficiency estimate was greater than 100% may be due, in part, to a low estimate for percentage microsclerotium germination; high propagule densities are required for these tests and this can lower percent germination in soil (9). Also, as developed later, infection courts other than the root tip may have played a role in root colonization.

Since the increases in the two infection rates, R_o and R_e, were somewhat similar, and since $\log_e (1/1-y)$ may be a good estimate of those observed infections that develop into necroses, it may be reasonable to develop the following estimate for efficiency of observed infection for necrosis:

Efficiency of observed infection for necrosis $= (\log_e (1/1-y)_{t_2})/(O_{t_1}) \times 100$ in which the numerator represents the estimated number of infections per plant at a comparable time, t_2; and the denominator represents the number of observed infections per plant at a given time, t_1, where $t_2 - t_1$ is equal to the incubation period minus the germination period and infection time estimate (= incubation period − [germination period + infection time]). We used $\log_e (1/1-y)$, estimated infections per plant, to calculate this efficiency estimate since we were unable to reliably count discrete lesions due to *C. crotalariae* on peanut roots in any of the tests conducted in this investigation. Based on the lesion growth-rate experiment and the data of Krigsvold (17), $t_2 - t_1$ was estimated to be 5 days. Infection efficiency estimates for the experiment reported in Fig. 1 ranged from 0.27 to 0.28% for the three 21-day periods. An efficiency of *inoculum* for necrosis estimate for VA-72-R plants after 3 wk, calculated with $\log_e (1/1-y)$, was 0.85%.

DISCUSSION

Multiple observed infections of *C. crotalariae* were found over all areas of asymptomatic fine, taproots, and lateral roots of field- and greenhouse-grown peanut plants. Many infections were located near the root tips which, according to the observations of Krigsvold (17), are probably the primary infection courts. Thus, as the root tip grows, the infection site becomes further removed from the root tip. Based on the root surface-sterilization results, most observed root infections probably were not limited to surface cortical cells.

Previous fungal root-colonization studies used root segments 2 cm (5,6,19) or smaller (19,20). For plant pathogens, overestimating the number of observed infections could result from plating short segments, instead of mostly uncut root systems, especially at high inoculum densities. Overestimation of the number of observed infections at high inoculum densities could cause the regression line to curve upward in the upper region of arithmetic inoculum-density plots. This was not observed here for second-order regression curves (Figs. 2 and 3).

The number of observed infections per asymptomatic peanut root system was generally more variable for field-grown plants than for greenhouse-grown plants. That some plants from the field plot were not colonized by *C. crotalariae* may have been due to the clumped or nonrandom *C. crotalariae* microsclerotial pattern found previously in this peanut field (24). For the greenhouse tests, microsclerotia-infested soil was thoroughly mixed previous to all experiments, which probably precluded clumping of inoculum.

The disease-progress study indicated that the infection rate, I_r°, for observed infections increased over the three 21-day periods of the experiment. R_e and R_o increased greatly over the same period. In contrast to I_r°, Vanderplank's (26) infection rate ($R \approx R_e$) does not allow for increased rates of host root length growth in simple-interest disease. The value of R_{ot}, where R_o is modified from Vanderplank's equation by using the number of observed infections per plant, instead of $\log_e (1/1-y)$, is equivalent to $I_{rl}^\circ l_t$, where l_t is the change in length of root per plant after any time, t. To our knowledge, I_r° is the first measurement of an infection rate for a root-infecting fungus that is based on observed root infections and is expressed on a unit-root-length and unit-inoculum basis. In inoculum-potential studies, I_r° should be more closely related to the

TABLE 4. Lengths and rates of necroses on peanut taproots caused by lesion and observed-root-infection isolates of *Cylindrocladium crotalariae* at 25 C

Isolate source	Isolate number	Length of necrosis[a] (mm)	Overall mean[b] (mm)	Rate of lesion development[a] (mm per day)	Overall mean[b] (mm per day)
Observed root infections	N3	4.3		0.2	
	N4	31.4		1.2	
	N10	20.0		0.8	
	N13	45.3	25.3 A	1.8	1.0 A
Root lesions	R13	31.4		1.2	
	R40	47.0		2.3	
	R39	31.6		1.2	
	R44	36.2	36.6 A	1.3	1.5 A

[a] Based on three replicates for each isolate on cultivar Florigiant peanut plants.
[b] Means followed by the same letter are not significantly different ($P = 0.05$).

effect of capacity factors on inoculum than R_o or R_e, since I_r° takes account of host growth rate. As indicated, I_r° increased over the period of the experiment, which suggests that root exudation or the number of infection sites per unit root length increased with time. This may result from root diameter growth and associated exudation (10) or from emergence of lateral and fine roots through the root cortex; there is more root branching as peanut root systems get older and these root emergence points may be sites of root exudation (12) and infection.

In absolute terms, the multiple-infection correction (1,26,27) greatly underestimated the number of observed root infections per plant, but appeared to be roughly proportional to it. Estimated infections never exceeded a mean of three per plant, whereas observed root infections reached over 400 times this value. This appeared to be so because most observed root infections did not progress to necroses within a short period of time; ie, incubation period minus the time required for microsclerotium germination and host infection. That most observed infections did not cause necrosis may have been due to low inoculum potential of individual microsclerotia (vs many microsclerotia on water agar strips in the lesion tests), differences in host resistance or disease proneness among plants (Florigiant is a multiline cultivar), and/or differences in pathogenicity among *C. crotalariae* isolates. The lack of necrosis resulting from observed infection is reflected in the low estimate of efficiency of infection for necrosis (avg, 0.28%), or efficiency of *inoculum* for necrosis, and this appeared to be much more limiting to disease development than was inoculum efficiency for observed infection, for which a high estimate was obtained. The latter is in agreement with the lack of appreciable *C. crotalariae* germ tube lysis in the peanut rhizosphere observed by Krigsvold (17). Use of estimated infections, from the proportion of necrotic roots, to calculate efficiency of infection is presented here as a preliminary approach to estimating efficiency of infection for necrosis, and may result in underestimation. As indicated, the opposite occurred for the efficiency of inoculum for observed infection estimates. To our knowledge no previous attempt has been made to calculate efficiency of infection or efficiency of inoculum (based on rhizosphere germination data) for root-infecting fungi.

The number of observed infections per plant, as well as observed infections per unit root length, increased in direct proportion to the inoculum density in the arithmetic plot. Although data points may be interpreted as fitting a curve with a decreasing slope, especially in Fig. 3, the $\log_{10}$-$\log_{10}$ plots of the same variables (Figs. 4 and 5) predict a straight-line relationship (3), since the slopes for each of the logarithmic plots were approximately 1.0. Furthermore, the results in Table 3 show the linear relationship to be the best fit, assuming that little confidence is placed in the highest inoculum density. The latter may have resulted in competition for, or overlapping of, infection sites. That the slope of the curve did not increase suggests that observed infection development from root-root contacts was probably not a factor at high inoculum densities. The inoculum density values required for 50% infection (ED_{50}) at 21 days were below 0.04 microsclerotia per gram of soil, since all plants examined at this inoculum density had observed infections.

The slope values of the two $\log_{10}$-$\log_{10}$ plots are close to 1.0 which suggests that the inoculum-host interaction follows a rhizosphere influence, as predicted by Baker et al (1,4). Other investigators (13,21,24) using $\log_{10}$ of estimated infections ($\log_e [1/1-y]$) plotted against $\log_{10}$ microsclerotia per unit of soil observed slope values closer to 0.67. Although two studies were field studies, where experimental conditions were not optimal, the 0.67 slope suggests a rhizoplane or root-contact influence (1,4). That Krigsvold (17) observed greater than 33% microsclerotium germination in the inner 1-mm layer of rhizosphere soil of both CBR-susceptible cultivar VA-72-R and CBR-resistant cultivar Argentine peanut root tips suggests that microsclerotium germination is restricted to near the root surface, but is not limited to contact of microsclerotia with the root surface. Further research is required to clarify why slopes of 0.67 or lower have been obtained in other studies. Possibly, only large microsclerotia or several microsclerotia in an organic matter particle that are in contact with the peanut root surface (as in the water-agar-strip tests), where exogenous nutrients (exudates) are high and the inoculum potential would be greatest, give rise to what Garrett (7) and Baker (1) consider to be *successful progressive* infections and *successful* infections, respectively. These result in symptoms and may be estimated by $\log_e (1/1-y)$. Underestimation of infections by $\log_e (1/1-y)$ does not appear to be a critical factor (1,2,26,27), as disease incidences were low in previous studies. In a field study, a clumped inoculum pattern in the field appeared to contribute to lowering $\log_{10}$-$\log_{10}$ slope values for this pathogen-host combination (24). Capacity factor differences may be important also (2).

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