Relationships Among Inoculum Density, Microsclerotium Size, and Inoculum Efficiency of *Cylindrocladium crotalariae* Causing Root Rot on Peanuts

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Use of trade names does not imply endorsement by the North Carolina Agricultural Research Service of the products named or criticism of similar ones not mentioned.
Journal Series Paper 8995 of the North Carolina Agricultural Research Service, Raleigh 27650.
The technical assistance of Joyce Hollowell and Jim Nichols, and helpful manuscript suggestions by B. B. Shew are acknowledged.
Accepted for publication 10 April 1984.

ABSTRACT

Black, M. C., and Beute, M. K. 1984. Relationships among inoculum density, microsclerotium size, and inoculum efficiency of *Cylindrocladium crotalariae* causing root rot of peanuts. Phytopathology 74:1128-1132.

Relationships between root rot severity on peanut (*Arachis hypogaea*) and inoculum density of *Cylindrocladium crotalariae* for different microsclerotia size categories were examined in greenhouse studies. Root rot severity at the same inoculum density was more severe for large microsclerotia (>150 μm minimum dimension). Cropping sequences of susceptible and resistant peanuts and of soybeans in infested field microplots for 2 or 4 yr did not consistently result in different microsclerotial sizes. On the average, microsclerotia in microplots were larger at low inoculum densities than at high inoculum densities.

Variation in severity of peanut (*Arachis hypogaea* L.) root rot caused by *Cylindrocladium crotalariae* (Loos) Bell & Sobers is partially explained by preseason inoculum densities of this pathogen in soil (3,7). Diomande and Beute (7,8) reported that when the plant parasitic nematodes *Meloidogyne* and *Criconemella* were present in soil with *C. crotalariae*, more variation in root rot severity was accounted for than by fungus inoculum density alone. Soil microflora, particle size, nutrients, temperature, and moisture as well as pathogen virulence also influence peanut root rot severity caused by *C. crotalariae* (3–5,15,16,19). Propagule size may also affect severity. Disease caused on bean (*Phaseolus vulgaris* L.) by *Rhizoctonia solani* was more severe with large than with small propagules (13).

Size of plant pathogen survival structures may vary under different management, environments, and/or with inoculum density. The reported mean size of microsclerotia (ms) of *C. crotalariae* was 52 × 74 μm in Virginia and 70 × 103 and 53 × 88 μm in two reports from North Carolina (7,20). Survival of ms was greater at low initial inoculum densities than at high initial inoculum densities of *C. crotalariae* (5).

Chuang and Ko (6) found an inverse linear relationship between the logarithm of propagule size and the logarithm of maximum reported population density among bacteria and fungi in soil. They concluded that a genetically determined relationship exists between mean propagule size and maximum density for a given species (6). Perhaps mean size varies among environments and among various population densities for individual species. Mean size variation within plant pathogenic species could be associated with variation in the efficiency of propagules in inducing disease and with variation in propagule longevity. Factors influencing propagule size within species have not been identified.

The objective of this study was to determine if size of ms of *C. crotalariae* is related to inoculum efficiency, and if variation for mean ms size occurs following different cropping sequences.

MATERIALS AND METHODS

Effect of ms size on inoculum efficiency. For test A, eight isolates of *C. crotalariae* were obtained from peanut root lesions and transferred every 3–4 mo on potato-dextrose agar (PDA) cultures for 12 mo before use. The isolates were grown on PDA for 4 wk, and ms were extracted separately for each isolate (18) and washed through five nested sieves with 150-, 106-, 75-, 53-, and 38-μm openings. The numbers of ms collected on each sieve were estimated (17) and for each of the five size ranges, equal numbers of ms from each isolate were combined in nonsterile Norfolk loamy sand free of *C. crotalariae* to give inoculum densities of 0, 0.08, 0.25, 0.8, 2.5, 8, and 25 ms/g soil. For each ms size × inoculum density combination, six plastic cylinders (inside diameter 3.5 cm, length 15.5 cm) with plastic mesh on one end were filled with infested soil. One 3-day-old Florigiant peanut seedling was transplanted into each cylinder and grown in a greenhouse at ~25 C with subirrigation. Root rot was visually estimated after 5 wk on a 0–5 scale: 0 for no lesions, 1 for few lesions on secondary roots and/or a few small lesions on the taproot, 3 for many lesions on secondary roots and many lesions on the taproot and with several secondary roots missing, and 5 for completely rotted roots with most secondary roots and part of the taproot missing. Ratings of 2 and 4 were for intermediate levels of severity. A randomized complete block design with six replications was used (tests A and B).

For test B, four isolates (isolated from peanut root lesions 1 mo before testing) were used separately to establish ms size × inoculum density combinations. Three ms size categories were obtained with nested 150-, 106-, and 53-μm sieves. As previously described, four inoculum densities (0, 0.5, 5, and 50 ms/g) for each isolate and for each ms size were established in soil free of *C. crotalariae*. One Florigiant seedling was transplanted into each cylinder.

Establishment, infestation, and sampling of field microplots. Microplots (76-cm diameter) were established (2) in April 1979 in a Norfolk loamy sand at Central Crops Research Station, Clayton, NC, to study effects of monoculture and crop rotation on severity of Cylindrocladium root rot of peanuts (3,5). Microsclerotia-PDA-mycelium suspensions prepared by blending were dispensed in microplots and mixed to a depth of 20 cm with shovels in 1979. Twelve isolates were used to infest soil (18) as composite inoculum or individually at ~35 ms/g soil in June 1979. Crops were planted in June 1979 and in May for 1980–1982 and managed as described (3,5). There were three plants per plot in 1979–1981 and six plants per plot in 1982. These populations of *C. crotalariae* were examined in this study for variation in mean sizes of ms.

Soil in microplots infested with a composite of 12 isolates was sampled in January 1981 as described below after two consecutive years of planting susceptible Florigiant peanuts (FF), resistant NC

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3033 peanuts (NN), or Ransom soybeans (SS) (3). There were eight microplots of each treatment (two microplots treated alike were in each of four replications). Soil was sampled in February 1983 after treatments of FFFF, NNNN, and SSNS in 1979–1982 (3). There were four microplots of each treatment and the assay was repeated with another portion of the sample.

Microplots infested with individual isolates were planted in 2- and 4-yr monoculture with susceptible Florigiant or resistant NC 3033 peanuts (5). Soil was sampled from four replications of each isolate × cultivar combination in January 1981 for isolates 110, 508, and 515 (one assay) and in February 1983 for isolates 205, 617, 621, and C12 (assay was repeated with another portion of the sample) (5).

Approximately 500 g soil were sampled from each microplot by inserting a 2-cm-diameter soil probe and withdrawing twenty-five 15-cm vertical cores. Samples were sieved (2.38-mm opening), mixed 1 min by shaking, and stored at ~25 C in closed polyethylene bags until assayed.

Determination of mean ms size. Soil was elutriated (17) and the fraction collected on the 425-μm opening sieve was discarded. The fraction collected on the 38-μm opening sieve was washed through nested 106-, 75-, 53-, and 38-μm sieves. The sample was washed through all sieves for 30 sec, the top sieve was removed, and the material on the remaining sieves was washed for 30 sec, etc. The fraction collected on each sieve was stirred continuously in 200 ml of water, one 10-ml subsample was mixed with 100 ml of semi-selective medium (17), and the agar suspension was poured into sterile petri plates (10 plates per subsample in 1981 and six per subsample in 1983). Colonies of *C. crotalariae* were counted after incubation of the cultures for 5 days.

The dimension of the sieve opening was considered to be the minimum dimension of ms retained on that sieve. In previous reports, the mean least diameter of ms was 60–70% of the mean greatest diameter (10,17,20). For simplification, we assumed that ms are spheres and mean ms size (volume) was estimated by $V = 4/3\pi r^3$, with the minimum value of r determined as one-half the size of sieve opening. The inoculum density (D) for the ith sieve subsample was calculated (17) and multiplied by the minimum V_i calculated for ms retained on that sieve. This product ($D_i \times V_i$) and D_i were summed by soil sample, and the mean ms size (S) calculated as $\Sigma (D_i \times V_i)/\Sigma D_i$.

RESULTS

The ratio of root rot severity to inoculum density decreased as inoculum density increased and nonlinear regression (PROC NLIN, Gauss-Newton Method) (21) was used for data analysis. Model selection was based upon reduction in residual error, improvement in coefficient of determination (R^2), and a near-random distribution of residuals. A Poisson equation was used with parameters modified from Vanderplank (22), $R = a(1-e^{-bD})$, in which R = root rot severity, a = estimated maximum root rot severity for the treatment, $e = 2.71828$ (base of natural logarithm), b = estimated slope, and D = mean inoculum density.

As ms size increased in test A, root rot was progressively more severe at all inoculum densities ($P = 0.05$) except that ms from 75- and 53-μm sieves had similar response curves (Fig. 1). Equations were $R_{150} = 2.3(1-e^{-0.46D})$, $R_{106} = 1.3(1-e^{-0.31D})$, $R_{75,53} = 0.8(1-e^{-0.55D})$, and $R_{38} = 3.3(1-e^{-0.01D})$. The effective dosages of inoculum to give 10% disease severity (ED_{10}, equivalent to the root rot severity rating of 0.5) were 0.53 ms/g for the 150-μm size category, 1.57 ms/g for the 106-μm category, 1.78 ms/g for the 75- and 53-μm size categories, and 16.43 ms/g for the 38-μm size category.

For all four isolates in test B, large ms from the 150-μm sieve caused greater root rot ($P = 0.05$) than smaller ms from 106- and 53-μm sieves, which were similar (Fig. 2). Curve equations were:

isolate 513, $R_{150} = 3.8(1-e^{-3.0D})$ and $R_{106,53} = 3.5(1-e^{-0.4D})$;

isolate 520, $R_{150} = 4.6(1-e^{-1.7D})$ and $R_{106,53} = 4.5(1-e^{-0.5D})$;

isolate 818, $R_{150} = 4.8(1-e^{-6.1D})$ and $R_{106,53} = 4.5(1-e^{-1.0D})$; and

isolate 819, $R_{150} = 4.8(1-e^{-6.0D})$ and $R^{106,53} = 4.1(1-e^{-1.9D})$.

In nearly every case root rot severity did not increase with inoculum densities above 8 ms/g. The effective dosages of inoculum to give 50% disease severity (ED_{50}, equivalent to the root rot severity rating of 2.5) for isolate 513 were 0.36 and 3.13 ms/g, respectively, for the 150-μm size category and for the combined 106- and 53-μm categories. Similarly, the corresponding ED_{50}s were 0.46 and 1.62 ms/g for isolate 520, 0.12, and 0.81 ms/g for isolate 818, and 0.12 and 0.50 ms/g for isolate 819.

The relationship between mean ms size and inoculum density was analyzed by using nonlinear regression analysis (PROC NLIN, Gauss-Newton Method) (21). A model ($S = aD^{-b}+c$) similar to Gregory's (9) model for dispersal gradients was found to fit the data. Parameters used in this report are S = mean ms size, D = inoculum density (including ms of all sizes), b = estimate of slope, c = estimate of asymptote of the curve (minimum ms size), and $(a + c)$ = estimate of mean ms size at $D = 1$ ms/g or at $b = 0$.

Among soil samples taken in 1981 from composite-infested microplots, samples with low inoculum densities tended to have large ms and samples with high inoculum densities generally had small ms (Fig. 3A) even though all samples had more than 7 ms/g of soil (as inoculum density decreased, mean size increased exponentially). No effect of crop sequence on ms size was detected and one equation describes the overall effect, $S = 3.5D^{-0.45}+1.5$ (Fig. 3A).

In 1981, for microplots infested with individual isolates, a similar relationship was observed (Fig. 3B) and ms size tended to be less for NC 3033 treatment (NN) than for Florigiant treatment (FF) at similar inoculum densities. The equations were $S_{FF} = 3.9D^{-0.50}+1.5$ and $S_{NN} = 3.9D^{-0.79}+1.5$. Residual error was not significantly ($P = 0.35$) less than for one-line linear models (Fig. 3A and B).

Similar relationships were observed between ms size and inoculum density for 1983 samples (Fig. 3C and D). In 1983, samples from composite-infested soil, ms size was similar for NC 3033 and soybean plots and these were smaller than for Florigiant plots at similar inoculum densities; ie, considering a separate response to Florigiant was a significant ($P = 0.08$) improvement in the nonlinear model (Fig. 3C) compared to a one-line linear model.

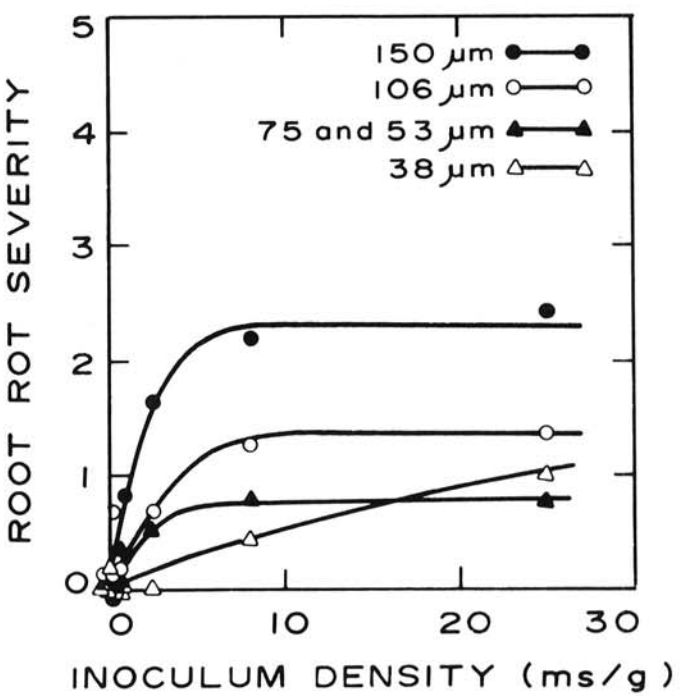

Fig. 1. Mean root rot severity (0 for no lesions, 5 for completely rotted roots) on Florigiant seedlings for microsclerotia (ms) of eight isolates of *Cylindrocladium crotalariae* separated on nested sieves with 150-, 106-, 75-, 53-, and 38-μm openings. Inoculum densities of 0, 0.08, 0.25, 0.8, 2.5, 8, and 25 ms/g soil were established for each ms size category. Root rot response to inoculum density was different ($P = 0.05$) among all comparisons except for ms from 75- and 53-μm sieves.

The equations were $S_{FFFF} = 2.3D^{-0.30}+1.5$ and $S_{NNNN,SSNS}= 1.7D^{-0.51}+1.5$.

In 1983, for individual isolate-infested soil, most NC 3033 plots had low inoculum densities and Florigiant had high inoculum densities. One curve described the response to both cultivars, $S = 1.5D^{-0.33}+2.3$ (Fig. 3D). Residual error was not significantly ($P = 0.21$) less than for a one-line linear model (Fig. 3D).

DISCUSSION

Increased ms size at various inoculum densities caused increased root rot severity in greenhouse experiments with a mixture of isolates (Fig. 1) and with individual isolates (Fig. 2). We conclude that large ms of *C. crotalariae* induced peanut root rot more efficiently than small ms.

"Inoculum efficiency" in this report indicates the relationship between root rot severity with various treatments and inoculum densities. Efficiency is the capacity to produce a given severity of root rot with a minimum number of ms. If following one treatment, root rot was more severe than following a second treatment at the same inoculum density, then the inoculum of the first treatment was considered to induce root rot more efficiently than inoculum of the second treatment. Lesions enlarge and coalesce as this root disease develops. Later, severely rotted portions of the root system disintegrate and are not recoverable from soil. Therefore, a root rot severity scale of 0–5 was used in rating severity rather than counting numbers of infections. "Inoculum efficiency" is used because we feel that severity of root rot reflects various probabilities (among treatments) of getting one lesion for each ms and also various lesion expansion rates (among treatments).

Root rot severity was described as a function of inoculum density by the Poisson equation, $R = a(1-e^{-bD})$. Estimates of a (maximum severity, assumed to be caused by many ms) and b (slope) under the various conditions in this study were positively correlated, and together they can be thought of as descriptions of inoculum efficiency. High values of a and b indicate high inoculum efficiency, and low values indicate low inoculum efficiency. In tests A (Fig. 1) and B (Fig. 2), a and b increased with increased ms size and when inoculum was from isolates of increased virulence. Test A was conducted under greenhouse temperatures higher than optimum for disease development, and isolates used in test A were probably less virulent due to length of time in culture. Consequently, estimates of a and b for test A were lower than for test B. Similar effects on a and b were observed within test B where isolate 513 was less virulent than 520, 818, or 819 (Fig. 2). Suboptimal soil temperature and moisture, suppressive soil microflora, and host resistance have been reported to reduce Cylindrocladium root rot severity (4,5,16). When evaluated over a range of inoculum densities, these factors should reduce estimates of a and b.

Madden (14) notes that estimates of parameters with nonlinear regression are often highly correlated in the "neighborhood" of the least squares solution. Therefore, any parameter (eg, a of the Poisson equation) correlated with another parameter (eg, b) does not have an exact biological meaning independent of that other parameter.

Some factor(s) other than inoculum density limited the maximum severity of disease (Figs. 1 and 2). Factors involved many have included competition among propagules for highly susceptible infection sites, lack of response to multiple infections (11,22), duration of the experiment, and/or level of fungus virulence. Plant defense mechanisms may limit the height of the plateau of the curve. Harris and Beute (12) placed one, or groups of two or four ms adjacent to peanut roots (somewhat similar to increased ms size in this study) and observed that plant defense mechanisms were more often overcome as the number of ms increased. The small ms of low virulence used in this study (Fig. 1) were apparently within a range of conditions (propagule size,

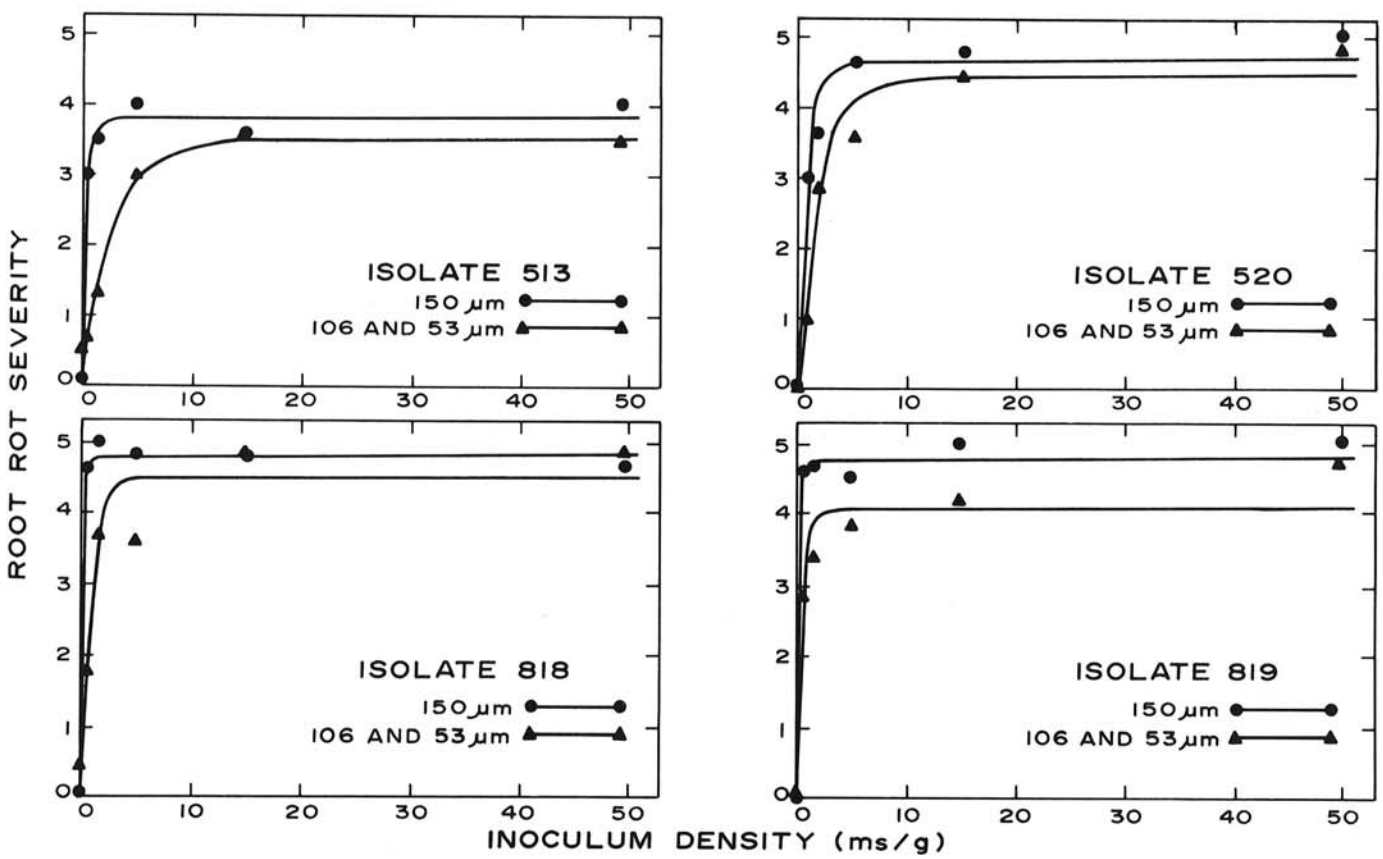

Fig. 2. Mean root rot severity (0 for no lesions, 5 for completely rotted roots) on Florigiant seedlings for microsclerotia (ms) of *Cylindrocladium crotalariae* (isolates 513, 520, 818, and 819) separated on nested sieves with 150-, 106-, and 53-µm openings. Inoculum densities of 0, 0.5, 5, and 50 ms/g soil were established for each ms size category. For each isolate, the root rot severity response to inoculum density for ms from the 150-µm sieve was greater ($P = 0.05$) than for ms from 106- and 53-µm sieves.

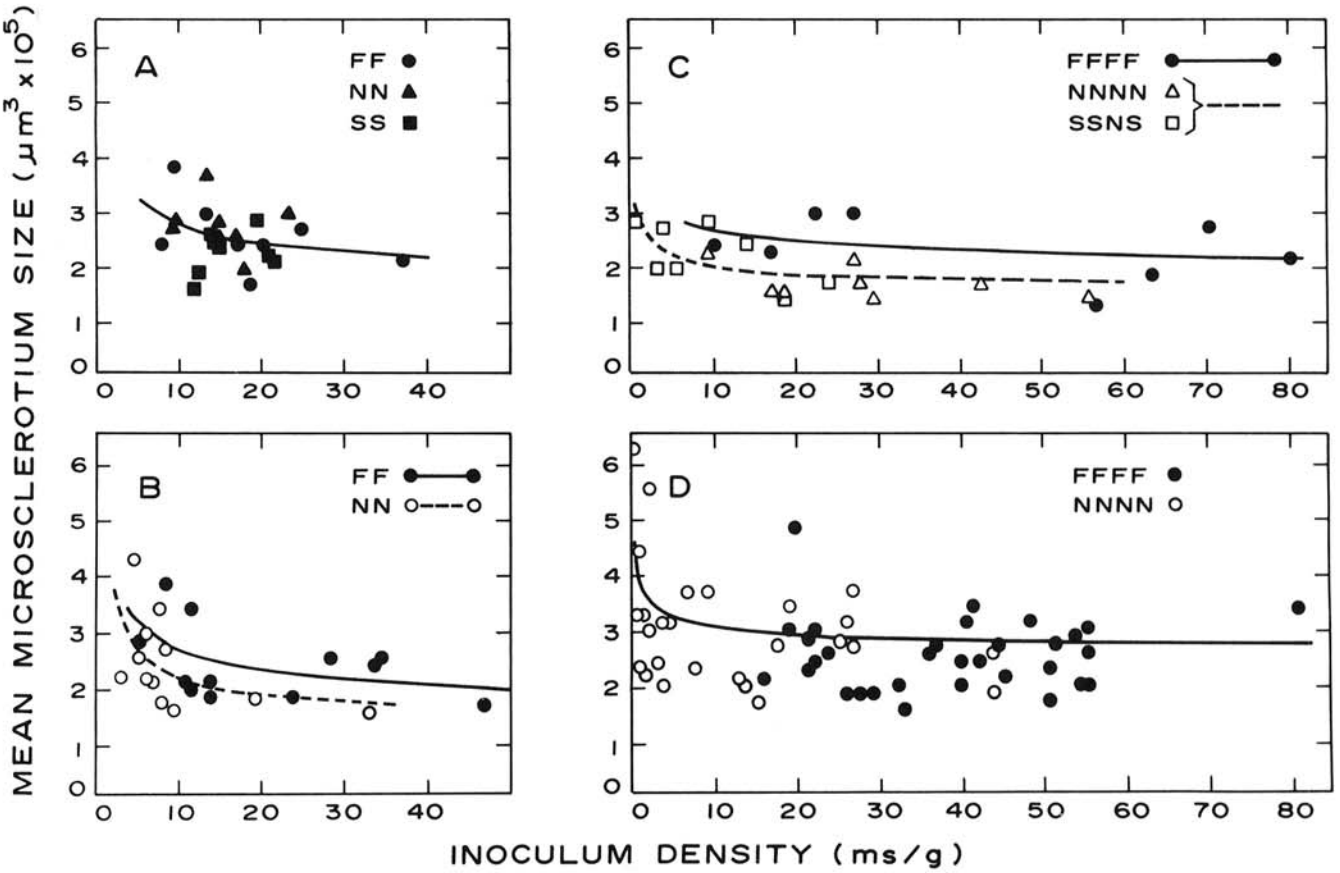

Fig. 3. Mean microsclerotium (ms) size of *Cylindrocladium crotalariae* in relation to inoculum density. Initial inoculum density was approximately 35 ms/g in June 1979. **A,** January 1981 following 2 yr of growing susceptible Florigiant peanuts (FF), resistant NC 3033 peanuts (NN), and soybeans (SS) in soil infested with a composite of isolates. All treatments had similar effects on ms size. **B,** January 1981 following 2 yr of growing Florigiant (FF) or NC 3033 (NN) in soil infested with individual isolates. **C,** February 1983 following 4 yr treatments of Florigiant (FFFF), NC 3033 (NNNN), or soybeans with one season of NC 3033 (SSNS), in soil infested with a composite of isolates. NNNN and SSNS had similar effects and are represented by one curve. **D,** February 1983 following 4 yr treatments of Florigiant (FFFF) or NC 3033 (NNNN) in soil infested with individual isolates. Both treatments had similar effects on ms size.

virulence, and time elapsed) under which defense mechanisms of Florigiant peanuts were effective.

Grogan et al (11) proposed that as distance increases between propagule and infection site, a continuum of effects exists, which expands the rhizoplane and rhizosphere effects proposed by Baker (1). As propagule size increases, infections may be initiated from greater and greater distances in soil (11), depending upon the pathogen. Results in this study of more severe root rot with increased ms size are compatible with the ideas of Grogan et al (11).

The host may influence the size of ms formed. Mean ms size tended to be smaller at similar inoculum densities for soybeans and/or for resistant NC 3033 peanuts than for susceptible Florigiant peanuts in two sets of soil samples (Fig. 3B and C), but this was not observed in two other sets of samples (Fig. 3A and D).

The larger mean size of ms that occurred in some samples at low inoculum densities (eg, <5 ms/g) complicates estimates of disease potentials from inoculum densities in soils. Following growth of partially resistant hosts or environmental conditions unfavorable for disease, densities of inocula of *C. crotalariae* may be low but composed of larger ms that are more efficient at inducing root rot than ms produced in more dense populations. Even at high inoculum densities, ms may be large if formed in a highly susceptible host, eg, Florigiant. There was also considerable variation in mean ms size not accounted for by either host or inoculum density. Therefore, prediction of disease severity from preseason inoculum density should take into account the ms size. Severe root rot can occur at low inoculum densities with a susceptible cultivar as seen in root rot severity curves with highly virulent isolates (Fig. 2). An infestation at very low inoculum densities may not be detected by sampling and assay, but for susceptible Florigiant peanuts, detection of any ms in soil indicates that some loss from this disease probably will occur (P. M. Phipps and M. K. Beute, *unpublished*).

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