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Interaction of dinitramine and dinoseb with *Cylindrocladium crotalariae* and the Cylindrocladium black rot (CBR) disease of peanut.[1]

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ABSTRACT

Axenic growth by *Cylindrocladium crotalariae* (Loos) Bell & Sobers in potato dextrose broth was suppressed significantly by dinoseb at 50 and 100 μg/mL, and Dyanap® (dinoseb + naptalam) at 100 μg/mL. High concentrations of either dinitramine or alachlor suppressed growth of only one of two *C. crotalariae* isolates tested. Benefin, diphenamid, vernolate, and 2,4 DB at rates up to 100 μg/mL failed to have similar effects on growth of either isolate. Dinitramine at rates up to 100 μg/g soil had no effect on survival of *C. crotalariae* microsclerotia (ms) in a Ruston or a Woodstown loamy fine sand. Dinoseb reduced ms populations significantly in Woodstown soil at 5, 10, 50 and 100 μg/g soil and in Ruston soil at rates of 50 and 100 μg/g soil. Soil type inoculum density, and herbicide dosage were demonstrated to be important interacting factors affecting CBR development in peanut. Greenhouse and field tests implicated dinitramine at 0.56 kg/ha and dinoseb at 1.68 kg/ha as herbicide treatments which can increase the severity of CBR in Florigiant peanut.

Key Words: *Arachis hypogaea* L., *Calonectria crotalariae* (Loos) Bell & Sobers, herbicides, microsclerotia, soil type.

Cylindrocladium black rot (CBR), caused by *Cylindrocladium crotalariae* (Loos) Bell & Sobers (5), is one of the most destructive soilborne diseases of peanut (*Arachis hypogaea* L.) in Virginia. Research in Virginia and North Carolina has provided considerable information on the biology of *C. crotalariae*. The development of CBR-resistant cultivars of peanut (14, 17) and strategies for use of soil fumigants (12) have shown the greatest promise for CBR control.

Herbicides used in peanut production may have significant effects on non-target organisms and plant disease. Dinoseb has been reported to suppress southern stem rot (2, 6) and Sclerotinia blight (15) of peanut. Dinitramine at relatively high rates has been shown to increase the resistance of both peanut and tomato to infection by *Sclerotium rolfsii* (9). Other reports on dinitramine (7, 18) have described significant increases in the severity of soilborne diseases of plants.

The present study was initiated to determine the influence of herbicides on *C. crotalariae* and the severity of CBR in peanut. The work reported describes a portion of the research included in a Ph.D. dissertation by the senior author (3).

Materials and Methods

Laboratory studies. Two pathogenic isolates of *C. crotalariae* from peanut were selected for growth tests and soil infestation. Growth responses were recorded as mycelial dry weight in herbicide-amended and non-amended potato-dextrose broth (PDB). Herbicides tested included alachlor, benefin, dinitramine, dinoseb, Dyanap® (a formulation containing one part dinoseb and two parts naptalam), diphenamid, vernolate and 2,4-DB. Stock solutions of each herbicide in sterile distilled water were added to PDB (25 ml) in 250-ml Erlenmeyer flasks to achieve herbicide concentrations of 1, 5, 10, 50 and 100 μg active ingredient (a.i.)/ml. Axenic suspensions of inoculum were prepared by comminuting mycelium from two 7-day-old PDB cultures in 300-ml sterile distilled water. Flasks were inoculated with 1.0 ml of the inoculum suspension, and incubated in darkness at 25 C without agita-

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tion for 10 days. Mycelium was collected by suction onto tared filter papers in Buchner funnels, dried at 70 C for 24 h, and weight of dry matter determined.

The effect of dinitramine and dinoseb on survival of *C. crotalariae* microsclerotia (ms) in soil was determined with two peanut soils having different physical properties (Table 1). Ms inoculum was produced by culture of isolates in a high C/N agar medium (4) and prepared for soil infestation by comminuting agar cultures for 2 min with a blender, washing the propagules for 2 min on a 74-μm mesh sieve, and suspending the sieve residue in water. Inoculum density was determined by counting ms in six replicate subsamples (0.1 ml) spread over gridded Millipore filters (3-cm dia.). Quantities of ms to achieve a density of 100 ms/g soil were then concentrated on the 74-μm mesh sieve, washed into soil, and the soil mixed for 10 min in polyethylene bags. Samples of infested soil weighing 144 g were placed in 9-cm dia. x 6-cm deep petri dishes with lids that permitted some gas exchange. Stock solutions of herbicides were prepared and pipetted (3 ml) onto soil to achieve concentrations of 1, 5, 10, 50 and 100 μg a.i./g soil. There were three replicates per treatment. Herbicides were mixed immediately into soil, and the containers placed in plastic boxes containing an open beaker of water to retard moisture loss. After 30 days incubation at 25 C, the soils were assayed for populations of ms by the procedure of Griffin (8).

Table 1. Physical properties of soils selected for infestation with *Cylindrocladium crotalariae* microsclerotia.

Name and textural class	Particle size distribution (%) [a] sand	silt	clay	Organic matter (%)	pH	Water potential (bars) -0.1	-0.33	-5.0	-15.0 Soil moisture (%)
Woodstown l.f.s. [b]	77.4	14.7	7.8	2.0	5.9	13.2	11.4	5.2	4.9
Ruston l.f.s.	83.2	12.6	4.2	1.0	6.2	10.0	8.0	2.3	2.2

[a] Determined by the hydrometer method.

[b] l.f.s. = loamy fine sand.

Greenhouse studies. Microsclerotia (ms) of three pathogenic isolates of *C. crotalariae* were utilized for infestation of the two soils described in Table 1. A portable motorized cement mixer was used to mix soil and inoculum for each experiment. After mixing for 20 min, infested soil was dispensed into 12-cm dia. plastic pots. Populations of ms in soil were confirmed by soil assay (8) prior to initiating each experiment.

Stock solutions of herbicides were prepared in water then diluted to a volume of 20 mL, poured over the soil surface, and incorporated in the upper 2.5 cm of soil. Treatments were replicated six times and temperature-controlled water baths were utilized to maintain soil temperature near 25 C (14). Florigiant peanut (two plants/pot) was utilized in all experiments unless otherwise indicated. After 8 weeks the severity of root and shoot symptoms, and fresh weights were recorded to assess plant growth. Twenty root samples (10/plant) from each replicate were assayed for *C. crotalariae*.

Several experiments were performed with dinitramine and dinoseb to provide some insight into the mechanism of herbicide enhancement of CBR development. The effects of dinitramine and dinoseb treatments on root and shoot growth were assessed with Florigiant peanut grown in non-infested Ruston and Woodstown soil. Peanut transplant studies utilizing selected combinations of treated and untreated soil either infested or non-infested with ms inoculum were performed also. In these trials, seed were germinated in soil with a specific combination of inoculum and herbicide treatment, then after 5 days the germlings were rinsed to remove adhering soil particles and transplanted to soil with a different combination of herbicide treatments. An additional experiment was performed to assess the impact of dinitramine at 0.56 kg/ha on expression of CBR resistance by NC 3033 peanut. Florigiant peanut was included in these evaluations as a reference standard.

Field microplot studies. Field tests were conducted in 1979 and 1980 at the Tidewater Research Center in Woodstown loamy fine sand previously planted to corn in even numbered years and peanuts in odd numbered years. Microplots were established by insertion of fiberglass barriers (0.3 cm thick, 60-cm high, 77-cm dia.) in soil to a depth of 45 cm, as described previously (13). Microsclerotia of *C. crotalariae* for infestation of soil were produced in an agar medium (4) and standardized quantities of 1.8×10^6 ms were mixed with 800 g of moist soil in polyethylene bags. The soil inoculum mixture was then mixed into the upper 15 cm of soil in each microplot, providing an estimated density of 15 ms/g soil. All microplots were infested with ms inoculum 4 weeks prior to planting, and soil assays were performed 3 weeks after soil infestation to verify inoculum densities in each microplot.

Florigiant peanut seed were planted in the microplots on 22 May 1979 and 10 May 1980. Seed were treated with Botec® (30% botran and 30% captan) at 0.25 g/kg seed and plant densities were standardized at three plants per plot to simulate field densities. Soil analyses indicated a pH of 5.7 and no deficiencies of major nutrients. Land-plaster (1120 kg calcium sulfate/ha) was applied to microplots during the first week of July in both years of this study. During 1979 foliar fungicides were not applied because of the absence of Cercospora leafspot; however, two applications of carbaryl (1.12 kg/ha) were made to control thrips and leafhoppers.

The same herbicides used in 1979 were again applied to the original microplots in 1980, but no supplementary ms inoculum was added. On 10 April 1980, twenty new microplots (test-2) were installed nearby and infested with *C. crotalariae* ms to repeat a portion of the 1979 experiment. Cultural practices in 1980 were similar to those in 1979 except for two foliar applications of benomyl (0.56 kg/ha) and monocrotophos (1.8 kg/ha) for leafspot and spider mite control, respectively.

The severity of above ground symptoms of CBR and the incidence of plants with perithecia of *Calonectria crotalariae*, the sexual stage of *C. crotalariae*, were recorded monthly during each growing season. At harvest (29 Sep 1979 and 1 Oct 1980), plants were inverted and the severity of root and pod rot rated on a 1-5 scale (1 = no symptoms; 5 = complete decay). Five 1.0-cm segments of tap roots from each plant were biopsied for *C. crotalariae*. Peanut pods were removed, air dried, and weighed to assess yield. Soil samples were collected after harvest and subsequently assayed to determine populations of *C. crotalariae* ms and plant parasitic nematodes.

Statistical analyses. Data collected were subjected to an analysis of variance and Duncan's multiple range test. Unless specified otherwise, reference to significant differences were at a 95% confidence level (or P = 0.05).

Results

Effect of herbicides on C. crotalariae.

Mycelial growth by two isolates of *C. crotalariae* was suppressed significantly only by dinoseb at 50 and 100 μg/mL, and Dyanap® at 100 μg/mL. Dinitramine at 50 and 100 μg/mL, and alachlor at 100 μg/mL suppressed growth of one isolate, but at similar concentrations these herbicides stimulated growth of the second isolate. Benefin, diphenamid, vernolate, and 2,4-DB at rates up to 100 μg/mL failed to have similar suppressive effects on growth of either isolate of *C. crotalariae*. All of these herbicides at one concentration or more produced slight to sometimes measurable stimulatory effects on mycelial growth.

Dinitramine and dinoseb were selected for further evaluation based on their demonstrated fungitoxic activity. Dinoseb reduced significantly the recovery of *C. crotalariae* ms from Ruston soil treated at rates of 50 and 100 μg/g soil, and from Woodstown soil treated at rates of 5, 10, 50, and 100 μg/g soil (Fig. 1). Dinitramine at rates up to 100 μg/g soil had no significant effect on recovery of ms from soil. Populations of ms in the untreated infested soils did not change significantly during the 30-day incubation period of these tests.

Effects of herbicides on CBR development under greenhouse conditions.

Greenhouse studies with artifically infested Woodstown soil indicated that dinitramine at 0.56 kg/ha and dinoseb at 1.68 kg/ha caused a significant increase in the severity of CBR of peanut (Fig 2). Shoot symptoms and signs of CBR appeared among plants grown in soils

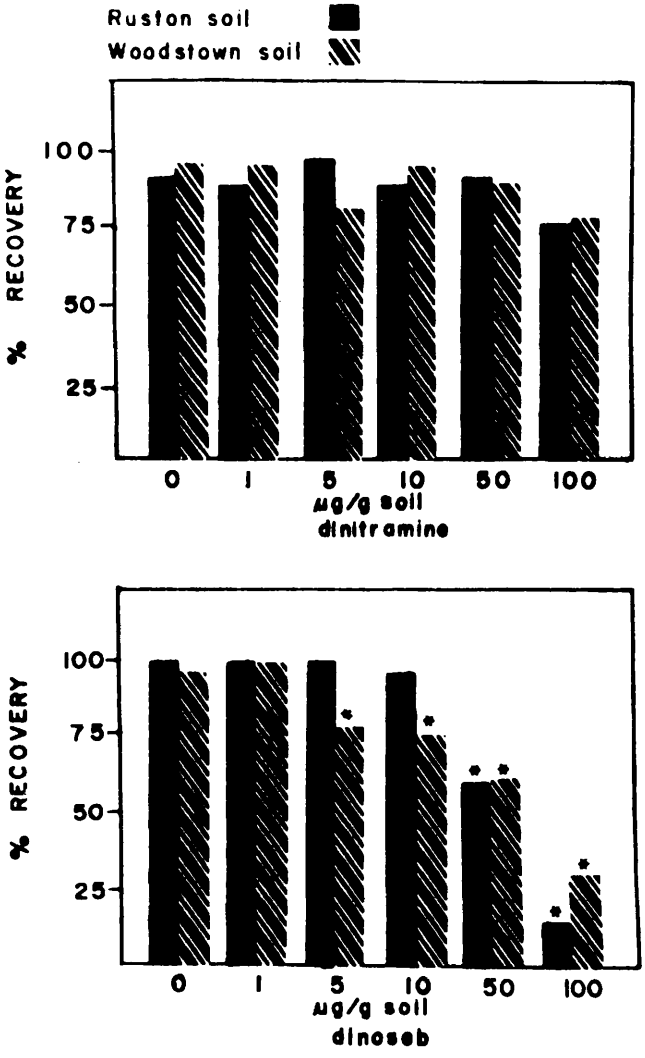

Fig. 1. Recovery of *Cylindrocladium crotalariae* microsclerotia from two loamy fine sand soils 30 days after treatment with dinitramine and dinoseb. Bars labeled with an asterisk are significantly different (P = 0.05) from the untreated check.

treated with these herbicides up to 3 weeks before symptoms were noted in untreated controls. The frequency of recovery of *C. crotalariae* from tap roots of plants was also greater for roots from herbicide treated soils than untreated soils. When Woodstown soil and Ruston soil were infested with ms inoculum to provide densities of 5 to 50 ms/g soil, CBR development in untreated soil was consistently more severe in Ruston soil (Fig. 3). Disease severity in both soils was affected significantly by inoculum density and herbicide treatment. The severity of root rot was increased significantly by dinitramine at 0.56 kg/ha in Ruston soil infested with 5 ms/g soil and in Woodstown soil infested with 50 ms/g soil (Fig. 3). At rates of 0.28 and 0.84 kg/ha, dinitramine had no significant effect on root rot within either soil type. In contrast to results of previous trials (Fig. 2), dinoseb treatments caused no increase in root rot severity regardless of inoculum density or soil type. A somewhat surprising result was the suppression of root rot severity by dinoseb at 6.72 kg/ha in Woodstown soil infested to a density of 5 ms/g soil.

Dinitramine treatments in non-infested soils were

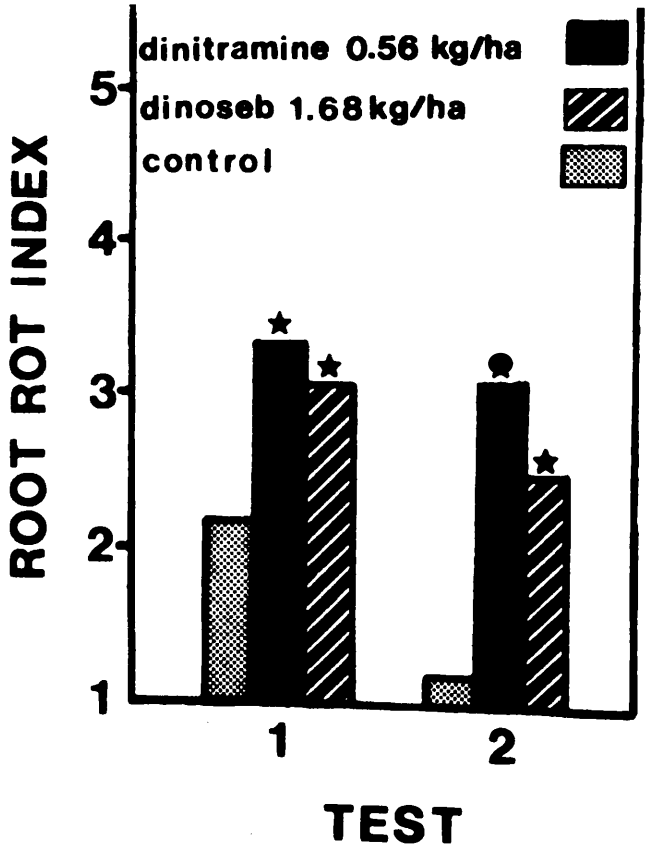

Fig. 2. The effect of dinitramine and dinoseb as pre-plant treatments in Woodstown soil on the severity of root rot of peanut caused by *Cylindrocladium crotalariae*. Root rot index: 1 = no root rot symptoms, 5 = roots completely decayed. Bars labeled with an asterisk are significantly different (P = 0.05) from the untreated check.

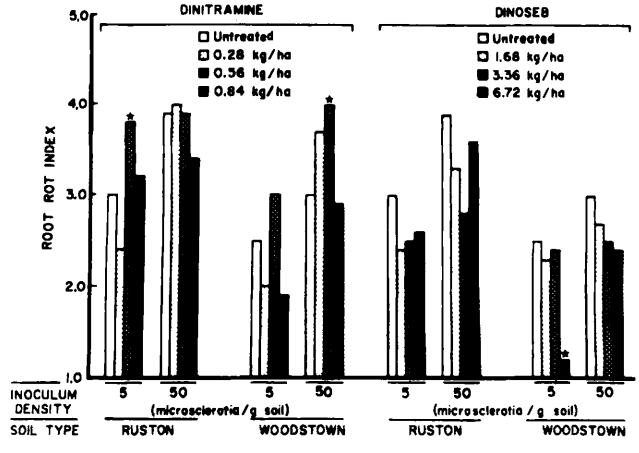

Fig. 3. The effect of soil-applied herbicide treatments and inoculum densities of *Cylindrocladium crotalariae* microsclerotia on development of Cylindrocladium black rot of peanut in Ruston and Woodstown loamy fine sand. Root rot index: 1 = no disease, 5 = severe disease. Bars labeled with a star denote a significant difference (P = 0.05) from untreated soil with the same inoculum level.

more suppressive to plant growth than dinoseb treatments and resulted in greatest levels of growth suppression in Woodstown soil (Fig. 4). Numerous stubby adventitious roots with swollen tips were observed in the hypocotyl and root/hypocotyl transition region of plants taken from dinitramine treated soil. Subsequent tests in

Ruston soil showed that dinitramine resulted in significant inhibition of root elongation soon after seed germination in treated soil. Mean root lengths after 5 days incubation in soil treated with dinitramine at 0.56 and 0.84 kg/ha were 56 and 46 percent of root lengths in untreated soils, respectively. Additionally, the diameter of roots in the region of elongation was increased as a result of soil treatment with dinitramine.

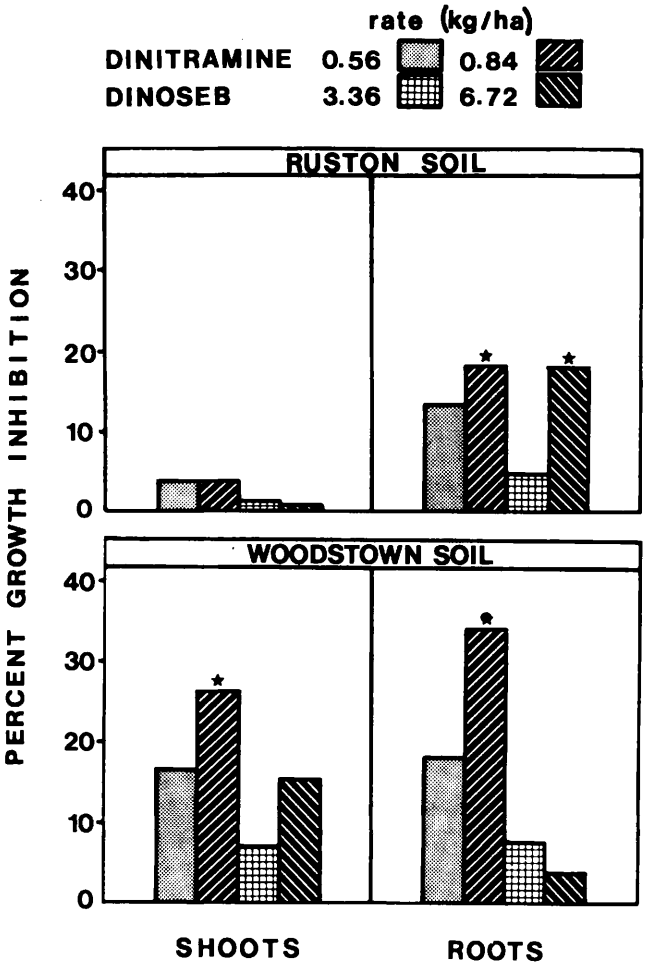

Fig. 4. The effect of dinitramine and dinoseb on root and shoot growth of Florigiant peanut in Ruston and Woodstown loamy fine sand. Bars labeled with a star denote levels of growth inhibition that are significantly different (P = 0.05) from growth in untreated soil.

In experiments to provide more insight as to the mechanism of CBR enhancement by dinitramine, symptoms of CBR were significantly more severe in plants from soil treated with dinitramine either before or after transplanting germlings to infested soil (Table 2). Growth assessments indicated that the presence of *C. crotalariae* and dinitramine in transplant soil resulted in the greatest reduction of plant dry weight. Dinitramine treatment of pre-transplant or transplant soil not infested with *C. crotalariae* resulted in some decrease in plant dry weights.

The expression of CBR resistance by NC 3033 peanut was not altered by soil treatment with dinitramine at 0.56 kg/ha. No visible shoot symptoms of CBR were present in NC 3033 peanut in either treated or untreated in-

Table 2. Assessment of host and pathogen effects of dinitramine at 0.56 kg/ha on development of Cylindrocladium black rot of peanut in a Ruston loamy fine sand.[1]

Herbicide treatment [2]		*C. crotalariae*	Root rot	Shoot symptom	% recovery	dry wt. (g)	
Pre-transplant soil	Transplant soil	inoculum in transplant soil	index (1-5) [3]	index (1-5) [3]	*C. crotalariae* from roots [4]	Shoots	Roots
untreated	untreated	(+)	2.9 B	1.6 B	83 B	6.6 B	2.9 CD
treated	untreated	(+)	4.0 A	2.7 A	98 A	6.6 B	2.2 DE
untreated	treated	(+)	4.1 A	2.8 A	98 A	5.9 B	1.8 E
untreated	untreated	(-)	1.0 C	1.0 C	0 C	9.8 A	4.5 A
treated	untreated	(-)	1.0 C	1.0 C	0 C	9.1 A	3.6 BC
untreated	treated	(-)	1.0 C	1.0 C	0 C	8.9 A	3.9 AB

[1] Data are the mean of six replications of two plants per 11.4-cm plastic pot 8 weeks after transplanting seedlings. Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

[2] Dinitramine was applied and incorporated into the upper 2.5 cm of soil. Microsclerotia (10 ms/g soil) were used to infest transplant soil as indicated. Pre-transplant soil was non-infested.

[3] 1 = no symptoms, 5 = tissues severely decayed or dead.

[4] Based on 20 root biopsy tissue samples (10 per plant) from the six replicates per treatment.

fested soil. Root rot symptoms were observed for eight of twelve plants of NC 3033 peanut in treated infested soil, but the overall severity was not significantly greater than root rot in untreated, infested soil. As in previous experiments, dinitramine treatment of soil at a rate of 0.56 kg/ha increased significantly the severity of shoot and root symptoms of CBR in reference standard pots of Florigiant peanut.

Field microplot tests. Successive treatment of infested soil with dinitramine at 0.56 kg/ha in 1979 and 1980 resulted in an increase in severity of CBR that proved significant in 1980 (Fig. 5). In 1979 the percent of plants exhibiting perithecia of *Calonectria crotalariae* in plots treated with dinitramine at 0.56 kg/ha was four times greater than in untreated, infested plots. A two-fold increase in the incidence of perithecia on plants was noted in plots treated with dinitramine at 0.84 kg/ha. In the second microplot test established in 1980, dinitramine at both 0.56 and 0.84 kg/ha resulted in significant increases in the severity of root rot associated with CBR. Although the results of 1979 and 1980 tests indicated that yield may be reduced as a result of dinitramine at 0.56 kg/ha, pod yields among treatments were not significantly different.

In the 1979 microplot study, the severity of CBR was not affected significantly by pre-plant treatments with dinoseb at rates up to 6.72 kg/ha (Fig. 5). Subsequent treatment of the same plots in 1980 with dinoseb at 1.68 kg/ha, however, caused an increase in the severity of root rot. A similar significant increase in root rot severity also occurred in soils of the second microplot test in 1980 where dinoseb at 1.68 kg/ha was used as a pre-plant soil treatment. As in 1979, dinoseb at 6.72 kg/ha as a pre-plant treatment had no significant effect on CBR severity in either 1980 test. Pre-emergence, surface-applied treatments with dinoseb (1.12 - 2.24 kg/ha) plus naptalam (2.24 - 4.48 kg/ha) as successive treatments in the 1979-1980 test also had no apparent effect on the severity of root rot. Because of the common practice of using dinoseb as a post-emergence treatment on peanuts for weed control, the 1.68 gk/ha rate was also evaluated as two mid-season applications in 1979 (Jul 7, Aug 28) and three mid-season applications in 1980 (Jul 2, Aug 6, Sep

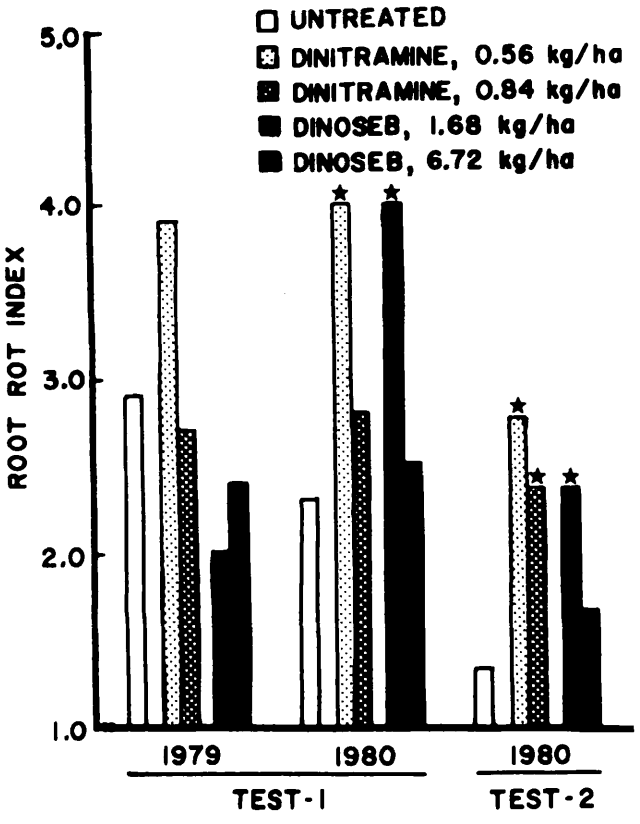

Fig. 5. The effect of soil-applied herbicides on development of Cylindrocladium black rot of peanut in field microplots of Woodstown soil. Root rot index: 1 = no disease, 5 = severe disease. Bars labeled with a star denote a significant difference (P = 0.05) from untreated soil in the same test and year.

4) in microplots untreated prior to planting and microplots treated with either dinitramine at 0.56 kg/ha or dinoseb at 6.72 kg/ha prior to planting. None of the postemergence treatments alone or in combination with preplant treatments had a significant effect on the severity of CBR. *C. crotalariae* was isolated from roots of all plants grown in microplots with ms infested soil in 1979 and 1980. Except for a few random isolations of *Sclerotium rolfsii*, no other pathogenic fungi were detected in biopsy tests. Nematode assay results subsequent to harvest in 1979 and 1980 showed low populations of ring (*Macroposthonia ornatum*) and root knot (*Meloidogyne hapla*) nematodes in all plots. Analyses of variance in nematode population data after square root transformation showed no significant differences in nematode counts as a result of herbicide treatments in either year.

Discussion

Previous reports (2,6,9,15) indicate that dinitramine and dinoseb can influence the development and severity of certain soilborne diseases of peanut. The maximum recommended rates for single applications of dinitramine and dinoseb in Virginia are 0.56 and 1.68 kg/ha, respectively. Assuming soil moisture was near field capacity (-0.1 bars) and herbicides were in the soil solution within the upper 7.5 cm of soil, dinitramine would be present at 5.0 and 3.8 μg/mL, and dinoseb would be present at 15.0 and 11.5 μg/mL in the Ruston and Woodstown soils, respectively. At reduced soil moisture levels or 50% field capacity, herbicide concentrations in the soil solution could theoretically show a two-fold increase. Based on results of mycelial growth studies in PDB, dinoseb concentrations could become a limiting factor in mycelial growth by *C. crotalariae* at concentrations near 50 μg/mL and higher. Dinitramine treatment of soil would not be expected to result in significant suppression of mycelial growth by *C. crotalariae* in soil, because it is applied at a lower rate and showed less activity than dinoseb in suppression of mycelial growth. The existence of differences in isolates with respect to sensitivity to herbicides was demonstrated with dinitramine and alachlor in PDB growth studies. These findings suggest that herbicide tolerant strains of plant pathogens may exist in soil and may be present in nature as a result of repeated use of certain herbicides.

Studies to determine the vertical distribution of *C. crotalariae* ms in July in naturally-infested, commercial peanut fields have shown lower numbers of germinable ms were present in the upper 2.5 cm of soil (Phipps and Beute, unpublished). Physical environmental factors (i.e. drying, freezing) have been reported to contribute greatly to reduced survival of ms near the soil surface (13,16). Results of the current study suggest that the common use of dinoseb at preemergence and subsequently may also reduce populations of ms near the soil surface.

The impact of dinitramine treatments on the severity of CBR under greenhouse conditions appears to be dependent on soil type, density of *C. crotalariae* ms in soil, and herbicide rate (Fig. 3). Increases in the severity of CBR following soil treatment with dinitramine at 0.56 kg/ha may be a result of the herbicide increasing the disease proneness of Florigiant peanut. Herbicide-induced predisposition of plants to disease was indicated by results following treatment of Woodstown soil having 50 ms/g soil and treatment of Ruston soil having 5 ms/g soil. Predisposition effects of dinitramine at 0.56 kg/ha were also suggested to be significant by transplanting peanut seedlings from treated soil to untreated, infested soil (Table 2). Based on the severity of disease in peanut seedlings exposed to dinitramine for only 5 days and subsequently transplanted to untreated soil, the susceptibility of roots to infection by *C. crotalariae* appears to be altered almost immediately after seed germination and probable uptake of dinitramine.

The failure of dinitramine at 0.84 kg/ha to increase CBR severity in greenhouse tests apparently was not related to fungitoxic effects. A similar conclusion was reported (7) in tests with low and high rates of dinitramine and the Rhizoctonia disease of cotton. Possibly the high rate of dinitramine exceeds a threshold beyond which predisposition effects are offset by hypersensitive host responses, such as wound periderm formation (10) and/or the production of phytoalexins. Mechanisms for increased susceptibility of peanut to CBR as a result of dinitramine at the low rate of 0.56 kg/ha may include alteration of the quality as well as quantity of root exudates and their effect on germination of microsclerotia inoculum in soil (11). The absence of any significant effect

of dinitramine on CBR development in NC 3033 peanut indicates that the resistance mechanism in this cultivar is not altered by the herbicide.

No single rate of dinoseb increased the severity of CBR consistently in greenhouse tests. Dinoseb has been reported to leach readily from soils of low water holding capacity (1). This characteristic coupled with variable environmental conditions that affect transpiration and resulting demands for water in greenhouse tests may explain inconsistencies in greenhouse as well as field studies with dinoseb.

Field microplot studies with dinitramine and dinoseb supported previous conclusions that both herbicides can increase the severity of CBR. Although not apparent in 1979 data, low rates of both herbicides caused significant increases in CBR severity in two separate tests in 1980. Climatological records from a weather station ca. 50 m from the microplot tests showed major differences in rainfall patterns during the two growing seasons. In 1979 ca. 18 cm of rainfall occurred within the 2-week period after soil treatment. Amounts of rainfall totaling 0.51, 1.95, 4.52 and 0.23 cm were recorded in the 4 days immediately following application of pre-plant treatments in 1979. No rainfall occurred in the first 8 days after herbicide treatment of soil in 1980. Overall, the 1979 growing season exhibited near normal levels of rainfall, whereas the 1980 season was extremely dry and without rainfall most of June and August. Greater soil moisture resulting in leaching (1) of herbicides from soil was believed to account for the absence of significant herbicide effects on disease in 1979.

Data obtained in these studies indicate that dinitramine and dinoseb can significantly affect the development of CBR in peanut plants grown under greenhouse and field conditions. Environmental factors and their interaction with a host, pathogen and specific herbicide dosage are believed to be important determinants of how herbicides may affect CBR development. The results of the current study emphasize the importance of understanding non-target effects of pesticides and their interaction with environmental factors and plant pathogens in the biosphere of peanuts.

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