EFFECT OF HERBICIDES ON

CYLINDROCLADIUM CROTALARIAE AND THE

CYLINDROCLADIUM BLACK ROT (CBR) DISEASE

OF PEANUT

BY

James Albert Barron, III

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APPROVED:

P. M. Phipps, Chairman

G. D. Griffin

C. W. Roane

L. D. Moore

O. E. Rud

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Blacksburg, Virginia

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CHAPTER ONE

INTRODUCTION, LITERATURE REVIEW, OBJECTIVES

Cylindrocladium black rot.

Cylindrocladium black rot (CBR) of peanut (Arachis hypogaea L.) was first observed in southwestern Georgia in 1964 (4). Since 1970, CBR has often been associated with severe yield losses in Virginia-grown peanuts (12,13). The disease incitant, Cylindrocladium crotalariae (Loos.) Bell and Sobers, is a soilborne fungus which forms clusters of chlamydospore-like cells called microsclerotia (ms) within the cortex of colonized peanut roots. These are apparently released into soil upon decomposition of the root (39) and are believed to be the primary survival and infective propagules of this fungus in nature (23,33).

During periods of wet weather or high soil moisture, perithecia of Calonectria crotalariae (Loos.) Bell and Sobers can be found on stems, pegs and pods of infected plants just above and/or below the soil surface (4,28). The presence of perithecia provides positive identification of CBR disease which otherwise requires the culture of tissue samples on laboratory media for verification (28). When subjected to a sudden reduction in relative humidity, perithecia may forcibly eject large numbers of ascospores

which may serve as inoculum for disease spread (25,37). Since ascospores are extremely susceptible to dessication, and incapable of long-term survival in soil, it seems likely that they contribute only to within-field dissemination of the fungus (19). Long distance dissemination of Cylindrocladium crotalariae is thought to occur chiefly through the transport of infested soil on farm implements (24). Fragments of colonized plant debris expelled from peanut combines operating in infested fields also may transport this pathogen aerially for short distances (39). Air-dried peanut seeds from colonized plants apparently do not contain the pathogen (10).

Cylindrocladium crotalariae is primarily a root, peg and pod pathogen of the peanut plant. Usually, colonization results in a stunted, chlorotic plant that at first wilts on hot days. Infected plants may or may not die, depending on the environmental factors (4,28,38). Such symptoms are usually first observed in the field during the month of July in Virginia, but they may occur later in the season. Plants with chlorotic, wilted or dead foliage usually have severely blackened and rotted tap and lateral roots that are difficult to remove from the soil. The pegs and fruit may also exhibit dark brown lesions and widespread decay that ultimately results in loss of yield and quality. Similar symptoms occur in the greenhouse, but these may be observed

as early as 3-4 weeks after planting, when soil moisture is maintained at or near field capacity and soil temperature is held at 25 C (30).

The severity of CBR disease has been shown to be directly affected by: 1) inoculum density (31,34); 2) soil moisture and temperature (30); plant genotype (12,31), and the presence of certain plant-parasitic nematodes (8). Unfortunately, little is currently known about how these parameters interactively affect disease severity in the field environment.

It is not known how long ms of C. crotalariae may survive in field soil in the absence of suscept or other plants. Soil physical factors, however, are known to adversely affect propagule germinability. Roth et al. (36) found that numbers of germinable C. crotalariae ms recovered from naturally-infested field soil decreased significantly when the soil was incubated at 6 C. No ms were recovered from soils incubated at -10 C. Phipps and Beute (32) similarly found that populations of ms were reduced by 60% in soil incubated at 5 C for 5 wk. High soil temperatures (30 C) and low soil water potentials (-224 bars) also resulted in reduced recovery of the propagules from naturally-infested soil (14,15). Evidence that cropping practices affect populations of ms in the field has been presented by Phipps and Beute (32). Harvest-time ms

populations in microplots planted to peanut cultivars 'Florigiant' and 'Argentine' and soybean (Glycine max L.) cultivar 'Ransom' were 9.6, 5.2 and 3.7 times greater than pre-plant populations, respectively. In fallow soil and in soil planted to cotton, corn, and tobacco, ms populations declined significantly in 1975 and again slightly in 1976. Taylor (42) found in a three-year Virginia field study that environmental factors can have an important influence on populations of C. crotalariae ms.

At the present time, control strategies for CBR disease have not been reported in the literature. Microsclerotia are difficult to eradicate from infested soil using soil fungicides and biocides (26,29,43,45). Complete kill of the fungus in soil has been demonstrated only in laboratory experiments (44) and not in the field (29). Since severe CBR disease can occur in commercial peanut cultivars at inoculum densities as low as 0.5 ms/g soil (31), it will probably be necessary to integrate eradication procedures with a shift to less susceptible cultivars in order to achieve acceptable control. Screening programs in North Carolina and in Virginia have resulted in the identification of peanut breeding lines that are resistant to CBR (12,31,46). A concerted effort by scientists in both states is underway to develop agronomically acceptable CBR-resistant cultivars that could be grown on farms with a history of this disease.

Herbicide peanut disease interactions.

During the past fifteen years, the use of herbicides for weed control has become a universal crop management practice in Virginia and other states. Nearly 100% of all peanut acreage in the United States is treated with herbicides each year (2). In Virginia, there are currently ten compounds registered for herbicidal use in peanuts (40). These are listed in Table 1A. The development and use of selective herbicides has had tremendous impact on peanut production in the United States. Without herbicides, it would be difficult to achieve peanut yields and quality standards that meet today's market requirements. Peanuts are less adapted to mechanical cultivation for weed control than most other crop plants (18). Therefore, it is not likely that grower dependence on herbicides will decrease in the future.

Soilborne plant pathogens and other microorganisms are particularly vulnerable to herbicidal influence (1,2). This is true because, regardless of the way in which a herbicide is initially applied, it will eventually be introduced, in one form or another, into the soil (2,20). Currently, of the ten herbicides registered for peanut weed control in Virginia, eight are routinely applied directly to the soil surface; three of these are normally soil incorporated

Table 1 A. Commonly used herbicides in Virginia peanut production.

Common Name	Manufacturer	Trade Name	Type Formulation[a]	% Active Ingredient	Time of Application[b]	Rate (kg a.i./ha)[c]
alachlor	Monsanto	Lasso 4EC	EC	43.0	AC, PRE	3.36 - 4.48
benefin	Elanco	Balan LC	EC	19.4	PPI	1.25 - 1.68
bentazon	BASF-Wyandotte	Basagran	EC	42.0	PO	0.84 - 1.68
dinitramine	U. S. Borax	Cobex 2EC	EC	25.0	PPI	0.28 - 0.56
dinoseb	Dow Chemical	Premerge-3	EC	31.8	AC, PO	0.84 - 1.68
dinoseb + naptalam	Uniroyal Chemical	Dyanap	EC	22.3 11.5	AC	0.84 - 1.68 2.24 - 4.48
diphenamid	Tuco-Upjohn	Enide 50W	WP	50.0	PO	2.24
vernolate	Stauffer Chemical	Vernam 7E	EC	88.5	PPI	2.24 - 2.80
2,4-DB	Amchem Products	Butyrac 200	EC	26.0	PO	0.22 - 0.28

[a] EC= emulsifiable concentrate; WP= wettable powder.

[b] AC= at cracking; PPI= pre-plant incorporated; PO= postemergent; PRE= preemergent.

[c] Recommended for use in the Virginia Pest Management Guide 1 (40).

immediately after application (40). Consequently, there exists a rather high potential for herbicide effects (beneficial or detrimental) on the soil microflora of this region. Little is known about how herbicides affect the pathogenic and non-pathogenic microflora associated with the peanut plant.

It is almost inevitable that herbicides should affect plants and microorganisms in addition to the weeds against which they are used (21). In fact, herbicides are now known to influence significantly the diseases of many crop plants (1). According to Katan and Eshel (20), the way in which a herbicide might affect a specific disease bears little relation to the type of plant, herbicide and pathogen involved. So many factors are altered when herbicides are used that pinpointing their effects on a specific suscept-pathogen interaction is often very difficult (22).

Research with herbicides for weed control in peanuts began in several states about 1949 (18). During the early 1950's, Chappell (6) screened 24 compounds for herbicidal efficacy in Virginia peanut fields. In these tests he found dinoseb to be one of the most promising preemergence materials for future use in peanuts. Dinoseb had also performed well in North Carolina peanut fields (41).

In 1954, Chappell and Miller (7) noticed that peanut plants in field plots sprayed with dinoseb were generally

larger and healthier than those in unsprayed plots, even when all weeds were removed from both by hand. In laboratory tests, they determined that dinoseb was very toxic to several species of plant-pathogenic fungi, including Sclerotium rolfsii Sacc., the causal agent of southern stem rot of peanut. The herbicide was also found to be lethal to adult sting nematodes (Belonolaimus gracilis Steiner, 1949) at concentrations in excess of 10 ug/ml (7). Chappell and Miller (7) observed in 1955 at four locations that the severity of southern stem rot of peanut was always less in field plots sprayed with dinoseb than in those which were unsprayed but cultivated for weed control. They also observed that symptoms of peanut leafspot disease (Cercospora arachidicola Burkh.) and sting nematode injury (B. gracilis) were somewhat suppressed in plots treated with the herbicide. It was concluded that the observed suppression of disease was related to the use of dinoseb, although no evidence was presented that the herbicide possessed fungicidal or nematicidal properties under field conditions.

In 1956, Garren and Duke (11) included dinoseb in their investigations on control of southern stem rot. From the data obtained in their studies, it was concluded that dinoseb may have provided some additional disease control in excess of that obtained by moldboard plowing surface organic

matter at planting time and by using the "non-dirting" method of in season cultivation for weed control. Garren (9) later concluded that the additional disease control associated with dinoseb application was not sufficient to justify its recommendation as a control measure for southern stem rot of peanut in Virginia.

Sesone, like dinoseb, was extensively tested for herbicidal efficacy in the early 1950's (18). It was later used as a preemergence herbicide in Virginia and Georgia, often in conjunction with dinoseb. In Georgia, the use of sesone was somtimes associated with poor emergence of peanut plants in treated areas (5). Boyle et al. (5) conducted tests in the greenhouse to determine if the herbicide alone had a direct effect on emergence or whether it interacted with soilborne pathogens to result in unusually poor stands of peanuts. Seeds were planted in field soils infested with Rhizoctonia solani Kuhn and S. rolfsii and either treated or not treated with sesone. The soils were amended with varying amounts of organic matter beforehand to simulate environments in which low, moderate and high potentials for severe seedling disease would exist. Under conditions associated with optimum seedling emergence (low disease potential) the use of sesone had no detrimental effect on emergence. Under conditions of either moderate or severe disease potential, seedling emergence was substantially

reduced in soils treated with sesone relative to the untreated controls. Boyle et al. (5) concluded that sesone interacted with these pathogens to cause poor emergence, however, they did not propose a mechanism for this observation. Sesone at field rates did not substantially affect the axenic growth of S. rolfsii and four other soilborne plant-pathogenic fungi in culture (7). This herbicide was used in several states as recently as 1971, but it has since been replaced by newer, more effective compounds (18).

During the 1960's several new herbicides became available to Virginia peanut growers. Osborne et al. (27) conducted a field test in 1971 on the effects of certain herbicides and nematicides on pod rot of peanut. Disease incidence (% rotted pods) was significantly lower in the untreated plots (7.5%) than in those which received any of the chemical treatments. Herbicides used and the resulting percentages of disease incidence were: nitralin, 16.2; dinoseb + naptalam 14.6; vernolate, 17.6; benefin, 16.9; alachlor, 18.8; benefin + vernolate, 22.4; and 2,4-DB, 32.2. Unfortunately, the results reported here were not verified by tests in subsequent years.

In 1972, Backman et al. (3) noted that peanuts grown in field plots treated with a preemergence spray of oxadiazon yielded better than those grown in unsprayed but weed-free

plots. They later observed that the number of peanut plants with visible symptoms and signs of infection by S. rolfsii was significantly reduced when oxadiazon alone at 3.3 kg/ha or oxadiazon + dinoseb treatments were applied. Fluorodifen, dinoseb and flourodifen + dinoseb did not reduce disease severity. In laboratory tests oxadiazon was shown to be non-toxic to S. rolfsii in axenic culture. The herbicide did not induce gross anatomical changes in treated peanut stem tissue, but it reduced foliar growth of the peanut significantly in the field. Backman et al. concluded that S. rolfsii was suppressed either by unfavorable subcanopy microclimate conditions or by an imbalance in soil antagonists resulting from herbicide treatment.

Certain dinitroaniline herbicides have been found to influence significantly plant diseases incited by soilborne pathogens (16). With greenhouse and field experiments, Grinstein et al. (17) determined that dinitramine significantly reduced the severity of southern stem rot of peanut. In a naturally infested field in Israel, dinitramine at 3.0 kg/ha resulted in a 60% reduction of disease severity in peanut. In the greenhouse, peanut seeds were germinated in dinitramine-treated soil then washed and transplanted to herbicide-free soil infested with the pathogen. The number of diseased plants grown from seedlings which underwent pre-treatment with dinitramine at

1.0 ug/g soil was 40% lower than when untreated seedlings were used. It was proposed that dinitramine increased the resistance in peanut to colonization by S. rolfsii. Dinitramine was also shown to be relatively non-toxic in culture to the pathogen, S. rolfsii, and the soil antagonist of the pathogen, Trichoderma harzianum Rifai aggr.

Sclerotinia blight, incited by Sclerotinia minor Jagger is now considered the most severe disease of peanut in Virginia (P.M. Phipps, 1980, personal communication). Porter and Rud (35) recently found that the growth of S. minor on potato dextrose agar amended with 1.0, 3.0, and 5.0 µg/ml dinoseb was significantly suppressed. In a two-year field test they also showed that multiple postemergence applications of dinoseb or dinoseb + naptalam suppressed disease and increased peanut yields significantly. The herbicides were not effective when applied once preemergence during both years or as single postemergence applications during 1977 (35). Porter and Rud concluded that dinoseb, under certain conditions, directly inhibits the growth and parasitic development of S. minor under field conditions, however, no data supporting this contention was presented.

Objectives

The purpose of this research was to determine the effects of herbicides used in Virginia peanut production on Cylindrocladium black rot (CBR) of peanut. Specific objectives were to assess the effects of selected herbicides on:

A. Axenic growth of C. crotalariae

B. Severity of CBR in greenhouse and field tests; and

C. Soil populations of C. crotalariae ms.

Portions of experiments comprising objectives A and B were repeated using isolates of S. minor and S. rolfsii, the incitants of Sclerotinia blight and southern stem rot of peanut, respectively. Other workers have shown that certain herbicides influence the development and severity of these diseases (3,7,35).

Literature Cited

1. Altman, J., and C. L. Campbell. 1977. Effects of herbicides on plant diseases. Ann. Rev. Phytopathol. 15:361-385.

2. Anderson, W. P. 1977. Weed science:principles. West Publishing Co., New York, NY. 598 pp.

3. Backman, P. A., R. Rodriguez-Kabana, and G. A. Buchanan. 1977. Interactions of oxadiazon and dinoseb with stem rot in peanuts. Weed Sci. 25:260263.

4. Bell, D. K., and E. K. Sobers. 1966. A peg, pod and root necrosis of peanuts caused by a species of Calonectria. Phytopathology 56:1361-1364.

5. Boyle, L. W., E. W. Hauser, and J. T. Thompson. 1958. The combined effect of a herbicide and disease on the emergence of peanut seedlings. Weeds 6:461-464.

6. Chappell, W. E. 1956. Screening tests on peanuts, soybeans and castor beans. Proc. So. Weed Conf. 9:57-60.

7. Chappell, W. E., and L. I. Miller. 1956. The effects of certain herbicides on plant pathogens. Plant Dis. Reptr. 40:52-56.

8. Diomande, M., and M. K. Beute. 1979. Influences of Meloidogyne hapla and Macroposthonia ornata on the effectiveness of microsclerotial inoculum of Cylindrocladium crotalariae in causing black rot (CBR) on peanuts. Phytopathology 69:1-A3. (Abstr.).

9. Garren, K. H. 1959. An evaluation of the role of dinoseb in "non-dirting" control for peanut stem rot. Plant Dis. Reptr. 43:665-667.

10. Garren, K. H. 1973. Further studies on Cylindrocladium black rot of peanuts in Virginia. J. Amer. Peanut Res. Ed. Assoc. 5:31-32.

11. Garren, K. H., and G. B. Duke. 1957. The peanut stem rot problem and a preliminary report of interrelations of "non-dirting" weed control and other practices to stem rot and yield of peanuts. Plant Dis. Reptr. 41:424-431.

12. Garren, K. H., and T. A. Coffelt. 1976. Reaction to Cylindrocladium black rot in Virginia-type peanut cultivars. Plant Dis. Reptr. 60:175-178.

13. Garren, K. H., D. M. Porter, and A. H. Allison. 1971. Cylindrocladium black rot of peanuts in Virginia. Plant Dis. Reptr. 55:419-421.

14. Graham, P. J. 1980. Relationship between peanut canopy temperature and Cylindrocladium black rot development and effects of soil temperature and water potential on the germinibility of Cylindrocladium crotalariae microsclerotia. M.S. Thesis. Va. Polytech. Inst. and State Univ. 108 pp.

15. Griffin, G. J., D. A. Roth, and N. L. Powell. 1978. Physical factors that influence the recovery of microsclerotium populations of Cylindrocladium crotalariae from naturally-infested soils. Phytopathology 68:887-891.

16. Grinstein, A., J. Katan, and Y. Eshel. 1976. Effect of dinitroaniline herbicides on plant resistance to soilborne pathogens. Phytopathology 66:517-522.

17. Grinstein, A., Y. Elad, J. Katan, and I. Chet. 1979. Control of Sclerotium rolfsii by means of a herbicide and Trichoderma harzianum. Plant Dis. Reptr. 63:823-826.

18. Hauser, E. W., P.W. Santelman, G. W. Buchanan, and O. E. Rud. 1973. Controlling weeds in peanuts. Pages 310-385 in: Peanuts-Culture and Uses. Amer. Peanut Res. and Ed. Assoc., Stillwater, OK. 684 pp.

19. Hwang, S. C., and W. H. Ko. 1975. Biology of conidia, ascospores and microsclerotia of Calonectria crotalariae in soil. Phytopathology 65:393-398.

20. Katan, J., and Y. Eshel. 1973. Interactions between herbicides and plant pathogens. Residue Rev. 45:145-177.

21. Kavanagh, T. 1969. The influence of herbicides on plant disease. I. Temperate fruit and hops. Sci. Proc. Royal Dublin Soc. Ser. B, Vol 2(19):179-190.

22. Kavanagh, T. 1974. The influence of herbicides on plant disease. II. Vegetables root crops and potatoes. Sci. Proc. Royal Dublin Soc. Ser. B, Vol 3(18):251-265.

23. Krigsvold, D. T., and G. J. Griffin. 1975. Quantitative isolation of Cylindrocladium crotalariae microsclerotia from naturally-infested peanut and soybean fields. Plant Dis. Reptr. 59:543-546.

24. Krigsvold, D. T., K. H. Garren, and G. J. Griffin. 1977. Importance of peanut field cultivation and soybean cropping in the spread of Cylindrocladium crotalariae within and among peanut fields. Plant Dis. Reptr. 61:495-499.

25. Linderman, R. B. 1974. Ascospore discharge from perithecia of Calonectria theae, C. crotalariae, and C. kyotensis. Phytopathology 64:567-569.

26. Morton, C. S. 1979. Studies on Cylindrocladium black rot of peanut in South Carolina. Ph. D. dissertation, Clemson University, Clemson, SC. 84 pp.

27. Osborne, W. W., O. E. Rud, C. Harris, K. M. Hameed, R. Pristou, R. C. Lambe, J. A. Fox, and L. Sill. 1973. Evaluation of certain herbicide-nematocide treatments on the incidence of peanut pod rot. Pages 32-38 in: Results-Peanut disease control. Information Note 128, Dept. Plant Pathol. and Physiol. Va. Polytech. Inst. and State Univ., Blacksburg, VA.

28. Phipps, P. M. 1978. Cylindrocladium black rot. Control Series 167, Virginia Polytechnic Institure and State University Extension Division, Blacksburg, VA.

29. Phipps, P. M., and J. A. Barron. 1981. Efficacy of soil fumigants in control of CBR of peanut in Virginia. Fungicide-Nematicide Tests 36:(in press).

30. Phipps, P. M., and M. K. Beute. 1977. Influence of soil temperature and moisture on the severity of Cylindrocladium black rot in peanut. Phytopathology 67:1104-1107.

31. Phipps, P. M., and M. K. Beute. 1977. Sensitivity of susceptible and resistant peanut cultivars to inoculum densities of Cylindrocladium crotalariae microsclerotia in soil. Plant Dis. Reptr. 61:300-303.

32. Phipps, P. M., and M. K. Beute. 1979. Population dynamics of Cylindrocladium crotalariae microsclerotia in naturally-infested soil. Phytopathology 69:240-243.

33. Phipps, P. M., M. K. Beute, and K. R. Barker. 1976. An elutriation method for quantitative isolation of Cylindrocladium crotalariae microsclerotia from peanut field soil. Phytopathology 66:1255-1259.

34. Phipps, P. M., M. K. Beute, and B. A. Hadley. 1977. Relationship of inoculum density and time to incidence of Cylindrocladium black rot of peanut in naturally-infested fields. Proc. Amer. Peanut Res. Ed. Assoc. 9:51. (Abstr.).

35. Porter, D. M., and O. E. Rud. 1980. Suppression of Sclerotinia blight of peanuts with dinitrophenol herbicides. Phytopathology 70:720-722.

36. Roth, D. A., G. J. Griffin, and P. J. Graham. 1979. Low temperature induces decreased germinability of Cylindrocladium microsclerotia. Can. J. Microbiol. 25:157-162.

37. Rowe, R. C., and M. K. Beute. 1975. Ascospore formation and discharge by Calonectria crotalariae. Phytopathology 65:393-398.

38. Rowe, R. C., M. K. Beute, and J. C. Wells. 1973. Cylindrocladium black rot of peanuts in North Carolina - 1972. Plant Dis. Reptr. 57:387-389.

39. Rowe, R. C., S. A. Johnston, and M. K. Beute. 1974. Formation and dispersal of Cylindrocladium crotalariae microsclerotia in infected peanut roots. Phytopathology 64:1294-1297.

40. Rud, O. E. 1980. Weed control in peanuts. Pages 243-277 in: Virginia Pest Management Guide 1. Extension Division, Virginia Polytechnic Institute and State University, Blacksburg, VA.

41. Shaw, W. C., E. T. York, and W. C. Gregory. 1951. The effect of chemicals on weeds and the stand and yield of peanuts. Proc. So. Weed Conf. 4:112-120.

42. Taylor, J. D. 1980. Influence of crop rotation, minimum tillage in a soybean rotation, and peanut cultivars on the populations of Cylindrocladium crotalariae (Loos.) Bell and Sobers in field soils. Ph.D. Dissertation. Va. Polytech. Inst. State Univ. 128 pp.

43. Thies, W. G., and R. F. Patton. 1971. Evaluation of Cylindrocladium root rot control by fumigation. Forest Sci. 17:323-331.

44. Umechuruba, C. I., G. J. Griffin, and M. G. Hale. 1978. Effect of sodium azide on survival of Cylindrocladium microsclerotia and lettuce seeds in soil clods of high and low clay content. Plant Dis. Reptr. 62:1-5.

45. Womble, B. A., and K. H. Garren. 1978. Sodium azide offers some promise for control of Cylindrocladium black rot (CBR) of peanuts. Proc. Amer. Peanut Res. Ed. Assoc. 10:53. (Abstr.).

46. Wynne, J. C., R. C. Rowe, and M. K. Beute. 1975. Resistance of peanut genotypes to Cylindrocladium crotalariae. Peanut Sci. 2:54-56.

CHAPTER TWO

EFFECTS OF HERBICIDES ON AXENIC GROWTH OF *CYLINDROCLADIUM CROTALARIAE* AND SURVIVAL OF MICROSCLEROTIUM POPULATIONS IN TWO SOILS.

Introduction

The spectrum of activity of an individual herbicide is not necessarily limited to species of higher plants (1). Many of the organic herbicides used today are known to be actively metabolized by soil bacteria, fungi and actinomycetes (3). Earlier reviews (5,9,12) have reported that no significant changes in total populations of soil microorganisms occur when herbicides are applied to soil at normal rates. These reviews, however, did not consider the effects of specific chemicals on individual species of the microflora. Investigations concerning the effects of herbicides on mixed populations of soil microorganisms are numerous (12), but relatively little is known about how populations of specific soilborne plant pathogens are affected. Notable exceptions are reports by Tang, et al. (24), Percich and Lockwood (18) and Filho and Dhingra (11). Much more is known about how herbicides affect the growth, morphology and physiology of microorganisms in laboratory culture.

Growth inhibition and stimulation of several soilborne plant-pathogenic fungi in culture by herbicides has been observed (16). In many cases, a relationship exists between effects in culture and effects on disease severity (2). Chappell and Miller (10) and Backman, et al. (6) have found dinoseb to be both fungitoxic to Sclerotium rolfsii Sacc. in culture and suppressive to the peanut stem rot disease in the field. Oxadiazon was not toxic to this fungus in axenic culture, but like dinoseb, it suppressed the development of stem rot in field tests (6). Recently, Porter and Rud (20) found that dinoseb was fungitoxic to Sclerotinia minor Jagger and multiple postemergence applications of this herbicide suppressed development of Sclerotinia blight of peanut in the field.

Cylindrocladium black rot (CBR), caused by Cylindrocladium crotalariae (Loos.) Bell and Sobers, is now considered one of the most destructive diseases of the peanut (Arachis hypogaea L.) in Virginia (13). The fungus survives in soil primarily as microsclerotia (ms) formed in the cortex of infected peanut roots (17,21). Although this disease has received much attention by scientists in Virginia and North Carolina, there are currently no effective control measures available (19).

Dinoseb, dinitramine and several other herbicides have been extensively used for weed control in Virginia peanut

production (21). These chemicals have also been shown to have significant effects on soilborne diseases of the peanut and other crops (2,6,16,20). The present study was initiated to determine the direct effects of dinoseb, dinitramine and other herbicides on axenic growth of C. crotalariae, and to determine the effects of dinoseb and dinitramine on microsclerotium populations of this fungus in field soils. Growth tests were also conducted with S. minor and S. rolfsii for comparison to C. crotalariae and the results of other workers (7,10,20).

Materials and Methods

Growth Tests.

The growth response of all fungi was recorded as mycelial dry weight in herbicide-amended and non-amended potato-dextrose broth (PDB). Herbicides tested included alachlor, benefin, dinitramine, dinoseb, dinoseb + naptalam, diphenamid, vernolate, and 2,4-DB (see Tables 1A and 1B for detailed information on these herbicides). Stock solutions of each herbicide were prepared by adding precise amounts of the formulated materials to sterile distilled water just before use. Potato-dextrose broth (PDB) was prepared by the following method. Potato slices (200 g) were placed in 500 ml distilled water and autoclaved at 103.4 kPa for 45 min.

Table 1 B. Chemical names of herbicides used in laboratory, greenhouse and field experiments. [1]

Common Name	Chemical Name
alachlor	2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide
benefin	N-butyl-N-ethyl-α,α,α-trifluoro-2,6,-dinitro-P-toluidine
dinitramine	N^3,N^3-diethyl-2,4-dinitro-6-trifluoromethyl-m-phenylene diamine
dinoseb	2-sec-butyl-4,6-dinitrophenol
diphenamid	N, N-dimethyl-2,2-diphenylacetamide
naptalam	N-1-napthylpthalamic acid
vernolate	S-propyl dipropylthiocarbamate
2,4-DB	4-(2,4-dichlorophenoxy) butyric acid

[1] As approved by the Weed Science Society of America (2).

The resulting infusion was strained through three layers of cheesecloth, diluted to 1000 ml with distilled water and amended with 20 g dextrose. The medium was then dispensed into 250 ml Erlenmeyer flasks (25 ml/flask), autoclaved at 103.4 kPa for 15 min and cooled. The pH of PDB was determined to be 5.9 $\pm$ 0.05. Quantities of stock solutions of herbicides required to achieve final concentrations of 1, 5, 10, 50 and 100 µg a.i./ml PDB were then aseptically transferred to each of four replicate flasks. Herbicide stock solutions were found to have no effect on the pH of PDB.

The isolates of all test fungi were determined to be highly pathogenic to the peanut in previous tests. To initiate an experiment, each flask was inoculated with 1.0 ml of mycelial suspension. Axenic suspensions of fungi were prepared by comminuting mycelium from two seven-day old PDB cultures in 300 ml sterile distilled water. The initial mycelial dry weight in each ml of inoculum averaged 15.0 mg/ml. Inoculated medium in flasks was incubated without shaking at 25 C in a growth chamber in darkness for 10 days. Growth at this time was determined by collecting the mycelium on tared filter papers which were then dried at 70 C for 24 h and weighed to the nearest 0.1 mg.

Soil population studies.

The effects of herbicides on survival of C. crotalariae ms were determined by assays to determine populations of microsclerotia in herbicide-amended and unamended soils before and after a 30 day incubation period. The population assay used was that of Griffin (14). Quantities of two peanut field soils with different physical properties (Table 2) were collected from the A horizon of soil in fields where CBR had not been observed previously. Soils were passed through a 2 mm sieve and stored at 25 C until infestation.

Microsclerotia used for infestation of soil were obtained by culture of C. crotalariae on a high C/N agar medium. The medium was prepared (monobasic potassium phosphate, 1.0 g; anhydrous magnesium sulfate, 0.5 g); potassium chloride, 0.5 g; dextrose, 100.0 g; casein hydrolysate, 0.05 g; agar, 20.0 g; dist. water, 1000 ml; 1.0 ml of a micronutrient solution supplying 0.05 mg Mn^{+2}, 0.2 mg Zn^{+2}, and 0.1 mg Fe^{+3}), adjusted to pH 6.5, autoclaved (103.4 kPa for 15 min), poured into 9-cm Petri dishes (25 ml/dish) and cooled. Each plate was inoculated with 5.0 ml of mycelial suspension (prepared as for growth studies using 10-day-old potato-dextrose agar cultures), then incubated in darkness for 4-6 wk at 25 C. Microsclerotia were freed from the agar by comminuting the contents of plates for 2 min with a blender, then the propagules were washed with tap

Table 2. Physical properties of peanut field soils used for laboratory experiments.

Soil Name	Textural Class	Particle size Distribution[1] Sand (%)	Silt (%)	Clay (%)	Organic Matter (%)	pH	Water potential (bars) -0.1 Soil moisture (%)	-0.33 Soil moisture (%)	-5.0 Soil moisture (%)	-15.0 Soil moisture (%)
Woodstown	L.F.S[2]	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

[1] Determined by the hydrometer method

[2] Loamy fine sand

water for 2 min on a 74 μm sieve to remove mycelial fragments, and suspended in additional water in a beaker. The propagule suspension was standardized by counting the number of ms in six-0.1 ml aliquots on gridded Millipore filters (3.0-cm dia.). Desired quantities of ms were concentrated on the 74 μm sieve and washed into soil to achieve specific population densities per g of soil.

Each soil sample to be assayed by Griffin's procedure was first washed with running water on a 25 μm sieve for 5 min to reduce levels of undesired microorganisms before plating on the selective medium (14). A soil washing tower (SWT) was designed to eliminate the need to hand wash soil on sieves (Fig. 1). The SWT consisted of a galvanized sheet metal cylinder (80-cm high x 20-cm dia.) with plumbing for supplying water to an adjustable spray nozzle. The SWT diameter was slightly less than the inner diameter of the standard sieve frame (20.3 cm) to provide a water-impermeable, yet moveable interface between the two. A copper rod (3 mm-dia.) was soldered around the outside edge of the SWT, 7.5 mm above the bottom, to prevent the device from sliding downward when placed on the sieve and to provide stability. Water was directed from the supply (a garden hose attached to a cold water faucet) to the spray nozzle through 12.7-mm copper tubing and soldered fittings assembled as illutrated. An in-line adjustable valve was

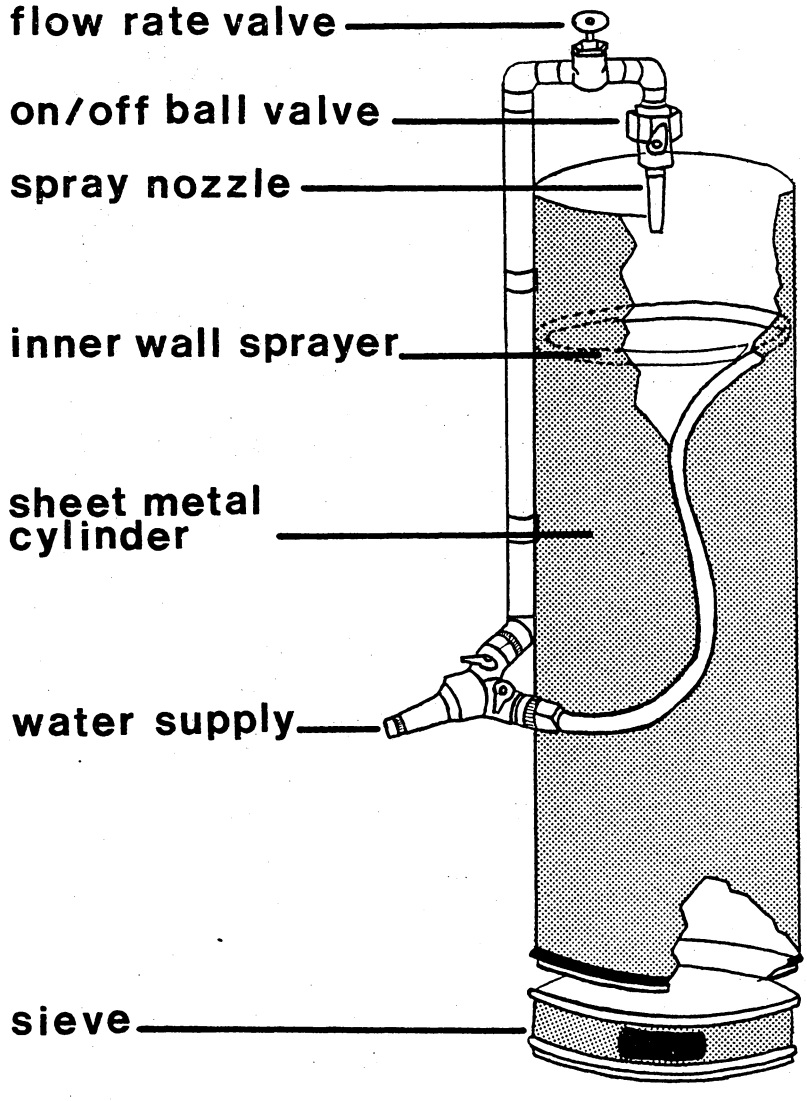


Fig. 1. Diagram of the soil-washing tower (SWT) used to process soil samples for Cylindrocladium crotalariae microsclerotium population assays.

installed to simplify flow rate adjustments and the separate on/off ball valve was used to start and stop washing without having to readjust flow rate. During washing, soil often splashed from the sieve upwards and adhered to the inside wall of the SWT. An auxiliary spray element (inner wall sprayer) was made by bending a section of 9.5-mm copper tubing to form a loop sealed at one end. Holes 1 mm in diameter were drilled 3-mm apart on the lower side of the loop and the element was installed by positioning the loop along the inside wall of the cylinder so that the straight end could be passed through a hole and connected to a water supply outside. When water was directed to this element via a separate on/off ball valve, the inside wall of the SWT was rinsed, and any soil particles remaining were flushed onto the sieve. All experiments were repeated at least once. Data were subjected to analysis of variance and means were compared by Duncan's multiple range test.

Results

Effects of herbicides on mycelial growth.

The axenic growth of two isolates of C. crotalariae (1 and 2) after 10 days incubation in PDB amended with alachlor, dinitramine, dinoseb and dinoseb + naptalam at 1, 5, and 10 µg/ml was not significantly suppressed (P=0.05)

below that in non-amended PDB (Table 3,4). Growth of isolate 1 was suppressed significantly in the presence of 1, 5, and 10 µg/ml of benefin and vernolate and with 1 and 5 µg/ml 2,4-DB. Only benefin at 10 µg/ml suppressed significantly growth of the second isolate. When the rates of alachlor, benefin, vernolate and 2,4-DB were increased to 50 and 100 µg/ml, growth response was not generally correlated with herbicide concentration. Dinoseb at 50 and 100 µg/ml and dinoseb + naptalam at 100 µg total active ingredient/ml suppressed significantly the growth of each isolate. Growth of isolate 2 was suppressed significantly by dinitramine at 50 and 100 µg/ml.

Of particular interest was the growth response of C. crotalariae in PDB amended with dinitramine and dinoseb at rates of 1, 5, and 10 µg/ml. Under these conditions, the mycelial growth of isolate 2 was significantly greater (P=0.05) than controls (Table 4). At 5 and 10 µg/ml dinoseb or dinitramine, growth of isolate 1 was also increased (although not significantly) (Table 3). Slopes of the concentration-response curves representing isolate 1 and isolate 2 for dinitramine and dinoseb at 0, 1, 5, and 10 µg/ml were not significantly different (P=0.05) from one another. Individual isolate responses were then pooled and expressed as a percent of the untreated controls. Regression analysis of the pooled responses indicated that

Table 3. Effect of herbicides on axenic growth of Cylindrocladium crotalariae (isolate 1) in culture[1].

Rate (µg a.i./ml)	Herbicide							
	alachlor	benefin	dinitramine	dinoseb	dinoseb + naptalam	diphenamid	vernolate	2,4-DB
0	93.2 B	93.2 A	93.2 CD	93.2 A	93.2 B	93.2 AB	93.2 B	93.2 A
1	85.6 B	78.2 B	88.1 D	92.2 A	111.6 A	86.2 B	74.7 C	76.3 B
5	81.3 B	80.6 B	95.8 CD	112.0 A	109.5 A	85.7 B	72.5 C	77.2 B
10	82.7 B	76.3 B	100.0 C	110.8 A	116.1 A	83.4 B	76.8 C	91.7 A
50	114.1 A	85.5 AB	133.8 B	15.1 B	118.0 A	84.5 B	83.4 BC	92.6 A
100	123.4 A	87.5 AB	153.9 A	8.1 B	55.6 C	100.1 A	120.1 A	94.7 A

[1] Growth expressed as the mean dry weight in milligrams of four replicate cultures grown for ten days in herbicide-amended and non-amended potato-dextrose broth at 25 C.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 4. Effect of herbicides on axenic growth of Cylindrocladium crotalariae (isolate 2) in culture[1].

Rate (µg a.i./ml)	Herbicide							
	alachlor	benefin	dinitramine	dinoseb	dinoseb + naptalam	diphenamid	vernolate	2,4-DB
0	187.0 AB	187.0 AB	187.0 B	187.0 A	187.0 B	187.0 A	187.0 C	187.0 C
1	193.8 AB	194.4 A	199.7 A	191.7 A	210.6 A	187.6 A	199.7 BC	197.6 BC
5	202.9 A	178.2 BC	205.8 A	196.5 A	209.7 A	199.8 A	195.2 C	209.6 AB
10	188.3 AB	172.1 C	209.2 A	196.1 A	219.0 A	186.0 A	212.9 AB	203.0 AB
50	177.7 B	193.3 A	168.3 C	91.2 B	211.6 A	193.2 A	219.8 A	168.3 D
100	153.6 C	198.3 A	166.8 C	47.1 C	126.0 C	188.7 A	216.0 A	217.0 A

[1] Growth expressed as the mean dry weight in milligrams of four replicate cultures grown for ten days in herbicide-amended and non-amended potato dextrose broth at 25 C.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

growth of C. crotalariae was increased in proportion to the concentration of dinitramine and dinoseb within a range of 1-10 µg/ml, according to a linear model. The r^2 values for linear regression were 0.8 and 0.7, respectively, for dinitramine and dinoseb. Regression lines are plotted in Fig. 2. Of the other herbicides tested, only dinoseb + naptalam induced a similar growth response in both isolates of C. crotalariae (Table 3,4).

Mycelial growth by S. minor cultures harvested from PDB amended with either alachlor, benefin, diphenamid or vernolate was often, but not always greater than mycelial growth in unamended PDB (Table 5). The response was statistically significant (P=0.05) in some cases. Dinitramine, dinoseb + naptalam and 2,4-DB at low rates also stimulated significantly the growth of S. minor. In response to these materials, growth increase was proportional to concentration within a range of 1-10 µg/ml. Growth was stimulated by dinoseb at 1 and 5 µg/ml but completely suppressed at higher concentrations. Dinoseb + naptalam at 50 and 100 µg/ml was also extremely toxic to this fungus.

The growth of S. rolfsii was suppressed significantly by dinoseb at 1.0 µg/ml and essentially prevented at higher concentrations (Table 6). Dinoseb + naptalam inhibited growth at 50 and 100 µg/ml. Alachlor, benefin, diphenamid

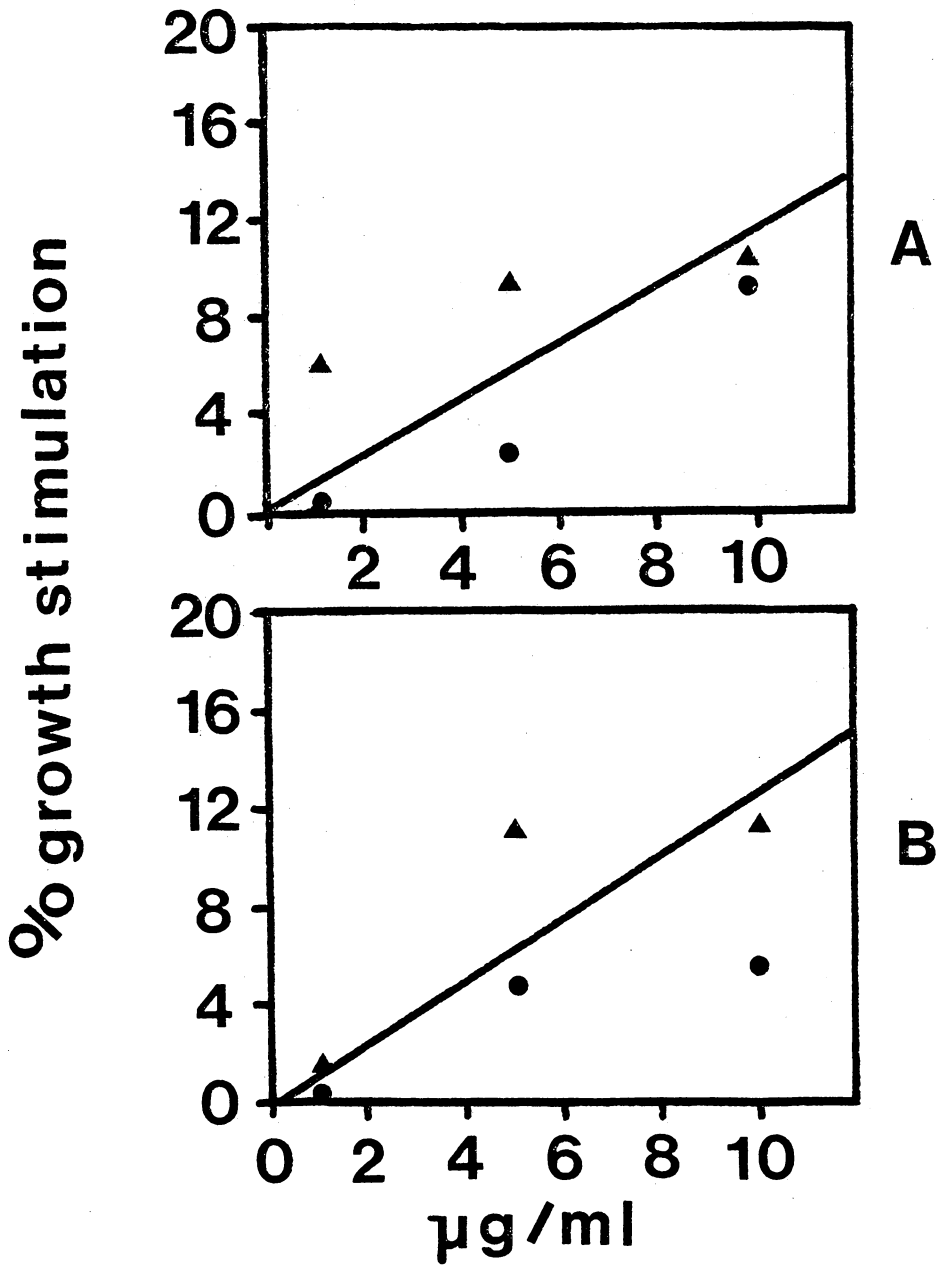


Fig. 2. Effect of dinitramine (A) and dinoseb (B) on axenic growth of Cylindrocladium crotalariae in culture. Lines represent regression equations calculated for linear models (r^2 dinitramine = 0.8; r^2 dinoseb = 0.7). Points represent the mean response of four replicate cultures (expressed as a % in excess of the untreated control, or % growth stimulation) observed for isolate 1 (dots) and isolate 2 (triangles), which were combined for this analysis.

Table 5. Effect of herbicides on axenic growth of Sclerotinia minor in culture[1].

Rate (µg a.i./ml)	Herbicide							
	alachlor	benefin	dinitramine	dinoseb	dinoseb + naptalam	diphenamid	vernolate	2,4-DB
0	104.3 D	104.3 C	104.3 E	104.3 C	104.3 D	104.3 C	104.3 B	104.3 D
1	93.7 D	130.0 B	141.3 D	129.8 B	115.9 C	130.0 B	93.7 B	213.9 C
5	127.4 C	126.6 BC	203.0 C	206.2 A	130.0 B	150.3 A	102.2 B	289.5 B
10	138.1 C	125.7 BC	257.7 A	6.9 D	146.5 A	149.3 A	112.1 AB	324.2 A
50	161.5 B	138.3 B	222.3 B	6.0 D	11.9 E	121.9 B	117.1 AB	323.2 A
100	174.4 A	173.4 A	223.2 B	6.4 D	7.4 E	152.4 A	124.2 A	318.1 A

[1] Growth expressed as the mean dry weight in milligrams of four replicate cultures grown for ten days in herbicide-amended and non-amended potato-dextrose broth at 25 C.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 6. Effect of herbicides on axenic growth of Sclerotium rolfsii in culture[1].

Rate (µg a.i./ml)	Herbicide							
	alachlor	benefin	dinitramine	dinoseb	dinoseb + naptalam	diphenamid	vernolate	2,4-DB
0	286.0 A	286.0 A	286.0 A	286.0 A	286.0 A	286.0 A	286.0 A	286.0 A
1	259.0 BC	231.3 BC	244.1 B	201.1 B	256.8 B	264.3 AB	262.7 AB	251.4 B
5	269.7 AB	221.1 CD	162.9 C	19.7 C	238.6 B	260.8 B	250.3 B	256.2 B
10	272.0 AB	242.0 B	159.9 C	15.7 C	210.2 C	273.9 AB	238.0 B	256.2 B
50	242.2 C	207.3 DE	156.2 C	18.0 C	17.3 D	222.2 C	251.2 B	261.4 B
100	234.7 C	200.5 E	123.3 C	15.6 C	16.3 D	215.3 C	239.5 B	18.1 C

[1] Growth expressed as the mean dry weight in milligrams of four replicate cultures grown for ten days in herbicide-amended and non-amended potato-dextrose broth at 25 C.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

and 2,4-DB resulted in growth suppression that was detectable, but not of a high magnitude except for 2,4-DB at 100 µg/ml. Although mycelial growth was suppressed to levels that were proportionate to increased rates of dinitramine, this herbicide was far less toxic to S. rolfsii than dinoseb or dinoseb + naptalam. In no case was mycelial growth of this fungus stimulated by the herbicides tested.

Herbicide effects on survival of C. crotalariae ms in soil.

Since dinitramine and dinoseb were the only herbicides that consistently affected growth of C. crotalariae at rates corresponding to field use, they were selected for study in the following tests. Quantities of a standardized water suspension of ms were added to soil samples of known weight and moisture content to achieve a population density of ca. 100 ms/g. Soils were mixed for 10 min in plastic bags to distribute the propagules evenly. Infested soil samples of each type weighing 144.0 g (dry weight) were placed in 9-cm x 6-cm deep-welled glass Petri dishes fitted with lids that permitted some gas exchange. Stock solutions of dinitramine and dinoseb were prepared with water. Quantities of stocks required to achieve soil concentrations of 1, 5, 10, 50 and 100 µg a.i./g soil were diluted to 3.0 ml with water and poured onto the soil surface. There were three replicates

per treatment. The herbicides were immediately mixed into the soils with a spatula and the loosely-covered dishes were placed in covered plastic culture boxes along with an open beaker of water to prevent moisture loss. After 30 days, the soils were mixed again, tested for moisture content gravimetrically, and assayed for populations of ms using the procedure of Griffin (14), with the modified soil washing procedure.

There were no significant differences (P=0.05) in the numbers of C. crotalariae ms recovered from unamended soils before and after a 30 day incubation period. Results of population assays performed on infested soils incubated after treatment with dinitramine at 1, 5, 10, 50 and 100 µg/g soil indicated that the herbicide had no significant effect on survival of ms in soil (Table 7). Slight reductions in rates of recovery of ms were noted for each soil type treated with dinitramine at 100 µg/ml relative to untreated soils, but these levels were not significantly different from initial populations. Dinoseb reduced significantly the recovery of ms populations from Ruston soil at 50 and 100 µg/g soil and from Woodstown soil at 5, 10, 50 and 100 µg/g soil. Soil type apparently influenced the sensitivity of ms to dinoseb (Table 7). Approximately ten times more dinoseb was required to reduce populations significantly in the Ruston soil than in the Woodstown soil (Table 7).

Table 7. Influence of dinitramine and dinoseb on recovery of Cylindrocladium crotalariae microsclerotia in a Ruston and a Woodstown loamy fine sand. [1]

Herbicide	Soil	Rate (µg/g soil) 0	1	5	10	50	100
dinitramine	Ruston	90.6 A	88.6 A	96.7 A	88.5 A	91.7 A	84.4 A
	Woodstown	93.3 AB	93.5 AB	81.2 B	96.4 A	88.7 AB	87.4 AB
dinoseb	Ruston	100.0 A	100.0 A	100.0 A	94.9 A	59.7 B	15.9 C
	Woodstown	97.6 A	100.0 A	78.0 B	76.0 B	60.2 C	31.6 D

[1] Means represent three replicate soil samples treated with herbicides and incubated for 1 mo at 25 C in darkness. Values for recovery are the percentages of initial populations prior to application of herbicides. Initial populations were 98.9 microsclerotia/g in Ruston soil and 90.4 microsclerotia/g in Woodstown soil.

Means for each soil type followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

Discussion

Results indicated that mycelial growth of C. crotalariae was inhibited consistently only when exposed to 50 and 100 µg/ml of dinoseb and dinoseb + naptalam, respectively. The maximum recommended rates for single applications of dinitramine and dinoseb in Virginia are 0.56 and 1.68 kg/ha, respectively (Table 1) (22). Concentrations of dinitramine and dinoseb in the uppermost 7.5 cm of soils treated at these rates would theoretically not exceed 2.5 and 15.0 µg/g soil water (Table 8). At rates in a nutrient medium (PDB) which approximate these soil concentrations, dinitramine, dinoseb and dinoseb + naptalam consistently enhanced growth of C. crotalariae and might be expected to increase the severity of CBR of peanut. Growth of S. minor was also enhanced by dinitramine, dinoseb + naptalam, and 2,4-DB at the same rates, but dinoseb was fungitoxic to both S. minor and S. rolfsii at these rates in PDB. Dinoseb at low rates has been shown to inhibit linear growth of S. minor and S. rolfsii on amended agar media (6,7,10,20). In contrast to the present results, Porter and Rud (20) reported the rate of linear growth of S. minor mycelium to be suppressed by dinoseb + naptalam and 2,4-DB at rates which enhanced growth in this study. The use of acetone as

Table 8. Estimated concentrations of herbicides in soil after surface application and incorporation. [1]

Rate		Concentration	
kg/ha	lb/A	µg a.i./g soil	µg a.i./g soil water[2]
0.56	0.5	0.50	5.0
0.84	0.75	0.75	7.5
1.68	1.5	1.50	15.0
3.36	3.0	3.00	30.0
6.72	6.0	6.00	60.0

[1] Calculations assume that herbicides are uniformly distributed within the upper 7.5 cm of the soil profile.

[2] Soil moisture assumed is 10%.

a solvent for preparing stock solutions to amend media with these herbicides may explain the increase in sensitivity to herbicide concentrations observed by Porter and Rud.

A mechanism for herbicide-induced growth enhancement or inhibition could not be determined by the tests conducted. The concentration of herbicides, when compared to dextrose and other nutrients in amended PDB was relatively low. In cases of enhanced growth, it appears doubtful that herbicides would be significant carbon or nitrogen sources when more readily-available forms of these elements were present in higher concentrations. The observed responses (stimulatory and inhibitory) may have in part been due to surfactants used in formulating the herbicides. Tween-80, a non-ionic surfactant, is known to increase significantly the growth rate of certain fungi (8). Other surfactants inhibit growth (23). The identity of pesticide formulation adjuvants is proprietary information that is not often available to researchers.

Dinitramine had no effect on inoculum densities of C. crotalariae in the two soils tested. Dinitramine at low rates stimulated growth of C. crotalariae in culture and it might be expected to temporarily stimulate microsclerotium germinability although this was not determined here. Tang, et al. (24) found that trifluralin, a related herbicide, enhanced production and germinability of chlamydospores of

Fusarium oxysporum f. sp. vasinfectum (Atk.) Snyd. and Hans. in soil at concentrations of 0.6-40.0 µg/g.

Dinoseb reduced significantly the density of C. crotalariae ms in Woodstown soil but not in Ruston soil at rates consistent with grower usage. In similar experiments, Filho and Dhingra (11) found that dinoseb reduced populations of Macrophomina phaseolina (Tassi.) Goid. by 61% in a sandy loam and by 96% in a sandy clay loam soil after a 10 day incubation period. The Woodstown soil used in these tests contained twice the organic matter and 3.6% more clay than the Ruston soil (Table 2). Dinoseb and its cationic alkanolamine salt are readily leached from highly porous soils (i.e. Ruston) but are more tightly held with increasing percentages of clay and organic matter (i.e. Woodstown) (3). The increased number of binding sites provided by the additional colloidal materials in Woodstown soil may provide for interactions between dinoseb and C. crotalariae ms that are not allowed in the other soil. It is also possible that soil populations of microorganisms antagonistic to C. crotalariae or capable of degrading dinoseb completely or to a more toxic analogue may be involved. The fact that dinoseb, at equivalent concentrations, stimulated axenic growth of the fungus and reduced populations of ms in the Woodstown soil cannot presently be explained. It is recognized that many more

variables operate in soil than in sterile nutrient solutions. In any case, the magnitude of population reduction resulting from dinoseb at rates comparable to use patterns in peanut production does not justify its use as a soil fungicide against C. crotalariae.

Previous reports (6,10,15,20) indicate that dinitramine and dinoseb-containing herbicides influence significantly certain soilborne diseases of the peanut. Results presented and discussed in this chapter are summarized as follows: 1) dinitramine and dinoseb tended to enhance the axenic growth of C. crotalariae at rates corresponding to field management practice, and 2) dinoseb treatments reduced significantly numbers of germinable C. crotalariae ms in soil, whereas dinitramine had no effect. Although dinoseb appeared to be fungitoxic to ms, no herbicide used in these studies demonstrated a recognizable potential for use as a fungicide in CBR disease control programs. The effect of dinitramine and dinoseb on development of CBR in peanut is the subject of a later chapter.

Literature Cited

1. Alexander, M. 1977. Introduction to soil microbiology. John Wiley and sons. New York. 467 pp.

2. Altman, J., and C. L. Campbell. 1977. Effects of herbicides on plant diseases. Ann. Rev. Phytopathol. 15:361-385.

3. Anderson, W. P. 1977. Weed Science: Principles. West Publishing Co. New York. 598 pp.

4. Anonymous. 1979. Herbicide Handbook of the Weed Science Society of America. Weed Sci. Soc. Amer. Champaign, ILL. 479 pp.

5. Audus, L. J. 1960. Microbiological breakdown of herbicides in soils. Pages 1-19 in: E. K. Woodford and G. R. Sagar (Eds.), Herbicides and the soil. Blackwell Scientific Publications. Oxford, UK.

6. Backman, P. A., R. Rodriguez-Kabana, and G. A. Buchanan. 1977. Interactions of oxadiazon and dinoseb with stem rot in peanuts. Weed Sci. 25:260-263.

7. Bain, D. C. 1961. Effect of various herbicides on some soil fungi in culture. Plant Dis. Reptr. 45:814-817.

8. Binder, F. L. 1974. Differences in the uptake of glucose and glycerol by Tieghemiomyces parasiticus in axenic culture. Can. J. Botany 52:2571-2574.

9. Bollen, W. B. 1961. Interactions between pesticides and soil microorganisms. Ann. Rev. Microbiol. 15:69-91.

10. Chappell, W. E., and L. I. Miller. 1956. The effects of certain herbicides on plant pathogens. Plant Dis. Reptr. 40:52-56.

11. Filho, E. S., and O. D. Dhingra. 1980. Effect of herbicides on survival of Macrophomina phaseolina in soil. Trans. Br. Mycol. Soc. 74:61-64.

12. Fletcher, W. M. 1960. The effect of herbicides on soil microorganisms. Pages 20-62 In: E. K. Woodford and G. R. Sagar (Eds.), Herbicides and the soil. Blackwell Scientific Publications. Oxford, UK.

13. Garren, K. H. 1973. Further studies on Cylindrocladium black rot of peanut in Virginia. Jour. Amer. Peanut Res. Ed. Assoc. 5:31-32.

14. Griffin, G. J. 1977. Improved selective medium for isolating Cylindrocladium crotalariae microsclerotia from naturally-infested soils. Can. J. Microbiol. 23:680-683.

15. Grinstein, A., Y. Elad, J. Katan, and I. Chet. 1979. Control of Sclerotium rolfsii by means of a herbicide and Trichoderma harzianum. Plant Dis. Reptr. 63:823-826.

16. Katan, J., and Y. Eshel. 1973. Interactions between herbicides and plant pathogens. Residue Rev. 45:145-177.

17. Krigsvold, D. T., and G. J. Griffin. 1975. Quantitative isolation of Cylindrocladium crotalariae microsclerotia from naturally-infested peanut and soybean fields. Plant Dis. Reptr. 59:543-546.

18. Percich, J. A., and J. L. Lockwood. 1975. Influence of atrazine on the severity of Fusarium root rot of pea and corn. Phytopathology 65:154-159.

19. Phipps, P. M. 1978. Cylindrocladium black rot. Control Series 167. Va. Polytech. Inst. State Univ. Extension Div., Blacksburg, VA.

20. Porter, D. M., and O. E. Rud. 1980. Suppression of Sclerotinia blight of peanut with dinitrophenol herbicides. Phytopathology 70:729-722.

21. Rowe, R. C., S. A. Johnston, and M. K. Beute. 1974. Formation and dispersal of Cylindrocladium crotalariae microsclerotia in infected peanut roots. Phytopathology 64:1294-1297.

22. Rud, O. E. 1980. Weed Control in peanuts. Pages 243-277 in: Virginia Pest Management Guide 1. Extension Div. Virginia Polytech. Inst. and State Univ. Blacksburg, VA.

23. Steiner, G. W., and R. D. Watson. 1965. The effect of surfactants on growth of fungi. Phytopathology 55:1009-1012.

24. Tang, A., E. A. Curl, and R. Rodriguez-Kabana. 1970. Effect of trifluralin on inoculum density and spore germination of Fusarium oxysporum f. sp. vasinfectum in soil. Phytopathology 60:1082-1086.

CHAPTER THREE

EFFECTS OF DINITRAMINE AND DINOSEB ON CYLINDROCLADIUM BLACK ROT OF PEANUT

Introduction

Cylindrocladium black rot (CBR), incited by Cylindrocladium crotalariae (Loos.) Bell and Sobers (7) is one of the most destructive soilborne diseases of peanut (Arachis hypogaea L.) in Virginia (12). Intensive efforts by workers in Virginia and in North Carolina have provided considerable information on the biology of C. crotalariae, but no effective and/or economical control measures for CBR. Efforts to develop CBR-resistant peanut cultivars (29) and certain soil fumigant treatments (24) have shown some promise for control of this disease.

Herbicides of several types are now used extensively in peanut production (see Table 1A) (27). Herbicides, like other pesticides may have significant effects on non-target organisms and plant diseases (1, 17). Evidence that dinoseb suppresses the severity of certain diseases caused by soilborne plant pathogens has been presented (4,9,11,25). Chappell and Miller (9) and Backman et al (4) have demonstrated that dinoseb can reduce the severity of southern stem rot of peanut (Sclerotium rolfsii Sacc.).

Recently, Porter and Rud (25) showed that dinoseb can also suppress the severity of Sclerotinia blight of peanut (Sclerotinia minor Jagger.) and result in a significant yield increase. Dinoseb has been demonstrated to have in vitro fungitoxicity against several plant pathogenic fungi (4,9,11,25), but this fungitoxicity has not been directly related to disease suppression. Dinoseb has been reported to increase the severity of certain diseases of snap bean (Phaseolus vulgaris L.) and soybean (Glycine max L.) (26,30).

Dinitramine, a dinitroaniline herbicide, can increase or decrease the severity of certain plant diseases, depending on the plant and pathogen involved (11,14). Grinstein et al. (14) reported that the peanut was rendered resistant to southern stem rot disease by dinitramine in greenhouse and field tests. However, dinitramine increased significantly the severity of Rhizoctonia disease in cotton (10) and Fusarium root rot in navy bean (P. vulgaris) (30).

Non-target effects of peanut production practices on diseases must be understood if economical and effective control strategies for CBR are to be developed. The present study was initiated to determine the influence of dinitramine and dinoseb on the severity of CBR in peanut under greenhouse conditions. Data from in vitro experiments suggest that these two herbicides may have a significant

effect on the development of CBR and on the biology of C. crotalariae in soil. Furthermore, these herbicides have been demonstrated to have a significant effect on development of other soilborne diseases of the peanut (14,25). A preliminary report has been published on a portion of the work reported here (5).

Materials and Methods

Properties of soils.

Quantities of soil free of C. crotalariae were collected in late February and early March from the A horizon of two peanut fields in southeastern Virginia (separated by ca. 0.8 km), and stored in plastic-lined cans in a greenhouse. The percentage composition of sand, silt, clay, and organic matter and percent soil moisture at selected water potentials were determined for each soil and recorded in Table 2 (Chapter 2). Personnel at the soil physics laboratory at V.P.I.& S.U. classified one soil as Ruston loamy fine sand and the other as Woodstown loamy fine sand.

Infesting soils.

Microsclerotia (ms) of three C. crotalariae isolates from severely diseased peanut roots were produced on a high

C/N agar medium as described previously (Chapter 2). The same three isolates were used for all greenhouse experiments to ensure uniform disease development. A water suspension of the propagules was standardized by counting the number of ms in six-0.1 ml aliquots on gridded Millipore filters. Volumes of standardized suspension were concentrated on a 74 um sieve and washed into pre-weighed portions of soil to achieve desired population densities of ms in soil. Soil and inoculum were mixed for 20 min in a portable, motor-driven cement mixer to provide uniform distribution of ms in soil, then dispensed into 11.4-cm plastic pots (ca. 1300 g dry weight of soil/pot) each with a single hole to permit drainage. Populations were confirmed by soil assay using the procedure of Griffin (13).

Application of herbicides.

Stock solutions of herbicides were prepared with tap water to obtain the desired rates of dinitramine (0.28, 0.56, and 0.84 kg/ha) and dinoseb (1.68, 3.36, and 6.72 kg/ha). The required quantity of herbicide stock solution was diluted to a final volume of 20.0 ml with water, poured over the surface of soil in each pot, then incorporated into the upper 2.5 cm of soil with a large spatula. In one test, dinoseb was applied 4 wks after planting to simulate a late-season postemergence (layby) treatment.

Assessment of herbicide effects on disease severity.

Six replicate pots prepared identically comprised a single treatment. After herbicides were applied, the pots were placed in temperature-controlled water baths (set to maintain 25 ± 2 C) each equipped with watertight submerged manifolds to facilitate drainage of excess water and prevent soil saturation (Fig. 3). Four hand-selected seeds of 'Florigiant' peanut were treated (0.25 g Botec /kg seed) and planted in each pot. Subsequent to emergence, seedlings were thinned to two seedlings/pot for all experiments unless described otherwise. Soil in each pot was irrigated at intervals necessary to maintain soil moisture near field capacity during each test (23).

After 8 wk, plants were carefully removed from pots. The roots were washed to remove soil and then blotted with paper towels to remove excess water. Severity of both root rot and top symptoms were rated using a scale of 1 to 5 (1= no symptoms, 5= dead plant) (Fig. 4). After recording the fresh weight of shoots and roots, twenty root biopsy tissue samples were collected at random from each replicate (10/plant). Tissues were surface-disinfested for 2 min in 0.5% NaOCl, then placed on an agar medium developed for selective isolation of C. crotalariae (13). The extent of root colonization was expressed as the percent of root segments which yielded the fungus after 6 days incubation at 25 C.

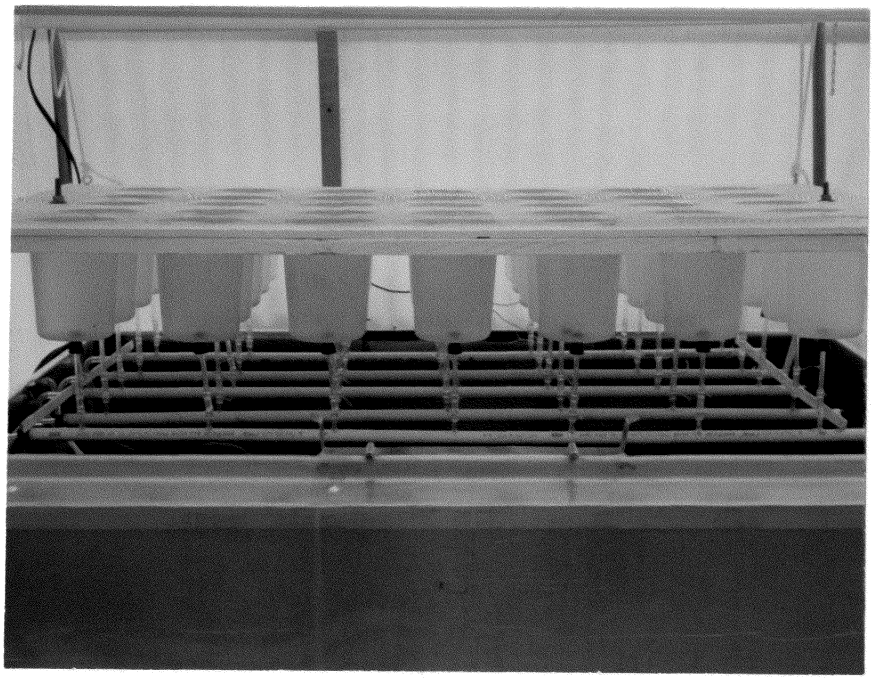


Fig. 3. Watertight manifold system used in greenhouse temperature baths to facilitate drainage of excess irrigation water from pots.

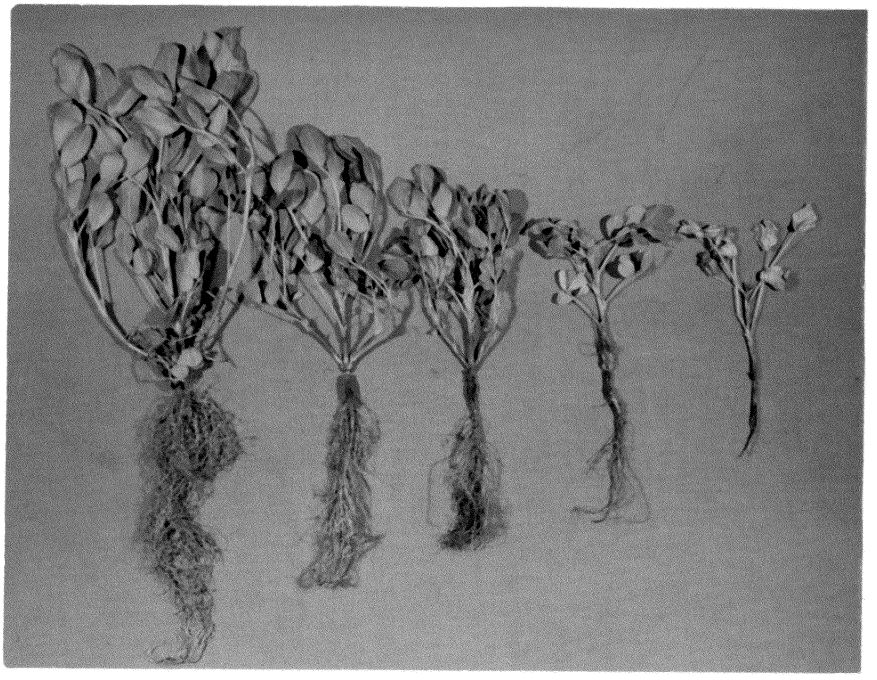


Fig. 4. Illustration of numerical scale used to rate peanut plants for severity of root rot and top symptoms caused by Cylindrocladium crotalariae. From left to right, the plants shown here were rated 1, 2, 3, 4, and 5, respectively. (1 = no symptoms; 5 = severe symptoms).

Data were subjected to analysis of variance and means were compared by Duncan's multiple range test (3). All experiments were repeated at least once.

Results

Influence of herbicides on disease severity.

In initial tests, dinitramine at a rate of 0.56 kg/ha or dinoseb at 1.68 kg/ha increased significantly (P=0.05) the severity of CBR after 8 wks in Woodstown soil infested to a density of 30 ms/g (Table 9). When roots of plants were examined, it was consistently observed that those grown in infested soil receiving either herbicide treatment were more severly rotted and had a higher percentage of colonized root segments than the infested controls. An illustration of the effects of dinitramine on CBR is presented in Fig. 5. Symptoms and signs of CBR among plants grown in infested soil treated with dinitramine often appeared 2-3 wk in advance of controls (data not shown).

Preliminary evidence that untreated, infested Woodstown soil supported low levels of disease was observed (Table 9). Further experiments with herbicides required knowledge of this effect. Quantities of Woodstown soil and Ruston soil (which has less organic matter and more sand than Woodstown soil) were infested to densities of 5 and 50 ms/g soil and

Table 9. Effect of dinitramine and dinoseb on the severity of Cylindrocladium black rot of peanut in Woodstown loamy fine sand.[1]

Treatment[2]	Root rot index[3] (1-5)	
Infested soil (30 ms/g)	Experiment 1	Experiment 2
control	2.1 B	1.2 C
dinitramine 0.56 kg/ha PPI	3.2 A	3.0 A
dinoseb 1.68 kg/ha PPI	3.0 A	2.3 B
dinoseb 3.36 kg/ha PPI + 1.68 kg/ha layby	N.D.	1.9 B
Noninfested soil		
control	1.3 C	1.1 C

[1] 'Florigiant' peanut plants were grown from seed in non-sterile field soil infested with Cylindrocladium crotalariae microsclerotia for 8 wks, then harvested and rated for disease severity.

[2] PPI = pre-plant incorporated; layby = applied once postemergence 4 wks after planting.

[3] Means represent 6 replicates (2 plants/replicate); 1 = no symptoms, 5 = severe rot. N.D. = not determined.

Means in columns followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

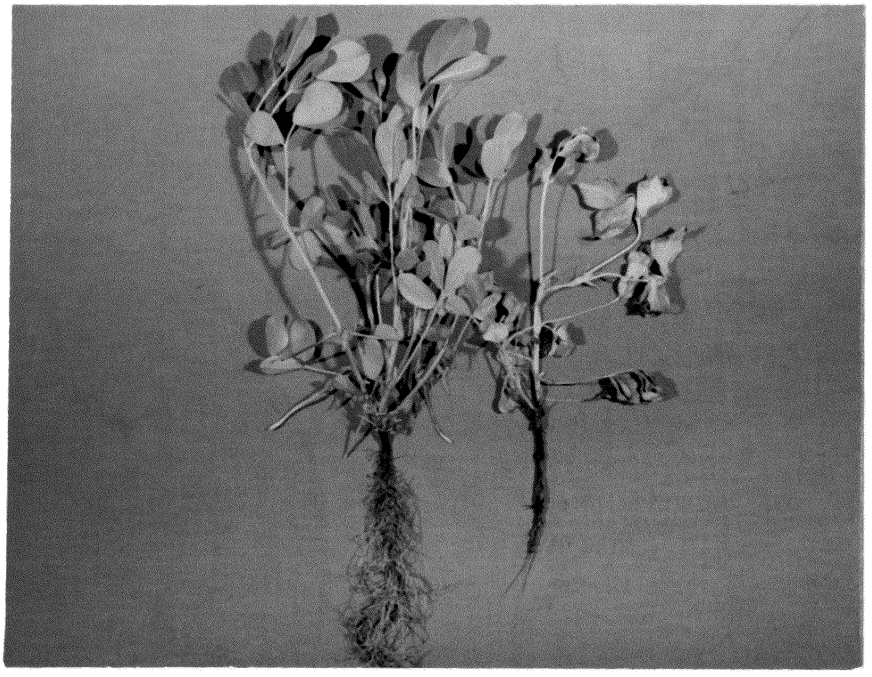


Fig. 5. Effect of dinitramine at 0.56 kg/ha on the severity of Cylindrocladium black rot in peanut. Plants shown were grown in Ruston loamy fine sand infested with Cylindrocladium crotalariae microsclerotia to a density of 5 ms/g soil which was either treated with the herbicide (right) or untreated (left).

planted with seed of Florigiant peanut as described earlier. Soil temperature was maintained at 25 C for 8 wk as in previous tests. Symptoms of CBR in 8-week-old plants were consistently more severe in Ruston than in Woodstown soil at both inoculum densities (Fig. 6). However, only at a density of 50 ms/g was this difference significant (P=0.05). Root rot, top symptom severity, and percent of colonized root segments was similar for plants grown in Woodstown soil infested at 50 ms/g and Ruston soil infested at 5 ms/g (Fig. 6).

Influence of herbicides, inoculum densities and soil types on CBR.

Dinitramine at 0.28, 0.56 and 0.84 kg/ha and dinoseb at 1.68, 3.36, and 6.72 kg/ha were applied to Ruston and Woodstown soil infested with ms to provide densities of 5 and 50 propagules/g soil for these tests. The severity of root rot was increased significantly (P=0.05) in Ruston soil that was infested with 5 ms/g soil and treated with dinitramine at 0.56 kg/ha (Table 10). The herbicide did not increase disease severity in Ruston soil infested with 50 ms/g soil or when applied at a rate of 0.84 kg/ha to either soil with any level of ms densities (Table 10,11). At a density of 50 ms/g, dinitramine at 0.56 kg/ha increased root rot significantly among plants grown in the Woodstown soil.

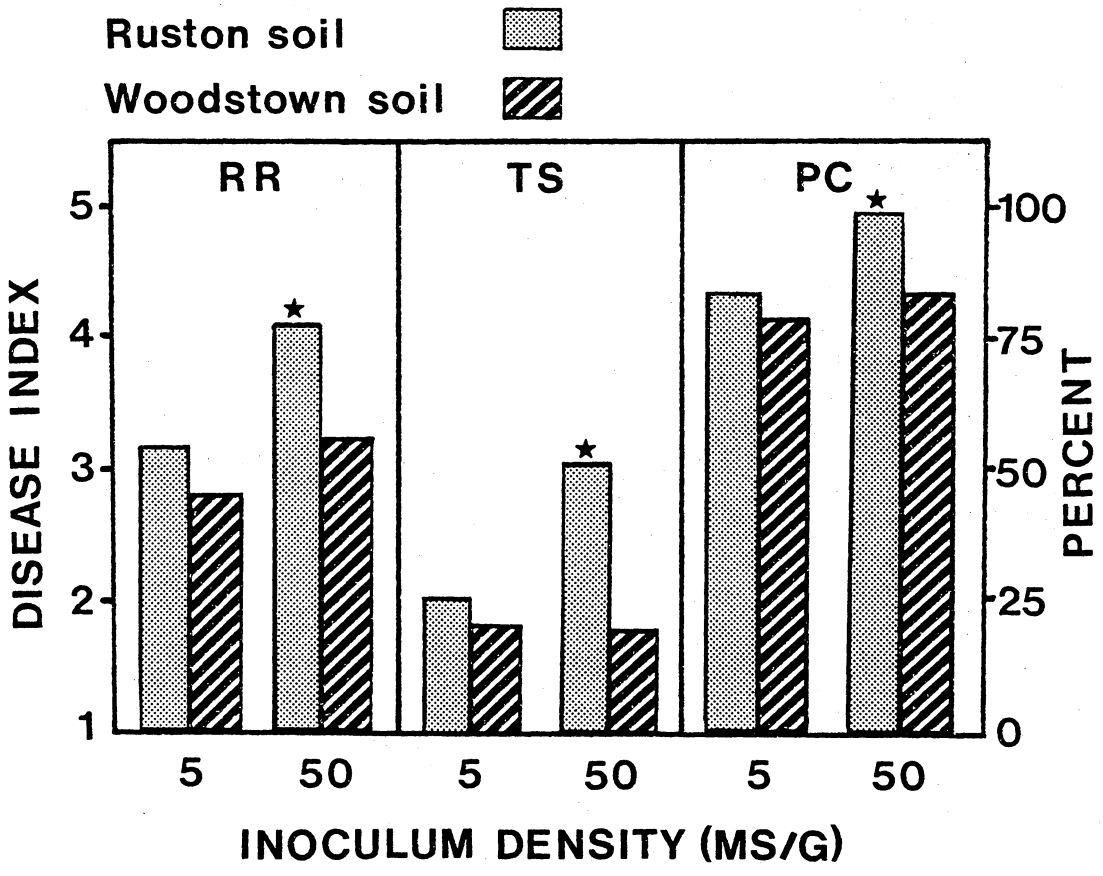


Fig. 6. Influence of soil type and inoculum density on the severity of Cylindrocladium black rot of peanut. RR = root rot index; TS = top symptoms index; PC = percent of colonized root segments. Bars labeled with a star denote means that are significantly different (P=0.05) between soil types.

Table 10. Influence of dinitramine, dinoseb and densities of Cylindrocladium crotalariae microsclerotia on the severity of Cylindrocladium black rot of peanut in a Ruston loamy fine sand.[1]

Inoculum density (ms/g)	Herbicide and rate[2] (kg/ha)	Root rot index[3] (1-5)	% Colonized root segments	Top symptoms index[4] (1-5)	Growth (fresh wt.) (grams)	
					Shoots	Roots
0	none applied	1.0 E	0.0 G	1.0 D	11.7 A	3.7 A
5	dinitramine 0.28 PPI	2.4 D	56.8 DEF	1.4 ABCD	10.5 AB	2.5 AB
5	dinitramine 0.56 PPI	3.8 A	71.0 BCD	2.6 ABC	8.4 ABC	2.0 AB
5	dinitramine 0.84 PPI	3.2 ABCD	67.5 CD	2.4 A	9.2 ABC	2.1 AB
5	dinoseb 1.68 PPI	2.4 D	38.5 F	1.3 CD	9.8 ABC	3.4 A
5	dinoseb 3.36 PPI	2.5 D	59.5 DEF	1.3 BCD	10.8 AB	3.0 AB
5	dinoseb 6.72 PPI	2.6 CD	42.6 EF	1.4 ABCD	10.6 AB	2.7 AB
5	none applied	3.0 BCD	60.6 DE	2.6 ABC	8.9 ABC	2.2 AB
50	dinitramine 0.28 PPI	4.0 A	100.0 A	2.6 AB	8.1 BC	2.0 AB
50	dinitramine 0.56 PPI	3.9 A	99.1 A	2.0 ABCD	9.6 ABC	1.6 B
50	dinitramine 0.84 PPI	3.4 ABC	90.5 AB	1.5 ABCD	9.1 ABC	2.1 AB
50	dinoseb 1.68 PPI	3.3 ABC	98.0 A	2.0 ABCD	9.1 ABC	3.1 AB
50	dinoseb 3.36 PPI	2.8 BCD	84.0 ABC	1.6 ABCD	9.7 ABC	2.2 AB
50	dinoseb 6.72 PPI	3.6 AB	95.5 A	1.6 ABCD	9.3 ABC	1.9 AB
50	none applied	3.9 A	100.0 A	2.6 AB	6.9 C	1.6 B

[1] 'Florigiant' peanut plants were grown from seed in non-sterile field soil infested with Cylindrocladium crotalariae microsclerotia for 8 wks, then harvested and rated for disease severity.

[2] PPI = pre-plant incorporated.

[3] 1 = no symptoms, 5 = severe rot.

[4] 1 = no symptoms, 5 = dead plant.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 11. Influence of dinitramine, dinoseb and densities of Cylindrocladium crotalariae microsclerotia on the severity of Cylindrocladium black rot of peanut in a Woodstown loamy fine sand.[1]

Inoculum density (ms/g)	Herbicide and rate[2] (kg/ha)	Root rot index[3] (1-5)	% Colonized root segments	Top symptoms index[4] (1-5)	Growth (fresh wt.) (grams)	
					Shoots	Roots
0	none applied	1.0 G	0.0 E	1.0 C	12.0 A	4.0 A
5	dinitramine 0.28 PPI[1]	2.0 DE	9.3 DE	2.0 B	10.9 ABC	3.4 ABC
5	dinitramine 0.56 PPI	3.0 BC	38.6 CD	1.9 BC	10.9 ABC	4.1 A
5	dinitramine 0.84 PPI	1.9 EF	28.3 C	1.6 BC	10.3 ABCD	3.9 A
5	dinoseb 1.68 PPI	2.3 CDE	26.8 CD	1.5 BC	11.5 AB	3.3 ABC
5	dinoseb 3.36 PPI	2.4 CDE	20.3 CDE	1.6 BC	10.6 ABC	3.6 AB
5	dinoseb 6.72 PPI	1.2 FG	19.0 CDE	1.2 BC	10.8 ABC	4.1 A
5	none applied	2.5 CDE	23.5 CD	1.6 BC	11.1 ABC	3.2 ABC
50	dinitramine 0.28 PPI	3.7 AB	92.3 AB	3.2 A	6.5 EF	2.7 BC
50	dinitramine 0.56 PPI	4.0 A	96.6 A	3.0 A	6.0 F	2.5 C
50	dinitramine 0.84 PPI	2.9 CD	90.3 AB	1.9 BC	8.3 CDEF	3.9 A
50	dinoseb 1.68 PPI	2.7 CDE	85.6 AB	1.8 BC	7.4 DEF	3.0 ABC
50	dinoseb 3.36 PPI	2.5 CDE	91.6 AB	2.0 B	8.8 BCDEF	3.6 AB
50	dinoseb 6.72 PPI	2.4 CDE	91.3 AB	1.8 BC	10.0 ABCD	3.2 ABC
50	none applied	3.0 BC	72.6 B	1.8 BC	9.2 ABCDE	3.4 ABC

[1] 'Florigiant' peanut plants were grown from seed in non-sterile field soil infested with Cylindrocladium crotalariae microsclerotia for 8 wks, then harvested and rated for disease severity.

[2] PPI = pre-plant incorporated.

[3] 1 = no symptoms, 5 = severe rot.

[4] 1 = no symptoms, 5 = dead plant.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

Differences in top symptom severity and percentages of colonized root segments also reflected these trends (Table 10,11). In contrast to initial results (Table 9), dinoseb had no effect on CBR in either soil type at the inoculum densities used in these experiments.

Effect of dinitramine on peanut and the severity of CBR.

Peanut seeds were planted in portions of noninfested Ruston soil that was untreated or treated with dinitramine (0.56 kg/ha) as described earlier. After 5 days, the seedlings from untreated, noninfested soil were transplanted to pots containing infested (10 ms/g soil) and noninfested Ruston soil with and without dinitramine. At the same time, seedlings from dinitramine-treated, noninfested soil were washed with water to remove herbicide-containing soil particles and transplanted to pots of infested (10 ms/g soil) or noninfested Ruston soil which received no herbicide. After 8 wk, symptoms of CBR were significantly more severe ($P=0.05$) in plants grown from seedlings pre-treated with dinitramine than in those not pre-treated and transplanted to unamended, infested soil (Table 12). Dinitramine applied to infested soil at 0.56 kg/ha as in previous tests also increased significantly the severity of CBR. The two methods used to introduce dinitramine into the host-pathogen system (seedling pre-treatment and treatment

Table 12. Effect of dinitramine treatments on peanut and the severity of Cylindrocladium black rot in a Ruston loamy fine sand.

Pre-transplant seedling treatment[1]	Transplant soil herbicide treatment[2]	Inoculum (10 ms/g)	Root rot index[3] (1-5)	% Colonized root segments	Top symptoms index[4] (1-5)	Growth (fresh wt.) (grams)	
						Shoots	Roots
none	none	(+)	2.9 B	83.0 B	1.6 B	6.6 B	2.9 CD
PTH	none	(+)	4.0 A	98.1 A	2.7 A	6.6 B	2.2 DE
none	yes	(+)	4.1 A	97.6 A	2.8 A	5.9 B	1.8 E
none	none	(-)	1.0 C	0.0 C	1.0 C	9.8 A	4.5 A
PTH	none	(-)	1.0 C	0.0 C	1.0 C	9.1 A	3.6 BC
none	yes	(-)	1.0 C	0.0 C	1.0 C	8.9 A	3.9 AB

[1] PTH = seed of 'Florigiant' peanut germinated in non-sterile soil treated with dinitramine (0.56 kg/ha) for 5 days before transplanting; none = seed germinated in non-amended soil.

[2] yes = transplant soil treated with dinitramine (0.56 kg/ha).

[3] 1 = no symptoms, 5 = severe rot.

[4] 1 = no symptoms, 5 = dead plant.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

of infested soil) resulted in nearly identical levels of disease increase (Table 12).

Influence of dinitramine and dinoseb on growth of peanut.

Quantities of non-sterile Ruston and Woodstown soil free of C. crotalariae were placed in pots on a greenhouse bench. Dinitramine at 0.56 and 0.84 kg/ha and dinoseb at 3.36 and 6.72 kg/ha were applied to soil and incorporated. Seeds of Florigiant peanut were planted and plants were grown for 8 wk under prevailing daylight and temperature conditions (22-28 C). Weights of shoots and roots were then recorded for each plant and root systems were examined for evidence of herbicide injury. Data were expressed as the percent of growth inhibition relative to plants grown in herbicide-free soils.

Dinoseb and dinitramine suppressed root growth more in Woodstown than in Ruston soil (Fig.7). Root growth in response to dinitramine at 0.56 kg/ha was inhibited by 18.5% (significant at P=0.05) in the Woodstown soil and by 13.3% in the Ruston soil. At 0.84 kg/ha dinitramine suppressed root growth by 33.3% (significant at P=0.05) and by 17.7% in the respective soils. Dinoseb at 3.36 kg/ha suppressed root growth by 7.4% in Woodstown soil and by only 4.4% in the Ruston soil. At 6.72 kg/ha dinoseb suppressed root growth in Ruston soil by 17.7%, however, growth suppression in

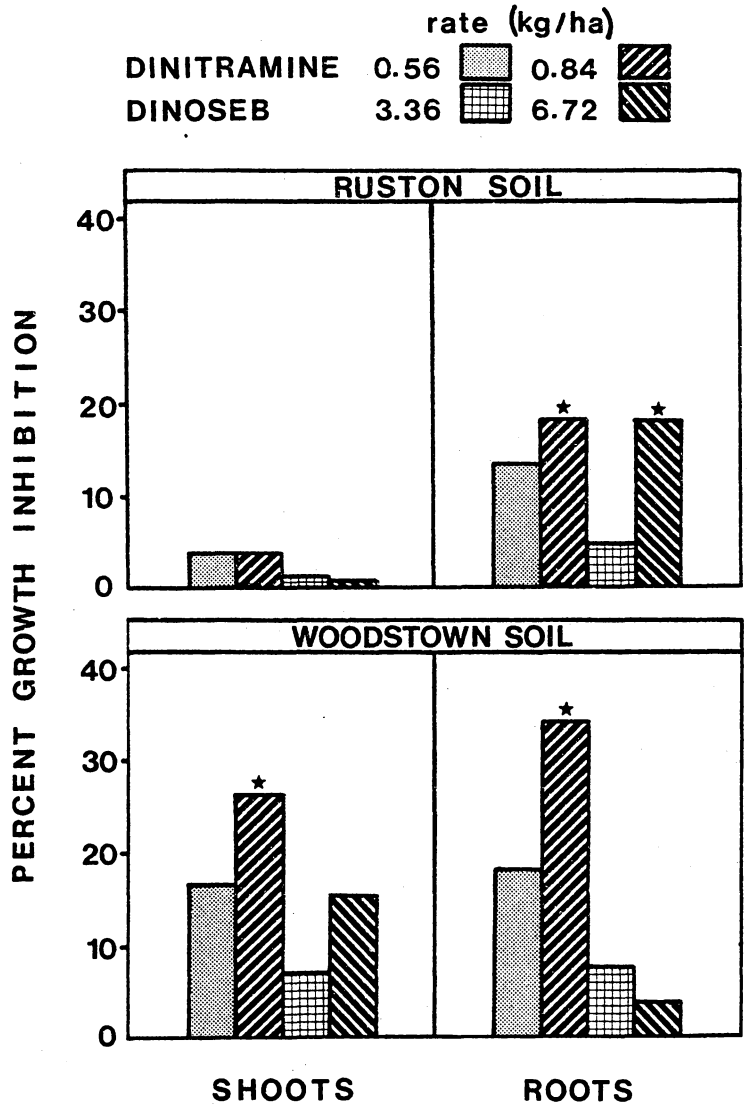


Fig. 7. Influence of dinitramine, dinoseb, and soil type on growth of 'Florigiant' peanut. Growth was expressed as a percent relative to untreated controls. Bars labeled with stars denote means that are significantly different (P=0.05) from others in the same category.

Woodstown soil was minimal (3.0%). Growth of shoots appeared to be unaffected by dinitramine and dinoseb in the Ruston soil but was suppressed in the Woodstown soil treated with these herbicides (Fig. 7).

The hypocotyl and root/hypocotyl transition region of plants grown for 8 wk in soils treated with dinitramine at both rates characteristically produced numerous short adventitious roots with swollen tips (Fig. 8). This condition was most pronounced at the high rate of dinitramine (0.84 kg/ha).

In preliminary tests, dinitramine appeared to cause inhibition of root elongation soon after seed germination. For additional support of this conclusion, seed of Florigiant peanut were planted in pots of non-sterile Ruston soil either untreated or treated with dinitramine at 0.56 and 0.84 kg/ha. After 5 days, seedlings were removed, washed, and root lengths were measured. The average length of 10 roots from untreated soil (5.0 cm) was significantly greater ($P=0.05$) than average root lengths from soil treated with dinitramine at 0.56 and 0.84 kg/ha (2.8 and 2.3 cm, respectively). Additionally, the diameter of roots at the region of maximum elongation appeared larger as a result of herbicide treatment (Fig. 9).

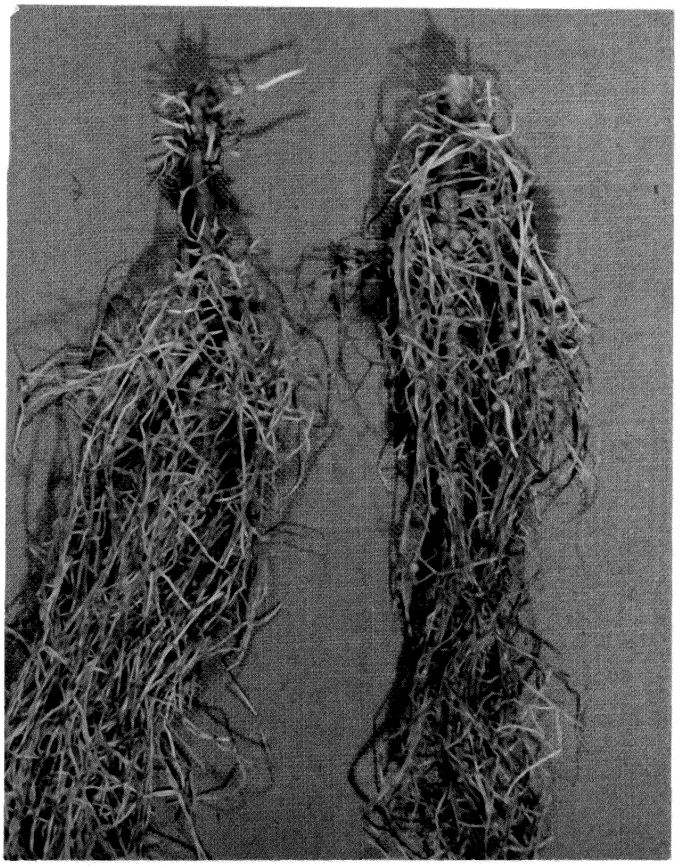


Fig. 8. 'Florigiant' peanut roots after 8 wk growth in Ruston loamy fine sand treated with dinitramine at 0.56 kg/ha (left) and untreated (right).

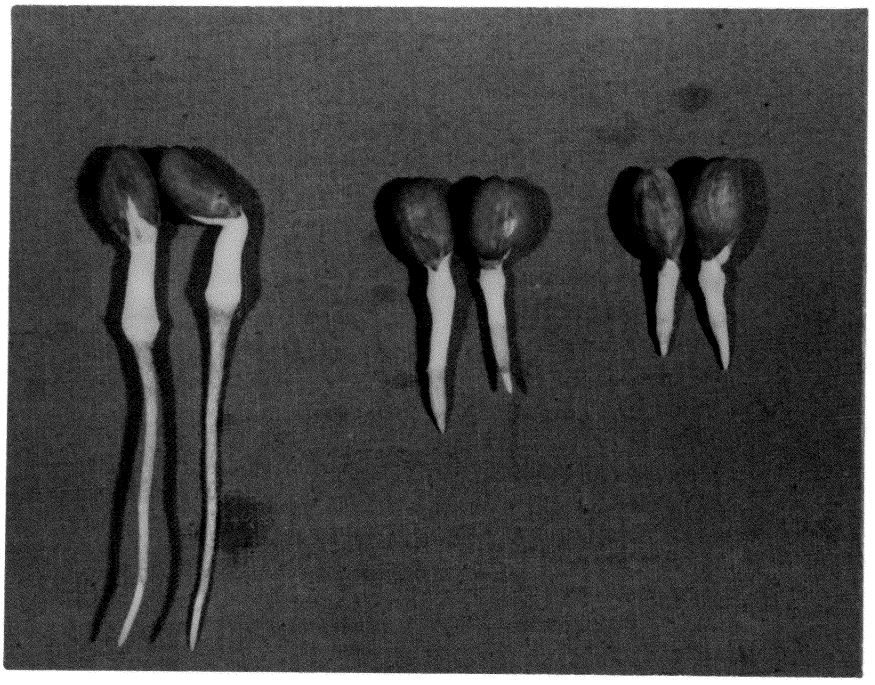


Fig. 9. Roots of 'Florigiant' peanut after five days growth in Ruston loamy fine sand treated with dinitramine. On left, untreated soil. Center, 0.56 kg/ha dinitramine. On right, 0.84 kg/ha dinitramine.

Effects of dinitramine on resistance of 'NC3033' peanut to CBR.

An experiment was initiated to compare the effects of dinitramine on disease severity in 'NC3033', a CBR-resistant cultivar (29), and the CBR-susceptible cultivar, Florigiant. A quantity of Ruston soil was infested to a density of 10 ms/g and treated with dinitramine at 0.56 kg/ha as described earlier. Seeds of NC3033 and Florigiant were then planted. After 8 wk there were no detectable symptoms of CBR in NC3033 grown in infested soil free of the herbicide (Table 13). Root rot was slight, but present in eight of twelve plants of NC3033 grown in infested soil treated with the herbicide. Symptoms of CBR in Florigiant grown in untreated, infested soil were less severe than in other experiments, but significantly higher (P=0.05) than non-infested controls. As in previous experiments, dinitramine at 0.56 kg/ha increased significantly the severity of CBR in Florigiant peanut (Table 13).

Discussion

The data presented indicate that dinitramine applied at 0.56 kg/ha, but not at 0.84 kg/ha, to soils infested with C. crotalariae results in a significant increase in severity of CBR in the susceptible peanut cultivar Florigiant. Although

Table 13. Effects of dinitramine on severity of Cylindrocladium black rot in peanut cultivars 'Florigiant' and 'NC 3033'.[1]

Cultivar	Inoculum (10 ms/g)	Soil herbicide treatment[2]	Root rot index[3] (1-5)	Top symptoms index[4] (1-5)
Florigiant	(+)	none	2.0 B	1.3 B
Florigiant	(+)	yes	3.4 A	2.9 A
Florigiant	(-)	yes	1.0 C	1.0 B
Florigiant	(-)	none	1.0 C	1.0 B
NC 3033	(+)	none	1.0 A	1.0 A
NC 3033	(+)	yes	1.5 A	1.0 A
NC 3033	(-)	yes	1.0 A	1.0 A
NC 3033	(-)	none	1.0 A	1.0 A

[1] Plants were grown from seed in non-sterile Ruston loamy fine sand infested with Cylindrocladium crotalariae microsclerotia for 8 wks, then harvested and rated for disease severity.

[2] Yes = soil received a pre-plant treatment of dinitramine at a rate of 0.56 kg/ha.

[3] 1 = no symptoms, 5 = severe rot.

[4] 1 = no symptoms, 5 = dead plant.

Means for each cultivar in columns followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

symptoms of CBR were noted for several plants of NC3033 peanut, dinitramine did not appear to affect significantly the resistance of this cultivar to infection by C. crotalariae (Table 13).

The increase in severity of CBR in Florigiant peanut following dinitramine (0.56 kg/ha) soil treatments was influenced by soil type and initial densities of C. crotalariae ms in soil (Table 10,11). In the Ruston soil, a significant increase in disease severity as a result of dinitramine treatment occurred only at an inoculum density of 5 ms/g (Table 10). At a density of 50 ms/g in Ruston soil, the resulting severe level of disease development was believed to have precluded demonstration of any enhancement effects due to herbicide treatment (Table 10). In contrast to Ruston soil, a significant increase in disease resulted from dinitramine treatment in the Woodstown soil only at inoculum densities of 30 and 50 ms/g and not at 5 ms/g (Table 9,11). Data from other experiments showed that Woodstown soil was somewhat suppressive to the development of CBR (Fig. 6). Effects of dinitramine at 0.56 kg/ha in Woodstown soil with high densities of ms can be explained if the herbicide acts to alter the disease proneness of the peanut. Under this assumption, the disease suppressiveness of Woodstown soil at the high but not at the low inoculum density is more than compensated for by herbicide-induced predisposition of the plant to infection.

Dinoseb at 1.68 kg/ha increased significantly (P=0.05) the severity of CBR in two tests with Woodstown soil infested to a density of 30 ms/g (Table 9). Disease increases after dinoseb treatment did not occur in other tests with ms densities of 5 and 50 ms/g Woodstown soil or at any inoculum density in Ruston soil (Table 9,10,11). Dinoseb may leach readily from soils of low water holding capacity and organic matter content (i.e. Ruston), but is more tightly held in the upper soil profiles with increasing amounts of organic matter and clay (i.e. Woodstown) (2). Dinoseb also is an inhibitor of asymbiotic nitrogen fixation and overall soil respiration (11,28), and it reduces significantly soil populations of certain bacteria and algae (20,28). The prolonged presence of dinoseb in the rhizosphere soil of peanuts growing in Woodstown soil as compared to Ruston soil may have resulted in the observed increase in disease severity. At a concentration of 5 µg/g in Woodstown soil, dinoseb reduced the number of germinable C. crotalariae ms by 19% (Table 7) (Chapter 2). Dinoseb may still increase disease in Woodstown soil in spite of its toxicity to C. crotalariae if it concommitantly reduces either host resistance or populations of soil competitors of the fungus to a greater extent (17). Dinoseb has been found to cause a significant increase in the severity of Fusarium root rot of navy bean (Fusarium solani f. phaseoli Burkh.)

(30), and in Rhizoctonia disease of snapbean (26). In both cases, dinoseb apparently reduced host resistance to infection and/or colonization (26,30). Additional research will be required to illucidate the mechanism(s) of dinoseb-induced increase of CBR in peanut as well as diseases of other crops.

Neubauer and Avizohar-Hershenson (21) found that pre-treatment of cotton (Gossypium hirsutum L.) seedlings with trifluralin resulted in a significant increase of Rhizoctonia disease. With infested Ruston soil in the current study, it was found that dinitramine at 0.56 kg/ha increased CBR significantly when used either to pre-treat peanut seedlings or when applied to infested soil before transplanting seedlings or planting seed (Table 10,12). Furthermore, levels of disease severity observed in response to the two methods of herbicide treatment were not significantly different (P=0.05) from one another (Table 12). If the primary effect of dinitramine is to increase the aggressiveness of C. crotalariae, it would seem that the level of disease severity in pre-treated seedlings would be less than that in untreated seedlings transplanted to infested soil treated with the herbicide. Dinitramine is absorbed in small quantities and translocated throughout the tissues of soybean roots (8). Trace amounts of dinitramine absorbed by the pre-treated peanut roots would not be

expected to exert a direct influence on C. crotalariae until after the fungus had penetrated the tissues and initiated colonization. Based on these observations, dinitramine apparently acts initially to increase the susceptibility of peanut roots to CBR. Dinitramine at low rates stimulated growth of C. crotalariae mycelium in axenic culture (Chapter 2) and may as a result also influence the aggressiveness of this fungus as it colonizes the peanut root.

It was observed consistently that the severity of CBR was significantly increased by dinitramine at a rate of 0.56 kg/ha, but not at 0.84 kg/ha (Table 10,11). El-Khadem et al. (10) recently observed that dinitramine at 0.43 and 0.88 kg/ha increased the severity of Rhizoctonia disease in cotton, but a rate of 1.41 kg/ha had no effect. Dinitramine exhibited no tendency to suppress mycelial growth of C. crotalariae in axenic culture or to reduce the germinability of C. crotalariae ms in soil at rates equivalent to either 0.56 or 0.84 kg/ha (Table 3,4,7). Decreased disease severity at 0.84 kg/ha dinitramine was evidently not due to direct fungitoxic effects. The herbicide did cause growth disturbances in Florigiant peanut roots, particularly at the high rate, that were similar to those reported by Bayer et al. (6) for cotton roots treated with trifluralin (Fig. 7). Trifluralin is a herbicide resembling dinitramine in that it increases the severity of Rhizoctonia disease in cotton

(10,21), and in snapbean (26). Apparently, by increasing the rate of dinitramine from 0.56 to 0.84 kg/ha, the level of herbicide-induced plant stress exceeded a threshold beyond which predisposition effects were compensated for by host responses, which could include wound periderm formation (16) or the production of phytoalexins. Phytoalexin production can be induced in many plants by certain chemical treatments (15,19). Several phytoalexins have been isolated from peanut tissue (18), but whether these compounds were responsible for the observations reported here is unknown.

Detailed knowledge of the mechanisms through which dinitroaniline herbicides increase host susceptibility to soilborne pathogens is lacking. These herbicides are known to influence significantly the physiology of growth and development in crop plants even when used as recommended in weed control programs (22). In this paper, herbicide-induced disturbances in the physiology of the peanut root are proposed as possible components of a mechanism by which dinitramine increases the susceptibility of this plant to infection by C. crotalariae. Further investigation will be necessary to confirm this theory.

Literature Cited

1. Altman, J., and C. L. Campbell. 1977. Effects of herbicides on plant diseases. Ann. Rev. Phytopathol. 15:361-385.

2. Anonymous. 1979. Herbicide Handbook of the Weed Science Society of America. Weed Sci. Soc. Amer., Champaign, ILL. 479 pp.

3. Anonymous. 1979. Statistical analysis system user's guide. Sas Institute, Inc., Raleigh, NC 27605. 494 pp.

4. Backman, P. A., R. Rodriguez-Kabana, and G. A. Buchanan. 1977. Interactions of oxadiazon and dinoseb with stem rot in peanut. Weed Sci. 25:260-263.

5. Barron, J. A., and P. M. Phipps. 1980. Effect of dinitramine and dinoseb on Cylindrocladium black rot (CBR) of peanut. Proc. Amer. Peanut Res. Ed. Soc. 12:40 (Abstr.).

6. Bayer, D. E., C. L. Foy, T. E. Mallory, and E. G. Cutter. 1967. Morphological and histological effects of trifluralin on root development. Amer. J. Bot. 54:945-952.

7. Bell, D. K., and E. K. Sobers. 1966. A peg, pod and root necrosis of peanuts caused by a species of Calonectria. Phytopathology 56:1361-1364.

8. Belles, W. S., and R. A. Smith. 1973. Dinitramine uptake, translocation and metabolism in soybeans. Proc. Weed Sci. Soc. Amer. (1973):61 (Abstr.).

9. Chappell, W. E., and L. I. Miller. 1956. The effects of certain herbicides on plant pathogens. Plant Dis. Reptr. 40:52-56.

10. El-Khadem, M., M. Zahran, and M. K. El-Kazzaz. 1979. Effect of the herbicides trifluralin, dinitramine and fluometuron on Rhizoctonia disease in cotton. Plant Soil 51:463-470.

11. Fletcher, W. M. 1960. The effect of herbicides on soil microorganisms. Pages 20-62 in: E. K. Woodford and G. R. Sagar (eds.), Herbicides and the soil. Blackwell Scientific Publications, Oxford, UK.

12. Garren, K. H., and T. A. Coffelt. 1976. Reaction to Cylindrocladium black rot in Virginia-type peanut cultivars. Plant Dis. Reptr. 60:175-178.

13. Griffin, G. J. 1977. Improved selective medium for isolating Cylindrocladium crotalariae microsclerotia from naturally-infested soils. Can. J. Microbiol. 23:680-683.

14. Grinstein, A., Y. Elad, J. Katan, and I. Chet. 1979. Control of Sclerotium rolfsii by means of a herbicide and Trichoderma harzianum. Plant Dis. Reptr. 63:823-826.

15. Hargreaves, J. A. 1979. Investigations into the mechanism of mercuric chloride stimulated phytoalexin accumulation in Phaseolus vulgaris and Pisum sativum. Physiol. Plant Pathology 15:279-287.

16. Johnston, S. A., and M. K. Beute. 1975. Histopathology of Cylindrocladium black rot of peanut. Phytopathology 65:649-653.

17. Katan, J., and Y. Eshel. 1973. Interactions between herbicides and plant pathogens. Residue Rev. 45:145-177.

18. Keen, N. T. 1975. The isolation of phytoalexins from germinating seeds of Cicer arietinum, Vigna sinensis, Arachis hypogaea, and other plants. Phytopathology 65:91-92.

19. Kuc, J. 1972. Phytoalexins. Ann. Rev. Phytopathol. 10:207-232.

20. Lewis, J. A., G. C. Papavizas, and T. S. Hora. 1978. Effects of some herbicides on microbial activity in soil. Soil Biol. Biochem. 10:137-141.

21. Neubauer, R., and Z. Avizohar-Hershenson. 1973. Effect of the herbicide trifluralin on Rhizoctonia disease in cotton. Phytopathology 63:651-652.

22. Parka, S. J., and O. F. Soper. 1977. The physiology and mode of action of the dinitroaniline herbicides. Weed Sci. 25:79-87.

23. Phipps, P. M., and M. K. Beute. 1977. Influence of soil temperature and moisture on the severity of Cylindrocladium black rot in peanut. Phytopathology 67:1104-1107.

24. Phipps, P. M., and J. A. Barron. 1981. Efficacy of soil fumigants in control of CBR of peanut in Virginia. Fungicide-Nematicide Tests 36:(in press).

25. Porter, D. M., and O. E. Rud. 1980. Suppression of Sclerotinia blight of peanut with dinitrophenol herbicides. Phytopathology 70:729-732.

26. Romig, W. R., and M. Sasser. 1972. Herbicide predisposition of snapbean to Rhizoctonia solani. Phytopathology 62:785-786 (Abstr.).

27. Rud, O. E. 1980. Weed control in peanuts. Pages 243-277 in: Virginia Pest Management Guide 1. Extension Division, Virginia Polytechnic Institute and State Univ., Blacksburg, Va.

28. Vlassak, K. K., A. H. Heremans, and A. R. Van Rossen. 1976. Dinoseb as a specific inhibitor of nitrogen fixation in soil. Soil Biol. Biochem. 8:91-93.

29. Wynne, J. C., R. C. Rowe, and M. K. Beute. 1975. Resistance of peanut genotypes to Cylindrocladium crotalariae. Peanut Sci. 2:54-56.

30. Wyse, D. L., W. F. Meggitt, and D. Penner. 1976. Effect of herbicides on the development of root rot in navy bean. Weed Sci. 24:11-15.

CHAPTER FOUR

EFFECTS OF HERBICIDES ON DEVELOPMENT OF CYLINDROCLADIUM BLACK ROT OF PEANUT IN FIELD MICROPLOTS

Introduction

Cylindrocladium crotalariae (Loos.) Bell and Sobers causes a root, peg, and pod necrosis of the peanut (Arachis hypogaea L.), known as Cylindrocladium black rot (CBR) (4). For several years after it was discovered in Virginia (8), the incidence of CBR increased at an alarming rate. Currently, CBR limits the productivity of many peanut farms in this region (14). Microsclerotia (ms) of the fungus, which form in cortical tissues of colonized peanut roots (23), are apparently the primary survival, dispersal and infective propagules of C. crotalariae in soil (13,18).

Populations of ms are known to decline significantly when infested soil is subjected to high (8) or low temperatures (22), low soil water potentials (8), certain crop rotations (18,26), or certain soil fumigants (16). However, methods for consistently reducing field populations of ms to levels below the reported threshold for severe disease in commercial peanut varieties (0.5 ms/g soil) (17) have not been developed. Presently, there are no effective CBR control strategies reported (15).

Recently, the use of herbicides has been recognized as an important factor affecting the incidence and severity of plant diseases (1,12). Dinoseb (6) and oxadiazon (3) were found to suppress the severity of southern stem rot in peanut caused by Sclerotium rolfsii Sacc. Dinoseb and dinoseb + naptalam were shown to reduce significantly the severity of Sclerotinia blight of peanut (Sclerotinia minor Jagger) in field experiments (20). Grinstein et al. (10) reported that dinitramine increased the resistance of peanut plants to infection by S. rolfsii in the greenhouse and in the field. In contrast to these reports, dinitramine and dinoseb were found to increase significantly the severity of CBR in greenhouse tests (Chapter 3). While such results are often useful, it is recognized that they have more meaning when supplemented with field results of a similar nature.

The purpose of this study was to determine the effects of dinitramine and dinoseb-containing herbicides on the severity of CBR and on soil populations of C. crotalariae in a field test environment. The effects of certain herbicides on development of Sclerotinia blight, caused by S. minor, and southern stem rot, caused by S. rolfsii, were assessed in microplots in order to compare and contrast results with those of other workers (3,20).

Materials and Methods

The field site and installation of microplots.

Experiments were conducted in a peanut field at the Tidewater Research and Continuing Education Center, Suffolk, Va. Based on physical properties determined of several replicate soil samples (78.8% sand, 12.9% silt, 8.3% clay), the field soil was classified as a Woodstown loamy fine sand. Microplot barriers (77-cm diameter) were constructed from fiberglass sheets (0.3-cm thick, 60-cm wide, 245-cm long) (19). A circular pit of soil was excavated (77-cm diameter x 45-cm deep) by hand for each microplot. Fiberglass barriers were inserted in soil, leaving 15 cm above ground as a splash barrier, then the soil horizons were carefully replaced (Fig 10).

Soil assays for C. crotalariae

From each microplot, 8 vertical soil cores (2 x 15 cm) were collected with a soil sampling tube and placed in plastic bags. Sample bags were closed with rubber bands and stored in darkness at room temperature (24-26 C) for 2-3 wk. Just prior to assay, samples were mixed thoroughly by hand then two subsamples/sample were weighed and subsequently dried at 105 C for 24 h to determine moisture content. Populations of C. crotalariae ms were determined by the

Fig. 10. Photograph of the field test site, located at the Tidewater Research and Continuing Education Center, Suffolk, VA, showing several newly-installed microplots.

method of Griffin (9), utilizing the modified soil washing procedure described in Chapter 2.

Infesting microplot soil with C. crotalariae.

In 1975 and 1977, several peanut plants growing in the field selected for use exhibited symptoms and signs of CBR (K. H. Garren, 1978, personal communication). Initial soil assays in 1979 detected C. crotalariae in this field at very low inoculum densities (0.1-0.5 ms/g soil). To ensure optimum disease development, microplots were infested with supplementary inoculum 1 mo before planting time. Microsclerotia of C. crotalariae used to infest soil were obtained by the method of Phipps et al. (19). Standardized quantities of 1.8×10^6 ms in 800 g soil were prepared (Chapter 3) and mixed into the upper 15 cm of soil in each microplot to provide an inoculum density of 15 ms/g soil. Microsclerotium densities in each microplot were verified 2-3 wk later by soil assay (9).

Infesting microplot soil with sclerotia of S. minor and S. rolfsii.

A medium was prepared for producing sclerotia of S. minor and S. rolfsii that contained 95% air-dried soil and 5% cornmeal (w:w). The medium was placed in 9-cm Petri dishes (50 g/plate) and moistened with distilled water (25

ml/plate). Plates were autoclaved at 103.4 kPa for 30 min on each of two consecutive days to ensure sterility of soil and when cool, each was inoculated with a 5-mm mycelial disk of either S. minor or S. rolfsii from potato-dextrose agar cultures. After 2 wk, sclerotia of each fungus were collected by washing the contents of plates on a 600-um sieve with water. The number of sclerotia produced per plate was standardized for each fungus by counting all propagules collected from each of 5 replicate plates. Quantities of S. minor or S. rolfsii sclerotia were collected and mixed into the upper 7.5 cm of soil in microplots to provide inoculum densities of 0.12 and 0.03 sclerotia/g soil, respectively, approximately 3 wks before planting in 1979.

Application of herbicides

A backpack sprayer, pressurized with carbon dioxide and equipped with a hand-held single-nozzle spray boom was used to apply herbicides (Fig. 11). The sprayer was calibrated to deliver 750 l/ha at a pressure of 137.9 kPa with a D2-13 (disc-core combination) nozzle assembly during a period of 8 sec. Herbicide stock solutions were prepared with water to achieve treatment specifications. Unless otherwise indicated, herbicides were sprayed just before planting and incorporated immediately into the upper 7.5 cm of soil.

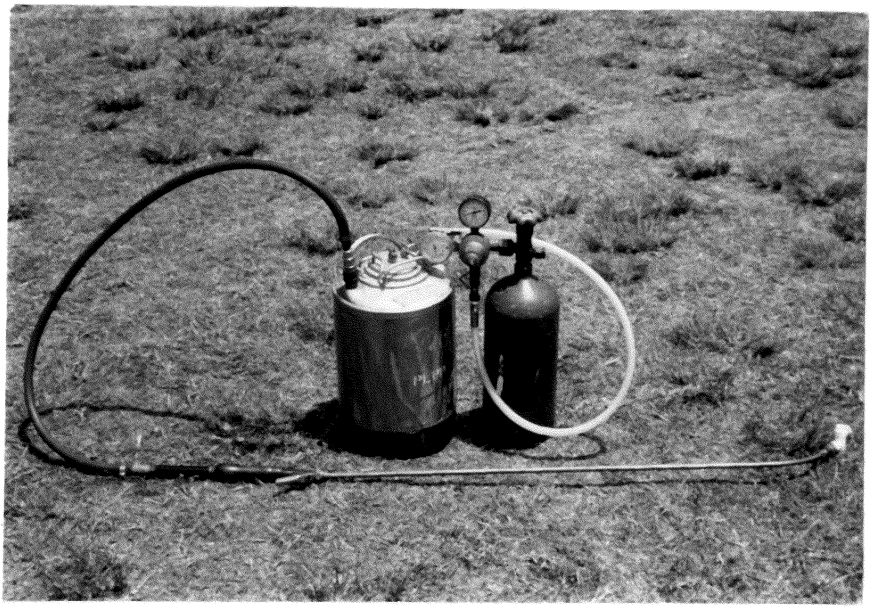


Fig. 11. Sprayer assembly used to apply herbicides to microplots.

Treatments were replicated four times in a randomized complete-block design.

Cultural practices.

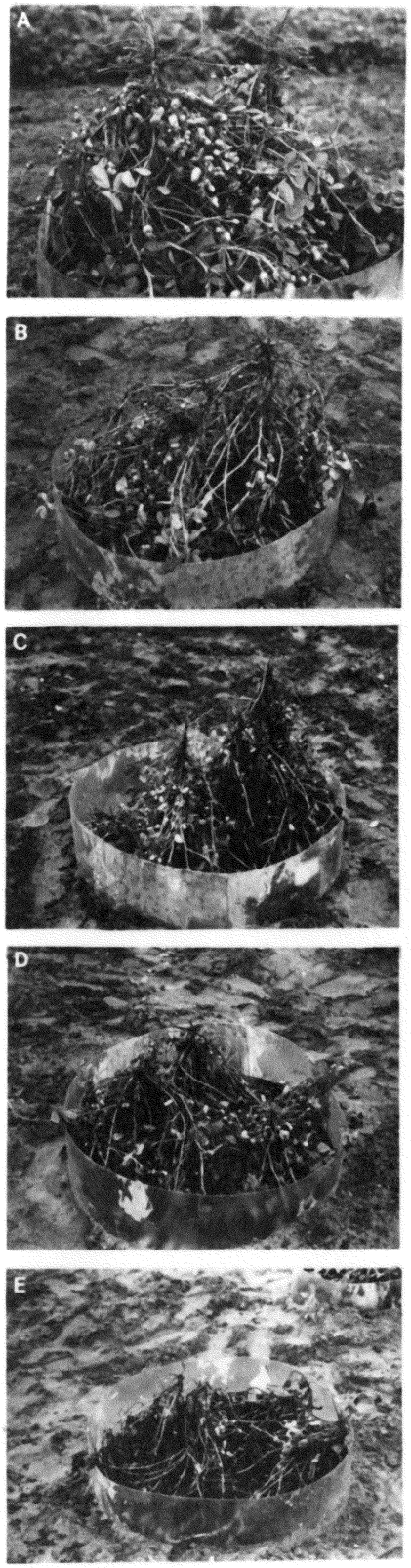
On 22 May 1979, 12 hand-selected seeds of 'Florigiant' peanut were treated (0.25 g Botec/kg seed) and planted in each microplot. After emergence, the number of plants was reduced by thinning to three per microplot. Weeds within microplots were controlled by hand but those occurring in alleyways between the barriers were controlled by tillage or with regular tank mix sprays of Lasso + Dyanap (4.6 + 14.0 L/ha). The concentrations of major plant nutrients in microplots at planting time in 1979 were (µg/g soil): P, 21.0; K, 53.0; N, 35; Ca, 528; Mg, 54.0, Zn, 0.8, and Mn, 1.1, as determined by the V.P.I.& S.U. extension soil testing laboratory. The soil pH was 5.7. No lime or nitrogen-containing fertilizers were applied to microplot soils, but during the first week of July, landplaster (1120 kg calcium sulfate/ha) was applied to each microplot. After landplaster was applied, each microplot was irrigated with 7.6 l of water to simulate 13 mm (0.5 inches) of rainfall. During 1979, no foliar fungicides were applied to peanuts, but two applications of carbaryl (1.12 kg/ha) were made to control Japanese beetles and tobacco thrips.

The same herbicides used in 1979 were again applied to the original microplots in 1980, but no supplementary inoculum was added to these plots in the second year of the test. On 10 April 1980, twenty new microplots were installed nearby and infested with C. crotalariae (15 ms/g soil) to repeat, as accurately as possible, a portion of the 1979 experiment. Peanut seed were planted in all plots on 10 May 1980. Cultural practices followed were similar to those of 1979 except for applications of benomyl (0.56 kg/ha) and azodrin (1.16 l/ha) for leafspot and spider mite control, respectively.

Assessment of herbicide effects on disease severity.

The number of plants with above-ground symptoms and signs of CBR were recorded monthly during each growing season. At harvest, (29 September 1979 and 1 October 1980), all plants were rated for severity of above-ground (top) symptoms, then dug and inverted. The severity of root and pod rot was rated on a 1-5 scale (Fig. 12) (1= no symptoms; 5= completely decayed). Five 1.0-cm root segments were collected from each plant for tissue biopsy to verify the presence of C. crotalariae. Biopsy tissues were surface-disinfested for 2 min in 0.5% NaOCl and placed on an agar medium designed to selectively isolate this pathogen (9). All fruit were picked, dried to 8% moisture and weighed.

Fig. 12. A-E. Illustration of the severity of root rot caused by Cylindrocladium crotalariae in microplots. Root rot ratings for these plots were A = 1; B = 2; C = 3; D = 4; and E = 5. (1 = no symptoms; 5 = completely decayed).


A
B
C
D
E

The incidence of Sclerotinia blight and stem rot was determined by recording the number of plants exhibiting symptoms and signs (sclerotia and/or mycelium) that are characteristic of these diseases. Disease severity was rated on a scale of 1-5 wherein, 1= no symptoms, 2= 25% of branches with lesions, 3= 50% of branches with lesions, 4= 75% of branches with lesions and some wilted branches, and 5= all branches with lesions and wilted. Disease incidence and severity assessments were recorded monthly during each growing season. The plants were dug and inverted, and pods were rated for severity of rot. Pods were then picked, dried to 8% moisture, and weighed.

Results

Effects of herbicides on severity of CBR.

In 1979, the severity of pod rot caused by C. crotalariae was increased significantly (P=0.05) by dinitramine at a rate of 0.56 kg/ha (Table 14). Substantial increases (not significant at P=0.05) in root rot and top symptom severity and suppressed yield also occurred in response to this treatment. The percentage of plants with perithecia of Calonectria crotalariae was three times greater in microplots treated with dinitramine at 0.56 kg/ha than plants in infested, untreated microplots. The severity

Table 14. Effects of herbicides on the severity of Cylindrocladium black rot of peanut in microplots (1979).[1]

Treatment and rate[2] (kg/ha)	Plants with perithecia (%)	Top symptoms index[3] (1-5)	Root rot index[4] (1-5)	Pod rot index[4] (1-5)	Yield (grams)
Infested soil					
control	16.6	3.0 AB	2.9 ABC	2.2 B	204.8 BC
dinitramine 0.56 PPI	66.6	3.7 A	3.9 A	3.6 A	160.0 C
dinitramine 0.84 PPI	33.3	2.2 ABCD	2.7 ABC	2.1 B	251.9 ABC
dinitramine 0.56 PPI + dinoseb 3.36 PPI	41.6	2.3 ABCD	2.9 ABC	2.5 B	191.2 BC
dinitramine 0.56 PPI + dinoseb 6.72 PPI	25.0	2.3 ABCD	2.9 ABC	2.6 AB	189.3 BC
dinoseb 1.68 PPI	33.3	2.2 ABCD	2.0 C	1.8 C	227.6 ABC
dinoseb 3.36 PPI	25.0	2.8 ABC	2.7 ABC	2.3 B	239.9 ABC
dinoseb 6.72 PPI	25.0	2.0 BCD	2.4 BC	2.0 BC	271.7 AB
dinoseb 1.12 + naptalam 2.24 PPI	25.0	1.6 CD	2.9 ABC	2.0 BC	217.0 BC
dinoseb 2.24 + naptalam 4.48 PPI	50.0	2.6 ABC	3.4 AB	2.9 AB	179.0 BC
dinoseb 6.72 PPI + 1.68 laybys	16.6	2.7 ABC	2.4 BC	2.0 BC	181.3 BC
dinoseb 1.68 laybys	33.3	2.4 ABCD	3.0 ABC	2.5 B	185.2 BC
dinitramine 0.56 PPI + dinoseb 1.68 PPI	33.3	2.3 ABCD	2.6 BC	2.3 B	244.0 ABC
Noninfested soil					
control	0.0	1.0 D	1.0 D	1.1 C	327.9 A

[1]Seed of 'Florigiant' peanut were planted on 22 May 1979 in microplots infested with Cylindrocladium crotalariae microsclerotia (ms) to a density of 15 ms/g soil. Plants were harvested and rated for disease severity on 29 September 1979.

[2]PPI = pre-plant incorporated; laybys = postemergence treatments applied on 7 July and 28 August 1979.

[3]1 = no symptoms, 5 = dead plant.

[4]1 = no symptoms, 5 = completely decayed.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

of top symptoms, root rot and pod rot among plants in microplots treated with dinitramine at 0.84 kg/ha and with dinitramine in combination with dinoseb was not significantly different from untreated plots (Table 14).

Dinoseb applied at rates of 1.68, 3.36, or 6.72 kg/ha before planting or as two 1.68 kg/ha late-season postemergence (layby) treatments did not influence consistently the severity of CBR in 1979 (Table 14). Soil treatment with dinoseb + naptalam also had no detectable effects on disease development.

In 1980, the same herbicide treatments were applied to the original microplots except that the number of layby applications of dinoseb was increased from two to three (Table 14,15). The severity of root and pod rot at harvest among plants in the original microplots (Test-1, 1980) was significantly increased (P=0.05) by soil treatment with dinitramine at a rate of 0.56 kg/ha (Table 15). In addition, dinoseb at 1.68 kg/ha significantly increased the severity of CBR in this test (Table 15). Both herbicide treatments also resulted in yield losses that were substantially (but not significantly) in excess of the infested controls. The effects of dinitramine (0.56 kg/ha) and dinoseb (1.68 kg/ha) on CBR of peanut in 1980 are illustrated in Fig. 13. As in 1979, other herbicide treatments resulted in no consistent effects on disease development.

Table 15. Effects of herbicides on the severity of Cylindrocladium black rot of peanut in microplots (Test-1, 1980).[1]

Treatment and rate[2] (kg/ha)	Top symptoms index[3] (1-5)	Root rot index[4] (1-5)	Pod rot index[4] (1-5)	Yield (grams)
Infested soil				
control	2.4 ABC	2.3 B	2.0 C	189.2 B
dinitramine 0.56 PPI	2.8 AB	4.0 A	3.6 AB	111.7 BC
dinitramine 0.84 PPI	2.5 ABC	2.8 AB	2.6 BC	147.7 BC
dinitramine 0.56 PPI + dinoseb 3.36 PPI	2.9 AB	2.8 AB	1.9 C	190.7 B
dinitramine 0.56 PPI + dinoseb 6.72 PPI	2.9 AB	2.7 AB	2.3 C	156.5 BC
dinoseb 1.68 PPI	3.6 A	4.0 A	3.8 A	106.0 BC
dinoseb 3.36 PPI	2.5 ABC	2.7 AB	2.1 C	197.0 B
dinoseb 6.72 PPI	1.8 BC	2.5 B	2.0 C	176.7 BC
dinoseb 1.12 + naptalam 2.24 PPI	2.5 ABC	2.7 AB	2.2 C	172.5 BC
dinoseb 2.24 + naptalam 4.48 PPI	2.6 ABC	2.4 B	2.0 C	114.7 BC
dinoseb 6.72 PPI + 1.68 laybys	2.7 AB	3.0 AB	2.3 C	106.7 BC
dinoseb 1.68 laybys	3.0 AB	2.3 B	1.8 C	91.7 C
dinitramine 0.56 PPI + dinoseb 1.68 PPI	2.4 ABC	2.7 AB	2.3 C	137.2 BC
Noninfested soil				
control	1.0 C	1.0 C	1.7 C	378.7 A

[1]Seed of 'Florigiant' peanut were planted on 10 May 1980 in microplots that were used in the 1979 test. Plants were harvested and rated for disease severity on 1 October 1980.

[2]PPI = pre-plant incorporated; laybys = postemergence treatments applied on 2 July, 6 August and 4 September 1980.

[3]1 = no symptoms; 5 = dead plants.

[4]1 = no symptoms; 5 = completely decayed.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

Fig. 13. A-D. Effect of dinitramine and dinoseb on the severity of Cylindrocladium black rot of peanut in microplots during 1980. A. Plants grown in noninfested soil with no herbicide treatment. B. Plants grown in soil infested with Cylindrocladium crotalariae microsclerotia with no herbicide treatment. C. Plants grown in infested soil + dinitramine at 0.56 kg/ha. D. Plants grown in infested soil + dinoseb at 1.68 kg/ha.


A
B
C
D

Newly installed and infested microplots (Test-2, 1980) were treated before planting seed with dinitramine at 0.56 and 0.84 kg/ha and with dinoseb at 1.68 and 6.72 kg/ha. Treatments were replicated four times in a randomized complete block design. In Test 2, soil treatments of dinitramine (0.56 and 0.84 kg/ha) and dinoseb (1.68 kg/ha) also resulted in a significant increase (P=0.05) of root rot severity (Table 16). Associated with these treatments were an increase in pod rot severity and suppressed yield (not significant at P=0.05). Dinoseb applied at a rate of 6.72 kg/ha resulted in a significant suppression of yield (P=0.05) in the new microplots of 1980, but there was no evidence that this occurrence was related to an interaction between dinoseb and C. crotalariae (Table 16).

Herbicide effects on populations of C. crotalariae ms and nematodes in soil.

In both years of the test, soil samples were collected from each microplot at planting time and again shortly after harvest and assays for populations of C. crotalariae ms were performed by the procedures described previously. In addition, nematodes were extracted (5) from one 250-ml subsample/sample from each microplot, and the numbers of all plant-parasitic species were counted.

Table 16. Effects of herbicides on the severity of Cylindrocladium black rot of peanut in microplots (Test-2, 1980).[1]

Treatment and rate[2] (kg/ha)	Top symptoms index[3] (1-5)	Root rot index[4] (1-5)	Pod rot index[4] (1-5)	Yield (grams)
Infested soil				
control	1.00 B	1.33 B	2.20 AB	163.5 B
dinitramine 0.56 PPI	1.83 A	2.78 A	2.87 A	158.2 BC
dinitramine 0.84 PPI	1.41 AB	2.37 A	2.20 AB	150.0 BC
dinoseb 1.68 PPI	1.00 B	2.37 A	2.41 A	122.2 BC
dinoseb 6.72 PPI	1.08 B	1.66 B	1.58 BC	104.2 C
Noninfested soil				
control	1.0 B	1.0 B	1.0 C	378.7 A

[1]Seed of 'Florigiant' peanut were planted on 10 May 1980 in microplots that were installed on 10 April 1980 and infested with Cylindrocladium crotalariae microsclerotia (ms) to a density of 15 ms/g soil on 15 April 1980. Plants were harvested and rated for disease severity on 1 October 1980.

[2]PPI = pre-plant incorporated.

[3]1 = no symptoms, 5 = dead plant.

[4]1 = no symptoms, 5 = completely decayed.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

Populations of C. crotalariae ms in microplots designated for herbicide treatment at planting time in 1979 averaged 9.1 (Std. Dev.= ±5) ms/g soil. Harvest-time ms populations for 1979 and 1980 are presented in Table 17A. Populations of ms in 1979 microplots that had received certain herbicide treatments (dinitramine at 0.56 kg/ha, dinoseb at 3.36 kg/ha, and dinoseb + naptalam at 2.24 + 4.48 kg/ha) were substantially higher than controls at harvest. However, according to analysis of variance and analysis of covariance, none of these increases were significant (P=0.05).

The mean ms population of the original microplots sampled on 8 May 1980 in preparation for the repeat experiment (Test-1) was 28.9 (Std. Dev.= ±37) ms/g soil. In 1980, harvest-time ms populations in Test-1 microplots treated with certain herbicides were increased when compared to controls, but, as in 1979, there were no significant (P=0.05) treatment effects detected (Table 17A).

In contrast to the results of Test-1, herbicide treatments of Test-2 appeared to retard the buildup of ms populations (Table 17A). Soil assays after harvest showed that ms populations in microplots of this test treated with dinitramine at 0.84 kg/ha and dinoseb at 1.68 and 6.72 kg/ha were significantly lower (P=0.05) than controls.

Table 17A. Harvest-time soil populations of Cylindrocladium crotalariae microsclerotia (ms) in microplots according to herbicide treatment.[1]

Herbicide and rate (kg/ha)[2]	Year					
	1979		1980			
			Test-1		Test-2	
	MSH[3]	P[4]	MSH	P	MSH	P
control	20.5 A	--	75.3 AB	--	253.3 A	--
dinitramine 0.56 PPI	50.2 A	0.36	115.0 AB	0.47	109.5 AB	0.08
dinitramine 0.84 PPI	21.2 A	0.99	26.5 B	0.28	56.9 B	0.02
dinitramine 0.56 PPI + dinoseb 3.36 PPI	41.5 A	0.51	45.2 B	0.42	--	--
dinitramine 0.56 PPI + dinoseb 6.72 PPI	36.5 A	0.60	35.2 B	0.22	--	--
dinoseb 1.68 PPI	47.5 A	0.38	98.7 AB	0.72	77.9 B	0.04
dinoseb 3.36 PPI	71.0 A	0.12	65.2 AB	0.75	--	--
dinoseb 6.72 PPI	35.4 A	0.61	26.8 B	0.28	28.0 B	0.01
dinoseb 1.12 + naptalam 2.24 PPI	14.0 A	0.84	163.2 A	0.07	--	--
dinoseb 2.24 + naptalam 4.48 PPI	74.7 A	0.09	89.1 AB	0.70	--	--
dinoseb 6.72 PPI + 1.68 layby (2x)	49.1 A	0.37	--	--	--	--
dinoseb 6.72 PPI + 1.68 layby (3x)	--	--	78.8 AB	0.90	--	--
dinoseb 1.68 layby (2x)	43.2 A	0.46	--	--	--	--
dinoseb 1.68 layby (3x)	--	--	116.0 AB	0.49	--	--
dinitramine 0.56 PPI + dinoseb 1.68 PPI	20.5 A	0.98	119.5 AB	0.35	--	--

[1]Mean initial populations (ms/g): 1979, 9.1 ±5; 1980 Test 1, 28.9 ±37; 1980 Test 2, 11.9 ±3.5.

[2]PPI = pre-plant incorporated; layby (2x) = 2 postemergence treatments; layby (3x)= 3 postemergence treatments.

[3]MSH = mean microsclerotium populations (expressed in ms/g) after harvest of four replicate microplots per treatment. Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

[4]P = probability that the least squares mean population for each treatment = the least squares mean population of controls, according to the analysis of covariance.

Nematode assay results at harvest in 1979 and 1980 showed that populations of ring (Macroposthonia ornatum Raski. de Grisse and Loof, 1965) and root knot (Meloidogyne hapla Chitwood, 1949) nematodes were present in all plots during each growing season, however, there were no clear indications that herbicides influenced significantly these populations (Table 17B). Other species of nematodes were detected only at low levels and infrequently.

Effects of herbicide treatments on Sclerotinia blight and stem rot.

On 28 August 1979, greater that 90% of the peanut plants in untreated soils infested with S. minor exhibited symptoms of Sclerotinia blight (Table 18). At harvest (29 September 1979) disease incidence was 100% in the untreated controls (Table 18). Pre-plant soil treatments of dinoseb at 1.68, 3.36, and 6.72 kg/ha had no influence on the incidence or severity of Sclerotinia blight in 1979 (Table 18). Disease incidence on 28 August and 29 September 1979 appeared to be slightly (but not significantly) reduced in microplots which received postemergence applications of dinoseb (1.68 kg/ha) on 10 July and 28 August 1979.

The incidence of southern stem rot of peanut at harvest (29 September 1979) in untreated soil infested with S. rolfsii was nearly 75% (Table 19). Pre-plant (3.36 and 6.72

Table 17B. Pre-plant and harvest-time soil populations of Macroposthonia ornatum and Meloidogyne hapla in microplots as related to herbicide treatment.[1]

Herbicide and rate (kg/ha)[2]	Year									
	1979				1980					
					Test-1				Test-2	
	Macroposthonia ornatum		Meloidogyne hapla		Macroposthonia ornatum		Meloidogyne hapla		Macroposthonia ornatum	
	Pre-plant	Harvest	Pre-plant	Harvest	Pre-plant	Harvest	Pre-plant	Harvest	Pre-plant	Harvest
control	0.0 A	90.0 A	2.5 A	15.0 A	40.0 A	172.5 BC	5.0 A	97.5 A	32.5 A	897.5 A
dinitramine 0.56 PPI	0.0 A	122.5 A	0.0 A	17.5 A	47.5 A	197.5 BC	0.0 A	77.5 A	5.0 A	720.0 A
dinitramine 0.84 PPI	0.0 A	107.5 A	0.0 A	12.5 A	60.0 A	367.5 B	0.0 A	75.0 A	10.0 A	547.5 A
dinitramine 0.56 + dinoseb 3.36 PPI	0.0 A	222.5 A	0.0 A	0.0 A	92.5 A	217.5 BC	2.5 A	40.0 A	--	--
dinitramine 0.56 + dinoseb 6.72 PPI	0.0 A	230.0 A	0.0 A	12.5 A	112.5 A	372.5 B	0.0 A	77.5 A	--	--
dinoseb 1.68 PPI	0.0 A	97.5 A	0.0 A	7.5 A	92.5 A	67.5 C	2.5 A	37.5 A	0.0 A	1085.0 A
dinoseb 3.36 PPI	0.0 A	105.0 A	0.0 A	17.5 A	97.5 A	320.0 BC	10.0 A	107.5 A	--	--
dinoseb 6.72 PPI	0.0 A	225.0 A	0.0 A	27.5 A	102.5 A	615.0 A	0.0 A	290.0 A	20.0 A	815.0 A
dinoseb 1.12 + naptalam 2.24 PPI	2.5 A	130.0 A	0.0 A	15.0 A	85.0 A	365.0 B	0.0 A	272.5 A	--	--
dinoseb 2.24 + naptalam 4.48 PPI	0.0 A	107.5 A	0.0 A	50.0 A	60.0 A	245.0 BC	7.5 A	57.5 A	--	--
dinoseb 6.72 PPI + 1.68 layby (2x)	0.0 A	195.0 A	0.0 A	22.5 A	--	--	--	--	--	--
dinoseb 6.72 PPI + 1.68 layby (3x)	--	--	--	--	72.5 A	317.5 BC	0.0 A	65.0 A	--	--
dinoseb 1.68 layby (2x)	2.5 A	190.0 A	0.0 A	22.5 A	--	--	--	--	--	--
dinoseb 1.68 layby (3x)	--	--	--	--	47.5 A	237.5 BC	7.5 A	65.0 A	--	--
dinitramine 0.56 + dinoseb 1.68 PPI	0.0 A	142.5 A	0.0 A	32.5 A	50.0 A	162.5 BC	7.5 A	145.0 A	--	--

[1]Population means represent four replicate microplots and are expressed as numbers of nematodes/250 cc of soil extracted by the sugar-flotation procedure. Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

[2]PPI= pre-plant-incorporated; layby (2x)= 2 postemergence treatments; layby (3x)= 3 postemergence treatments.

Table 18. Effect of dinoseb on the severity of Sclerotinia blight of peanut in microplots (1979).[1]

Treatment and rate[2] (kg/ha)	Disease incidence[3] (%) 8/28/79	Disease severity index[4] (1-5) 8/28/79	Disease incidence (%) 9/29/79	Disease severity index (1-5) 9/29/79	Pod rot index[5] (1-5) 9/29/79	Yield (grams) 9/29/79
Infested soil						
control	91.5 A	2.5 A	100.0 A	3.2 A	1.1 AB	206.7 BC
dinoseb 1.68 PPI	66.1 A	2.3 A	100.0 A	3.2 A	1.8 A	190.6 BC
dinoseb 3.36 PPI	100.0 A	2.8 A	100.0 A	3.4 A	1.8 A	163.5 C
dinoseb 6.72 PPI	83.0 A	2.2 A	100.0 A	3.2 A	1.2 AB	238.0 B
dinoseb 1.68 laybys	75.0 A	2.5 A	83.2 A	3.5 A	1.3 AB	196.8 BC
Noninfested soil						
control	0.0 B	1.0 B	0.0 B	1.0 B	1.0 B	327.9 A

[1]Seed of 'Florigiant' peanut were planted on 22 May 1979 in microplots infested with sclerotia (sc) of Sclerotinia minor to a density of 0.12 sc/g soil. Plants were harvested on 29 September 1979.

[2]PPI = preplant incorporated; laybys = postemergence treatments applied on 7 July and 28 August 1979.

[3]Percentage of plants with symptoms and signs of Sclerotinia blight.

[4]1 = no symptoms, 5 = severe disease (see text).

[5]1 = no symptoms, 5 = completely decayed.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 19. Effect of dinoseb on the severity of southern stem rot of peanut in microplots (1979).[1]

Treatment and rate[2] (kg/ha)	Disease incidence[3] (%) 8/29/79	Disease severity index[4] (1-5) 8/29/79	Disease incidence (%) 9/29/79	Disease severity index (1-5) 9/29/79	Pod rot index[5] (1-5) 9/29/79	Yield (grams) 9/29/79
Infested soil						
control	83.2 A	2.6 A	74.5 AB	2.0 A	2.2 A	148.7 A
dinoseb 3.36 PPI	91.5 A	2.7 A	49.5 B	2.3 A	2.0 A	170.9 A
dinoseb 6.72 PPI	66.5 A	2.6 A	79.0 A	2.7 A	2.3 A	158.6 A
dinoseb 1.68 laybys	91.5 A	2.6 A	83.2 A	2.3 A	2.5 A	166.7 A
Noninfested soil						
control	0.0 B	1.0 B	0.0 B	1.0 B	1.0 A	327.9 B

[1]Seed of 'Florigiant' peanut were planted on 22 May 1979 in microplots infested with sclerotia (sc) of Sclerotium rolfsii to a density of 0.03 sc/g soil. Plants were harvested on 29 September 1979.

[2]PPI = pre-plant incorporated; laybys = postemergence treatments applied on 7 July and 28 August 1979.

[3]Percentage of plants with symptoms and signs of stem rot.

[4]1 = no symptoms, 5 = severe disease (see text).

[5]1 = no symptoms, 5 = completely decayed.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

kg/ha) and postemergence (1.68 kg/ha) applications of dinoseb did not influence consistently the incidence and severity of this disease in 1979 (Table 19).

Microplots infested with S. minor and S. rolfsii for 1979 tests were used again for 1980 experiments without additional inoculum. In laboratory tests conducted during the 1979 growing season, low rates of dinitramine and 2,4-DB stimulated growth of S. minor in axenic culture (Table 5) and dinitramine suppressed growth of S. rolfsii (Table 6). These two herbicides were applied to microplots in 1980 in place of the pre-plant dinoseb treatments made in 1979 (Table 18,19) to evaluate their effects on disease severity.

Disease assessments made on 20 August 1980 and at harvest (1 October 1980) showed that the incidence and severity of Sclerotinia blight of peanut in untreated soil were generally lower than those recordings made on similar dates in 1979 (Table 20). Treatments with dinoseb did not consistently influence the incidence and severity of Sclerotinia blight in 1980 (Table 20). None of the herbicide treatments resulted in significant effects on this disease as reflected by severity readings at harvest. However, soil treatments with dinitramine (0.56 kg/ha) and postemergence applications of 2,4-DB (0.28 kg/ha) appeared to result in some disease suppression (not significant at P=0.05). On 20 August 1980, the percentage of diseased

Table 20. Influence of herbicides on the severity of Sclerotinia blight of peanut in microplots (1980).[1]

Treatment and rate[2] (kg/ha)	Disease incidence[3] (%) 8/20/80	Disease severity index[4] (1-5) 8/20/80	Disease incidence (%) 10/1/80	Disease severity index (1-5) 10/1/80	Pod rot index[5] (1-5) 10/1/80	Yield (grams) 10/1/80
Infested soil						
control	41.2 A	1.4 A	41.5 A	1.4 A	1.0 A	308.0 AB
dinitramine 0.56 PPI	33.2 AB	1.5 A	24.7 A	1.1 A	1.08 A	293.0 AB
2,4-DB 0.28 laybys	0.0 B	1.0 A	16.5 A	1.1 A	1.0 A	367.2 A
dinoseb 3.36 PPI + 1.68 laybys	24.7 AB	1.3 A	41.5 A	1.4 A	1.0 A	301.0 AB
dinoseb 1.68 laybys	41.2 A	1.4 A	25.0 A	1.3 A	1.33 A	259.2 B
Noninfested soil						
control	0.0 B	1.0 A	0.0 A	1.0 A	1.0 A	378.7 A

[1]Seed of 'Florigiant' peanut were planted on 10 May 1980 in microplots used for the 1979 test. Plants were harvested on 1 October 1980.

[2]PPI = pre-plant incorporated; laybys = postemergence treatments applied on 2 July, 6 August, and 4 September 1980.

[3]Percentage of plants with symptoms and signs of Sclerotinia blight.

[4]1 = no symptoms, 5 = severe disease (see text).

[5]1 = no symptoms, 5 = completely decayed.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

plants in untreated plots was 41.2, but no evidence of Sclerotinia blight was found in plots treated with 2,4-DB at this time (Table 20). At harvest, the number of diseased plants and the mean disease severity index in 2,4-DB-treated plots were less and yields were higher (not significant at P=0.05) in relation to controls.

Herbicide treatments had no apparent influence on the development of southern stem rot in the 1980 microplots (Table 21). Although the percentage of diseased plants and the mean disease severity index were generally higher in herbicide-treated microplots than in the untreated controls (Table 21), these differences were not significant. Pod rot indices and yield data recorded from these microplots were not significantly different for treatments.

Discussion

The severity of CBR in peanut was increased in 1979 and in 1980 by treatment of soil in microplots with dinitramine at a rate of 0.56 kg/ha (Table 14,15). A higher rate of the herbicide (0.84 kg/ha) was found to have no effect on disease during either year of the test. These observations are consistent with greenhouse results discussed earlier, in which there was a similar relationship between dinitramine and the severity of CBR (Chapter 3). In the second year of

Table 21. Influence of herbicides on the severity of southern stem rot of peanut in microplots (1980).[1]

Treatment and rate[2] (kg/ha)	Disease incidence[3] (%) 8/20/80	Disease severity index[4] (1-5) 8/20/80	Disease incidence (%) 10/1/80	Disease severity index (1-5) 10/1/80	Pod rot index[5] (1-5) 10/1/80	Yield (grams) 10/1/80
Infested soil						
control	33.0 AB	1.5 AB	66.5 A	1.7 AB	1.66 AB	125.5 B
dinitramine 0.56 PPI	66.0 A	2.0 A	58.0 A	1.9 A	1.91 A	215.7 B
2,4-DB 0.28 laybys	57.7 A	1.7 A	91.5 A	2.0 A	1.54 AB	177.0 B
dinoseb 3.36 PPI + 1.68 laybys	66.2 A	2.0 A	74.5 A	2.2 A	1.41 AB	206.5 B
Noninfested soil						
control	0.0 B	1.0 B	0.0 B	1.0 B	1.0 B	378.7 A

[1]Seed of 'Florigiant' peanut were planted on 10 May 1980 in microplots used for the 1979 test. Plants were harvested on 1 October 1980.

[2]PPI = pre-plant incorporated; laybys = postemergence treatments applied on 2 July, 6 August, and 4 September 1980.

[3]Percentage of plants with symptoms and signs of stem rot.

[4]1 = no symptoms, 5 = severe disease (see text).

[5]1 = no symptoms, 5 = completely decayed.

Means in columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

the two-year field test and in microplots installed in 1980, dinitramine at the low rate increased the severity of CBR to a higher degree than observed in 1979 (Table 14,15,16). This herbicide is normally applied to soil at low rates ($\leq$0.56 kg/ha) (24) and is relatively non-persistent (11). For this reason, it is believed that dinitramine increases the susceptibility of young plants to infection. In previously described greenhouse tests, dinitramine appeared to increase the susceptibility of peanut seedings to CBR (Table 12).

In 1979, dinoseb had no significant effect on the development of CBR (Table 14). In 1980, however, pre-plant soil treatments of dinoseb at a rate of 1.68 kg/ha increased significantly (P=0.05) the severity of disease occurring in both microplot experiments (Table 15,16). In greenhouse tests, (Chapter 3), dinoseb at 1.68 kg/ha similarly increased the severity of CBR in a soil collected near the field site which had very similar physicochemical properties. Dinoseb is known to increase the severity of diseases in other crops (1). Romig and Sasser (21) found that dinoseb increased the susceptibility of snapbean (Phaseolus vulgaris L.) to infection by Rhizoctonia solani Kuhn.

Differences in the effects of dinitramine and dinoseb on CBR in 1979 and 1980 microplot tests can possibly be

explained by environmental differences. Climatological data collected at a monitoring facility located less that 50 m from the test site revealed that there were large differences in average rainfall patterns between the growing seasons of 1979 and 1980 (Table 22,23). In 1979, herbicides were applied to microplots on 18 May, and peanut seed were planted on 22 May. Between 19 May and 5 June 1979, approximately 18 cm of rainfall were recorded (Table 22). Dinoseb can leach rapidly from sandy soils as a result of heavy rainfall (2). It is possible that much of the dinoseb initially applied was dissipated before the peanut seed had germinated. This could account for the failure of dinoseb treatments to increase CBR in 1979. Dinitramine is not subject to leaching but the herbicide may lose activity in wet soil (25). Apparently, enough dinitramine remained following the rainy period at planting time in 1979 to increase the susceptibility of seedlings to infection.

In 1980, herbicides were applied on 9 May and peanut seed were planted the subsequent day. No rainfall was recorded after planting until 18 May (Table 23). Between 18 May and 31 May, 8 cm of rainfall occurred but none was recorded again until 26 June. Moisture conditions during this period would not likely result in herbicide activity in soil of the same magnitude believed to occur in 1979. It is believed that these conditions provided for retention of

Table 22. Daily precipitation recorded at the Tidewater Research and Continuing Education Center, Suffolk, VA, for the months of April-October, 1979.[1]

Day of Month	Precipitation (cm)						
	April	May	June	July	Aug.	Sept.	Oct.
1	-	-	0.86	0.50	0.40	-	1.21
2	-	-	0.81	-	-	-	0.15
3	-	-	-	-	-	0.20	0.05
4	2.33	0.23	2.43	-	-	2.00	-
5	2.97	0.58	1.65	0.10	-	1.90	-
6	-	-	-	-	-	12.30	0.11
7	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-
9	-	-	-	-	0.05	-	0.08
10	0.05	-	-	-	-	-	-
11	-	0.84	-	-	-	-	0.71
12	-	-	2.23	-	0.17	-	0.05
13	0.33	1.77	-	-	2.08	-	-
14	3.86	7.36	-	-	-	0.30	1.00
15	-	2.71	-	-	-	-	-
16	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-
18	-	-	-	1.54	-	-	-
19	-	3.20	-	0.17	0.33	-	-
20	-	0.66	-	4.80	0.28	-	-
21	-	-	-	-	0.86	0.66	-
22	-	-	1.52	0.22	0.50	0.50	-
23	-	0.51	-	0.71	0.08	1.54	-
24	0.05	1.95	-	1.09	-	1.11	2.33
25	0.05	4.52	1.39	2.89	-	0.60	-
26	0.73	0.23	-	-	-	0.30	-
27	2.28	-	-	0.71	-	-	-
28	0.91	0.81	-	0.50	0.05	-	-
29	0.63	-	-	-	0.32	0.20	-
30	-	0.02	-	1.37	0.15	0.66	-
31	-	-	-	-	-	-	-
Total	14.91	25.37	10.89	14.75	5.76	22.27	5.98
Average	8.00	9.57	11.57	15.44	15.13	10.94	8.61

[1] Data compiled by D. L. Hallock, research agronomist.

Table 23. Daily precipitation recorded at the Tidewater Research and Continuing Education Center, Suffolk, VA, for the months of April-October, 1980.[1]

Day of Month	Precipitation (cm)						
	April	May	June	July	Aug.	Sept.	Oct.
1	-	-	-	-	-	-	3.42
2	-	0.13	-	-	-	-	0.13
3	-	-	-	-	-	-	-
4	-	-	-	1.01	-	-	0.43
5	0.25	-	-	-	-	-	-
6	-	-	-	1.78	0.22	1.11	-
7	-	-	-	-	-	0.76	-
8	0.13	-	-	-	-	-	-
9	0.30	-	-	0.02	-	-	-
10	0.78	-	-	0.96	-	-	-
11	-	-	-	0.33	-	-	-
12	-	-	-	-	-	-	0.58
13	-	-	-	0.55	-	-	-
14	0.94	-	-	-	-	-	-
15	1.11	-	-	-	0.13	-	-
16	-	-	-	-	-	2.36	-
17	-	-	-	-	-	-	-
18	-	2.00	-	1.27	-	0.25	-
19	-	-	-	-	0.22	-	3.93
20	-	2.38	-	-	0.10	-	0.33
21	-	1.27	-	-	-	-	-
22	-	-	-	-	-	-	-
23	-	-	-	-	-	0.05	-
24	-	0.86	-	1.01	-	-	-
25	-	-	-	-	-	0.18	4.31
26	0.13	1.27	0.05	-	-	0.02	0.20
27	-	-	0.02	-	-	-	-
28	2.50	-	-	-	-	-	-
29	0.76	-	-	-	-	-	0.17
30	0.45	-	0.76	-	-	0.15	1.01
31	-	0.10	-	-	-	-	0.30
Total	7.35	8.01	0.83	6.93	3.03	2.52	14.81
Average	8.10	10.55	11.53	15.40	15.10	10.8	8.5

[1] Data compiled by D. L. Hallock, research agronomist.

dinitramine and dinoseb in treated soils during the relatively dry conditions of 1980 and resulted in the observed effects of these herbicides on CBR.

Soil assays of samples from 1979 and 1980 Test-1 microplots showed that the population dynamics of C. crotalariae ms were not consistently affected by herbicide treatments (Table 17A). However, microsclerotium production appeared to be retarded by dinitramine and dinoseb in the Test-2 (1980) microplots (Table 17A). Soil treatment with dinitramine (1 to 100 µg/g soil) in laboratory tests resulted in no reductions in populations of ms (Table 7). Low ms populations at harvest in microplots treated with dinitramine (0.84 kg/ha) and dinoseb (6.72 kg/ha) may have been due to herbicide effects on root growth and/or the production of ms by the colonizing fungus. Dinitramine and dinoseb inhibited growth of peanut roots significantly (P=0.05) in greenhouse tests (Fig. 9,11).

Data obtained in this study indicate that dinitramine and dinoseb may have a significant effect on the development of CBR in peanut plants grown under field conditions. It is believed that the effects of these herbicides on CBR can be influenced significantly by environmental factors that affect their persistence and activity in soil.

In 1979 and in 1980, pre-plant and postemergence treatments with dinoseb had no significant (P=0.05) effects

on development of Sclerotinia blight of peanut in microplots (Table 18,20). However, postemergence applications of 2,4-DB in 1980 appeared to suppress disease severity (although not significantly) to the extent that further experiments may be justified (Table 20). Porter and Rud (20) found in their 1977 and 1978 field experiments that multiple postemergence applications of dinoseb suppressed Sclerotinia blight and increased yields significantly. Results reported here (Table 18,20) and in Chapter 2 provide little, if any support to the findings of these workers, but it is recognized that environmental conditions may account for variation in field test results from year to year.

None of the herbicides selected for study were found to influence consistently the development of southern stem rot of peanut in microplots during either year of the test (Table 19,21). Grinstein et al. (10) found that soil treatment with dinitramine suppressed the development of stem rot disease in Israel. However, the rate of dinitramine used in their field tests (3 kg/ha) would not be feasible under Virginia conditions due to phytotoxic effects (O. E. Rud, 1981, personal communication). The present study emphasizes that knowledge of the non-target effects of pesticides on the biosphere can be significant and must be considered in the development of effective disease control programs.

Literature Cited

1. Altman, J., and C. L. Campbell. 1977. Effects of herbicides on plant diseases. Ann. Rev. Phytopathol. 15:361-385.

2. Anonymous. 1979. Herbicide Handbook of the Weed Science Society of America. Weed Sci. Soc. Amer. Champaign, Ill. 479 pp.

3. Backman, P. A., R. Rodriguez-Kabana, and G. A. Buchanan. 1977. Interactions of oxadiazon and dinoseb with stem rot in peanut. Weed Sci. 25:260-263.

4. Bell, D. K., and E. K. Sobers. 1966. A peg, pod and root necrosis of peanut caused by a species of Calonectria. Phytopathology 56:1361-1364.

5. Byrd, D. W., C. J. Nusbaum, and K. R. Barker. 1966. A rapid flotation-sieving technique for extracting nematodes from soil. Plant Dis. Reptr. 50:954-957.

6. Chappell, W. E., and L. I. Miller. 1956. The effects of certain herbicides on plant pathogens. Plant Dis. Reptr. 40:52-56.

7. Garren, K. H., D. M. Porter, and A. H. Allison. 1971. Cylindrocladium black rot of peanuts in Virginia. Plant Dis. Reptr. 55:419-421.

8. Graham, P. J. 1980. Relationship between peanut canopy temperature and Cylindrocladium black rot development and effects of soil temperature and water potential on the germinability of Cylindrocladium crotalariae microsclerotia. M. S. Thesis. Va. Polytech. Inst. State Univ., Blacksburg, Va. 108 pp.

9. Griffin, G. J. 1977. Improved selective medium for isolating Cylindrocladium crotalariae from naturally-infested soils. Can. J. Microbiol. 23:680-683.

10. Grinstein, A., Y. Elad, J. Katan, and I. Chet. 1979. Control of Sclerotium rolfsii by means of a herbicide and Trichoderma harzianum. Plant Dis. Reptr. 63:823-826.

11. Jacques, G. L., and R. G. Harvey. 1979. Persistence of dinitroaniline herbicides in soil. Weed Sci. 27:660-665.

12. Katan, J., and Y. Eshel. 1973. Interactions between herbicides and plant pathogens. Residue Rev. 45:145-177.

13. Krigsvold, D. T., and G. J. Griffin. 1975. Quantitative isolation of Cylindrocladium crotalariae microsclerotia from naturally-infested peanut and soybean fields. Plant Dis. Reptr. 59:543-546.

14. Krigsvold, D. T., K. H. Garren, and G. J. Griffin. 1977. Importance of peanut field cultivation and soybean cropping in the spread of Cylindrocladium crotalariae within and among peanut fields. Plant Dis. Reptr. 61:495-499.

15. Phipps, P. M. 1978. Cylindrocladium black rot. Control series 167, Va. Polytech. Inst. State Univ. Extension Div., Blacksburg, Va.

16. Phipps, P. M., and J. A. Barron. 1981. Efficacy of soil fumigants in control of CBR of peanut in Virginia. Fungicide-Nematicide Tests 36: (in press).

17. Phipps, P. M., and M. K. Beute. 1977. Sensitivity of susceptible and resistant peanut cultivars to inoculum densities of Cylindrocladium crotalariae microsclerotia in soil. Plant Dis. Reptr. 61:300-303.

18. Phipps, P. M., and M. K. Beute. 1979. Population dynamics of Cylindrocladium crotalariae microsclerotia in naturally-infested soil. Phytopathology 69:240-243.

19. Phipps, P. M., M. K. Beute, and B. A. Hadley. 1977. A microsclerotia-infested soil technique for evaluating pathogenicity of Cylindrocladium crotalariae isolates and black rot resistance in peanut. Proc Amer. Phytopathol. Soc. 4:146. (Abstr.).

20. Porter, D. M., and O. E. Rud. 1980. Suppression of Sclerotinia blight of peanut with dinitrophenol herbicides. Phytopathology 70:720-722.

21. Romig, W. R., and M. Sasser. 1972. Herbicide predisposition of snapbean to Rhizoctonia solani. Phytopathology 62:785-786. (Abstr.).

22. Roth, D. A., G. J. Griffin, and P. J. Graham. 1979. Low temperature induces decreased germinability of Cylindrocladium microsclerotia. Can. J. Microbiol. 25:157-162.

23. Rowe, R. C., S. A. Johnston, and M. K. Beute. 1974. Formation and dispersal of Cylindrocladium crotalariae microsclerotia in infected peanut roots. Phytopathology 64:1294-1297.

24. Rud, O. E. 1980. Weed control in peanuts. Pages 243-277 in: Virginia pest management guide 1. Extension Div. Va. Polytech. Inst. State Univ., Blacksburg, Va.

25. Savage, K. E. 1978. Persistence of several dinitroaniline herbicides as affected by soil moisture. Weed Sci. 26:465-471.

26. Taylor, J. D. 1980. Influence of crop rotation, minimum tillage in a soybean rotation, and peanut cultivars on the populations of Cylindrocladium crotalariae (Loos.) Bell and Sobers in field soils. Ph.D. Dissertation. Va. Polytech. Inst. State Univ. 128 pp.

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EFFECT OF HERBICIDES ON *CYLINDROCLADIUM CROTALARIAE* AND THE CYLINDROCLADIUM BLACK ROT (CBR) DISEASE OF PEANUT.

by

James Albert Barron, III

(ABSTRACT)

The effects of herbicides on axenic growth of *Cylindrocladium crotalariae* and on the development of CBR in the greenhouse and in the field was studied. In laboratory tests, dinitramine at 1, 5, and 10 ug/ml increased axenic growth of two isolates of *C. crotalariae* in herbicide-amended potato-dextrose broth (PDB). Dinoseb and dinoseb + naptalam at the same rates also increased growth, whereas alachlor, benefin, diphenamid, vernolate and 2,4-DB were found to have no consistent effect. Treatment of infested soil with dinitramine at 1, 5, 10, 50, and 100 ug/g soil had no significant effects on populations of *C. crotalariae* microsclerotia (ms) in a Ruston or a Woodstown loamy fine sand. Dinoseb reduced ms populations significantly (P=0.05) in Woodstown soil at 5, 10, 50, and 100 ug/g soil and in Ruston soil at rates of 50 and 100 ug/g soil.

In greenhouse tests, soil treatment with dinitramine at 0.56 kg/ha but not at 0.84 kg/ha increased significantly (P=0.05) the severity of CBR in Ruston and in Woodstown soil. In Ruston soil, dinitramine increased the severity of CBR only at an inoculum density of 5 ms/g soil, but in

CBR only at an inoculum density of 5 ms/g soil, but in Woodstown soil, the herbicide resulted in disease increase only at 50 ms/g soil. Additional tests showed that disease severity was increased when peanut seedlings were pre-treated with dinitramine and transplanted to herbicide-free infested soil. Dinitramine treatment was postulated to alter growth and development processes in 'Florigiant' peanut in a way to result in increased susceptibility to CBR. Dinitramine treatment of soil did not increase disease severity in 'NC3033' peanut, a CBR-resistant cultivar. Dinoseb at 1.68 kg/ha increased significantly (P=0.05) the severity of CBR in Woodstown soil, but not in Ruston soil. Higher rates of dinoseb (3.36 and 6.72 kg/ha) were found to have no effect.

Field tests were conducted in microplots (77-cm diameter) to determine the effects of herbicides on CBR. The severity of pod rot caused by C. crotalariae was increased significantly (P=0.05) by pre-plant soil treatment with dinitramine at 0.56 kg/ha, but not at 0.84 kg/ha in 1979. Although not significant (P=0.05), substantial increases in root rot and top symptom severity occurred in 1979 in response to this treatment. Pre-plant soil treatments with dinoseb at 1.68, 3.36, and 6.72 kg/ha did not affect disease development in microplots in 1979. In 1980, dinitramine (0.56 kg/ha) increased the severity of

both root and pod rot significantly (P=0.05) in two separate microplot tests. Dinoseb at 1.68, but not at 3.36 or 6.72 kg/ha also increased significantly the severity of CBR in these tests. Soil assay results indicated that herbicide treatments had no consistent effect on populations of C. crotalariae ms, Macroposthonia ornatum or Meloidogyne hapla in microplots.

These results provide evidence that certain herbicides can affect the development and severity of CBR in peanut. Furthermore, the present study emphasizes that knowledge of the non-target effects of pesticides on the biosphere can be significant and must be considered in the development of effective disease control programs.