Effect of the Herbicide Ethalfluralin on Net Blotch Disease of Peanut Pods

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ABSTRACT

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Five field experiments and one container experiment were conducted during three successive years to evaluate the effect of ethalfluralin and vernolate on the incidence and severity of net blotch disease of peanut (*Arachis hypogaea*) pods in which unspecified actinomycetes seem to be involved. In all experiments, the incidence of diseased pods (range 38–86%) was significantly higher in the plots treated with a mixture of the two compounds than in the control plots without herbicides (range 25–48%). The range of disease severity ranked on a 0–10 rating scale was 0.44–2.13 and 0.25–0.83 in herbicide-treated and control plots, respectively. Disease incidence and severity on pods in soil treated with fenamiphos and metham-sodium, with and without herbicides, were similar to the levels observed in the control plots without herbicides. Application of methyl bromide, alone or with herbicides, reduced the disease considerably. Ethalfluralin alone increased disease incidence, and this increase was not affected by the addition of vernolate. Ethalfluralin was moderately toxic to bacteria but not to fungi or actinomycetes in growth media; vernolate was not toxic to any of the microorganisms. Infestation of soil with mixed cultures of actinomycetes isolated from diseased pods reproduced net blotch disease symptoms on the pods.

Interactions between herbicides and plant pathogens have been well documented. The main cause of this phenomenon is that the biological activity of pesticides may extend beyond the effects on the target organisms. Thus, herbicides may increase or decrease plant diseases (1–3,6–8,10–14,16–18,21,23). Substituted dinitroanilines are selective, wide-spectrum herbicides, which are used extensively in vegetable and field crops.

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Cases of increase (1,8,17,20) or decrease (6,11–14) in diseases caused by a soilborne pathogen, upon treatment with these herbicides, have been reported.

Net blotch of peanut (*Arachis hypogaea* L.) pods is a previously undescribed disease. Various unspecified isolates of actinomycetes were consistently isolated from the affected pod tissue and pathogenicity tests were started (19).

Net blotch disease of peanut pods in Israel was a sporadic and negligible blemish for many years (Z. R. Frank, *personal observation*). However, in recent years there has been an increase of the disease to various levels on different farms, totalling approximately 200 of the 1,000 ha planted to peanuts in that region. In that area, the growers use a mixture of two herbicides: ethalfluralin (dinitroaniline) and vernolate, a carbamate. It was suspected that the increase in the disease was related to the application of these herbicides. The purpose of this study was to evaluate the effect of these herbicides on the incidence of net blotch and to determine whether the disease is caused by biotic or abiotic agents.

MATERIALS AND METHODS

Field and container studies. Three field experiments in two successive years (1987 and 1988) were performed to study the effect of a mixture of the herbicides ethalfluralin and vernolate with and without a nematicide (fenamiphos) and of soil disinfestants (metham-sodium and methyl bromide) on the net blotch disease of peanut pods. The treatments tested were untreated control, fenamiphos, metham-sodium, and methyl bromide, each applied with and without herbicides. Two experiments (experiments 1 and 3) were in a sandy soil, with pH 7.9 and 10% (v/v) field capacity, and one (experiment 2) was in a sandy soil, with pH 7.6 and 15% field capacity. In two other field experiments (experiments 4 and 5) conducted in the third year (1989) and in one experiment (experiment 6) carried out in containers (50 cm diameter, 100 cm high), the effect of each herbicide on disease incidence was studied. The treatments tested were control without herbicides, ethalfluralin, vernolate, and a mixture of the herbicides. The soil used in experiments 4–6 was sandy, with pH 7.9 and 15% field capacity. The pH was measured in soil saturation extract.

The experimental design for each experiment was a randomized complete block. Treatments were replicated five or six times, each replicate consisting of a 12 × 12 m plot (field experiment) or one container, 50 cm diameter × 100 cm high.

Peanut cv. Shulamit was sown in late April or early May of each year according to standard agronomic practices (22). At maturity, pods from 2 m^2 of each replicate plot were harvested manually, 100-pod samples were scored for net blotch on each pod, and the percentage of diseased pods and the disease severity rating were determined. Disease severity of each pod was rated on a visual, nonlinear scale of 0–10, where 0 = a clean pod and 10 = a pod completely covered with net blotch, as exemplified by Figure 1. The disease severity for each sample of 100 pods was calculated by multiplying the number of diseased pods in each category by the appropriate score, adding the products, and dividing the result by 100.

Disease progress on peanut pods in control and herbicide-treated plots was determined by harvesting three plants from each replicate at each sample date and estimating disease level for all pods present on the plants. Sampling was started 80 and 65 days after seeding in experiments 3 and 5, respectively.

Herbicides, nematicide, and soil disinfestants. Herbicides used as emulsifiable concentrates were ethalfluralin (32% a.i.) and vernolate (82% a.i.), each at 3,000 ml/ha. The herbicides were applied preplant and immediately incorporated into the top 7 cm of soil. Methyl bromide (98% a.i.) was applied at 50 g/m^2 in experiment 1 as a hot gas and in experiments 2 and 3 as a cold gas by injection into the soil. Metham-sodium (32% a.i.) was applied through the sprinkler irrigation system at 500 L/ha, with water sufficient to wet the top 30 cm of soil (5). The nematicide fenamiphos (10% a.i.) was applied at 60 kg/ha before planting and 45 days after planting. The application was followed by irrigation to incorporate the chemical into the soil.

Effect of herbicides on soil microorganisms. The toxicity of the herbicides to soil microorganisms was tested on herbicide-amended media. Herbicides (technical grade) were added to the test media just before solidification, at a final concentration of 0, 1.5, 3.0, 15, and 30 µl/L. Then 1-ml aliquots from 10-fold aqueous dilutions of a 5-g soil sample collected from control plots (experiment 4) were placed on medium in each petri dish. Fungi were counted both on potato-dextrose agar (PDA) amended with 250 mg/L of dihydrostreptomycin, which sustains the profuse growth of fungi such as *Mucor* and *Rhizopus* spp., and on that amended medium supplemented with 25 mg/L of pentachloronitrobenzene (PCNB), which suppresses the growth of these molds (4) and, thereby, facilitates enumerating other fungi. Plates were incubated at 25 C for 36 hr for molds and 96 hr for other fungi. Bacteria were plated on nutrient agar medium and actinomycetes on a medium (9), as modified by G. Kritzman, which contained 0.6 g/L of Bacto peptone, 0.6 g/L of Bacto proteose, and 10 mg/L of nalidixic acid. The nalidixic acid was dissolved in 5 ml of 0.01 N NaOH and added before pouring the medium into dishes (G. Kritzman, ARO, *personal communication*). Plates were incubated at 25 C for 5 and 10 days for bacteria and actinomycetes, respectively.

The effect of herbicides on populations of soil microorganisms was studied by collecting soil samples in July and October 1989 from the treated plots in experiment 5 and assaying them for bacteria and actinomycetes as described earlier. Soil samples were composed of 10 subsamples per plot collected from the top 15 cm of soil.

Infestation of soil. Thirty isolates of actinomycetes that originated from diseased pods were each plated on nutrient agar medium. To isolate actinomycetes, symptomatic tissue of dry pods was ground in a Wiley mill, model 3383, with a 20-mesh screen. Samples of ground pods were suspended in water agar (0.1%) and then dispersed on the selective medium. These isolates were divided at random into three groups of 10 isolates each, homogenized, and mixed with natural or steamed soil 3 days before peanuts were planted. The same amount of the medium, without actinomycetes mixed into the soil, was used as a control. The soil used for growing peanut plants was collected from a field with a history of net blotch which was disinfested a year previously with methyl bromide at 50 g/m^2 to eliminate potential causal organisms. There were four replicates for each treatment. Peanuts were grown in 10-L buckets each containing 8 L of soil placed in a screenhouse at ambient temperatures of approximately 20–30 C.

Analysis of variance for the percentage of diseased pods was performed after arcsine-square transformation of the data.

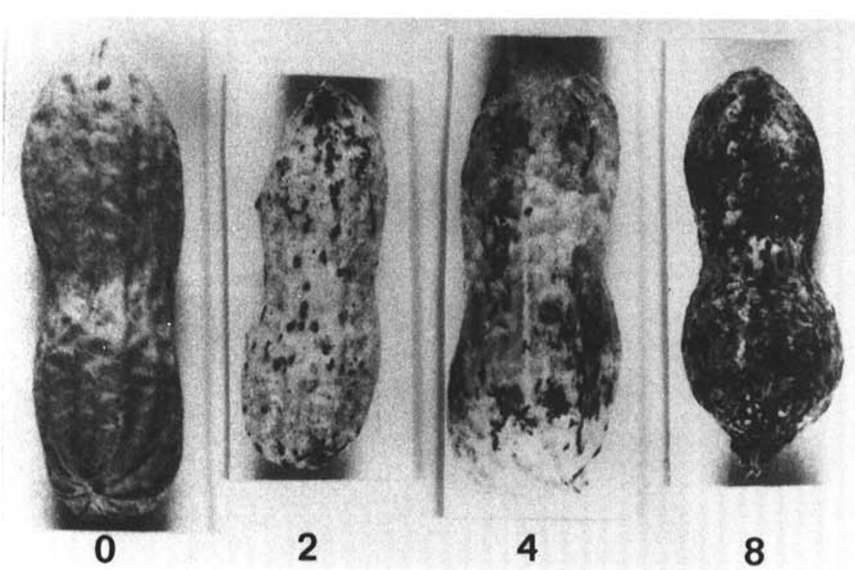

Fig. 1. Severity of net blotch disease on peanut pods, rated on a visual, nonlinear scale of 0–10 where 0 = healthy pod and 10 = pod completely covered with net blotch. Peanut pods (left to right) with disease severity of 0, 2, 4, and 8, respectively.

Table 1. Effect of a mixture of ethalfuralin and vernolate (E + V), with and without nematicide or soil disinfestants, on net blotch disease of peanut pods[w]

	Experiment					
	Diseased pods (%)[x]			Disease severity[x,y]		
Treatment	**1**	**2**	**3**	**1**	**2**	**3**
Control	25.0	32.0 bc[z]	41.0 c	0.52 b	0.57 b	0.76 bc
E+V	38.0 a	84.0 a	86.0 a	0.70 a	2.13 a	1.95 a
Fenamiphos	21.0 b	27.0 c	42.0 c	0.47 b	0.38 bc	0.65 c
Fenamiphos + (E + V)	25.0 b	38.0 b	51.0 b	0.52 b	0.75 b	0.78 b
Metham-sodium	...	30.0 bc	32.0 d	...	0.48 c	0.51 c
Metham-sodium + (E + V)	...	35.0 bc	42.0 c	...	0.57 b	0.77 bc
Methyl bromide	3.3 c	0.2 d	0.2 e	0.14 c	0.02 d	0.02 d
Methyl bromide + (E + V)	3.5 c	0.2 d	1.0 e	0.15 c	0.02 d	0.02 d

[w] Three field experiments were conducted in sandy soils during two successive years.
[x] Values are means of six replicates in experiment 1 and five replicates in experiments 2 and 3.
[y] Assessed on a scale of 0–10 where 0 = a clean pod and 10 = a pod completely covered with net blotch.
[z] Within columns, values followed by a common letter do not differ significantly according to Duncan's multiple range test ($P = 0.05$).

RESULTS

Effect of herbicide mixture, nematicide, and soil disinfestants on net blotch disease. Application of a mixture of the herbicides ethalfluralin and vernolate increased disease incidence and severity significantly compared with the control plots without herbicides in three field experiments (Table 1). The increase in disease incidence caused by the herbicide mixture ranged from 52 to 162%, and the increase in disease severity ranged from 35 to 273% over the control. Fenamiphos and metham-sodium only slightly reduced disease incidence and severity compared with the control plots; however, they significantly reduced disease levels compared with plots treated with the herbicides. Methyl bromide with and without herbicides reduced the disease to negligible levels.

Effect of ethalfluralin or vernolate on disease incidence. Vernolate had no significant effect on disease compared with the control (Table 2). In contrast, ethalfluralin, with and without the addition of vernolate, significantly increased disease incidence by 32–50% and disease severity by 76–287% over the control.

Disease progress during the growth season. The disease level was already high at the first date of sampling, 80 and 65 days after sowing in experiments 3 and 5, respectively (Table 3), when the oldest pods were approximately 45 and 30 days old, respectively. At the first sampling, disease level was 62–100% and 71–81% of the maximal value in the control and herbicide treatments, respectively. The respective increases in disease percentage by the herbicide mixture of the first sampling in experiments 3 and 5 were 71 and 85%. This increase was significant and evident throughout the sampling period.

Effect of ethalfluralin and vernolate on soil microorganisms. Vernolate was not toxic to the soil microorganisms tested: fungi, bacteria or actinomycetes as tested in herbicide-amended media. Ethalfluralin was not toxic to fungi or actinomycetes but was moderately toxic to bacteria at concentrations of 15 and 30 μl/L, reducing the population by 56 and 48%, respectively. Populations of bacteria and actinomycetes in soil were evaluated twice in July and October 1989 in experiment 5. In the first sampling, populations of bacteria in the control, vernolate, ethalfluralin, and vernolate + ethalfluralin mixture were 62×10^5, 49×10^5, 33×10^5, and 38×10^5 per gram of soil, respectively. The respective populations of actinomycetes were 20×10^5, 22×10^5, 28×10^5, and 31×10^5 per gram of soil. In both cases, the two treatments that included ethalfluralin were significantly different ($P = 0.05$) from the other two treatments. In the second sampling, the difference between treatments was not significant.

Soil infestation tests. Net blotch disease on peanut pods developed after soil infestation with actinomycetes (Table 4). A similar level of disease incidence was obtained upon infestation with the three groups of isolates tested in a natural soil. Higher disease incidence was recorded in the infested natural soil than in the steamed soil. Reisolation from inoculated, diseased pods yielded actinomycetes.

DISCUSSION

Application of ethalfluralin alone or in a mixture with vernolate for weed control before peanut sowing increased the incidence and severity of net blotch disease on peanut pods. Vernolate alone had no measurable effect on the disease. This disease is effectively controlled by fumigation with methyl bromide, even in the presence of herbicides. An increase or decrease in plant diseases attributable to herbicide application depends on the specific combination of herbicide, host, pathogen, and soil organisms. A similar percentage of diseased pods was recorded at various time intervals during the growth season (Table 3). Therefore, it is suggested that the symptoms develop on young pods a short time after gynophores penetrated into the soil. Variability in disease level at various sampling dates was probably attributable to existing microconditions that affected disease occurrence.

Table 2. Effect of ethalfluralin and vernolate, separately and in combination, on net blotch disease on peanut pods

	Experiment					
	Diseased pods (%)[x]			Disease severity[x,y]		
Treatment	4	5	6	4	5	6
Control	41 b[z]	42 b	48 b	0.25 b	0.40 b	0.83 b
Vernolate (V)	42 b	40 b	42 b	0.33 b	0.49 b	0.80 b
Ethalfluralin (E)	54 a	65 a	67 a	0.46 a	1.16 a	1.73 a
V + E	57 a	68 a	72 a	0.44 a	1.55 a	2.00 a

[x] Figures are means of six replicates each. Experiments 4 and 5 were conducted in the field and experiment 6 in containers filled with infested field soil and placed outdoors.
[y] Assessed on a scale of 0–10, where 0 = a clean pod and 10 = a pod completely covered with net blotch.
[z] Within columns, values followed by the same letter do not differ significantly according to Duncan's multiple range test ($P = 0.05$).

Table 3. Incidence of net blotch disease on peanut pods at various times after seeding in control plots and plots treated with a mixture of ethalfluralin (E) and vernolate (V)

	Diseased pods (% ± SE)[y]			
	Experiment 3		Experiment 5	
Days after seeding	Control	E + V	Control	E + V
65	...	...	26 ± 4[z]	48 ± 3
70	...	...	36 ± 3	58 ± 5
80	41 ± 5	70 ± 7	28 ± 4	59 ± 3
90	44 ± 8	86 ± 3	...	...
100	36 ± 9	89 ± 2	28 ± 5	68 ± 4
110	53 ± 6	88 ± 1	28 ± 4	66 ± 1
120	46 ± 3	91 ± 2	25 ± 5	56 ± 3
140	41 ± 3	86 ± 3	42 ± 1	68 ± 2

[y] Values are means of five replicates in experiment 3 and six replicates in experiment 5.
[z] At each sampling date, the herbicide treatment was significantly different ($P = 0.05$) from the comparable control according to t test.

Table 4. Effect of soil infestation with mixtures of strains of actinomycetes on the development of net blotch disease on peanut pods

	Natural soil		Steamed soil	
Treatment	Diseased pods[y] (%)	Disease severity	Diseased pods[y] (%)	Disease severity
Control	2.8 b	0.03 b	0.0 c	0.00 c
Strains (1–10)	33.0 a	0.24 a	25.0 a	0.20 a
Strains (11–20)	38.0 a	0.48 a	27.0 a	0.20 a
Strains (21–30)	41.0 a	0.28 a	9.0 b	0.02 b
Mean[z]	28.7	0.26	15.3	0.16

[y] Values are means of four replicates each. Within columns, values followed by the same letter do not differ significantly according to Duncan's multiple range test ($P = 0.05$).
[z] For comparison between soils of any strains used, the LSD (5%) = 4.8 for disease percent and 0.06 for disease severity.

Several investigators have examined the effect of dinitroaniline herbicides on soilborne pathogens and found that these herbicides may increase (1,8,20) or decrease (6,11–14) disease incidence caused by pathogens. From our study, it seems that the routine application of ethalfluralin in peanut fields resulted in the gradual increase of net blotch disease of peanut pods from a negligible level to one causing a major problem. Although many studies showed an increase in disease incidence attributable to herbicide applications, in only a limited number of these studies was the phenomenon demonstrated under field conditions (7,23).

Elimination of the disease by soil fumigation with methyl bromide suggests that biotic agents are involved in the disease syndrome, rather than the disease being the result of a direct effect of the herbicide on peanut pods. Unsuccessful control of the disease by fenamiphos suggests that nematodes do not play a key role in inducing disease symptoms.

Soil infestation with actinomycetes cultures (derived from diseased pods) resulted in the development of disease symptoms, and actinomycetes were reisolated from the symptomatic tissue. These findings suggest that the actinomycetes play a role in inducing net blotch disease symptoms on peanut pods. Inoculation with mixtures of actinomycete isolates orginating from diseased pods induced greater disease incidence in natural soil than in steamed soil. It is possible that the disease is caused by a complex of organisms which includes actinomycetes. The soil-treatment interaction was highly significant ($P = 0.01$) for both disease percentage and severity (Table 4).

Ethalfluralin is more toxic to bacteria than to actinomycetes in culture and it increased populations of actinomycetes in soil, with a concomitant decrease in populations of bacteria. One possible mechanism of disease increase by ethalfluralin is a shift in the microbial population toward an increase in actinomycetes at the expense of other organisms. Helmeczi et al (15) reported that the total number of bacteria and fungi in soil was affected after the application of ethalfluralin, i.e., their number first increased, dropped in midseason, and returned at the end of the growing season to its original level before ethalfluralin had been applied. The toxicity of dinitroaniline herbicides to fungi depends on the herbicide and the tested fungus (10). In all of our field experiments, the previous peanut crop had been harvested about 18 mo before peanuts were planted in the experimental plots. Because a relatively high disease incidence (25–48%) was reached in the untreated control plots, this indicates that the pathogen(s) can survive in the soil for at least 18 mo.

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