RESEARCH REPORTS

Vertical and lateral movement and nematicidal activity of fenamiphos applied to soil by trickle irrigation

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Summary

A bioassay with a fungal-feeding nematode, *Aphelenchus avenae*, proved useful in determining whether nematicidal concentrations of fenamiphos were present in soil. The nematode multiplied readily on *Rhizoctonia solani* in petri plates containing potato-dextrose agar mixed with nematicide-free soil, but the number of nematodes was reduced by at least 80% when the soil contained more than about 0.5 μg fenamiphos g^{-1} soil. Since galling on tomato plants caused by *Meloidogyne javanica* was inhibited by similar concentrations of fenamiphos, the *A. avenae* bioassay provided an indication of the likely reaction of root-knot nematodes in the soil under test. When used to monitor the vertical and lateral movement of fenamiphos applied by trickle irrigation, the bioassay showed that nematicidal concentrations of fenamiphos were obtained at depths of 30 cm but were never achieved 20 cm or more laterally from the emitter. Observations that fenamiphos eliminated root galling on tomato only in a cylindrical zone beneath an emitter with a radius of about 15 cm provided further evidence of the poor lateral movement of fenamiphos when applied through trickle irrigation systems.

Introduction

Fenamiphos is one of several non-volatile nematicides which are nemastatic in action rather than nematoxic (Van Gundy and McKenry 1977). Concentrations of 0.02–2 μg fenamiphos ml^{-1} suppress egg hatch and reduce the movement and infectivity of *Meloidogyne javanica* juveniles, but nematodes and eggs resume normal activity when the nematicide is removed (McLeod and Khair 1975; Greco and Thomason 1980). Fenamiphos also persists in soil for relatively short periods. Concentrations of 5–10 μg fenamiphos g^{-1} soil were recorded immediately after it was applied at 9 kg ha^{-1}, but 30–60 days later concentrations were less than 1 μg g^{-1} (Johnson *et al.* 1981, 1982).

When applied as a single dose at planting, non-persistent nematicides such as fenamiphos provide good nematode control in short-term annual crops, but annual or biannual applications to perennial crops generally have proved ineffective. Strategies in which application methods, dosage and timing are chosen with the aim of maintaining relatively low concentrations of the nematicide in soil throughout the growing season are likely to be needed for such crops. Trickle irrigation is an ideal vehicle for applying multiple nematicide treatments and nematicides applied by trickle irrigation have given encouraging results in some recent field trials (Apt 1981; Garabedian and Van Gundy 1982, 1985; McKenry and Buzo 1984; Radewald *et al.* 1985). In addition, this method of application has potential for reducing the contamination of ground water by nematicides and for reducing the cost of nematode protection.

Since little is known about the distribution and persistence of nematicides applied in this manner, this paper describes the development of a bioassay method for detecting the presence of nematicidal concentrations of fenamiphos in soil, and outlines its use in studying the lateral and vertical movement of the nematicide and its persistence when applied to field soil through individual emitters.

Materials and methods

Development of the bioassay

Since the fungal-feeding nematode *Aphelenchus avenae* Bastian and the fungus *Rhizoctonia solani* Kuhn had been used to compare the efficacy of non-volatile nematicides in agar plates (Hague *et al.* 1983), a preliminary experiment was carried out to determine whether this procedure could be modified for use with soil. The experiment was also established to determine the effect of inoculum level on the number of nematodes recovered at various times after inoculation. Soil (40 g) in petri plates (9 cm diam.) was autoclaved at 103 kPa and 121°C for 20 minutes and then 15 ml of sterilized molten potato dextrose agar (PDA) was added and the plates vibrated gently to mix the agar and soil. After the agar had solidified, plates were inoculated with a disc (5 mm diam.) of *R. solani* and four replicate plates were inoculated with either 40 or 800 *A. avenae* extracted under sterile conditions from a culture of *R. solani* on PDA by means of a Baermann funnel. The plates were incubated at 28°C and after 3, 6, 9, 12 and 15 days nematodes in four replicates of each inoculum density were extracted from the soil–agar mixture by a Whitehead tray (Whitehead and Hemming 1965) and counted.

To determine whether this procedure could be used to differentiate between different concentrations of fenamiphos, soil containing 0, 0.01, 0.1, 1 and 10 μg fenamiphos g^{-1} dry soil was prepared by adding the appropriate quantity of Nemacur 400 (40% w/v fenamiphos). Five replicate bioassay plates were prepared as described above using 40-g samples of each soil, and each plate was then inoculated with 100 *A. avenae*. After the plates had been incubated for 15 days at 28°C, nematodes were extracted and counted. Because it was not known whether autoclaving affected the activity of fenamiphos, another five replicate samples of each treatment were prepared with non-autoclaved soil and bioassayed in the same way.

For all subsequent experiments, a standard bioassay method was used. The test soil was autoclaved before agar was added, plates were inoculated by transferring an agar disc (5 mm diam.) containing 100–200 *A. avenae* from cultures of the nematode maintained on *R. solani*, and plates were incubated for 12 days before nematodes were extracted and counted. In each experiment, soil known to be free of nematicide was bioassayed in the same manner and the level of nematicidal activity in the soil under test estimated by expressing the reduction in numbers of *A. avenae* in the test soil as a percentage of the mean number of nematodes in nematicide-free soil.

Using this standard bioassay, three similar experiments were carried out to determine whether the multiplication of *A. avenae* on *R. solani* was inhibited by fenamiphos at concentrations similar to those that affected root-knot nematode. In the first experiment, fenamiphos was incor-

porated into soil at concentrations of 0, 0.1, 1 and 10 μg g^{-1} dry soil and four replicate samples of each treatment bioassayed with *A. avenae*. Four additional replicate soil samples containing the same concentrations of fenamiphos were added to pots (10 cm diam.) and a tomato (cv. Floradade) seedling was planted. Each pot was inoculated with 1000 *Meloidogyne javanica* eggs extracted from egg masses with sodium hypochlorite (Hussey and Barker 1973) and the number of galls on each plant were counted after plants had grown in a glasshouse for 28 days. The experiment was repeated with fenamiphos concentrations of 0, 0.1, 0.2, 0.5, 1, 2 and 5 μg g^{-1} dry soil (experiment 2) and 0, 0.1, 0.4, 0.7, 1, 4 and 7 μg g^{-1} dry soil (experiment 3).

Field experiments

Field studies were carried out at the Granite Belt Horticultural Research Station, Applethorpe, Qld, on a grey, sandy loam soil (87% sand, 8% silt, 5% clay) with an impermeable clay horizon at approximately 40 cm depth. The soil contained almost no organic matter and had a pH of 5.6 and a water holding capacity of 26%. In February 1985, two irrigation lines each having 24 Keyclip 2 l h^{-1} emitters were set up on the soil surface so that water entered the soil at a single point beneath the emitter. A tomato seedling (cv. Floradade) was planted next to each emitter, and the appropriate quantity of Nemacur 400 was then metered into each line so that either 1.25 ml or 0.625 ml Nemacur 400 (0.5 or 0.25 g fenamiphos) and 5 l water was applied through each emitter. Polyethylene sheeting was suspended over the area to allow air circulation but prevent rain falling on the plots and approximately 5 l water $week^{-1}$ was applied through the emitters for the first 5 weeks, increasing to 15 l $week^{-1}$ for the next 5 weeks. Soil temperatures at a depth of 20 cm varied from 22–26°C at the start of the experiment to 16–19°C at the end.

Immediately after application of the chemical and 2, 4, 6, 8 and 10 weeks later, the tomato plants were removed and the soil around four replicate emitters was sampled. Soil was collected with an Oakfield tube (2 cm diam.) from nine points: 0, 10 and 20 cm from the emitter and at depths of 0–10, 10–20 and 20–30 cm. Eight additional samples of soil known to be free of nematicide were also collected and the 80 samples (two nematicide concentrations × nine sampling positions × four replicates + eight control samples) were bioassayed by the standard method.

The experiment was repeated the following summer, commencing in October 1985, but this time the plots were not covered. Soil temperatures at a depth of 20 cm varied from 15–20°C at the start of the experiment to 20–24°C at the end. Irrigation water (approximately 5 l $week^{-1}$) was applied through the emitters and a total of 190 mm rain fell at approximately regular intervals during the course of the experiment. An additional six emitter sites for each nematicide treatment were included to determine the effects of the treatments on galling caused by root-knot nematode. Before the nematicide treatments were applied and the tomato seedlings were planted, a cylinder of soil (approximately 60 cm diam. and 40 cm deep) was removed from under each emitter, chopped roots of tomato containing egg masses of *Meloidogyne javanica* were evenly mixed with it, and then the soil was replaced and consolidated. Plants were treated in the same manner as those in the rest of the experiment and roots were examined for galling after 12 weeks.

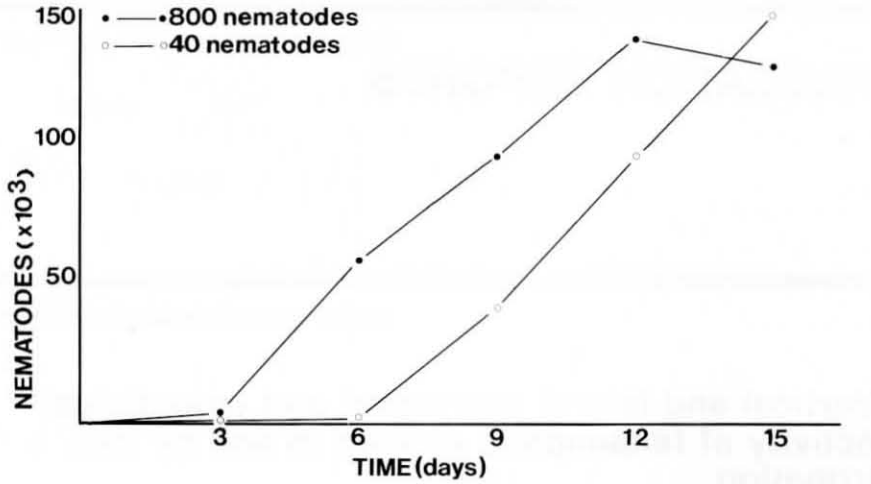

Figure 1 Effect of inoculum level and incubation time on population increase of *Aphelenchus avenae* on *Rhizoctonia solani*.

Table 1 Effect of fenamiphos concentration and autoclaving on the multiplication of *Aphelenchus avenae* on *Rhizoctonia solani*

μg fenamiphos g^{-1} soil	No. of nematodes (×10³) Not autoclaved	Autoclaved
10	0.3 (5.9)[A]	3.0 (8.0)[A]
1	7.2 (8.9)	16.6 (9.7)
0.1	197.6 (12.2)	246.0 (12.4)
0.01	196.7 (12.2)	178.1 (12.1)
0	140.5 (11.9)	211.0 (12.3)
L.S.D. ($P=0.05$)	(1.1)	

[A]Equivalent means with transformed means ($\log_e$ [number of nematodes + 1]) in parentheses.

Results

When PDA was mixed with nematicide-free soil, *R. solani* grew well enough to enable *A. avenae* to increase to more than 10^5 nematodes per plate in 10–15 days. The rate of population increase was similar on plates inoculated with both 40 and 800 nematodes, but at the lower inoculum density, nematode numbers per plate lagged by about 3 days (Figure 1). Concentrations of 0.1 and 0.01 μg fenamiphos g^{-1} had little effect on the multiplication of *A. avenae*, but multiplication was inhibited at concentrations of 1 and 10 μg g^{-1} (Table 1). Autoclaving soil containing fenamiphos did not appear to affect the activity of the

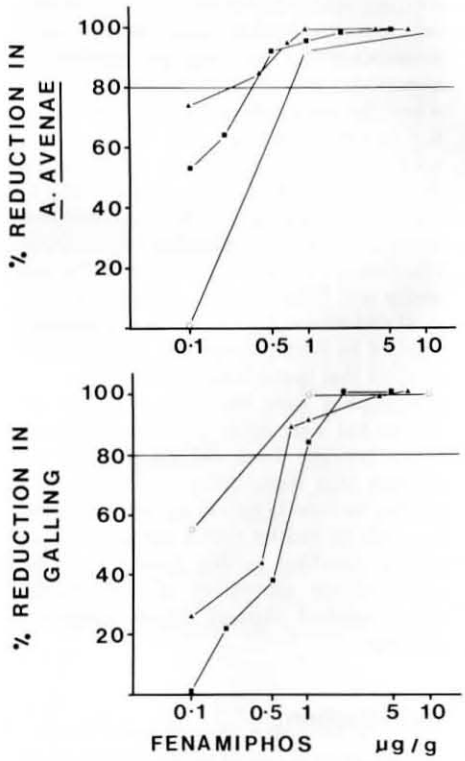

Figure 2 Reduction in number of *Aphelenchus avenae* or galls caused by *Meloidogyne javanica* as a percentage of the number of nematodes or galls in nematicide-free soil, as affected by fenamiphos concentration. ▲, ■ and o represent data from separate experiments.

nematicide markedly because there were significantly more nematodes in autoclaved soil than in non-autoclaved soil only at a concentration of 10 $\mu g\ g^{-1}$ (Table 1).

Experiments comparing the effect of fenamiphos on *A. avenae* and *M. javanica* showed that the two species were inhibited by similar concentrations of the nematicide. When the concentration of fenamiphos was greater than about 0.5 $\mu g\ g^{-1}$ soil, the number of *A. avenae* in bioassay plates and the number of galls induced by *M. javanica* on tomato was reduced by more than 80% (Figure 2).

When the bioassay was used to monitor the lateral and vertical movement of fenamiphos applied to the soil surface by trickle irrigation, data on the number of *A. avenae* multiplying in soil collected at several points distributed horizontally and vertically from the emitter was obtained. A factorial analysis of variance based on three levels of horizontal distance and three levels of vertical distance was carried out at weeks 0, 2, 4, 6, 8 and 10 using a natural logarithm (x + 1) transformation of the number of nematodes and is presented in Tables 2 and 3. In the two field experiments, the dominant effect was horizontal distance. Nematode numbers in soil collected beneath emitters generally were significantly lower than numbers at 10 cm and always significantly less than numbers at 20 cm. Vertical distance effects occurred mainly at early sampling times in experiment 1, when there were significantly fewer nematodes in soil collected near the surface than in soil from depths of 20–30 cm.

A diagram showing the sampling points in the field experiments where nematicidal activity was sufficient to reduce populations of *A. avenae* in the bioassay by 80–90% or 90–100% compared with nematicide-free soil, is presented in Figure 3. In both experiments, nematicidal concentrations occurred immediately under the emitter and laterally for distances of 10 cm, but were never obtained 20 cm from the emitter. Nematicidal activity persisted for 6–8 weeks in the first experiment and for at least 10 weeks in the second experiment, the zone of activity tending to be larger and to persist for a longer period at the higher of the two application rates. Observations of galling caused by *M. javanica* confirmed the poor lateral movement of fenamiphos. Roots beneath the emitters were free of

Table 2 The number of *Aphelenchus avenae* ($\log_e$ [number of nematodes + 1]) in bioassay plates containing soil collected every 2 weeks at various horizontal and vertical distances from drippers through which 1.25 or 0.625 ml fenamiphos was applied at week 0 (experiment 1)

	0.625 ml/dripper				*1.25 ml/dripper*			
Vertical distance (cm)	**Horizontal distance (cm)** 0	10	20	**Means (V)**	0	10	20	**Means (V)**
				Week 0				
0–10	5.02	7.35	8.14	6.84	4.76	6.53	8.52	6.60
10–20	5.05	8.23	9.31	7.53	5.03	8.60	9.05	7.56
20–30	7.95	9.21	8.28	8.48	6.60	8.88	8.89	8.12
Means (H)	6.01	8.26	8.58		5.46	8.00	8.82	
		LSD = 1.45^				LSD (H) = 0.84	LSD (V) = 0.84	
				Week 2				
0–10	5.67	5.27	10.15	7.03	4.18	3.49	10.17	5.95
10–20	5.75	9.03	9.63	8.14	4.99	5.22	10.18	6.80
20–30	8.07	9.51	9.71	9.10	6.85	8.16	9.86	8.29
Means (H)	6.50	7.94	9.83		5.34	5.62	10.07	
		LSD = 1.51				LSD = 0.99		
				Week 4				
0–10	6.65	6.46	9.66	7.59	5.06	4.62	9.50	6.39
10–20	2.03	7.04	9.28	6.12	2.22	4.46	9.55	5.41
20–30	8.38	8.88	8.89	8.72	3.52	6.95	9.30	6.59
Means (H)	5.69	7.46	9.28		3.60	5.35	9.45	
		LSD = 1.09				LSD (H) = 1.72		
				Week 6				
0–10	8.03	8.47	9.75	8.75	6.53	2.74	9.78	6.35
10–20	7.36	8.77	8.29	8.14	5.35	5.89	8.87	6.70
20–30	8.76	8.77	9.49	9.01	6.83	7.64	8.94	7.80
Means (H)	8.05	8.67	9.18		6.24	5.42	9.20	
		LSD (H) = 0.81				LSD = 2.22		
				Week 8				
0–10	8.88	8.83	8.59	8.77	6.51	6.77	8.68	7.32
10–20	6.62	8.92	8.85	8.13	5.39	7.06	9.53	7.33
20–30	8.12	9.01	9.11	8.75	7.33	8.62	8.67	8.21
Means (H)	7.88	8.92	8.85		6.41	7.49	8.96	
		LSD (H) = 0.73				LSD = 1.34		
				Week 10				
0–10	7.76	8.13	8.42	8.11	5.47	6.81	8.28	6.85
10–20	7.87	8.55	8.22	8.21	3.99	8.01	8.08	6.69
20–30	7.98	8.19	8.32	8.16	6.68	7.97	8.15	7.60
Means (H)	7.87	8.29	8.32		5.38	7.60	8.17	
		LSD (H) = 0.22				LSD = 0.89		

^When the interaction between horizontal and vertical distances was significant ($P = 0.05$), the LSD between each horizontal × vertical distance mean is given. Otherwise, LSD (H) ($P = 0.05$) refers to differences between horizontal distance means and LSD (V) ($P = 0.05$) refers to differences between vertical distance means.

galls, but roots that grew past a point some 15 cm laterally from the emitter were heavily galled.

Discussion

Although fenamiphos can be extracted from soil and analyzed by gas chromatography (Johnson *et al.* 1981), the preparation of samples for analysis is time consuming and the procedure requires access to a well-equipped laboratory. Also, such analytical techniques measure the concentration of the nematicide at a particular point in time and biological activity must be predicted by extrapolating from the results of field, glasshouse or laboratory studies with known nematicide concentrations.

Bioassays using *A. avenae* provide a simple and inexpensive means of determining whether nematicidal concentrations of fenamiphos are present in soil during the 12-day incubation period. Since *A. avenae* and *M. javanica* are affected by similar concentrations of fenamiphos, the *A. avenae* bioassay could be used to predict whether *M. javanica* was likely to invade roots and produce galls in a particular test soil. Our results suggested that in soils where multiplication of *A. avenae* was reduced by 80% compared with that in untreated soil, a similar reduction in galling caused by *M. javanica* could be expected. It is interesting that fenamiphos appears to affect *A. avenae* and *M. javanica* at much the same concentrations. Fenamiphos acts by impairing sensory behaviour and neuromuscular activity (Wright 1981) and consequently affects egg hatch, movement, invasion and feeding. These processes are common to the life cycles of both nematodes.

When the bioassay was used to monitor the movement and activity of fenamiphos in soil following its application through a trickle irrigation system, the nematicide was found to move adequately in a vertical but not in a horizontal direction. The wetted zone extended 40 cm laterally from the emitter but nematicidal concentrations were never achieved 20 cm from the emitter. The fact that tomato roots were free of galls caused by root-knot nematode only in a cylindrical zone with a radius of 15 cm beneath the emitter also indicated inadequate lateral movement of fenamiphos.

Table 3 The number of *Aphelenchus avenae* ($\log_e$ [number of nematodes + 1]) in bioassay plates containing soil collected every 2 weeks at various horizontal and vertical distances from drippers through which 1.25 or 0.625 ml fenamiphos was applied at week 0 (experiment 2)

	0.625 ml/dripper				*1.25 ml/dripper*			
Vertical distance (cm)	**Horizontal distance (cm)** 0	10	20	**Means (V)**	0	10	20	**Means (V)**
				Week 0				
0–10	4.23	7.69	8.30	6.74	4.68	8.83	8.29	7.27
10–20	3.93	7.74	8.90	6.86	3.83	7.84	9.03	6.90
20–30	4.50	7.27	8.96	6.91	4.66	7.24	9.09	7.00
Means (H)	4.22	7.57	8.72		4.39	7.97	8.80	
		LSD = 1.26^				LSD (H) = 0.97		
				Week 2				
0–10	4.94	7.14	8.17	6.75	4.54	7.45	8.15	6.71
10–20	4.61	6.17	8.88	6.55	3.53	5.42	6.10	5.02
20–30	4.34	5.11	8.89	6.11	4.86	5.81	8.41	6.36
Means (H)	4.63	6.14	8.65		4.31	6.22	7.55	
		LSD (H) = 1.12				LSD (H) = 1.43		
				Week 4				
0–10	2.98	7.70	9.58	6.76	4.12	8.01	8.93	7.02
10–20	4.28	7.04	9.12	6.81	3.96	5.44	9.70	6.37
20–30	5.52	5.53	8.50	6.52	3.06	6.18	8.46	5.90
Means (H)	4.26	6.76	9.07		3.72	6.54	9.03	
		LSD (H) = 1.71				LSD (H) = 1.19		
				Week 6				
0–10	3.39	8.75	8.91	7.02	4.45	8.20	8.59	7.08
10–20	2.76	8.03	7.53	6.11	4.22	7.84	8.62	6.90
20–30	2.45	6.76	9.74	6.32	3.44	5.45	9.38	6.09
Means (H)	2.86	7.85	8.72		4.03	7.16	8.86	
		LSD (H) = 3.0				LSD (H) = 1.28		
				Week 8				
0–10	3.16	8.65	8.49	6.77	1.67	8.41	7.46	5.85
10–20	4.07	7.89	8.76	6.91	4.43	7.71	8.99	7.04
20–30	4.25	7.27	9.41	6.98	4.83	4.21	7.47	5.50
Means (H)	3.83	7.93	8.89		3.64	6.78	7.97	
		LSD (H) = 0.81				LSD = 1.73		
				Week 10				
0–10	2.73	8.05	9.23	6.67	1.31	6.93	8.87	5.71
10–20	1.64	8.92	8.95	6.50	3.16	7.23	8.84	6.41
20–30	2.97	7.18	9.19	6.45	2.86	5.07	8.74	5.56
Means (H)	2.45	8.05	9.12		2.45	6.41	8.82	
		LSD (H) = 1.16				LSD (H) = 1.19		

^When the interaction between horizontal and vertical distances was significant ($P = 0.05$), the LSD between each horizontal × vertical distance mean is given. Otherwise, LSD (H) ($P = 0.05$) refers to differences between horizontal distance means.

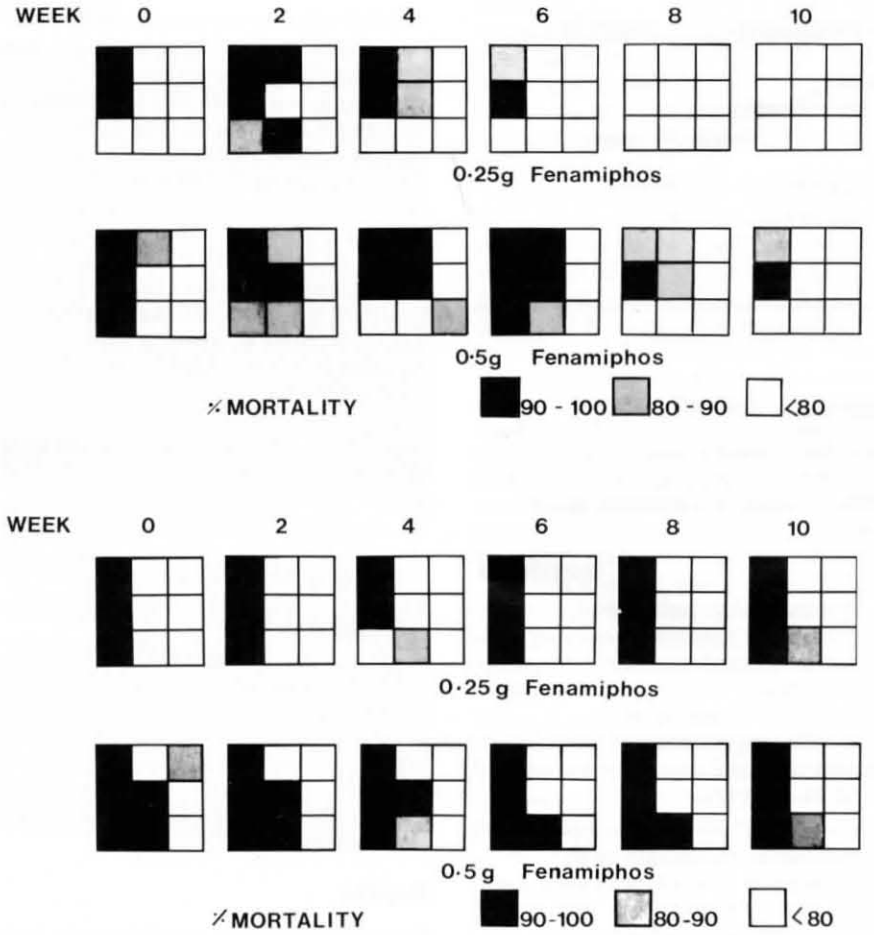

Figure 3 Vertical and lateral distribution of nematicidal activity, as determined by mortality of *Aphelenchus avenae* in bioassay plates, 0–10 weeks after 0.25 or 0.5 g fenamiphos was applied through emitters in two field experiments. (The left, middle and right-hand columns in each nine-squared box represent sampling points 0, 10 and 20 cm from an emitter, while the top, middle and bottom rows represent sampling points 0–10, 10–20 and 20–30 cm deep.) Data from experiment 1 (top) and experiment 2 (bottom).

Although this problem might be expected to limit the usefulness of trickle irrigation as a vehicle for applying fenamiphos to soil, yield increases have been obtained using this method of application (McKenry and Buzo 1984; Radewald *et al.* 1985).

Our results therefore raise questions about the proportion of the root system which must be protected from nematode attack in order to obtain a plant response. Also, previous assumptions that pesticides applied through trickle irrigation systems are evenly distributed throughout the wetted zone (Keng and Van der Gulik 1985; Keng *et al.* 1985) do not appear to be justified. It is essential that the distribution pattern of a nematicide is determined before the quantities needed to achieve particular concentrations in various parts of the wetted zone can be calculated. Such distribution patterns may differ in soils of different texture.

Fenamiphos applied by trickle irrigation persisted at nematicidal concentrations in soil for a shorter time in the first field experiment than the second, perhaps because higher soil temperatures at the time of application enhanced its rate of degradation. However, its period of effectiveness appeared to be similar to that observed in other soils where different methods of application had been employed (Homeyer 1971; Johnson *et al.* 1981). This suggests that in trickle irrigated perennial cropping systems, where several applications of nematicide are likely to be needed every year, fenamiphos need not be applied more than once every 6–8 weeks.

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