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BIOLOGICAL CONTROL OF LESION NEMATODE

PRATYLENCHUS PENETRANS (COBB)

A Thesis Presented

By

Vincent W. Saka

Submitted to the Graduate School of the

University of Massachusetts in partial fulfillment

of the requirements for the degree of

MASTER OF SCIENCE

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Plant Pathology

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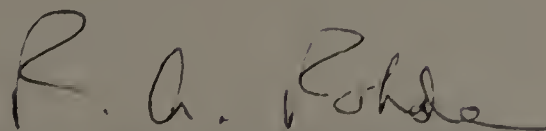
Approved as to style and control by:



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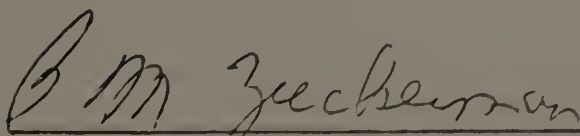

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ABSTRACT

Two batches of waste mycelium (1 and 2) produced in the manufacture of organic acids and antibiotics were added to sandy, loam soil in greenhouse pots and field plots at 50, 100 and 200 tons/acre. Significantly fewer *P. penetrans* were found in bean and tomato roots grown in soil containing high (200 tons/acre) rates of mycelium. The breakdown of mycelium in the field plots was accompanied by large numbers of free-living nematodes but would not support *P. penetrans* when they were added to the same soil. The antagonistic activity of mycelium to *P. penetrans* was observed one year after it was added to the field soil.

Six weeks after inoculation with 3000 *P. penetrans*, fewer nematodes were recovered from marigold than chrysanthemum, aster, pyrethrum and tomato. Feeding on marigold and aster ceased 12 hr after inoculation, and lesions on aster were darker than other hosts. Extracts from marigold consistently repelled *P. penetrans*. Repellent activity was found in three UV-fluorescent spots.

INTRODUCTION

Biological control is defined as "any condition under which or practice whereby survival or activity of a pathogen is reduced through the agency of any other living organism (except man himself) with the result that there is a reduction in incidence of the disease by the pathogen" (Garrett, 1965) (11).

According to this definition biological control agents would include the use of trap crops, resistant varieties, antagonistic plants that secrete exudates toxic to nematodes and organic ammendments. Decomposition of organic matter would increase natural enemies such as predaceous fungi and also lead to production of compounds which are toxic to nematodes. The use of these agents, however, is limited in that very few resistant varieties and antagonistic plants are available and they have relatively low cash value.

Linford et al, 1938 (21) observed a significant reduction in root-knot nematode populations when he added pineapple green manure to the soil. They attributed this to stimulation of natural enemies. Lear, 1959 (20) working with large quantities of castor pomace, was able to observe a reduction of population of three nematode species due to an unknown toxic substance produced by the pomace. A 95% reduction in number of galls/plant was obtained in lespedeza-hay-treated soil after 30 weeks of incubation and was assumed to be caused by an increase in both predaceous fungi and predaceous nematodes (16).

Johnson (1959) found that volatile fatty acids were toxic to plant-

parasitic nematodes. He reported that mixtures of formic, acetic, propionic and butyric acids produced by Clostridium butyric in culture media would effectively immobilize rice stylet nematode, Tylenchorhynchus martini (15). His results are consistent with those of Sayre et al who also found that extracts obtained from rye and timothy hay plant residues decomposing in soil produced butyric acid which at pH range of 4.0 to 5.3 was selectively toxic to Meloidogyne incognita and Pratylenchus penetrans (28,32).

Other workers have shown that addition of organic materials to the soil reduced the populations of nematodes such as Tylenchus semipenetrans, M. incognita and P. penetrans due to the production of toxic compounds and stimulation of natural enemies (21,23,29). Addition of oil cakes, sawdust in combination with urea, soybean meal and cellulose to the soil have been reported to reduce P. penetrans, H. tabacum and root-knot nematode. These organic materials are believed to produce nitrogen compounds which are toxic to nematodes (24,39).

Recently chitin amendments and industrial mycelial residues and wood fibers were observed to suppress populations of Tylenchorhynchus dubius and P. penetrans and some soil borne fungi such as Fusarium solani f. phaseoli and Fusarium oxysporum f. conglutinans. These materials are believed to release ammonia and stimulate chitinase producers such as actinomycetes which subsequently attack soil pathogens (23,25,41).

Most recent reports on the use of organic material amendments come from Sitaramaiah et al (1974) who used distillates from margosa cake

(Azadirachta indica) and sawdust against M. javanica. They observed 96-100% larval mortality and phenolic compounds being released into the soil during decomposition were implicated as toxic substances against the nematodes (33).

Biological control has also involved the use of trap crops which are heavily invaded by parasitic larvae which do reach maturity and the total number of nematodes in the soil is reduced. Studies by Morgan (1925), Triffit(1929) and Ellenby (1951) and other workers showed that root exudates from some crucifers and nightshade stimulated hatching of eggs of H. rostochiensis and the roots were invaded by large numbers of larvae, however, cyst production was negligible (8,27,36).

In a few cases plants have been found to be actually antagonistic toward nematodes and some have been shown to produce materials in their roots that are toxic to nematodes. Steiner (1941) observed that larvae of root knot enter the roots of marigold but failed to develop into adults (35). His results were confirmed by Uhlenbrook et al (1957) who showed that by growing Tagetes, Pratylenchus populations were reduced by 90% (42,12). Rohde (1957) discovered that the fleshly storage roots of asparagus produce a glycoside that is toxic to Trichodorus christiei (31). Van der Linde (1956) has shown that with several species occurring singly or in mixed populations can be remarkedly reduced by growing Eragrostis curvula or Crotalaria spectabilis which are toxic to nematodes (20).

Some plants have been observed to produce phenolic compounds when

infected by nematodes and other pathogens. These phenolic compounds appear to be the principal factor involved in root lesion formation around the region of nematode attack (10,30). Wallace (1961) studied the nature and chemistry of discoloration in chrysanthemum leaves infected by the foliar nematode A. ritzemabosi. He concluded that leaf browning was due to the hydrolysis of chlorogenic and isochlorogenic to caffeic acid and quinic acid which are subsequently oxidized to quinones and later polymerized to the brown end products (37).

The accumulation of phenolic compounds in infected root tissues as a result of injury caused P. penetrans has been reported by many workers. In some cases an assay of infected areas has revealed that peroxidase activity was five times higher than areas in healthy roots (1,3,13). Chang (1969) demonstrated that chlorogenic acid isolated from necrotic areas on alfalfa,tomato and carrots infected by P. penetrans was able to repel P. penetrans and also reduced the respiration rate of this nematode species (4). These results are consistent with those of Hung and Rohde (13) who found a high concentration of chlorogenic acid in the resistant Nemared tomato roots. They thought that varietal differences in amount of free chlorogenic acid in the uninfected roots might explain differences in resistance to injury by M. incognita acrita and P. penetrans in varieties Nemared, Hawaii 7153 and B-5 which they used (13).

Preliminary observations showed that P. penetrans populations are suppressed by marigold and aster although some lesions are formed. The nematode population is also reduced in mycelium-amended soil. It was,

therefore, decided (a) to investigate the role of phenols in marigold and asters as a mechanism of resistance and (b) to investigate the effects of industrial mycelial residues on _P_. _penetrans._

MATERIALS AND METHODS

Greenhouse studies

Two batches of waste mycelium (I and II) produced in the manufacture of organic acids and antibiotics by Chas. Pfizer Company, Groton, Connecticut were added to steam sterilized sandy loam soil in greenhouse pots at the rate of 50, 100 and 200 tons/acre and each treatment was replicated five times. Non-amended soil served as the control. The rates were chosen after higher rates in which 200 tons was the lowest rate proven to be toxic to the indicator plants, pinto beans (Phaseolus vulgaris L.). Each pot was then inoculated with 5000 P. penetrans axenically cultured on alfalfa variety Saranac callus growing in nutrient agar, a modification of the method developed by Krusberg (17). The pots were left for one week in the greenhouse to allow mycelial breakdown before pinto beans were planted in them.

Seven week-old seedlings were harvested and five grams of roots from each were washed clean of soil and subsequently cut and placed in 200 ml jars with 5 ml of tap water, and tightly capped (Young) (43). After 24 hr the nematodes were removed and counted.

The experiment was then repeated with unsterilized field soil naturally infected with P. penetrans. Only three rates of waste mycelium were applied to this soil: 0, 50 and 200 tons/acre. Two days later, two-week-old tomato plants (Lycopersicon esculentum var. Marglobe) were transferred into the pots and were left to grow for seven weeks. After 7 weeks the roots were processed using the procedure described above. For maxi-

mum recovery, nematodes were extracted from roots for 4 days but were counted daily with fresh water added each time. Nematodes were also extracted from the soil.

Field Studies

In addition to greenhouse studies the effects of applying the industrial mycelium were studied in field plots. Before the addition of mycelium, soil in each of the 32 treatment sites was analyzed using the Baermann method (Fig. 1). Fresh mycelial waste was spread on the surface at the rate of 200 tons/acre and disked in with a rotary tiller. The mycelium was allowed to decompose for two weeks after which time the treated area was again analyzed for *P. penetrans*.

After the decomposition period, tomato plants (variety Bonny Best) about 9 inches high were transplanted in treated (designated at chitin area Fig. 1) as well as untreated areas. Three weeks later both roots and soil from amended and unamended areas were analyzed for nematode populations. Root and soil sampling was carried on for approximately 3 months at 2-3 week intervals until the tomatoes were all harvested.

After 2 and 3 months, soil from amended and unamended areas was potted in the greenhouse and inoculated with 900 *P. penetrans* from callus tissue. After 6 weeks the soil was processed using the procedure described above and surviving *P. penetrans* and free-living nematodes were counted.

Longevity Studies: Laboratory Studies

In order to study the longevity of mycelial activity, soil from amended

Fig. 1. Diagram representing untreated and treated field plots. Each plot was 30 x 80 ft in the field and divided into 16 rows 5 ft apart. Each row had 20 Bonny Best tomato plants, 10 plants in treated area. Treated area received 200 tons industrial mycelial waste/acre.

UNTREATED

CHITIN TREATED

and unamended field plots was brought to the laboratory and examined for P. penetrans. Soil was placed in 250 ml test tubes at the rate of 20 grams/tube. Each treatment, amended and unamended, was replicated 7 times. Approximately 40 P. penetrans from a tobacco field were added to each tube, capped with a plastic cap, and incubated at room temperature. The tubes were kept moist for 3 weeks and then examined for P. penetrans using the Baermann method.

Greenhouse Studies

To further determine long-term activity, tobacco field soil containing high population of P. penetrans was directly mixed with equal amounts of both amended and unamended soils and kept moist in the greenhouse for 3 weeks. The pots were then brought to the laboratory and the nematodes were extracted using the Jenkins Sugar Flotation Method (14).

In order to determine whether the effects of the mycelial product was biological or chemical, both amended and unamended soil was steam sterilized for 1-1/2 hrs and then potted. Unsterilized soil from both treatments served as checks in this experiment. Each treatment was replicated 7 times and inoculated with 600 P. penetrans from axenic callus tissues. After 5 weeks the pots were analyzed as described above. In a similar experiment the number of P. penetrans was increased to 1000/ pot and survivors were analyzed 10 and 20 days after inoculation.

MATERIALS AND METHODS, PART II

Greenhouse Studies

In order to study the development of P. penetrans in suitable and non-suitable hosts, two week old plants of marigold (Tagetes patula var. mixed color and Tall African), China aster (Callistephus chinensis), pyrethrum (Chrysanthemum coccineum), tomato (Lycopersicon esculentum var. Marglobe), and summer chrysanthemum (Chysanthemum maximum) were inoculated with 3000 P. penetrans from alfalfa callus tissue.

After 6 weeks the plants were harvested and 3 grams of roots per plant were washed free of soil and incubated in 200 ml jars with 5 ml tap water. The nematodes from roots were counted every 24 hrs for a period of 4 days.

The experiment was repeated using the same procedure, however, instead of counting nematodes, the roots were examined for visible lesions under a dissecting microscope (Fig. 2).

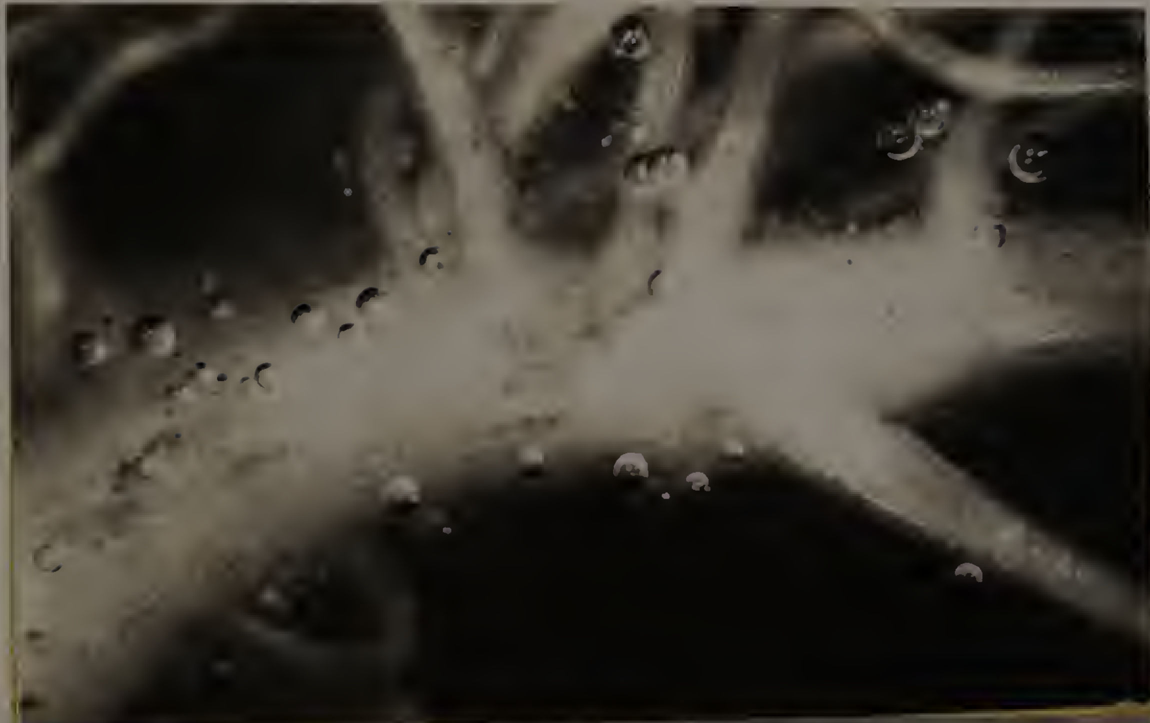
Feeding Observations

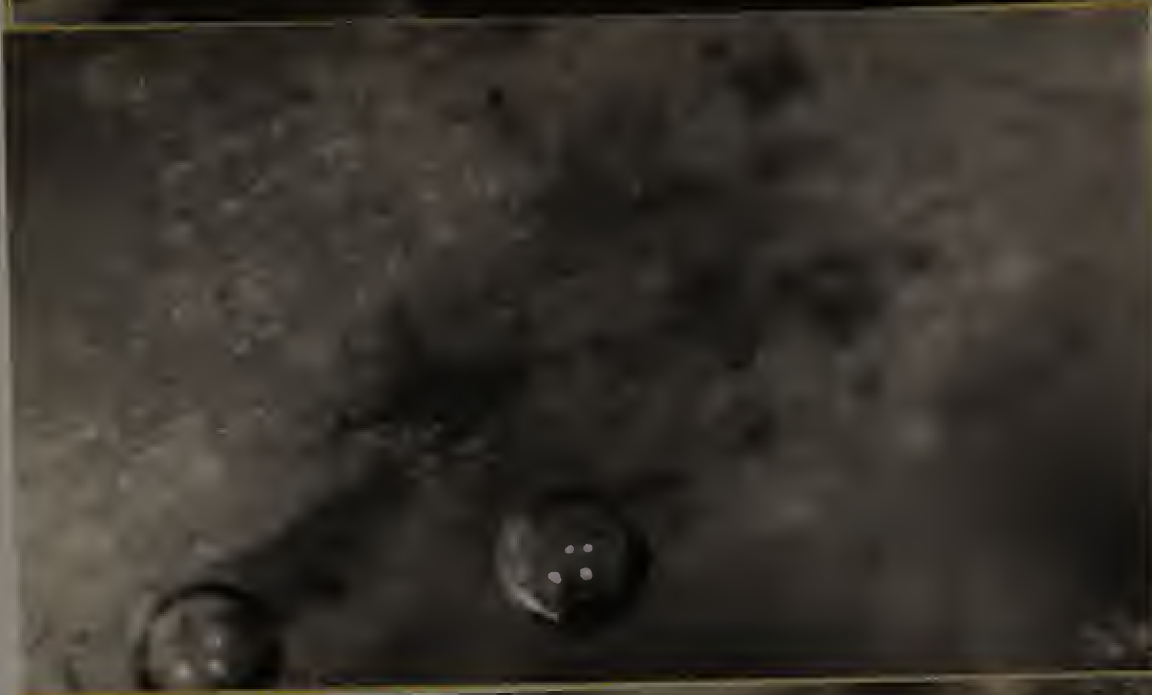
Seeds were sterilized in 2% sodium hypochlorite for 20 minutes and rinsed several times with sterilized distilled water. The seeds were germinated on 1% water agar in petri dishes. After 4 days the seedlings were aseptically transferred to 1% water agar slants and inoculated with approximately 60 P. penetrans. Feeding was observed every 6 hrs.

Nematicidal Activity

Ten grams fresh weight of roots and shoots from marigold and asters growing in the greenhouse were separately chopped up and placed in 100%

Fig. 2. Lesions caused by P. *penetrans* on marigold (A & B) and tomato (C) roots.


A


B

C

boiling methanol to extract phenols. The extracts were filtered through Whatman filter paper, #1 and taken to dryness in a rotary evaporator under reduced pressure at 50°. The residues were dissolved in 5 ml of distilled water and stored at -5° to -10° before they were tested for toxicity against P. penetrans.

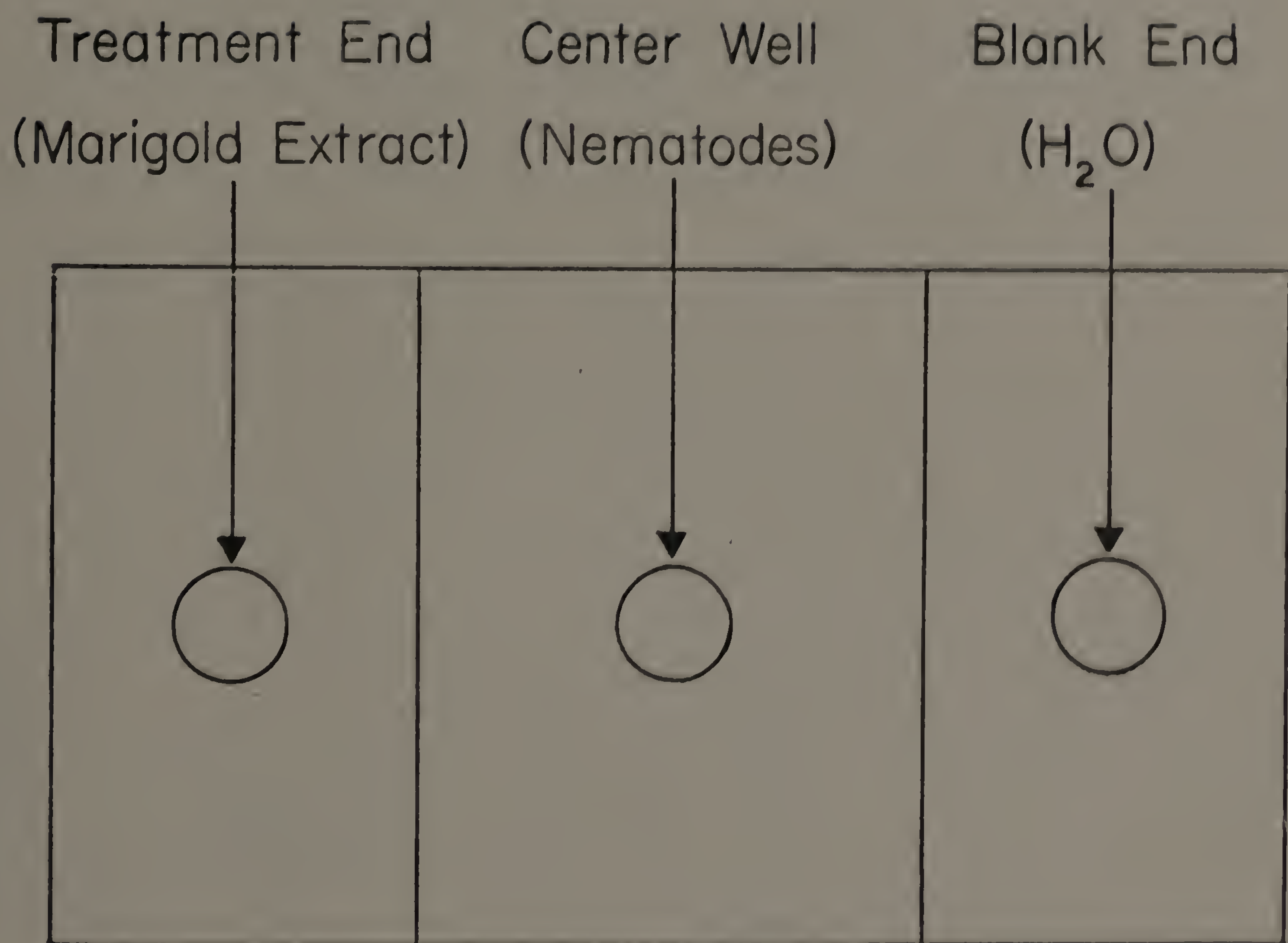
In order to test for toxicity a 1:1 and 1:10 dilution with distilled water was made from each stem and root extract. Distilled water served as the control. The dilutions plus the undiluted extract were placed in separate watch glasses. Thirteen active nematodes were added to each watch glass and observed frequently at 3 hour intervals. A nematode was considered dead if it did not move when touched with a needle.

Repellent Activity

Extracts were then tested for repellent action against P. penetrans. The method used was a slight modification from the one used by Lavallee and Rohde (18) and Chang (4). Polystyrene chambers (46 x 22 x 5 mm) marked on the undersurface into 14 mm end sections and an 18 mm center were filled with 1% water agar to the depth of about 5 mm. Using a number 3 cork borer a well was dug on each end section of the chamber after gelation and then sealed at the bottom with a drop of precooled 1% water agar.

After gelation one well on each chamber was filled with either marigold or aster extract and the other well on the opposite end was filled with distilled water as a check (Fig. 3). These treatments were replicated 6 times. The chambers were incubated for 24 hrs and then another well

Fig. 3. Movement of *P. penetrans* in agar in presence of marigold extract. Nematodes moved away from the center well toward H_2O one hour after inoculation. Actual dimensions of the chambers were 46 x 22 x 5 mm.


Treatment End
(Marigold Extract)
Center Well
(Nematodes)
Blank End
(H_2O)

was made in the center section and similarly sealed at the bottom. Approximately 60 P. penetrans from alfalfa callus tissues were pipetted to the center well with a Pasteur capillary pipette.

Nematodes were extracted by placing alfalfa callus tissues on a cotton plug in the upper part of a 15 ml centrifuge tube containing enough water to moisten the cotton (4,18). The tubes were then capped with aluminum foil and incubated for 24 hrs.

After addition of nematodes to the center well, excess water was removed with a pipette and the center well was filled with cool, but ungelled 1% water agar. Observations were made every 4 hrs after inoculation to see how many nematodes migrated to each end section.

Another experiment was done using only marigold extracts with 8 replicates and readings were taken every hour after the addition of the nematodes.

Biological Studies

A greenhouse study was undertaken to study the biology of P. penetrans in marigold. In one series of experiments, two-week-old marigolds were inoculated with 300 P. penetrans per plant. Tomatoes (var. Rutgers) were used for comparison. Each treatment was replicated 6 times. The plants were harvested after 5 weeks and 5 g of roots were incubated in 200 ml jars as described before. The nematodes were removed and counted. In another series of experiments the nematodes were increased to 1000 per plant. Half of the plants were examined 13 days after inoculation and the remaining plants were harvested 26 days later.

RESULTS AND DISCUSSION

Waste Mycelium

Waste mycelial residues significantly reduced the number of P. penetrans in bean and tomato roots grown in pots as shown in Tables 1 and 2. The effectiveness was particularly noticed in high rates. These results are in agreement with those of Miller et al, 1973 (24) who used material from the same source.

In the field soil, breakdown of the mycelia was accompanied by significantly larger numbers of free-living nematodes whereas P. penetrans populations decreased in the same soil (Table 3). Mankau et al (1969) observed an increase in mycophagous nematode populations when they amended the soil with cellulose, starch, chitin and dextrose (23, 25). These results indicate that one of the effects of mycelial residue breakdown may be stimulation of free-living nematodes which compete against P. penetrans. Toxin production may also be involved in the suppression of P. penetrans although its apparent effects were not observed.

Chitin when added to the soil has been shown to increase bacteria and actinomycetes. This was demonstrated by Dugan, 1973 (7,26) when he applied lobster shell to the soil; he noticed a decrease in Fusarium root rot of cucumbers and Rhizoctonia damping-off of cotton. He suggested that the lobster shell may preferentially favor chitinase producers notably actinomycetes, which presumably attack Fusarium. Miller et al (24) observed a reduction in Fusarium oxysporum f. sp. lycopersici

possibly due to stimulation of chitinase producers. Chitin is probably a general constituent of the egg-shell throughout the phylum, Nematoda (5). It is possible that the mycelial residues stimulates chitinase producers which are detrimental to P. penetrans eggs.

Organic nitrogen compounds have been shown to reduce P. penetrans in the soil (9,39,40). It is believed that breakdown of nitrogen containing substances in the soil via ammonification and nitrification results in production of ammonia, ammonium ions and subsequently nitrites and nitrates which would be operative against nematodes. Ammonia has been reported to be produced when the mycelial residues, containing 2% N, break down and its effect on plant parasitic nematodes has been demonstrated by a number of workers (9,24,41). It is, therefore, possible that ammonia may be playing a role in the suppression of P. penetrans (Table 4).

These mycelial residues are a promising agent of biological control not only as substrates that selectively stimulate microbial populations that compete with pathogens but because of their long lasting activity. Their activity was evident 12 months after application (Tables 4 and 5).

Resistant Plants

Six weeks after inoculation with 3000 P. penetrans, fewer nematodes were found in marigold roots than in tomato, aster, chrysanthemum and pyrethrum (Table 6), suggesting that marigold is not a host for P. penetrans. Nematodes ceased to feed on marigold and aster 12 hrs after inoculation (Table 7). Aster had larger and browner lesions than the other hosts.

Nematodes recovered from marigold roots did not differ from those recovered from tomato roots when they were examined and measured under light microscope.

Freshly expressed juice of both roots and shoots of marigold showed no toxicity when nematodes were added to it in watch glasses, suggesting that failure of nematodes to survive in marigold was due to a different mechanism.

The juices were also tested for repellent activity. Preliminary observations showed that more nematodes initially moved away from marigold extracts than from aster and water. *Pratylenchus penetrans* placed near drops of marigold extracts consistently moved away as indicated in Tables 9 and 10. Since the nematodes either stayed in one place or else moved equally to both sides of control chambers, movement of nematodes away from marigold extracts and toward water was evidence that they were actively repelled.

The extracts were separated chromatographically on thin layer chromatograms using BAW (6:1:2). The chromatograms were divided into 7 equal parts and eluted in water subsequent to testing for repellent activity. Three spots (Table 10) indicated some activity and these correspond to the spots which were fluorescent under the UV light.

Representative chromatograms were sprayed with 0.4% isatin in concentrated sulfuric acid to detect the presence of α -terthienyl compounds which have been previously reported to be present in marigold (2,6,12,42). Repellent spots did not give positive test for these compounds.

The nematodes were seen moving away from marigold extracts 1 hr after they were added to the center well. The results were similar to reports of Chang (1969) who found that lesion nematodes were repelled and their respiration was significantly reduced by oxidation products of chlorogenic acid (4). These results suggest that phenolic compounds are involved in marigold resistance against _P_. _penetrans_.

Phenolic compounds have been associated with nematode injury, including browning of plant tissues but their role both in resistant and susceptible hosts is not clearly understood (1,3,4,13). However, from these results, it appears that they may play an active part in denying or preventing endoparasitic nematodes like _P_. _penetrans_ from entering the roots.

Fig. 4. One dimensional thin layer chromatogram indicating three UV fluorescent spots in which repellent activity was found from the origin.

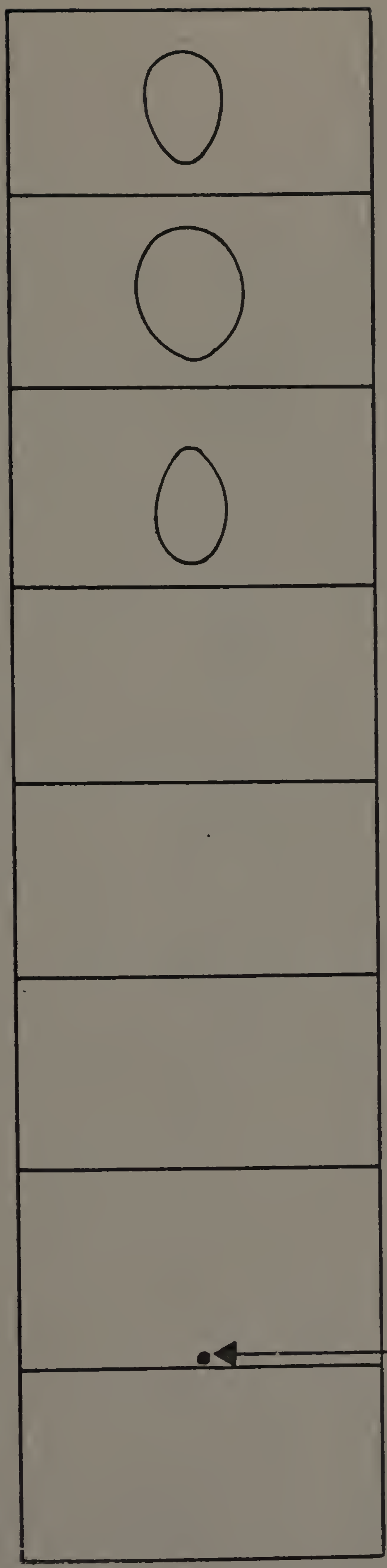

ORIGIN

Table 1. Numbers of P. penetrans recovered from bean roots growing in mycelium-amended soil in greenhouse pots 7 weeks after inoculation with 5000 nematodes. High, medium and low were 200, 100 and 50 tons/acre.

	Mycelium I[a]			Mycelium II			Control
	H	M	L	H	M	L	
	20	10	25	5	5	5	25
	0	0	50	10	20	55	50
	0	5	30	5	15	30	90
	0	5	5	0	15	10	15
Total	20	20	110	20	55	90	180
mean	5**	5**	27.25	5*	13.5*	22.5*	45

a = There was no significant difference between mycelia I & II.
** = Highly significant at 1% level.
* = Significant at 5% level.

Table 2. Numbers of _P_. _penetrans_ on tomato roots growing in mycelium-amended soil in greenhouse pots 7 weeks after inoculation with 5000 nematodes. High and low were 200 and 50 tons/acre.

	Mycelium I		Mycelium II		Control
	H	L	H	L	
	3	9	7	59	59
	4	31	22	51	102
	14	25	25	21	91
	11	21	16	31	121
	15	30	26	31	114
Total	47	116	96	199	487
mean	9.4**	23.4*	19.2	40.6	97.4

** = Highly significant at 1% level.
* = Significant at 5% level.

Table 3. Effects of mycelial residues on populations of P. penetrans and free-living nematodes in soil amended 3 months previously with 200 tons of waste mycelium per acre. 900 P. penetrans were added to each pot.

	Amended soil		Unamended soil	
	P. penetrans	Free-living	P. penetrans	Free-living
	0	2680	50	130
	0	2680	190	330
	0	3520	255	380
	0	3300	50	220
	5	2820	20	190
Total	5	15010	565	1250
mean	1	3000	113	250

Table 4. Survival of *P. penetrans* in soil amended 12 months previously with 200 tons waste mycelium per acre. Each test tube was inoculated with 50-60 nematodes.

	Amended soil	Unamended soil
	1	6
	3	4
	1	5
	3	5
	0	4
	0	6
	0	4
	0	3
	0	4
Total	8	41
mean	.888	4.44

Table 5. Comparison was made between amended unsterilized soil and amended sterilized soil. The soil was steam sterilized for one and one half hours and each pot was inoculated with 1000 P. penetrans. Counts were made after 10 and 20 days.

Day		Amended		Unamended	
		Unsterilized	Sterilized	Unsterilized	Sterilized
10		21	33	39	27
		16	57	45	39
		6	33	81	39
	Total	43	123	165	105
20		29	31	80	117
		23	53	53	52
		28	34	57	74
		25	69	57	52
	Total	105	187	247	295
Total		150	310	412	400
mean		21.25*	44.29	58.86	57.14

* = Significant at 5% level.

Table 6. Suitability of various host plants for survival of P. penetrans. Nematodes per 3 g roots 6 weeks after inoculation with 3000 nematodes per plant.

Replicates	Treatments				
	Chrysanthemum	Tomato	Pyrethrum	Aster	Marigold
1	125	149	10	62	0
2	101	84	19	48	0
3	115	87	28	35	0
4	63	23	85	20	0
5	82	105	2	31	4
Total	486	448	114	196	4
mean	97.2	89.6	22.8	39.2	0.8

Table 7. Feeding of P. penetrans on chrysanthemum, tomato, pyrethrum, aster and marigold.

Hours	Chry.	Tom.	Pyr.	Aster	Marigold
6	++	++	++	+	+
12	++	++	++	+	+
18	++	++	++	0	0
24	++	++	+	0	0

+ = Very few nematodes were seen feeding on these plants.
++ = Large numbers were feeding.
0 = No nematodes were seen around the roots.

Table 8. Movement of *P. penetrans* away from marigold root extracts 3 hrs. after 60 nematodes were added to each chamber.

	Treatments	
	Marigold root extracts	H_2O
	0	9
	0	6
	0	12
	0	2
	0	2
	0	6
	0	7
	0	14
	0	18
	0	20
Total	0	96
mean		9.6**

** = Highly significant at 1% level.

Table 9. Movement of *P. penetrans* in migration chambers as influenced by marigold root and stem extracts 3 hours after 60 nematodes were added to each chamber.

	Treatment					
	Root	H_2O	Stem	H_2O	Distilled H_2O (control) A	Distilled H_2O (control) B
	1	7	0	17	1	2
	0	8	0	20	0	0
	0	7	0	6	0	0
	0	8	0	20	1	2
	0	2	0	22	0	0
	0	8	0	11	0	0
	0	17	0	18	0	0
	1	18	0	18	1	1
Total[a]	2	73	0	130	3	4
mean	0.062*	9.125	0**	16.25	0.375	0.5

** = Highly significant at 1% level.

Table 10. Repellent activity of eluates from marigold extracts on thin layer chromatograms (Phosphocellulose) where BAW (Butanol acetic acid and water) was used as a solvent. Observations were taken at 3 hrs.

Eluates	Rf	Root Extracts(a)		Stem Extracts	
		Extracts	H_2O	Extracts	H_2O
1	0-.14	0	0	1	2
2	.14-.28	0	0	0	0
3	.28-.43	0	0	1	1
4	.43-.57	0	0	0	0
5	.57-.70	8	30(b)	3	29(b)
6	.70-.85	7	27(b)	10	30(b)
7	.85-1.00	7	27(b)	6	35(b)

a = Numbers of nematodes found in the extracts and H_2O. Each number is a total of six replicates.

b = Repellent activity was recovered in these units which correspond to fluorescent spots under UV light.

SUMMARY

Waste mycelium produced in the manufacture of organic acids and antibiotics was added to soil in greenhouse pots at the rate of 0, 50, 100 and 200/acre. Each treatment was replicated five times and inoculated with 5000 P. penetrans. The pots were then incubated for one week before pinto beans and tomatoes were planted in them.

Seven weeks later the seedlings were harvested and five grams of roots from each plant were incubated and nematodes were counted. Significantly fewer P. penetrans were found in the roots grown in mycelium-amended soil when compared with the control.

The mycelium was also tested in large field plots where it was added at the rate of 200 tons/acre. Before the addition of mycelium, the soil was analyzed for P. penetrans using the Baermann funnel method. Bonny Best variety tomatoes were used as indicator plants. Root and soil sampling was carried out for approximately 3 months. The treated soil developed abnormally large numbers of free-living nematodes but would not support P. penetrans.

The soil from the treated field was tested one year later both in the laboratory and greenhouse. The soil was placed in 250 ml test tubes and about 40 P. penetrans were added to each tube and incubated for 3 weeks. Very few nematodes were recovered from the tubes with treated soil. The experiment was repeated in the greenhouse with similar results.

The amended soil was then steam sterilized to see whether the effect was biological or chemical. Approximately 1000 nematodes were added

to each pot (7 replicates for each treatment). There was no significant difference between the sterilized and unsterilized amended soil suggesting that the effect of mycelium is possibly a combination of both microbial competition and chemical toxicity.

The host-parasite interaction in marigold, asters, pyrethrum, tomato and chrysanthemum was studied by inoculating greenhouse grown plants with 3000 P. penetrans. After 6 weeks the plants were harvested and lesion nematodes in 3 grams of roots per plant were recovered. Marigold had the lowest number of nematodes compared to intermediate hosts, pyrethrum and chrysanthemum and the good host, tomato.

In order to observe the length of time nematodes fed on hosts, seedlings were placed on 1% water agar in petri dishes and inoculated with about 60 specimens of P. penetrans. Feeding was observed every 6 hrs. The worms stopped feeding on marigold 12 hrs after inoculation whereas they fed on tomato for 24 hrs.

Marigold roots and tops were placed in 100% boiling methanol. The extracts were filtered, concentrated in a rotary evaporator, dissolved in 5 ml of distilled water, and kept at -5 to -10 C. The extracts were then placed in a watch glass and 13 nematodes were added to each drop. After 3 hrs all nematodes were found living.

The extracts were tested for repellent activity in polystyrene chambers (46 x 22 x 5 mm), marked on the undersurface into 14 mm end sections and 18 center sections were filled with water agar. Using a number 3 cork borer, a well was dug on each end section of the chamber after

gelation and then sealed with a drop of precooled 1% water agar. One well was filled with marigold extracts and the other one with distilled water. Twenty hours later a well was dug in the center and was similarly sealed at the bottom.

Approximately 60 active nematodes were added to the center well with minimum water and then sealed. Nematodes moved away from marigold extracts toward water 1 hr after inoculation.

The extract was separated on thin layer chromatograms using BAW (6:1:2) as solvent. The chromatograms were cut into sections and eluates were again tested for repellent activity and other chromatograms were sprayed with specific phenolic compound reagents. The fluorescent spots repelled the nematodes.

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APPENDIX

The analysis of variance as presented by Steel and Torrie was used to test differences between treatments and mycelia (34). Data F values were compared with those of Steel and Torrie. Orthogonal polynomial was used for comparison between treatments. To clarify the use of this method, the following example is given:

Table 1.

Mycelium I			Mycelium II			Control
H	M	L	H	M	L	
20	10	25	5	5	5	25
0	0	50	10	20	55	50
0	5	30	5	15	30	90
0	5	5	0	15	10	15
20	20	110	20	55	90	180

Total Sum Ei Ej Yij = 495

Total Uncorrected SS $E_i \; E_j \; Y_{ij}^2 = 21025$

Total Sum SS = $21025 \frac{(495)^2}{28}$

$= 21025 - 8750.8928 = 12750.1072$

Treatment SS $- \frac{(20)^2 + (20)^2 + (110)^2 + (20)^2 + (SS)^2 + (90)^2 + (180)^2 - C}{4}$

$14206.25 - 8750.8928 = 5455.358$

Within SS = Total ss - trt ss

$= 12750.1072 - 5455.358 = 7494.749$

Analysis of Variance

Source	df	ss	ms	F
Total	27	12750.1072	472.2262	1.3793
Treatment	6	5455.358	909.2233	2.6556 **
Within	21	7294.749	342.369	

Partition of SS among levels.

$$\text{Levels}_{ss} = \frac{(T_h)^2}{r_h} + \frac{(T_m)^2}{r_m} + \frac{(T_l)^2}{r_l} - \frac{(T_h + T_m + T_l)^2}{r_h\ r_m\ r_l}$$

$$L_{ss} = \frac{(40)^2 + (75)^2 + (200)^2}{8} - \frac{(315)^2}{24}$$

$$L_{ss} = 5903.1250 - 4134.3750 = 1768.7500$$

$$\text{Mycelia SS} = \frac{(150)^2 + (165)^2}{12} - \frac{(315)^2}{24}$$

$$4143.7500 - 4134.3750 = 9.3750$$

$$\text{Interaction SS} = \frac{(20)^2 + (20)^2 + (110)^2 + (SS)^2 + (20)^2 + (90)^2}{4} - M - 1 + (1)$$

$$6106.25 - 4143.7500 + 4134.375 + 4134.375 = 193.75$$

Comparison between control vs. treatment

$Q_1 = -1\ (T_h + T_m + T_1 + T_h + T_m + T_l) + 6\ (Tc)$

$Q_1 = (-315 + 1080)$

$D_1 = 4\ (42)$

$$\frac{Q_1^2}{D_1} = \frac{(765)^2}{168} = 3483.4821$$

Analysis of Variance

Source	df	SS	Ms	F
Treatment	6	5455.358	909.2333	2.61 *
Levels	2	1768.7500	844.375	2.5459 *
Mycelia	1	9.3750	9.3750	0.02698
M/L	2	193.7500	96.875	0.2788
Control vs Trt	1	3483.4821	3483.4821	10.0281 *
Within	21	7294.749	347.369	