Biological Control of *Meloidogyne javanica* with *Bacillus penetrans*

G. R. Stirling

Senior research officer (Nematology), Department of Agriculture, Loxton, S.A., 5333, Australia. Present address: Department of Primary Industries, Plant Pathology Branch, Meiers Road, Indooroopilly, Queensland, 4068, Australia.
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ABSTRACT

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Tomato roots containing females of the root-knot nematode, *Meloidogyne javanica*, infected with *Bacillus penetrans* were air-dried and finely ground to produce a powdery material heavily laden with spores of *B. penetrans*. When this material was incorporated into root-knot-nematode-infested field soil at rates of 212–600 mg per kilogram of soil, galling of tomato roots and the number of nematodes in the soil at harvest was reduced significantly. Nematode control was similar to that usually obtained with nematicides. Before the tomatoes were planted, treated soils were bioassayed for *B. penetrans* by adding juveniles of *M. javanica* and counting the number of juveniles encumbered with spores 24 hr later. Control was obtained in the field when at least 80% of the bioassayed juveniles were encumbered with 10 or more spores per nematode. Laboratory experiments showed that spore-encumbered juveniles were less able to invade roots than were unencumbered juveniles. Numbers penetrating roots decreased with increasing spore concentration and as the distance juveniles moved in soil increased. Spores of *B. penetrans* did not always germinate after adhering to juveniles and more than five spores were required per nematode to ensure infection. In pot experiments with grapes, there were significantly fewer root-knot nematodes in vineyard soil infested with *B. penetrans* than in similar soil without the pathogen, suggesting that *B. penetrans* increased naturally in vineyards to levels sufficient to reduce root-knot nematode populations.

The organism first described by Thorne (14) as *Duboscqia penetrans* and since named *Bacillus penetrans* (4), is an obligate pathogen of some plant-parasitic nematodes. Spores of populations which attack root-knot nematodes (*Meloidogyne* spp.) attach to the cuticle of second-stage juveniles in soil and germinate after the spore-encumbered juveniles enter roots and initiate feeding. A germ tube penetrates the cuticle and the pathogen then proliferates through the body of the developing nematode. Consequently, diseased nematodes do not reproduce and at maturity are filled with spores of the pathogen (10).

B. penetrans has the attributes of a successful biological control agent against root-knot nematodes (5,9), but its field potential has not been confirmed because of the difficulties encountered in

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culturing it in large quantities. Data on its efficacy is limited to a single greenhouse test (4). The pathogen is generally considered to act by preventing females from reproducing (5,9), but the number of spores required to ensure infection is not known. Although spore-encumbered juveniles are less motile than healthy juveniles (7), it is not known how many spores must attach before motility is affected or whether the infectivity of spore-encumbered juveniles is reduced. Without such information, the complex relationships between nematode movement, spore concentration in soil, nematode infectivity, and disease development cannot be understood.

This work aimed at assessing the potential of *B. penetrans* for the control of *Meloidogyne*, determining the mode of action of the pathogen, and improving our understanding of the factors affecting its efficacy as a biological control agent.

MATERIALS AND METHODS

Preparations of *B. penetrans* were produced by grinding air-dried tomato roots infested with diseased females of *Meloidogyne* (12). The potency of these preparations was compared by diluting each of them serially in water and determining the concentration (IC_{50}) at which 50% of the juveniles of *M. javanica* added were infested with spores 22 hr later (12). Second-stage juveniles of *M. javanica* with spores of *B. penetrans* attached were obtained by agitating nematodes in spore suspensions prepared by grinding dried root preparations in water with a mortar and pestle and then removing root debris by pouring the slurry through a 25-μm sieve.

A bioassay procedure was used to estimate the concentration of *B. penetrans* in soil. Two thousand second-stage juveniles of *M. javanica* in 2 ml of water were added to 25-g samples of air-dried soil. Nematodes were extracted 24 hr later by using a sieving and centrifugation technique modified from Caveness and Jensen (1), and the proportion of juveniles with spores attached was estimated in samples of 60–100 nematodes.

Roots were indexed for root-knot nematode damage as follows: 0, no galls; 1, 1–25% of the roots galled; 2, 26–50%; 3, 51–75%; 4, 76–99%; and 5, all the roots galled and rotting. Nematodes were extracted from roots in a mist chamber (11) or by incubation in plastic bags for 5 days (15). The number of juveniles of *Meloidogyne* in soil was determined by processing 200-g samples by a combination of Cobb's decanting and sieving method (2) and the Baermann funnel technique (11). Healthy females of *Meloidogyne* were identified by the presence of egg masses, diseased and immature females by the absence of egg masses. Samples of females without egg masses were checked for *B. penetrans* to obtain the proportion of immature and diseased nematodes; the total number of healthy and diseased females was then estimated. In experiments involving only one nematode generation, plants were harvested 5–6 wk after inoculation when healthy females had produced egg masses but few second generation juveniles had penetrated roots.

Addition of *B. penetrans* to tomato plants in pots. Sixty-four 1.5-L pots were filled with either sterile sand or with sand containing a preparation of *B. penetrans* (IC_{50} = 7 mg/L) at concentrations of 100, 200, or 400 mg per kilogram of dry sand. Half the pots were used to study the effect of *B. penetrans* on the infectivity of juveniles of *M. javanica*. A seedling of tomato (cultivar Q3) was planted in eight replicate pots of each soil and 1 wk later 1,000 juveniles were added to each pot. After 6 wk in a glasshouse, healthy and diseased females in roots were counted. The other half of the pots were used to examine the effect of *B. penetrans* on nematode population increase in roots already infected with healthy *M. javanica*. Tomato seedlings (cultivar Q3) growing in sand and inoculated 2 days previously with 1,000 juveniles of *M. javanica* were washed free of sand and transplanted individually to eight replicate pots containing 0, 100, 200, or 400 mg of the preparation of *B. penetrans* per kilogram of dry soil. Plants were harvested after 10 wk in a glasshouse and roots separated into those present at the time of transplanting (primary roots) and those produced later (secondary roots). Diseased and healthy females in secondary roots were counted. Also, juveniles were recovered from both primary and secondary roots in a mist chamber and counted.

Addition of *B. penetrans* to tomato plants in the field. Two experiments were established in fields heavily infested with root-knot nematodes to determine whether the nematode could be controlled by the addition of *B. penetrans*. At site 1, tomato (cultivar Q3) was grown under drip irrigation in a sandy soil. Five treatments (a dried-root preparation of *B. penetrans* [IC_{50} = 2.5 mg/L] at rates of 106, 212, and 424 mg per kilogram of dry soil, dried roots without *B. penetrans* [424 mg/kg], and an untreated control) were established on eight replicate plants in a randomized block design. At site 2, tomato (cultivar Burnley Gem) was grown under sprinkler irrigation in a sandy-loam soil. Treatments consisted of dried roots containing *B. penetrans* (IC_{50} = 18 mg/L) at 300 and 600 mg per kilogram of dry soil, dried roots without *B. penetrans* at 300 and 600 mg per kilogram of dry soil, granules containing 100 g of the nematicide fenamiphos per kilogram applied at a rate of 1 g active ingredient per square meter at planting (wk 0), fenamiphos (1 g/m^2) at 0, 4, and 8 wk, and an untreated control. The seven treatments were set out in a randomized block design with five single-plant replicates of each treatment.

At both sites, tomatoes were planted in the center of 50-cm-diameter plots with plants spaced 1.2 m × 1.2 m apart. *B. penetrans* was incorporated into soil 1 wk prior to planting by mixing the appropriate quantity of a dried root preparation with 120 kg of soil. The soil was removed from a cylindrical hole approximately 50 cm in diameter and 40 cm deep, mixed with the dried root material in a concrete mixer, and then returned to the hole. Granules containing fenamiphos were sprinkled on the surface of plots 50 cm in diameter and incorporated to a depth of 5 cm. At planting, soil cores were collected with a 2-cm-diameter Oakfield tube from plots treated with dried root preparations. The soil was air-dried and a 25-g subsample was bioassayed for *B. penetrans*. Yield and the dry weight of tops was measured at harvest and roots were indexed for root-knot nematode damage. Four soil cores were collected from each plot at depths of 0–30 cm, nematodes were extracted from a 200-g subsample and counted.

Naturally occurring *B. penetrans* on grape. In a preliminary laboratory experiment, DBCP (1,2-dibromo-3-chloropropane, Fumazone®), 1,3-D (1,3-dichloropropane, Telone II®), EDB (ethylene dibromide, Nemadi®), or dazomet (tetrahydro-3,5-dimethyl-2*H*-1,3,5-thiadiazine-thione, Basamid®) at 100 μg of active ingredient per gram of soil were added to soil infested with *B. penetrans* in sealed containers. After 24 hr, the containers were opened and the nematicides were allowed to dissipate. Juveniles of *M. javanica* were then added to the soil, or to similar soil that had not been treated with nematicides. When the nematodes were extracted, observed for *B. penetrans* and then inoculated to tomato plants, *B. penetrans* attached to and infected similar numbers of nematodes in nematicide-treated and -untreated soil.

The capacity of nematicides to kill nematodes without having a measurable effect on *B. penetrans* was utilized in the first of two experiments to determine whether levels of *B. penetrans* occurring naturally on grape were sufficient to reduce root-knot nematode populations. Soil known to contain *B. penetrans* and *M. javanica* was collected from around 60-yr-old grapes at Moorook, South Australia. The soil was either autoclaved for 3 hr or treated with the nematicides DBCP or 1,3-D at a rate of 100 μg/g soil. Root-knot nematodes were not detected in any of these soils 1 wk after treatment. In October 1979, a grape rootling (cultivar Cabernet Sauvignon) was planted in seven replicate 9-L pots of each soil and 1,000 juveniles of *M. javanica* inoculated into each pot. In May 1981, after plants had grown in a shadehouse for two seasons, soil was collected from each pot with an Oakfield tube, nematodes extracted and counted and soils bioassayed for *B. penetrans*. In May 1982, plants were harvested, tops and roots weighed, juveniles of *M. javanica* in soil and roots counted, and the proportion of diseased females of *Meloidogyne* was estimated. Soil was again bioassayed for *B. penetrans*.

In the second experiment, grape rootlings (cultivar Cabernet Sauvignon) were planted in sixteen 9-L pots filled with soil from the root zone of 25-yr-old grapes at Cooltong, South Australia. The same number of pots were filled with virgin soil of the same texture

from an adjacent area. The number of root-knot nematodes in each pot was estimated by counting nematodes in 200-g subsamples of soil and adjusting counts for the efficiency of the extraction technique. There were no nematodes in the virgin soil and an average of 820 nematodes per pot of vineyard soil. Therefore, 820 juveniles of *M. javanica* were added to the pots containing virgin soil. All the pots were placed in a shadehouse in October 1980 and at the end of the first and second growing seasons (May 1981 and 1982) eight plants from each treatment were harvested. Tops and roots were weighed, juveniles of *M. javanica* in soil and roots were counted and soil was bioassayed for *B. penetrans*. The proportion of diseased females of *M. javanica* was estimated in 1982.

Nematode movement, spore concentration, and disease. The infectivity of juveniles of *M. javanica* with differing numbers of spores attached was studied using spore-encumbered nematodes obtained by agitating juveniles in a suspension of *B. penetrans* spores. Approximately 2, 4, 6, 11, and 21 hr after agitation commenced (times t_1–t_5), the number of spores per nematode was estimated by examining a sample of 100 nematodes. Five hundred nematodes were then inoculated around the roots of six tomato (cultivar Q3) seedlings growing in 1.5-L pots in the glasshouse. Since the infectivity of juveniles may have been affected by agitation, a control group of nematodes was agitated in a suspension of dried roots that did not contain *B. penetrans*. At each sampling time, 500 nematodes without spores were added to another six replicate pots. Four weeks after inoculation the number of nematodes in each root system was counted.

To determine whether the infectivity of nematodes and their infection by *B. penetrans* was affected by the distance juveniles moved in soil and the spore concentration through which they moved, 500 juveniles of *M. javanica* were added at the top of a 25-mm-diameter tube which stood in a pot containing a tomato seedling (cultivar Q3) (Fig. 1). The tube was filled with soil treated with the same concentrations of *B. penetrans* used in field trial 1 (either 0, 106, 212, or 424 mg of a dried-root preparation of *B. penetrans* per kilogram of dry soil) and the length of the soil column was varied (either 1, 2, 4, 8, or 16 cm). Each distance × concentration treatment was replicated three times. Diseased and healthy females in roots were counted 32 days after the nematodes were inoculated.

To determine how many spores must adhere to a nematode to ensure infection, juveniles of *M. javanica* with one to five spores attached were identified at a magnification of ×100 and transferred individually to a tomato seedling in a 40-ml pot. Diseased and healthy females were counted 5 wk later.

TABLE 1. Infectivity of *Meloidogyne javanica* on tomato in soil containing *Bacillus penetrans*

B. penetrans (mg/kg soil)	Females per root system[a]	Healthy females per root system[a]	Females infected (%)
0	573 (6.34)	573 (6.34)	0
100	340 (5.77)	127 (4.76)	63
200	227 (5.40)	44 (3.75)	81
400	206 (5.28)	19 (2.84)	91
LSD ($P = 0.05$)	(0.30)	(0.41)	

[a] Numbers in parentheses transformed: $\log_e$ (no. nematodes +1).

RESULTS

Addition of *B. penetrans* to tomato in pots. When tomato plants growing in soil containing 0, 100, 200, or 400 mg of the preparation of *B. penetrans* per kilogram of dry soil were inoculated with juveniles of *M. javanica*, significantly fewer nematodes developed in the soils treated with *B. penetrans*. The number of females per root system decreased as the concentration of the pathogen increased and most of the females were diseased at the highest concentrations of *B. penetrans* (Table 1). When plants already infected with *M. javanica* were transplanted into soil containing *B. penetrans*, the pathogen had little effect on the next generation of nematodes if they invaded tissue surrounding the females producing them. There was no significant difference in the number of juveniles hatching from primary roots in soil containing 0, 100, 200, or 400 mg of the preparation of *B. penetrans* per kilogram of dry soil (Table 2). *B. penetrans* had a greater effect on the juveniles that migrated from the primary roots to infect other roots. These nematodes were invariably diseased and the number of juveniles hatching from secondary roots was reduced significantly (Table 2).

Addition of *B. penetrans* to tomato in the field. At the time of planting, bioassays of soil at site 1 which had been treated with *B. penetrans* showed that 89–96% of juveniles of *M. javanica* were infested with spores within 24 hr (Table 3). Nematodes had up to 50 spores attached in soil containing the highest concentration of *B. penetrans*. Spore concentrations were lower at site 2; 77% of the juveniles were infested with up to 10 spores in treatments containing 600 mg of the preparation of *B. penetrans* per kilogram of dry soil.

At both sites, incorporation of dried roots into soil had no effect on plant growth, yield, root-knot index, and root-knot nematode populations. Generally, preparations of dried roots containing *B.*

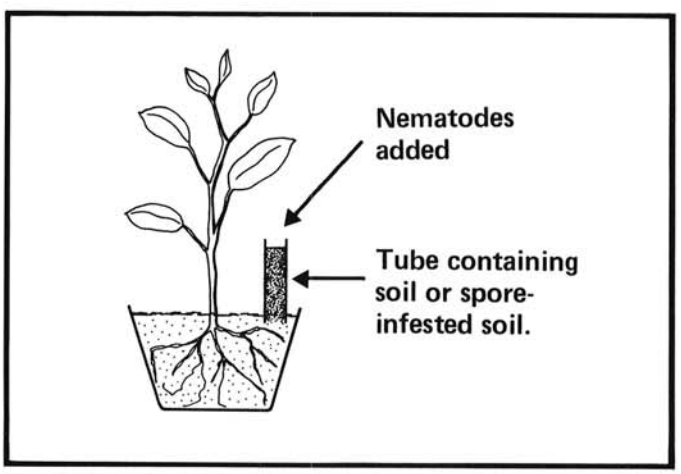

Fig. 1. Method used for studying the relationship between nematode movement, spore concentration, and disease.

TABLE 2. Increase in nematode populations on tomato roots in soil containing *Bacillus penetrans* after the primary roots were infected with healthy juveniles of *Meloidogyne javanica*

B. penetrans (mg/kg soil)	*Meloidogyne* juveniles per root system[a]		Females in secondary roots	Females infected in secondary roots (%)
	Primary roots	Secondary roots		
0	23,200 (9.94)	11,700 (9.26)	71	0
100	23,600 (9.98)	2,600 (7.63)	61	95
200	18,400 (9.72)	3,700 (7.87)	52	98
400	12,000 (9.21)	1,000 (6.32)	53	99
LSD ($P = 0.05$)	(n.s.)	(1.03)	12	

[a] Numbers in parentheses transformed: $\log_e$ (no. nematodes + 1).

penetrans significantly reduced root-knot nematode numbers in soil (Table 4). Also, analysis of root-knot indexes using Friedman's test (3) showed that galling in all plots treated with *B. penetrans*, except the 300-mg/kg treatment at site 2, was significantly different from the untreated control. In the 212- and 424-mg/kg treatments at site 1 and the 600-mg/kg treatment at site 2, 7 of 8, 6 of 8, and 4 of 5 replicates had root-knot indexes of 0 or 1 (Fig. 2). Some nematode control was obtained with fenamiphos, but root-knot indexes and nematode populations were significantly less with three applications than with a single application (Table 4). Plant growth was poor and variable at both sites because of inadequacies in the irrigation system and heat wave conditions during the growing season. Although top growth and yields were higher in plants growing in soil containing *B. penetrans* than in plants growing in untreated soil or soil amended with dried roots, the differences were not always significant.

Naturally occurring *B. penetrans* on grape. In the first of two experiments designed to determine the effect of naturally occurring *B. penetrans* on root-knot nematode populations, bioassays showed that levels of *B. penetrans* were reduced by autoclaving but were not affected by nematicides. In soil treated with DBCP or 1,3-D, 72–93% of the juveniles of *M. javanica* added were infested 24 hr later, compared to 7–10% in the autoclaved soil (Table 5). Approximately 60% of the females of *M. javanica* in roots growing in nematicide-treated soil were infected by *B. penetrans* compared with 1% in autoclaved soil. Plants grew equally well in all soils but root-knot nematode populations in soil in 1981 and roots in 1982 were significantly higher in autoclaved soil than in nematicide-treated soil.

In the second experiment, bioassays at the end of the 1981 and 1982 growing seasons showed that levels of *B. penetrans* were higher in vineyard soil than in virgin soil (Table 6). In vineyard soil, 20 and 19%, respectively, of the juveniles of *M. javanica* added were infested 24 hr later, compared with 2 and 5% in virgin soil. Root-knot nematode populations in soil and roots were significantly higher in virgin soil than in vineyard soil in both 1981 and 1982. In 1982, 34% of the females of *M. javanica* in roots from vineyard soil were infected by *B. penetrans*, whereas no diseased females were found in virgin soil. Plants grew equally well in both soils.

Nematode movement, spore concentration, and disease. When nematodes were agitated in a suspension containing *B. penetrans*, the spore load per nematode increased as the time of agitation increased. At the first sampling time (t_1) most nematodes carried 0–5 spores; at t_5, 85% of the nematodes had more than 40 spores attached (Table 7). The infectivity of uninfested and spore-infested nematodes was similar at sampling times t_1, t_2, t_3, and t_4 but at t_5 significantly fewer spore-encumbered nematodes invaded roots.

Data from the experiment in which nematodes were inoculated into tubes of varying length was transformed ($\log_e$ [no. nematodes +1]) and analyzed by analysis of variance. The total number of

TABLE 3. Bioassays for *Bacillus penetrans* at the time of planting at two field trial sites

	Nematodes infested[a] (%)	Spores per nematode (range)
Site 1		
Dried roots (424 mg/kg)	3	0–1
Dried roots + *B. penetrans* (106 mg/kg)	89	0–4
Dried roots + *B. penetrans* (212 mg/kg)	96	0–10
Dried roots + *B penetrans* (424 mg/kg)	93	0–50
Site 2		
Dried roots (300 mg/kg)	0	0
Dried roots (600 mg/kg)	0	0
Dried roots + *B. penetrans* (300 mg/kg)	54	0–3
Dried roots + *B. penetrans* (600 mg/kg)	77	0–10

[a] Mean of eight replicates.

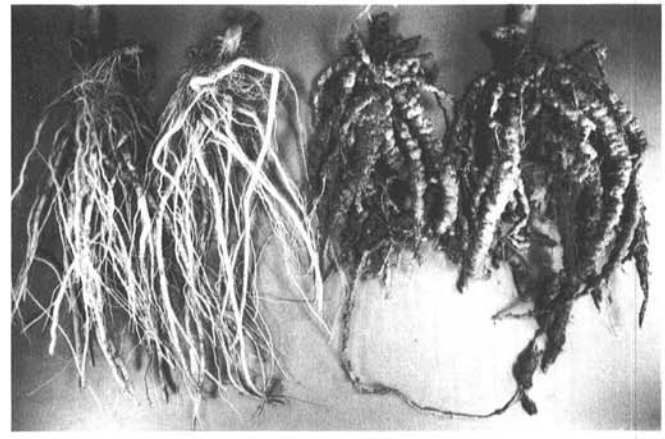

Fig. 2. Roots of tomato grown in soil infested with *Meloidogyne javanica* and treated with 600 mg of a preparation of *Bacillus penetrans* preparation per kilogram of dry soil (left) and grown in untreated soil (right).

TABLE 4. Effect of *Bacillus penetrans* and the nematicide fenamiphos on top growth and yield of tomato, root-knot index, and populations of *Meloidogyne*, at two field sites

	Dry wt. tops (g)	Fruit wt. (kg/plant)	Root-knot index	*Meloidogyne* juveniles/200 g soil[a]	
Site 1					
Dried roots + *B. penetrans* (106 mg/kg)	179	1.6	2.5	891	(6.22)
(212 mg/kg)	157	1.3	1.4	257	(4.77)
(424 mg/kg)	157	1.3	0.5	144	(4.41)
Dried roots (424 mg/kg)	113	0.9	4.8	1,231	(6.24)
Untreated control	88	0.9	4.9	1,091	(6.54)
LSD ($P<0.05$)	56	n.s.	***[b]		(1.37)
Site 2					
Dried roots + *B. penetrans* (300 mg/kg)	240	6.3	2.8	422	(5.84)
(600 mg/kg)	194	6.2	1.6	748	(5.52)
Fenamiphos (wk 0)	247	7.7	3.4	2,307	(7.11)
(wk 0,4,8)	222	6.4	1.0	177	(4.77)
Dried roots (300 mg/kg)	167	3.3	5.0	5,504	(8.57)
(600 mg/kg)	188	5.3	5.0	4,168	(8.57)
Untreated control	141	3.8	5.0	4,313	(7.77)
LSD ($P = 0.05$)	n.s.	n.s.	**[b]		(1.33)

[a] Numbers in parentheses transformed: $\log_e$ (no. nematodes + 1).
[b] Levels of significance according to Friedman's test (**, $P = 0.01$; ***, $P = 0.001$).

TABLE 5. Populations of *Meloidogyne javanica* and growth of grape in soil from a 60-yr-old vineyard after naturally occurring levels of *Bacillus penetrans* were reduced by autoclaving and retained following nematicide treatment

Treatment	Soil bioassay (% juveniles infested)		Fresh wt. (g)		*Meloidogyne* juveniles/200 g soil[a]		*Meloidogyne* juveniles/plant[a]	Females infected (%)
	1981	1982	tops	roots	1981	1982	1982	1982
DBCP	93	72	31	188	19 (2.87)	138 (4.61)	1,561 (7.28)	59
1,3-D	90	84	34	189	36 (3.27)	177 (4.78)	3,261 (7.99)	62
Autoclaved	10	7	36	213	104 (4.47)	259 (5.30)	5,973 (8.64)	1
LSD ($P = 0.05$)			n.s.	n.s.	(0.93)	(n.s.)	(0.48)	

[a] Numbers in parentheses transformed: $\log_e$ (no. nematodes + 1).

TABLE 6. Populations of *Meloidogyne javanica* and growth of grape in soil from a 25-yr-old vineyard naturally infested with *Bacillus penetrans* and in virgin soil

	Soil bioassay (% juveniles infested)	Fresh wt. (g)		Juveniles per plant[a]	Juveniles 200 g soil[a]	Females infected (%)
		tops	roots			
Year 1 (1981)						
Vineyard soil	20	6.4	16.1	1,934 (7.85)	176 (4.71)	...
Virgin soil	2	8.0	20.3	5,444 (9.06)	801 (6.32)	...
Year 2 (1982)						
Vineyard soil	19	6.5	41.2	1,681 (6.98)	102 (4.71)	34
Virgin soil	5	6.0	36.2	6,371 (8.65)	355 (5.52)	0
LSD ($P = 0.05$)		n.s.	5.6	(0.50)	(0.59)	

[a] Numbers in parentheses transformed: $\log_e$ (no. nematodes + 1).

TABLE 7. The infectivity of juveniles of *Meloidogyne javanica* infested with spores of *Bacillus penetrans*

Spore load class (spores/nematode)	Nematodes (%) in each spore load class at sampling time:				
	t_1	t_2	t_3	t_4	t_5
0–5	99	41	17		
6–10	1	44	79	1	
11–15		15	3	9	1
16–20			1	46	1
21–30				43	5
31–40				1	8
41–50					28
>50					57
Nematodes in roots					
Nematodes with spores	249	270	237	172	106
Nematodes without spores	229	208	254	246	290
	LSD ($P = 0.05$) = 83				

TABLE 8. Relationship between the number of spores of *Bacillus penetrans* attached to juveniles of *Meloidogyne javanica* and the number of females infected by the pathogen

Spores/nematode	Nematodes observed	Females infected	Females infected
1	26	7	27
2	21	11	52
3	21	15	71
4	19	14	74
5	21	19	90

nematodes in roots decreased as the length of the tube increased, this decrease being most apparent when soil in the tube contained *B. penetrans* (Fig. 3). Significantly fewer nematodes penetrated roots after moving through spore-infested soil than after moving the same distance through untreated soil, except at a distance of 1 cm. When the tube was filled with soil containing *B. penetrans*, a large proportion of the nematodes in roots were diseased, the proportion of diseased:healthy nematodes increasing as the distance moved in each concentration of *B. penetrans* increased (Fig. 3). Few nematodes penetrated roots after moving more than 4 cm through concentrations of 424 mg of the preparation of *B. penetrans* per kilogram of dry soil and those that did were invariably diseased.

Disease developed in 27% of the juveniles infested with only one spore (Table 8). The proportion of diseased females increased as the number of spores per nematode increased and most of the juveniles with five spores attached were infected.

DISCUSSION

This study confirmed the potential of *B. penetrans* for the biological control of root-knot nematode on annual crops. When *B. penetrans* was incorporated into soil prior to planting tomatoes, the reduction in crop damage and in nematode populations was similar to that usually achieved with nematicides.

The virtual absence of galls on tomato roots growing in soil containing relatively high concentrations of *B. penetrans* was unexpected, as previous life cycle studies (10) suggested that the pathogen acted by preventing reproduction by females. This work showed that *B. penetrans* also prevented juveniles of *M. javanica* from invading roots. The number of juveniles penetrating roots decreased as both the spore concentration and the distance moved in soil increased, and the infectivity of juveniles with more than 40 spores attached was reduced. The capacity of *B. penetrans* to prevent penetration by almost all juveniles moving more than 4 cm through spore-infested soil is significant when it is considered that juveniles of *M. javanica* may migrate up to 75 cm to invade roots (8).

In annual crops, the efficacy of *B. penetrans* depends largely on the spore concentration in soil. There must be enough spores present to prevent a large proportion of the juveniles of *Meloidogyne* from invading roots, and to ensure that most of the nematodes which penetrate roots become infected, and therefore do not reproduce. The pathogen is probably most effective against the juveniles which initially infect the plant, as they may move relatively long distances to roots (8) and are more likely to become encumbered with spores than are second- and later-generation juveniles, which hatch from eggs on the root surface and migrate relatively short distances to nearby roots. Nematodes invading the galled tissue which surrounds the females producing them are most likely to escape infection.

A laboratory bioassay in which juveniles of *Meloidogyne* were

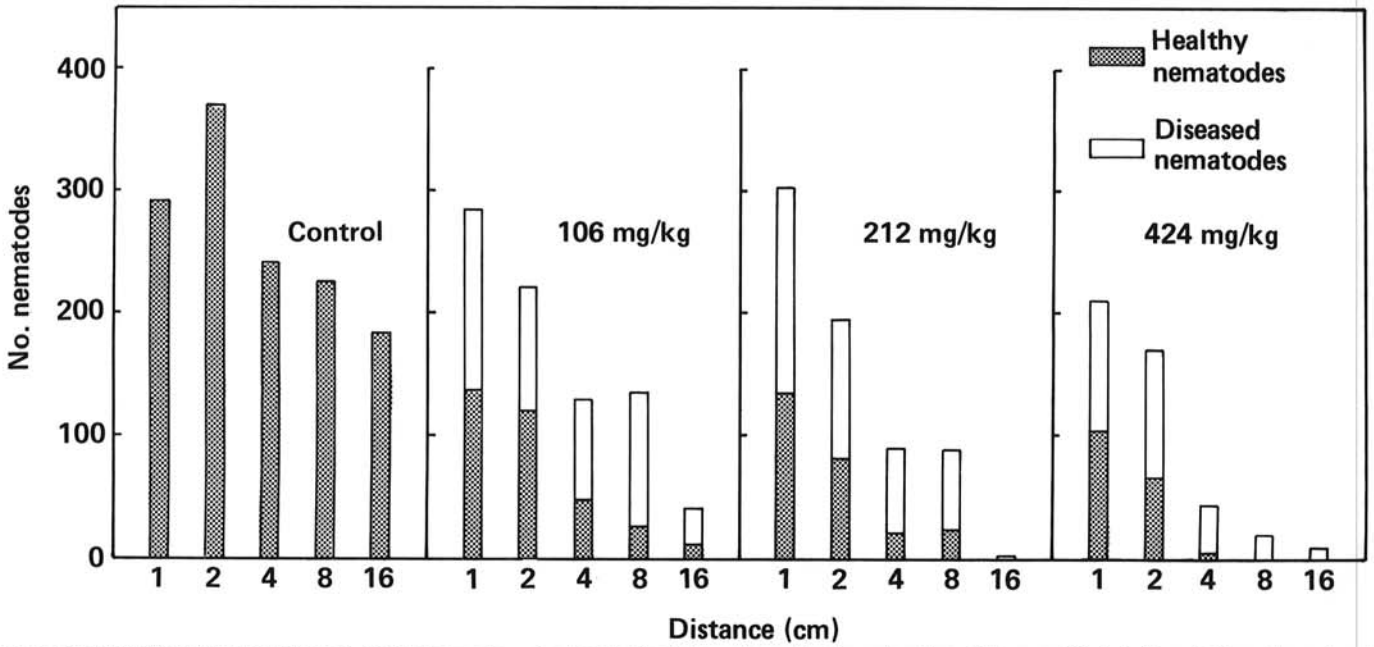

Fig. 3. Number of healthy and diseased *Meloidogyne javanica* developing in tomato roots after migrating distances of 1, 2, 4, 8, and 16 cm through soil containing 0, 106, 212, or 424 mg of a preparation of *Bacillus penetrans* per kilogram of soil.

added to soil and extracted 24 hr later proved useful for predicting whether levels of *B. penetrans* were sufficient to control root-knot nematode in the field. Good results were achieved with *B. penetrans* when more than 80% of the bioassay juveniles were infested with an average of about 10 spores per nematode.

The natural occurrence of *B. penetrans* in some vineyards in South Australia (13) provided the opportunity to study the effect of the pathogen on root-knot nematodes in a perennial cropping situation. Bioassays showed that levels of *B. penetrans* in soil from a 60-yr-old vineyard approached those used to control root-knot nematode on tomato. Less *B. penetrans* was detected in soil from around 25-yr-old grapes. When grapes were grown in pots in soil from these two vineyards, approximately 60 and 30%, respectively, of the females of *Meloidogyne* were diseased. Also, there were significantly fewer root-knot nematodes in soil infested with *B. penetrans* from vineyards than in similar soil without the pathogen. Over many years, *B. penetrans* apparently increased naturally to levels which reduced root-knot nematode populations on grape. There is insufficient information on economic thresholds for *Meloidogyne* on grape to determine whether such reductions are large enough to prevent crop losses in the field.

Sayre and Wergin (10) estimated that only 20% of the spores of *B. penetrans* germinated while the results presented here suggest that about 30% of the spores attached to juveniles initiated disease. The results of bioassays therefore need be interpreted with caution because spore-encumbered nematodes do not necessarily become infected. The capacity of spores to germinate probably depends on the environment to which spores are exposed and it may be possible to increase the potency of preparations of *B. penetrans* by changing the conditions under which they are prepared and stored. However, the optimum conditions for maintenance of viability and infectivity will not be defined until the factors affecting attachment and germination are better understood.

The ability of spores of *B. penetrans* to survive treatment with nematicides was first noted by Mankau and Prasad (6) and confirmed during this study. In practice, resistance to nematicides may prove to be one of the most useful attributes of the pathogen; *B. penetrans* and nematicides could be used together in management programs against root-knot nematodes.

Although these studies have shown that *B. penetrans* is a potentially useful biological nematicide, the inability to culture the pathogen in large quantities limits its commercial usefulness. The relatively small quantities of *B. penetrans* required for small-scale field trials can be produced using a culture technique in which the pathogen is increased in its nematode host (12). Minor increases in the yield of *B. penetrans* could undoubtedly be obtained by modifying this method but commercialization of the pathogen requires the development of in vitro culture techniques.

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