EFFECTS OF *PRATYLENCHUS NEGLECTUS*, *BACILLUS MEGATERIUM*, AND OIL RADISH OR RAPESEED GREEN MANURE ON REPRODUCTIVE POTENTIAL OF *MELOIDOGYNE CHITWOODI* ON POTATO

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ABSTRACT

Al-Rehiayani, S., S. L. Hafez, M. Thornton, and P. Sundararaj. 1999. Effects of *Pratylenchus neglectus*, *Bacillus megaterium*, and oil radish or rapeseed green manure on reproductive potential of *Meloidogyne chitwoodi* on potato. Nematropica 29:37-49.

The effects of *Pratylenchus neglectus*, *Bacillus megaterium* and oil radish or rapeseed green manure crops on *Meloidogyne chitwoodi* reproduction on potato were investigated in greenhouse and field microplot experiments. *B. megaterium* reduced the population densities of *M. chitwoodi* and *P. neglectus* by up to 50% on potato plants. There was a three-way interaction ($P < 0.05$) of *B. megaterium* × *P. neglectus* × green manure on *M. chitwoodi* population control on potato. The Population density of *M. chitwoodi* was suppressed by *B. megaterium* more in pots with green manure of oil radish or rapeseed than in pots with no green manure. *M. chitwoodi* generally suppressed *P. neglectus* numbers, but not in the presence of *B. megaterium* in pots amended with oil radish green manure. In field microplots, the main effects of green manure and nematode species on potato yield and nematode population density were significant ($P < 0.05$). Oil radish or rapeseed green manure treatments increased tuber yield compared to non-amended soil. Tuber yield from oil radish green manure treated plots was greater than from aldicarb treated plots. Tuber size was also greater in oil radish and rapeseed treatments than in aldicarb and non-amended treatments. Tuber yield from microplots inoculated with *M. chitwoodi* alone or in combination with *P. neglectus* was significantly lower than the non-inoculated control. Population densities of both the nematode species on potato plants were suppressed when they were inoculated together, compared to individual inoculation. The presence of *P. neglectus* also reduced tuber infection by *M. chitwoodi*.

Key words: Bacillus megaterium, biological control, *Brasssica campestris*, green manure, IPM, *Meloidogyne chitwoodi*, potato, *Pratylenchus neglectus*, *Raphanus sativus*, soil amendment.

RESUMEN

Al-Rehiayani, S., S. L. Hafez, M. Thornton y P. Sundararaj. 1999. Efecto de *Pratylenchus neglectus*, *Bacillus megaterium*, y del abono verde de rábano oleoso o colza en el potencial reproductivo de *Meloidogyne chitwoodi* en papa. Nematrópica 29:37-49.

El efecto de *Pratylenchus neglectus*, *Bacillus megaterium* y del abono verde de rábano oleoso o de colza en la reproducción de *Meloidogyne chitwoodi* en papa, fue investigado en experimentos realizados en el invernadero y en microparcelas. *B. megaterium* redujo hasta un 50%, la población de *M. chitwoodi* y de *P. neglectus* en las plantas de papa. Hubo una interacción triple significativa ($P < 0.05$) de *B. megaterium* × *P. neglectus* × abono verde en el control de la población de *M.chitwoodi* en papa. Interacciones significantes de *B. megaterium* × *P. neglectus* indicaron que la densidad de *M.chitwoodi* fue más reprimida en las macetas con el abono verde de rábano oleoso o de colza que en las macetas sin estos. La interacción *B. megaterium* × *M. chitwoodi* fue significante para la población de *P. neglectus* en macetas mejoradas con el abono verde de rábano oleoso, pero no con el abono verde de colza ni con el suelo sin mejorar. En las microparcelas, el efecto principal del abono verde o de las especies de nematodos, en el rendimiento de la papa y la población de nematodo fue significante ($P < 0.05$). Los tratamientos con el abono verde de rábano oleoso o colza aumentaron significativamente el rendimiento del tubérculo en comparación al suelo no mejorado. El rendimiento del tubérculo en las parcelas tratadas con el abono verde de rábano oleoso fue mayor que en la tratadas con aldicarb. El numero de tubérculos grandes obtenidos (50g), fue mayor en los tratamientos con rábano oleoso y colza que con aldicarb y el suelo sin mejoras. El rendimiento del tubérculo en las microparcelas inoculadas con *M. chitwoodi* solo o en combinación con *P. neglectus* fue significativamente menor que el control sin inocular. Las densidades poblacionales de ambas especies de nematodos fueron mutuamente suprimidas en las plantas de papa al inocularse juntas, en comparación a la inoculación individual. La presencia de *P. neglectus* también redujo la infección del tubérculo por *M. chitwoodi.*

Palabras claves: abono verde, *Bacillus megaterium, Brasssica campestris,* control biológico, mejora del suelo, *Meloidogyne chitwoodi,* papa, *Pratylenchus neglectus, Raphanus sativus.*

INTRODUCTION

Columbia root-knot nematode, *Meloidogyne chitwoodi* Golden, O'Bannon, Santo, and Finley, and root-lesion nematode, *Pratylenchus neglectus* (Rench) Filipjev and Schuurmans Stekhoven, are the predominant plant parasitic nematodes in Idaho potato (*Solanum tuberosum L.*) fields (Hafez *et al.*, 1992). *M. chitwoodi* is a serious pest and a major limiting factor for potato production in Idaho. It causes warts on the tuber surface and brown blemishes within the tubers, which reduces tuber value and marketability. Blemish ratings of potato tubers were found to be closely correlated with final field population levels of *M. chitwoodi* (Ferris *et al.*, 1994). *P. neglectus* does not cause blemishes on tubers and has negligible effects on potato yield in Idaho (Al-Rehiayani and Hafez, 1998; Hafez and Thornton, 1992). Competition between *M. chitwoodi* and *P. neglectus* occurs when both species are found together, and a higher population density of one species around the roots has the potential to lesser invasion by the other (Umesh *et al.*, 1994). The host plant and environmental conditions significantly influence the level of competition between these two species. When high populations of *P. neglectus* were present in a potato field infested with *M. chitwoodi, P. neglectus* suppressed the population of *M. chitwoodi* (Umesh *et al.*, 1994).

Green manure and nonhost crops are known to influence nematode populations in soil (Johnson *et al.*, 1992; Mojtahedi *et al.*, 1993a; 1993b; Rodríguez-Kábana *et al.*, 1992). Soil amendment with some green manure crops may cause significant decline in *M. chitwoodi* populations, but has minimal effects on *P. neglectus* populations (Mojtahedi *et al.*, 1991).

Potato plants are often colonized by several species of bacteria that may or may not be harmful to the host plant (Hooker, 1981). Some bacterial species enhance plant growth (Broadbent *et al.*, 1977; Burr *et al.*, 1978) and others suppress plant parasitic nematodes (Oostendorp and Sikora, 1989). Isolates of *Bacillus megaterium* de Bary 1884 are often found in healthy tissues of potato (Hooker, 1981) and in other plants (Weller, 1988) and reduce the negative impact of soil-borne plant pathogens (Liu and Sinclair, 1989; 1990). *Bacillus megaterium* B153-2-2 reduced symptoms caused by *Rhizoctonia solani* on soybean plants, enhanced plant growth, and increased the nodulation by *Bradyrhizobium* spp. on soybean seedlings (Liu and Sinclair, 1989; 1990). However, the effect of *B. megaterium* isolates on plant parasitic nematodes is unknown.

Currently, soil fumigation with Telone II (1,3-dichloropropene) is the most widely used method for *M. chitwoodi* control in potato fields. However, there are both environmental concerns and high chemical costs associated with nematicide use. Consequently, development of environmentally safe and effective alternatives for *M. chitwoodi* management is needed. Utilization of green manure crops in crop rotation for the suppression of *M. chitwoodi* populations is an alternative practice for nematode management. Recently, oil radish (*Raphanus sativus* L. cv. Trez) and rapeseed (*Brasssica campestris* L. cv. Humus) have been used as green manure crops for *M. chitwoodi* management in Idaho potato fields (Al-Rehiayani and Hafez, 1998). Although complete control of *M. chitwoodi* may not be obtained with a green manure crop alone, the efficacy of the green manure crop may be enhanced by the use of a biocontrol agent. We have evaluated an isolate of *B. megaterium* as a biological agent against *M. chitwoodi* and *P. neglectus.* In addition, we investigated the competitive influence of *P. neglectus* on *M. chitwoodi.* The objectives of this study were to: 1) evaluate the antagonistic activity of *B. megaterium* against *M. chitwoodi* and *P. neglectus*; 2) determine the effects of green manure and potato seed treatment with *B. megaterium* on the interaction between *P. neglectus* and *M. chitwoodi* on potato; and 3) determine the effects of green manure on potato yield in the presence of *M. chitwoodi* and *P. neglectus.*

MATERIALS AND METHODS

Nematode inoculum preparation: Columbia root knot nematode, *M. chitwoodi* race 2, and the lesion nematode, *P. neglectus*, were used throughout this study. The population of *M. chitwoodi* originated from a Parma, Idaho, potato field, and nematode inoculum was obtained from infected tubers (cv. Russet Burbank) that were kept in cold storage. Tubers were dipped in 0.26% NaOCl for 1 minute, rinsed and peeled to a depth of 10-12 mm. Peeled tuber tissue was then macerated using a mixer and placed on Baermann funnels in a mist chamber. Hatched juveniles were collected at 24-hr intervals for a period of 10 days. The *P. neglectus* population originated from a potato field in Parma, Idaho, and was cultured in the greenhouse on barley (*Hordeum vulgare* L. cv. Stepto). Juveniles and adult females of *P. neglectus* were extracted from barley roots. Washed roots were dipped in 0.26% NaOCl, cut into small pieces, placed in a mist chamber on a Baermann funnel and nematodes were collected daily for a period of two weeks.

Bacterium inoculum preparation: A *Bacillus megaterium* isolate provided by United Agri Products (Dr. Franklin Fronek) Greeley, Co. was maintained on nutrient agar (NA) medium in Petri dishes. Cell suspension (used as inoculum) was prepared by transferring a loop of freshly prepared bacterial cells grown on NA to a 1 000 ml Erlenmeyer flask containing 500 ml of a liquid nutrient medium (glycerin 10 ml, glucose 10 g, potassium phosphate 1 g, yeast extract 0.5 g, sodium chloride 0.5 g, and agar 4 g per liter; Thirumalachar and O'Brien, 1977). The liquid medium was sterilized in an autoclave for 25 min at 15 psi, cooled, and, inoculated under sterile conditions; flasks were then incubated for 7 days on a rotary shaker at 21°C. The population density of bacteria in the inoculum was determined by plating serial dilutions on Petri dishes containing NA and counting the number of colonies that formed.

Effects of bacteria on nematodes in the greenhouse: Three-week-old Russet Burbank potato plants were dipped in 25% (v/v) dilution of the bacterial cell culture (2.9×10^6 cfu/ml) for 5 minutes or in a diluted liquid medium (25% v/v) without bacteria (as control) and planted in plastic pots containing 500 cm^3 sandy loam soil infested with *M. chitwoodi* race 2 (450 nematodes/pot). Plants were arranged in a completely randomized design on a greenhouse bench and grown for seven weeks at 21-26°C. Plants received 14 hours of light per day supplemented with fluorescent lamps. Pots were watered daily and fertilized weekly with a commercial NPK solution (20-20-20) (50 ml/pot). At harvest, soil from each pot was separated from roots. The soil from each pot was placed in a three-liter pan with water. Roots were washed in a second pan to remove soil particles and the resulting suspension was added to the pan containing the soil and stirred thoroughly. Nematodes were extracted from the soil suspension by sieving (250 µm over 38 µm openings, respectively). The suspension was passed through the fine-mesh sieve three times to increase recovery of smaller nematodes. This sieving procedure was followed by sugar flotation (Jenkins, 1964). Individual root systems were chopped and placed on Baermann funnels in a mist chamber for 10 days and then oven dried (65°C) and weighed. Nematode suspensions were concentrated using a 25-µm sieve. Eggs and juveniles were counted from 1-ml aliquots and results were extrapolated to the total volume of the suspension. Final nematode population densities (Pf), nematodes per root system and per gram of dry root, and reproductive factors (Rf, where Rf = Pf/Pi) were subjected to analysis of variance. Final nematode population data were log transformed before analysis and LSD was used for mean separation when appropriate ($P < 0.05$).

In a second experiment, about 600 freshly hatched juveniles of *M. chitwoodi* in 2 ml water in test tubes were exposed to different concentrations of bacterial cell suspensions for 24 hr by adding 2 ml of a dilution series of bacterial suspensions (156 250, 312 500, 625 000, 1 250 000, and 2 500 000 cfu per tube). Control tubes received 2 ml of dilute sterile liquid medium (50% v/v). A mixed population of adult and juvenile *P. neglectus* (300 nematodes/tube) were exposed to the highest concentration of bacterial suspension. Nematode/bacteria suspension from each tube was added to three-week-old Russet Burbank seedlings grown in 200 cm^3 of sterile sand and soil mix (3:1). Plants were arranged in a completely randomized design on a greenhouse bench and grown for three weeks at 21-26°C with 14 hr of light per day. At harvest, the plants were uprooted and washed free of adhering soil particles. The root systems were stained by boiling for three min in a solution of Acid Fuchsin-lactoglycerol (Bird *et al.*, 1983) and then rinsed in tap water three times and destained for 24 hr in a solution of 50 ml distilled water plus 50 ml glycerol. Population data of *M. chitwoodi* were subjected to regression analysis. *P. neglectus* population data were subjected to analysis of variance, and LSD was used for mean separation.

Effects of green manures, bacteria and P. neglectus *on* M. chitwoodi *reproduction in the greenhouse*: Pots (7.6 L) filled with sandy loam soil (sand 74%, silt 21%, clay 5%, organic matter 1.5%, and pH 8.0) were inoculated with 10 000 *M. chitwoodi*, *P. neglectus* or Mc + Pn. Non-inoculated pots were maintained as untreated controls. Shoots from green house-grown oil radish and rapeseed were chopped into small pieces (1 cm long) and 200g of either plant was mixed with the soil in assigned pots. Control pots received no biomass. Pots were watered to enhance green manure decomposition. After seven days, a single certified potato seed tuber was planted in each pot. Potato seed tubers were dipped in 50% (v/v) dilute bacterial cell suspension (3.0 10^6 cfu/ml before dilution) for 10 minutes and then air dried at 21°C for 24 hr. Treatments were assigned randomly to five replicate blocks in a complete factorial arrangement. The following experimental factors were used: three types of green manure (oil radish, rapeseed, or no green manure); four levels of nematodes (no nematodes, Pn alone, Mc alone, or Pn + Mc); bacteria with 2 levels (present or absent). Plants were watered daily and fertilized every two weeks (200 ml/pot) with a commercial solution of N-P-K (20-20-20). Plants were grown at a soil temperature of 21-26°C and received 14 hours of light per day. The experiment was terminated eight weeks after planting and nematodes were extracted from soil and roots as described previously. Nematode population densities per root system and 500-cm^3 soil were subjected to analysis of variance. Effects of single and combined nematode and bacterial treatments on the nematode population on potato were assessed by comparing the nematode populations in roots and soil, and the total final population (Pf) per pot. The effects of nematode treatments were analyzed separately for each nematode species by analysis of variance. Means were separated using LSD when appropriate.

Effects of P. neglectus, M. chitwoodi, *and green manure, on potatoes in field microplots*: Field microplots at the University of Idaho Research & Extension Center in Parma, consisting of 19-L buckets (30 cm diameter × 35 cm deep; Pinkerton *et al.*, 1989) were used for an experiment that began in May, 1997 and was harvested in the first week of September, 1997. Five holes (each 2-cm in diameter) were cut at the bottom of each container to allow drainage and the bases of the containers were set 30 cm below the soil surface with containers spaced 1 m apart. Each microplot contained 16 L of soil (sand 46%, silt 44%, clay 10%, organic matter 1.1%, and pH 7.8) inoculated with Mc, Pn, Mc + Pn, or non-inoculated (control). Nematode densities were 15 000 *M. chitwoodi* and 16 000 *P. neglectus* per plot. Three weeks before potatoes were planted, control plots were fumigated with metam sodium (41.5 kg/ha). Treatments were assigned randomly to four replicate blocks in a complete factorial arrangement. The following factors were used: soil amendment with four levels (oil radish or rapeseed green manures, aldicarb, non-amended) and nematode species with four levels (Mc, Pn, Mc + Pn, or no nematodes). Shoots of oil radish cv. Trez and rapeseed cv. Humus grown in the greenhouse for two months were chopped and manually incorporated (300g/bucket) 15 cm deep in the appropriate microplots. Aldicarb was worked into soil with a trowel to a depth of 3 to 5 cm at 0.2g/microplot (22.4 kg a.i./ha) on a broadcast basis. A single potato seed tuber (55-65g) was planted in each microplot seven days after green manure incorporation. A drip irrigation system with a single emitter at each microplot was used to irrigate the microplots before planting, after germination and as needed throughout the growing season. Plants were fertilized weekly with a commercial solution of N-P-K (20-20-20) (500 ml/microplot), beginning three weeks after germination and ending three weeks before harvest. At harvest, tubers were separated manually from microplots. Tubers were weighed, graded for tuber size, and total tuber yield (g/microplot) and total number of tubers per microplot were determined. Potato tubers were divided into 2 categories based on size: <50g and >50g. Tubers from each microplot were hand peeled and examined for *M. chitwoodi* infection sites. A tuber with at least one infection site was considered infected and the percent of tubers infected per microplot was determined. Remaining roots were sieved from soil and 8-g-root samples were placed on a Baermann funnel in an intermittent mist chamber for nematode extraction for 10 days. Soil from each microplot was mixed thoroughly and a 500-cm^3 sample was processed for nematode extraction as described previously. Data were analyzed using analysis of variance and LSD was used for mean separation ($P < 0.05$). The effect of nematode treatments on nematode reproduction was analyzed separately.

RESULTS

Effects of bacteria on nematodes in the greenhouse: Compared to controls, a significant reduction in numbers of eggs and J2 of *M. chitwoodi* occurred in roots and in soil when potato plants were treated with bacterial suspension (Table 1).

B. megaterium reduced *M. chitwoodi* and *P. neglectus* infection of potato. The number of J2s of *M. chitwoodi* that penetrated potato roots was inversely related to the concentration of bacterial cell suspension (Fig. 1). Similarly, *P. neglectus* treated with a bacterial cell suspension of 2.5×10^6 cfu/ml were approximately half as numerous ($P < 0.05$) in the roots of potato (199 nematodes per root system) as those treated with the medium suspension (96 per roots system).

Effects of green manures, bacteria and P. neglectus *on* M. chitwoodi *reproduction in the greenhouse*: All treatments affected ($P < 0.05$) the population density of *M. chitwoodi* (Tables 2-3). Green manure and bacteria had the largest and most consistent overall effects, and *P. neglectus* interacted with *Bacillus megaterium* and green manures (Table 2). In pots with oil radish amendment, *B. megaterium* and *P. neglectus* reduced the final population of *M. chitwoodi* independently of one another. However, in pots with rapeseed amendment, *P. neglectus* increased the final population per pot of *M. chitwoodi* when *B. megaterium* was present. No interaction was detected in pots without green manure but the *B. megaterium* and *P. neglectus* main effects were significant (Table 3). *B. megaterium* significantly reduced the population of *M. chitwoodi* in roots and pots while *P. neglectus* reduced *M. chitwoodi* in soil and in pots. The effects of *B. megaterium* on *M. chitwoodi* were greatest in pots with soil amendments.

Table 1. Numbers of eggs and second-stage juvenile (J2) *Meloidogyne chitwoodi* race 2 produced on Russet Burbank potato seedlings coated with *Bacillus megaterium* cells.

Treatments	J2s per root system	J2s per gram dry root	J2s per 500 cm^3 soil	Eggs per 500 cm^3 soil	Nematode final population (Pf)/pot	Reproductive factor (Rf)[y]
Liquid medium only	3 006 a	4 171 a	564 a	4 854 a	8 424 a	19.1 a
Liquid medium + Bacteria[z]	1 462 b	1 549 b	150 b	2 688 b	4 300 b	9.8 b

Values are means of five replications. Means in each column followed by the same letter are not different ($P = 0.05$) according to LSD.
[y]Rf = (Pf/Pi).
[z]Bacterial cell concentration was 2.9 10^6 cfu/ml.

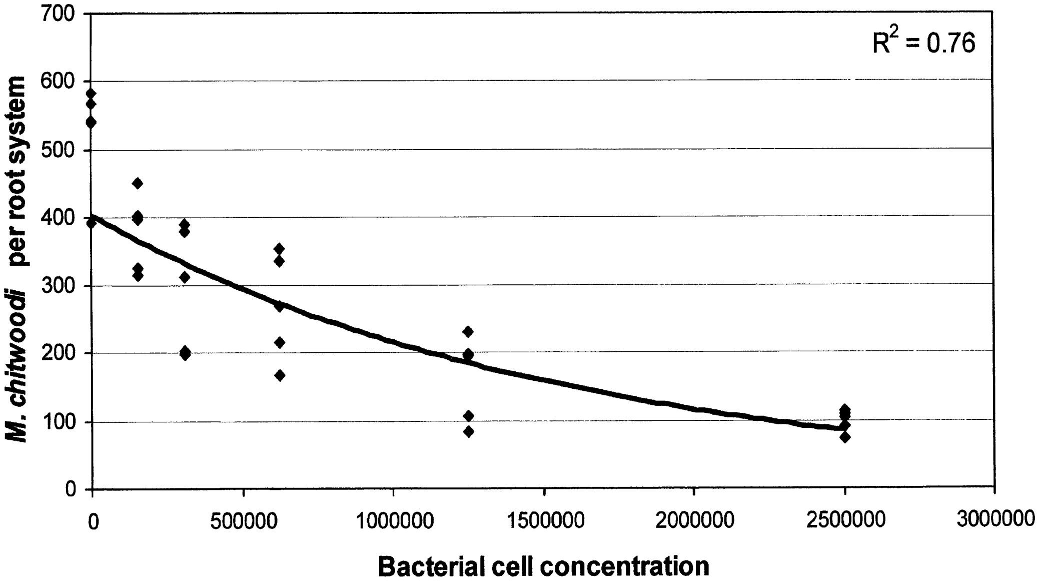

Fig. 1. Relationship between the number of second stage juveniles of *Meloidogyne chitwoodi* that penetrated roots of potato following 24 hours exposure to several concentrations of *Bacillus megaterium.*

Significant interaction was observed between *B. megaterium* and *M. chitwoodi* on *P. neglectus* populations in oil radish green manure treatments (Table 4). When inoculated independently, *B. megaterium* or *M. chitwoodi* significantly reduced the population of *P. neglectus.* However, there was no effect of *M. chitwoodi* when *B. megaterium* was present. The main effects of *B. megaterium* and *M. chitwoodi* on *P. neglectus* population density per pot were significant ($P < 0.05$) in unamended pots, while in rapeseed amended pots only *M. chitwoodi* reduced the numbers of *P. neglectus* (Table 5).

Effects of P. neglectus, M. chitwoodi, *and green manure, on potatoes in field microplots:* The main treatment effects on total tuber yield were significant and did not interact with soil amendment. *M. chitwoodi* alone or in combination with *P. neglectus* significantly reduced total tuber yields and the numbers of large tubers as compared to the non-infested control (Table 6). *P. neglectus* alone had no negative impact ($P > 0.05$) on tuber size or total tuber yield per microplot. Soil amended with oil radish or rapeseed green manure increased ($P <$ 0.05) total tuber yield and total number of large tubers compared to non amended soil. Oil radish green manure produced greater total tuber yields than did aldicarb, and both green manures increased tuber size compared to aldicarb.

No interaction occurred between the effects of soil amendment and *P. neglectus* on population densities of *M. chitwoodi* in roots or soil, or percentage tubers infected with *M. chitwoodi.* The main effect of each alone was significant (Table 7). The presence of *P. neglectus* reduced the population densities of *M. chitwoodi* in roots and soil, and reduced nematode tuber infection. All soil amendments reduced population densities of *M. chitwoodi* in roots and soil. Oil radish green manure and aldicarb reduced tuber infection while rapeseed did not.

Table 2. Effect of oil radish and rapeseed green manures, *Bacillus megaterium* and *Pratylenchus neglectus* on *Meloidogyne chitwoodi* population development on Russet Burbank potato.

	Juveniles per root system			Juveniles per 500 cm³ soil			Final population per pot[y]		
B. megaterium	–	+	t test	–	+	t test	–	+	t test
Oil radish green manure									
P. neglectus –	9 442	2 476	**[z]	2 288	1 158	**	27 716	11 740	**
P. neglectus +	1 752	1 630	NS	1 612	1 120 b	*	14 648	10 590	NS
t test	**	NS		**	NS		**	NS	
Rapeseed green manure									
P. neglectus –	19 760	5 805	**	1 568	602	**	32 304	10 622	**
P. neglectus +	8 950	10 570	NS	2 080	1 646	NS	25 590	23 738	NS
t test	**	NS		NS	**		*	**	

Values are means of five replications.
[y]Final population = Juveniles in roots + juveniles in soil + eggs in soil.
[z]**Significant difference ($P < 0.01$); *significant difference ($P < 0.05$); NS = nonsignificant difference detected by analysis of variance.

There was no interaction between the effects of soil amendment and *M. chitwoodi* on population densities of *P. neglectus* in roots or soil, but the main factor effects were significant (Table 8). *M. chitwoodi* reduced ($P = 0.05$) population densities of *P. neglectus* in roots and soil. All soil amendments reduced *P. neglectus* populations in soil. Aldicarb resulted in lower populations of *P. neglectus* than did either oil radish or rapeseed amendments, in both soil and roots.

DISCUSSION

Green manure crops, in combination with a biocontrol agent, may provide an

Table 3. Main effects of *Bacillus megaterium* and *Pratylenchus neglectus* on *Meloidogyne chitwoodi* population development on Russet Burbank potato growing in soil without green manure amendment.

Factor	Level	Juveniles per root system	Juveniles per 500 cm³ soil	Final population per pot[z]
B. megaterium	–	21 176 a	4 708 a	58 840 a
	+	16 589 b	3 779 a	46 821 b
P. neglectus	–	18 199 a	5 581 a	62 847 a
	+	19 566 a	2 906 b	42 814 b

Values are means of five replicates. In each column, means for each factor followed by same letter do not differ ($P < 0.05$) according to analysis of variance.
[z]Final population = Juveniles in roots + juveniles in soil + eggs in soil.

Table 4. Effects of *Bacillus megaterium* and *Meloidogyne chitwoodi* on *Pratylenchus neglectus* populations on Russet Burbank potato in pots amended with oil radish green manure.

	Nematodes per root system			Nematodes per 500 cm³ soil			Nematodes per pot[y]		
B. megaterium	–	+	t test	–	+	t test	–	+	t test
M. chitwoodi –	3 564	1 012	**	184	170	NS	5 404	2 712	**
M. chitwoodi +	1 356	688	*	247	238	NS	4 096	3 068	*
t test	**	NS		*	NS		*	NS	

Values are means of five replications.
[y]Final population = Juveniles and adults in roots + Juveniles and adults in 5 000 cm³soil
[z]**Significant difference ($P < 0.01$); *significant difference ($P < 0.05$); NS = nonsignificant difference detected by analysis of variance.

effective method to manage *M. chitwoodi* on potato without the use of nematicides. *B. megaterium,* applied as a seed treatment in the greenhouse, suppressed the population development of *M. chitwoodi* and in some instances of *P. neglectus.* Potato seedling treatment with *B. megaterium* resulted in *M. chitwoodi* infection levels that were half those in roots of untreated plants. This bacterium was also successful when applied to potato seed pieces and was effective against nematodes in soil amended with oil radish and rapeseed green manure.

Application of *B. megaterium* to potato seed pieces is easily accomplished and antagonistic activity probably occurs on

Table 5. Main effects of *Bacillus megaterium* and *Meloidogyne chitwoodi* on *Pratylenchus neglectus* population densities on Russet Burbank potato growing in rapeseed cv. Humus green manure amended and unamended soil.

Factor	Level	Nematodes per root system	Nematodes per 500 cm³ soil	Final population per pot[z]
		Rapeseed green manure soil amendment		
B. megaterium	–	2 525 a	298 a	6 619 a
	+	3 119 a	350 a	5 415 a
M. chitwoodi	–	4 227 a	343 a	7 657 a
	+	1 417 b	296 a	4 377 b
		No soil amendment		
B. megaterium	–	6 455 a	2 171 a	28 165 a
	+	5 650 a	745 b	13 100 b
M. chitwoodi	–	7 355 a	1 650 a	23 855 a
	+	4 750 b	1 266 a	17 410 b

Values are means of five replicates. In each column, means for each factor followed by the same letter do not differ ($P < 0.05$) according to analysis of variance.
[z]Final population = Juveniles and adults in roots + juveniles and adults in soil.

Table 6. Main effects of nematode species and soil amendment on tuber yield of Russet Burbank potato grown in field microplots infested with *Meloidogyne chitwoodi* and *Pratylenchus neglectus*.[y]

Treatments	Tuber number (size <50 g)	Tuber number (size 50 g)	Tuber wt (g/microplot)
Effect of nematode treatments			
None	12 a[z]	4 a	552.1 a
P. neglectus	12 a	3 ab	502.7 ab
M. chitwoodi	13 a	1 c	401.5 c
P. neglectus + *M. chitwoodi*	9 a	2 bc	436.6 bc
Effect of soil amendments			
None	12 a	2 b	415.5 c
Oil radish	11 a	3 a	537.3 a
Rapeseed	10 a	3 a	481.9 ab
Aldicarb	13 a	2 b	458.2 bc

[y]Microplots were infested with 15 000 *M. chitwoodi*, 16 000 *P. neglectus*, or both *P. neglectus* and *M. chitwoodi*. Soil was amended with oil radish or rapeseed green manure, aldicarb, or non-amended (None).
[z]Overall F tests for the analysis of variance were significant at $P < 0.05$. Means for each main factor followed by the same letter do not differ ($P = 0.05$) according to LSD.

the root surface during the initial phase of root infection by the nematode (Chao *et al.*, 1986; Oostendorp and Sikora, 1989). The ability to colonize roots and remain competitive in the rhizosphere are primary characteristics of a successful biocontrol

Table 7. Main effects of *Pratylenchus neglectus* and soil amendments on *Meloidogyne chitwoodi* numbers and percentage of infected tubers of Russet Burbank potato growing in microplots.[y]

Treatment	Juveniles per 8 g roots	Juveniles per 500 cm^3 soil	% infection of tubers
Effect of *P. neglectus*			
M. chitwoodi alone	107 a[z]	56 a	39.1 a
M. chitwoodi + *P. neglectus*	41 b	39 b	14.8 b
Effect of soil amendment			
None	174 a	85 a	27.9 a
Oil radish	48 b	35 b	16.3 b
Rapeseed	29 b	44 b	46.0 a
Aldicarb	46 b	26 b	17.0 b

[y]Microplots were infested with 15 000 *M. chitwoodi*, 16 000 *P. neglectus*, or both *P. neglectus* + *M. chitwoodi*. Soil was amended with oil radish or rapeseed green manure, Aldicarb, or non-amended (None).
[z]Overall F tests for the analysis of variance were significant at $P < 0.05$. Means for each main factor followed by the same letter do not differ ($P = 0.05$) according to LSD.

Table 8. Main effects of *Meloidogyne chitwoodi* and soil amendment on *Pratylenchus neglectus* numbers on Russet Burbank potato growing in microplots.[y]

Treatments	*P. neglectus* per 8 g roots	*P. neglectus* per 500 cm^3 soil
Effect of *M. chitwoodi*		
P. neglectus alone	1 480 a[z]	1 416 a
P. neglectus + *M. chitwoodi*	491 b	690 b
Effect of soil amendment		
None	1 177 a	1 961 a
Oil radish	1 135 a	1 010 b
Rapeseed	1 410 a	899 b
Aldicarb	220 b	343 c

[y]Microplots were infested with 15 000 *M. chitwoodi,* 16 000 *P. neglectus,* or both *P. neglectus* + *M. chitwoodi.* Soil was amended with oil radish or rapeseed green manure, Aldicarb, or non-amended (None).
[z]Overall F tests for the analysis of variance were significant at $P < 0.05$. Means for each main factor followed by the same letter do not differ ($P = 0.05$) according to LSD.

agent. *B. megaterium* forms spores that are resistant to unfavorable environmental conditions and are thus adaptable to formulation and application in the field. It is a good root colonizer and rhizosphere competitor and remains viable for extended periods (Liu and Sinclair, 1993).

Bacillus megaterium has been shown to be antagonistic to other soil-borne pathogens (Liu and Sinclair, 1993). For example, *B. megaterium* strain B153-2-2 suppressed *Rhizoctonia solani* activity and improved soybean root growth (Liu and Sinclair, 1989; 1990). Toxic metabolic products and antibiotic production by *Bacillus* spp. are often suggested as mechanisms of action in reducing soil-borne plant pathogen populations (Mankau, 1981). A toxin produced by *B. thuringiensis* was found to be harmful to *Meloidogyne incognita* (Mankau, 1981). A 15-minute exposure of *Caenorhabditis elegans* to *B. megaterium in vitro* resulted in nematode death (Andrew and Nicholas, 1976). The specific chemical and physical components of various soils that are necessary for the optimal growth of *B. megaterium* are unknown and require investigation for commercial development of this bacterium.

Because plant parasitic nematodes usually occur in polyspecific communities, multiple species interactions are likely to influence the severity of crop losses and the nematode population dynamics (Eisenback, 1985). Reproduction of *M. chitwoodi* or *P. neglectus* was suppressed in our experiments when they were present together. The finding that *P. neglectus* suppresses reproduction of *M. chitwoodi* on potato confirms a previous report that competition can occur between these two species (Umesh *et al.*, 1994). Other studies (Ferris *et al.*, 1994) indicate a close correlation between final population levels of *M. chitwoodi* and blemish ratings of potato tubers. In our microplot study, the presence of *P. neglectus* reduced the numbers of *M. chitwoodi* in roots and soil, which resulted in less tuber infection.

Increased potato yield and reduction in numbers of *M. chitwoodi* by oil radish or rapeseed green manure in these studies confirms the results of previous reports (Al-Rehiayani and Hafez, 1997; 1998). Green manure did not diminish the antagonistic potential of *B. megaterium.* Indeed, the antagonistic effect of the bacterium was more pronounced in the presence of oil radish or rapeseed green manure. Early studies showed that *B. megaterium* was among several Gram-positive bacteria isolated from the rhizosphere of well known green manure crops such as velvetbean and castor, and such bacteria may be responsible for reduction of nematode development on such crops (Kloepper *et al.*, 1992).

Although *B. megaterium* and *P. neglectus* can suppress *M. chitwoodi* populations, the effects appear to interact with soil conditions. In oil radish green manure pots, *B. megaterium* reduced *M. chitwoodi* population more in the presence of *P. neglectus* while in rapeseed green manure pots, *B. megaterium* reduced *M. chitwoodi* population more in the absence of *P. neglectus.* The reason for this difference is not understood. However, it could be because oil radish and rapeseed green manures influence communities of soil microorganisms differently.

Suppression of *M. chitwoodi* on potato by oil radish or rapeseed green manure, *B. megaterium,* and *P. neglectus* was evident in these studies. Although complete control of *M. chitwoodi* is unlikely to be achieved by biocontrol alone, more effective suppression can be obtained by combining oil radish green manure with *B. megaterium* in the presence of *P. neglectus.*

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