

## The Effect of Marigolds (*Tagetes* spp.) and Other Cover Crops on *Pratylenchus penetrans* and on Following Potato Crops

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**Abstract:** Root-lesion nematodes (primarily *Pratylenchus penetrans*) were monitored in two marigold cultivars (*Tagetes tenuifolia* cv. Nemakill and cv. Nemanon), annual ryegrass (*Lolium multiflorum* cv. Lental), red clover (*Trifolium pratense* cv. Florex), and soybean (*Glycine max* cv. Proteus), and in the following potato (*Solanum tuberosum* cv. Superior) crop during three growth sequences. Meadow fescue (*Festuca elatior* cv. Miner) and bee plant (*Phacelia tanacetifolia* cv. Gipha) were added to the trial in the second year. Black-eyed Susan (*Rudbeckia hirta*, unidentified cv.) and two additional marigold cultivars (*T. patula* ssp. *nana*, unidentified cv., and *T. erecta* cv. Crackerjack) were included in the final sequence. Population levels of root-lesion nematodes were consistently lower under marigolds compared to the other cover crops tested. Correspondingly, average potato tuber yields were significantly higher (8–14%) when potato followed marigolds. The highest levels of root-lesion nematodes occurred under red clover and soybean, and the average potato tuber yields were lowest following these crops.

**Key words:** crop sequence, lesion nematode, marigold, potato, *Pratylenchus penetrans*, *Tagetes* spp.

Potato (*Solanum tuberosum* L.) is cultivated annually on about 70,000 ha in the Maritime region of Canada (Prince Edward Island Department of Agriculture and Forestry, 1998). The root-lesion nematode, *Pratylenchus penetrans* (Cobb), is the dominant nematode parasite of potatoes in the region, and control of this species with nematicides has resulted in significant increases in tuber yields (Kimpinski, 1986; Kimpinski and Sanderson, 1989). Unfortunately, the nematicide, aldicarb, and its metabolites have been detected in ground water at a few locations in the Maritime provinces of Canada (Anonymous, 1987), and its use has been restricted. Furthermore, chemical control of nematodes, especially with fumigants, is often not economical in this region and, because the use of aldicarb was restricted, growers rarely use nematicides for nematode control in potatoes.

Since nematicides are expensive and may impact the environment, and because none of the potato cultivars that are currently grown in the region are resistant to root-

lesion nematodes, other forms of nematode management are necessary. The use of cover crops that suppress root-lesion nematode populations is an attractive alternative for growers. Marigolds (*Tagetes* spp.) have been shown to suppress certain nematode species (Alexander et al., 1999; Hackney and Dickerson, 1975; Ko and Schmitt, 1996; McSorley and Frederick, 1994; Ploeg, 1999; Riga and Potter, 1998; Siczka et al., 1991), and studies in Holland have shown that some *Tagetes* spp. suppress populations of *P. penetrans* by as much as 90% (Oostenbrink, 1960; Oostenbrink et al., 1957). Previous studies (Kimpinski, 1984; Kimpinski et al., 1984) in the Maritime region of Canada have shown that populations of root-lesion nematodes were lower under annual ryegrass (*Lolium multiflorum*) than under red clover (*Trifolium pratense*) or soybean (*Glycine max*), but data on the use of marigolds for root-lesion nematode control are not available for this region.

Our objectives were to determine the influence of marigolds and several other potential cover crops on the population levels of root-lesion nematodes and to evaluate the yield response of subsequent potato crops to these plant species.

### MATERIALS AND METHODS

Field trials were conducted during 1992–1995 at adjacent locations in each year at the

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Harrington Farm of the Agriculture and Agri-Food Canada Crops and Livestock Research Centre on Prince Edward Island. The soil type at each location was a fine sandy loam that averaged 70% sand, 20% silt, 10% clay, 2.8% organic matter, and a pH range of 5.2–6.2.

At each site in early May 1992, 1993, and 1994, the soil was moldboard-plowed 20 cm deep, disked twice to 15 cm deep, and harrowed 15 cm deep. Individual plots were 4 by 6 m, and cultural and fertilizer practices similar to those used in commercial production were followed for the cover crops such as annual ryegrass, red clover, and soybean (Atlantic Provinces Field Crop Guide, 1991). The advice of the seed suppliers was considered for the remaining cover crops that are not widely grown in this region. All the cover crops except annual ryegrass received a pre-emergence herbicide treatment of chlorthal-dimethyl.

Five cover crops were seeded on 3 June 1992: two marigold cultivars (*Tagetes tenuifolia* cv. Nemakill and cv. Nemanon), annual ryegrass cv. Lemtal, red clover cv. Florex, and soybean cv. Proteus. On 21 June 1993, the same cover crops were seeded at an adjacent site as in 1992; in addition, meadow fescue (*Festuca elatior* cv. Miner) and bee plant (*Phacelia tanacetifolia* cv. Gipha) were planted. On 27 June 1994 at an adjacent site, the same crops were seeded as in 1993; in addition, black-eyed Susan (*Rudbeckia hirta*, unidentified cv.) and two other marigold cvs. (*T. patula* ssp. *nana*, unidentified cv. and *T. erecta* cv. Crackerjack) were also seeded. The marigold cultivars, bee plant, and black-eyed Susan were cut and chopped in late October in each year. Red clover, annual ryegrass, and meadow fescue were cut to a height of about 8 cm in mid-August; soybeans were harvested in late October. The foliage from all plots was left on the surface over winter and disked in the spring about 3 weeks before planting potatoes.

Elite-III seed pieces of the potato cultivar Superior were planted at 25-cm intervals in four rows, 0.9 m apart, in each plot. Planting dates were 3 June 1993, 24 May 1994, and 16 May 1995. The cultivar Superior was used

because it is sensitive to damage from *P. penetrans* in the Maritime region (Kimpinski and McRae, 1988). A pre-emergence herbicide treatment of metribuzin was applied to all plots, and cultural and fertilizer practices used in commercial potato production were used (Atlantic Canada Potato Guide, 1993). In late July of each year, deltamethrin insecticide was applied at the recommended rate to foliage in all plots for control of Colorado potato beetles (*Leptinotarsa decemlineata*) and potato flea beetle (*Epitrix cucumeris*). Harvest dates were 10 October 1993, 7 October 1994, and 18 September 1995. Tubers were mechanically sized according to Canadian standards into small (38–57 mm), Canada No. 1 (58–88 mm), large (89–114 mm), and culls (<38 mm or >114 mm).

Soil nematode populations in each plot were assayed from a composite sample of 10 cores, 2.5-cm-diam. and 20-cm-deep, taken at random from each plot just prior to planting each year, and again on 4 November 1992, 12 October 1993, and 28 October 1994. In the subsequent potato crops, 10 cores were taken at random from each plot just prior to planting, and from the outside rows about 1 week prior to harvest. A 50-g subsample from each composite sample was placed in a modified Baermann funnel (Barker, 1985). Nematodes in roots were assayed by separating the roots from the fall soil samples and placing 10 g of tissue in a mist chamber (Hooper, 1986). After 7 days at 22 °C, nematodes that had emerged from soil or roots were counted, the residual material from each sample was dried for 48 hours at 100 °C, and the numbers of nematodes per kilogram of dry soil or per gram of dry root were recorded.

The experimental design each year was a randomized complete block with four replicates per treatment. The nematode data were transformed to logarithms ( $x + 1$ ) for analyses of variance (Genstat, 1993; Snedecor and Cochran, 1989) to assess the effects of the various crops on nematode populations and on yields in the subsequent potato crop. The nematode data are presented as back-transformed means in the tables. Duncan's multiple-range test (SAS Institute Inc.,

1985) was used to make comparisons between means. In addition, treatment sums of squares were partitioned into single-degree-of-freedom contrasts to examine differences between various cover crops (Steel and Torrie, 1960). Since error variances were homogenous, years were treated as fixed effects and, where applicable, combined-year analyses of variance were conducted.

## RESULTS

The predominant plant-parasitic nematode species in the cover crops and subsequent potato crops was the root-lesion nematode, *Pratylenchus penetrans*. In many cases this species accounted for more than 95% of the nematodes extracted from root samples, and it was also the main plant-parasitic species in soil. *Pratylenchus crenatus*, *Meloidogyne hapla*, *Merlinius* spp., *Tylenchorhynchus* spp., and *Helicotylenchus* spp. were detected in low numbers in soil (<200 nematodes/kg).

*Pratylenchus penetrans* was the only plant-parasitic nematode species that was present at levels above the damage threshold for potatoes (Kimpinski and McRae, 1988). The average population densities of *P. penetrans* in 1992, 1993, and 1994 when the cover crops were sown were 1,090, 1,240, and 1,040 per kg of soil, respectively. The average population densities of *P. penetrans* in

1993, 1994, and 1995 when potatoes were planted were 1,850, 1,320, and 1,000 per kg of soil, respectively.

Averaged over 3 years, the population levels of root-lesion nematodes in the 3-year analysis were lower in the marigold cultivars than in the other crops, and nematode numbers did not differ between the two marigold cultivars (Table 1). Nematode population densities were lower in annual ryegrass than in red clover and soybean. The yield of no. 1 tubers (58 to 88 mm) was about 8% higher when potatoes followed marigolds than when potatoes followed the other crops.

Two-year averages indicated that marigolds had the lowest root-lesion nematode population densities, and about 10% higher tuber yields in the subsequent potato crop when potatoes followed marigolds than when they followed the other crops (Table 2). Nematode population densities did not differ in the two marigold cultivars and were intermediate in meadow fescue and bee plant roots between the marigold cultivars and red clover and soybean. Tuber yields in the potato crop after meadow fescue or bee plant were also intermediate between yields that followed the marigolds and red clover or soybean.

In the single-year study, the four marigold cultivars were the most effective in suppressing root-lesion nematode populations (Table 3). Tuber yields averaged about 14%

TABLE 1. Root-lesion nematode<sup>a</sup> population densities in cover crops and subsequent potato crops, and tuber yields averaged over 3 years.

Treatment <sup>b</sup>	Cover crop		Potato cv. Superior			
			Number of nematodes			Tuber yield <sup>d</sup>
	(per kg soil)	per g root)	(per kg soil) <sup>c</sup>	(per kg soil) <sup>c</sup>	(per g root)	(Tons/ha)
MG cv. Nemakill	830a <sup>e</sup>	160a	870a	950a	1,170a	44.2a
MG cv. Nemanon	630a	150a	1,020a	1,200a	620a	43.4a
ARG cv. Lemtal	870a	430b	1,020a	1,410ab	1,660ab	42.1ab
RC cv. Florex	2,950b	1,620c	3,310b	2,570b	2,510b	39.1b
SB cv. Proteus	3,630b	5,620d	2,290b	2,690b	1,860ab	40.4b
Contrasts <sup>f</sup>	A,B	A,B,C	A,B	A	A	A

<sup>a</sup> Primarily *Pratylenchus penetrans*.

<sup>b</sup> MG = marigold, ARG = annual ryegrass, RC = red clover, SB = soybean.

<sup>c</sup> First and second columns are preplant and fall samples, respectively.

<sup>d</sup> Canada No. 1 (58 – 88 mm).

<sup>e</sup> Antilog means in a column followed by the same letter are not different ( $P \leq 0.05$ ) according to Duncan's multiple-range test.

<sup>f</sup> Significant differences ( $P \leq 0.05$ ): A = mean of marigold cultivars vs. mean of other crops; B = annual ryegrass vs. mean of red clover and soybean; C = red clover vs. soybean.

TABLE 2. Root-lesion nematode<sup>a</sup> population densities in cover crops and subsequent potato crops, and tuber yields averaged over 2 years.

Treatment <sup>b</sup>	Cover crop		Potato cv. Superior <sup>d</sup>			Tuber yield <sup>d</sup> (tons/ha)
	Numbers of nematodes					
	(per kg soil)	(per g root)	(per kg soil) <sup>c</sup>	(per kg soil) <sup>c</sup>	(per g root)	
MG cv. Nemakill	650ab <sup>c</sup>	190a	1,000ab	1,230a	1,170a	42.3a
MG cv. Nemanon	540ab	170a	700a	2,090ab	350a	41.6a
ARG cv. Lemtal	390a	600b	1,260ab	2,690ab	1,350a	38.5b
MF cv. Mimer	620ab	620b	760a	2,950ab	460a	39.4ab
BP cv. Gipha	2,690b	3,090c	1,480a	1,550a	1,200a	39.5ab
RC cv. Florex	3,090c	4,720cd	3,800b	3,980b	2,190a	35.7c
SB cv. Proteus	2,090ab	11,220d	1,120ab	3,800b	1,450a	37.3bc
Contrasts <sup>f</sup>	A,B	A,B		A,C	A	A,C

<sup>a</sup> Primarily *Pratylenchus penetrans*.<sup>b</sup> MG = marigold, ARG = annual ryegrass, MF = meadow fescue, BP = bee plant, RC = red clover, and SB = soybean.<sup>c</sup> First and second columns are preplant and fall samples, respectively.<sup>d</sup> Canada No. 1 (58 - 88 mm).<sup>e</sup> Antilog means in a column followed by the same letter are not different ( $P \leq 0.05$ ) according to Duncan's multiple-range test.<sup>f</sup> Significant differences ( $P \leq 0.05$ ): A = mean of marigold cultivars vs. mean of other crops; B = mean of annual ryegrass and meadow fescue vs. mean of bee plant, red clover, and soybean; C = bee plant vs. mean of red clover, and soybean.

higher after marigolds than after the other crops. There were no differences between the marigold cultivars except nematode population density in soil around *T. patula* roots was higher than around roots of the other marigold cultivars. The average nematode levels in annual ryegrass, meadow fescue, and black-eyed Susan were lower than the average values for bee plant, red clover, and soybean. Tuber yields were higher in potatoes grown after bee plant than after red clover or soybean, and yields were also higher after soybean than after red clover. There were no differences among cover

TABLE 3. Root-lesion nematode<sup>a</sup> population densities in cover crops in 1994 and in the potato crop in 1995, and tuber yield.

Treatment <sup>b</sup>	Cover crop		Potato cv. Superior			Tuber yield <sup>d</sup> (tons/ha)
	Numbers of nematodes					
	(per kg soil)	(per g root)	(per kg soil) <sup>c</sup>	(per kg soil) <sup>c</sup>	(per kg root)	
MG cv. Nemakill	650a <sup>c</sup>	200a	460ab	3,090b	1,550a	51.8a
MG cv. Nemanon	790ab	120a	400a	2,290b	370a	50.9a
MG cv. Crackerjack	950ab	250a	500ab	590a	580a	49.6ab
MG <sup>f</sup>	3,890bc	190a	460ab	3,550b	590a	52.8a
ARG cv. Lemtal	740ab	300a	1,700ab	6,760bc	2,510a	44.5b
MF cv. Mimer	1,050ab	410a	690ab	4,070b	580a	46.8b
BES <sup>f</sup> cv. Super	830ab	110a	710ab	890ab	320a	48.3ab
BP cv. Gipha	10,230cd	4,570b	1,950ab	7,240bc	1,905a	46.0b
RC cv. Florex	14,450cd	11,480bc	3,980b	12,880c	3,020a	38.4c
SB cv. Proteus	22,390d	20,420c	3,630b	6,460bc	2,190a	45.3b
Contrasts <sup>g</sup>	A,B,C	A,C	A,C	A,C,D		A,C,D,E

<sup>a</sup> Primarily *Pratylenchus penetrans*.<sup>b</sup> MG = marigold, ARG = annual ryegrass, MF = meadow fescue, BES = black-eyed Susan, BP = bee plant, RC = red clover, SB = soybean.<sup>c</sup> First and second columns are preplant and fall samples, respectively.<sup>d</sup> Canada No. 1 (58 - 88 mm).<sup>e</sup> Antilog means in a column followed by the same letter are not different ( $P \leq 0.05$ ) according to Duncan's multiple-range test.<sup>f</sup> *Tagetes patula* ssp. *nana* (unidentified cultivar).<sup>g</sup> Significant differences ( $P \leq 0.05$ ) between: A = mean of marigold cultivars vs. mean of other crops; B = *T. patula* ssp. *nana* vs. mean of other marigold cultivars; C = mean of annual ryegrass, meadow fescue, and black-eyed Susan vs. mean of bee plant, red clover, and soybean; D = bee plant vs. mean of red clover and soybean; E = red clover vs. soybean.

crops in the number of nematodes extracted from potato roots in the subsequent crop.

#### DISCUSSION

The marigold cultivars evaluated in this study were either poor hosts or perhaps antagonistic to *P. penetrans*. This is in agreement with observations from field studies in Holland (Oostenbrink, 1960) and Connecticut (Miller and Ahrens, 1969). In addition, similarity among the different marigold cultivars agreed with previous observations in the greenhouse (El-Zawahry et al., 1998), and our results indicating that red clover and soybean were good hosts for *P. penetrans* agreed with previous data from this region (Kimpinski, 1984; Kimpinski et al., 1984). The improvement in potato yield after marigold was also similar to results reported by others (Oostenbrink, 1960).

In this study, the 3-year average when potatoes were planted after soybean and red clover was approximately 2,600 root-lesion nematodes per kg of soil (derived from Table 1). This was only slightly greater than threshold values established for root-lesion nematode economic damage in potatoes (Kimpinski and McRae, 1988). Previous work with nematicides when populations at planting were 10,000 to 20,000 root-lesion nematodes per kg of soil improved potato yields by 25% (Kimpinski, 1986; Kimpinski and McRae, 1988; Kimpinski and Sanderson, 1989). If the initial root-lesion nematode populations had been higher in this study, the yield differences between marigold and red clover or soybean might have been greater.

The negative effect of the marigold cultivars on root-lesion nematodes is presumed to be caused by the nematicidal action of thiophenic compounds within the roots (Oostenbrink et al., 1957; Uhlenbroek and Bijloo, 1958; Chitwood, 1992). When tested in the laboratory or field, these compounds are often as potent as synthetic nematicides (Oostenbrink et al., 1957; Gommers, 1973). The presence of these compounds makes the marigold crop a useful tool in the management of endoparasites such as root-

lesion nematodes (Gommers and Bakker, 1988).

Currently, marigolds are of limited economic value to growers in the Maritime region of Canada, so a broad range of plant groups should be included in future work seeking an effective cover crop. Recently, Ferris and Zheng (1999) reported that aqueous extracts from 73 plant species killed either root-knot nematodes or root-lesion nematodes, or both. Because the potential exists to find an effective cover crop for root-lesion nematode management in the Maritime region, and because of the lack of a safe synthetic nematicide, continuing the search for natural plant products with nematicidal properties is justified.

#### LITERATURE CITED

- Alexander, S., C. Wladenmaier, and S. Sriharan. 1999. Reduction of soil lesion nematode, *Pratylenchus penetrans*, by marigold as a sequential crop in rotation with tomato and potato. Program Guide and Abstracts, American Society of Parasitologists and the Society of Nematologists, Monterey, CA, July 6–9, 1999. Pp. 150–151.
- Anonymous. 1987. Concerns about aldicarb. Pesticide information, vol. 9 (1), Scientific Information Retrieval Section, Research Branch, Agriculture Canada, Ottawa.
- Atlantic Canada Potato Guide. 1993. Publication 1300/93. Agdex 257/13, Atlantic Provinces Agriculture Services Steering Committee.
- Atlantic Provinces Field Crop Guide. 1991. Publication No. 100. Agdex 100.32, Atlantic Provinces Agricultural Services Coordinating Committee.
- Barker, K. R. 1985. Nematode extraction and bioassays. Pp. 19–35 in K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise on *Meloidogyne*, vol. 2. Methodology. Raleigh, North Carolina: North Carolina State University Graphics.
- Chitwood, D. J. 1992. Nematicidal Compounds from plants. Pp. 185–204 in H. N. Nigg and D. Seigler, eds. Phytochemical resources for medicine and agriculture. New York: Plenum Press.
- El-Zawahry, A. M., T. H. A. Olthof, and J. W. Potter. 1998. The nematicidal effects of *Tagetes* spp. on the final population of *Pratylenchus penetrans*. *International Journal of Nematology* 8:117–122.
- Ferris, H., and L. Zheng. 1999. Plant sources of Chinese herbal remedies: Effects on *Pratylenchus vulnus* and *Meloidogyne javanica*. *Journal of Nematology* 31:241–263.
- Genstat, 1993. Genstat 5 Committee of the Statistics Department, Genstat 5 (Release 3). Rothamsted Experimental Station, Oxford Science Publications, Oxford, UK: Clarendon Press.

- Gommers, F. J. 1973. Nematicidal principles in *Compositae*. Mededelingen Landbouwhogeschool 73 (17):ii + 71.
- Gommers, F. J., and J. Bakker. 1988. Physiological diseases induced by plant responses or products. Pp. 3–22 in G. O. Poinar and H. B. Jansson, eds. Diseases of nematodes, vol. I. Boca Raton, FL: CRC Press.
- Hackney, R. W., and O. J. Dickerson. 1975. Marigold, castor bean, and chrysanthemum as controls of *Meloidogyne incognita* and *Pratylenchus alleni*. Journal of Nematology 7:84–90.
- Hooper, D. J. 1986. Extraction of nematodes from plant material. Pp. 51–58 in J. F. Southey, ed. Laboratory methods for work with plant and soil nematodes. Reference Book 402, Ministry of Agriculture, Fisheries, and Food. London: Her Majesty's Stationery Office.
- Kimpinski, J. 1984. Plant-parasitic nematodes in soybeans. Forage Notes 28:21–23.
- Kimpinski, J. 1986. Effects of aldicarb and oxamyl on *Pratylenchus penetrans* and potato yields. Canadian Journal of Plant Pathology 8:189–192.
- Kimpinski, J., H. T. Kunelius, and C. B. Willis. 1984. Plant-parasitic nematodes in temperate forage grass and legume species in Prince Edward Island. Canadian Journal of Plant Pathology 6:160–164.
- Kimpinski, J., and K. B. McRae. 1988. Relationship of yield and *Pratylenchus* spp. population densities in Superior and Russet Burbank potato. Annals of Applied Nematology 2:34–37.
- Kimpinski, J., and J. B. Sanderson. 1989. Effects of aldicarb, aldoxycarb, and oxamyl on potato tuber yields and root lesion nematodes. Canadian Journal of Plant Science 69:611–615.
- Ko, M. P., and D. P. Schmitt. 1996. Changes in plant-parasitic nematode populations in pineapple fields following inter-cycle cover crops. Journal of Nematology 28:546–556.
- McSorley, R., and J. J. Frederick. 1994. Response of some common annual bedding plants to three species of *Meloidogyne*. Supplement to Journal of Nematology 26(4S):773–777.
- Miller, P. M., and J. F. Ahrens. 1969. Influence of growing marigolds, weeds, two cover crops, and fumigation on subsequent populations of parasitic nematodes and plant growth. Plant Disease Reporter 53:642–646.
- Oostenbrink, M. 1960. *Tagetes patula* L. als voorvruchtvan enkele land- en tuinbouwgewassen op zand- en dalgrond. Mededelingen van de Landbouwhogeschool en de Opzoekingsstations van de Staat te Gent, 25:1065–1075. Helminthological Abstracts, vol. 30, No. 2387.
- Oostenbrink, M., K. Kuiper, and J. J. s' Jacob. 1957. *Tagetes* als Feindpflanzen von *Pratylenchus*-Arten. Nematologica 2(S):424–433.
- Ploeg, A. T. 1999. Greenhouse studies on the effect of marigolds (*Tagetes* spp.) on four *Meloidogyne* species. Journal of Nematology 31:62–69.
- Prince Edward Island Department of Agriculture and Forestry. 1998. 1997 Agricultural Statistics, vol. 31.
- Riga, E., and J. W. Potter. 1998. Marigolds as biological control agents of plant nematodes. Nematologica 44:568–569 (Abstr.).
- SAS Institute Inc. 1985. SAS procedures guide for personal computers, version 6 edition. SAS Institute Inc.: Cary, NC.
- Sieczka, J. B., D. D. Moyer, B. B. Brodie, and J. M. Kossowski. 1991. Marigold rotation studies on Long Island. Cornell University, New York State IPM Publication 112:132–137.
- Snedecor, G. W., and W. G. Cochran. 1989. Statistical methods. Ames, Iowa: Iowa State University Press.
- Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. New York: McGraw-Hill.
- Uhlenbroek, J. H., and J. D. Bijloo. 1958. Investigations on nematocides. II. Structure of a second nematocidal principal isolated from *Tagetes* roots. Recueil des Travaux Chimiques Pays-Bas 78:382–390.