EFFECT OF FALLOW AND ROOT DESTRUCTION ON SURVIVAL OF ROOT-KNOT AND ROOT-LESION NEMATODES IN INTENSIVE VEGETABLE CROPPING SYSTEMS

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ABSTRACT

C. Ornat, S. Verdejo-Lucas, F. J. Sorribas, and E. A. Tzortzakakis. 1999. Effect of fallow and root destruction on survival of root-knot and root-lesion nematodes in intensive vegetable cropping systems. Nematropica 29:5-16.

Changes in population densities of root-knot nematodes after short-term clean-fallow periods between successive crops were estimated in intensive vegetable cropping systems for greenhouse and outdoor crops. The effect of root destruction during fallow on population decline of *Meloidogyne arenaria* race 2 and *Pratylenchus neglectus* was also determined following a greenhouse-grown bean crop. The average rate of root-knot nematode survival between subsequent crops was 0.50. Survival rates were negatively correlated with increased initial population densities but were not correlated with the length of fallow periods. Fallowing combined with root destruction was more effective (P = 0.05) than fallowing alone in reducing population densities of *M. arenaria* race 2 and *P. neglectus*.

Key words: Meloidogyne arenaria, nematode decline, *Pasteuria* group, population dynamics, *Pratylenchus neglectus*, vegetable crops.

RESUMEN

C. Ornat, S. Verdejo-Lucas, F. J. Sorribas y E. A. Tzortzakakis. 1999. Efecto del barbecho y la destrucción de raíces en la supervivencia de nematodos agalladores y lesionadores en agricultura intensiva. Nematrópica 29:5-16.

Se determinó los cambios en las densidades de población de nematodos agalladores después de periodos cortos de barbecho entre cultivos sucesivos en sistemas agrícolas de producción intensiva de invernadero y aire libre. También se determinó el efecto de la destrucción de las raíces sobre la declinación de las poblaciones de *Meloidogyne arenaria* raza 2 y *Pratylenchus neglectus* después de un cultivo de judía de invernadero. La supervivencia media de los nematodos agalladores fue de 0,5 entre cultivos sucesivos. La tasa de supervivencia estaba correlacionada negativamente con las densidades iniciales de población, pero no se encontró correlación con los periodos de barbecho. La reducción de las densidades de población de *M. arenaria* raza 2 y *P. neglectus* en las parcelas donde se combinó el barbecho con la destrucción de las raíces fue mayor (P = 0.05) que en las parcelas en las que sólo se realizó barbecho.

Palabras claves: Cultivos hortícolas, declinación, dinámica de poblaciones, grupo *Pasteuria, Meloidogyne arenaria, Pratylenchus neglectus.*

INTRODUCTION

Vegetable crops are grown intensively for fresh market in the Mediterranean basin. These intensive production systems are feasible because the climate is characterized by mild winters and warm summers. Growers have developed specialized crop sequences that provide marketable crops that are economically attractive, and that take advantage of suitable geographic locations within the Mediterranean basin. Usually, two to three crops are cultivated at the same site from spring through winter, hence there is little time left between crops. The reasons for intensive cropping frequency are mainly economic and include the high cash values of the crops and the need to ensure farm subsistence by providing income to the farm year round.

The nematodes that cause the most serious damage in horticultural crops are the root-knot nematodes, *Meloidogyne* spp., of which, *M. javanica* (Treub) Chitwood, *M. incognita* (Kofoid & White) Chitwood and *M. arenaria* (Neal) Chitwood are the most common species in the Mediterranean basin (Lamberti, 1981; Ibrahim, 1985). They cause considerable yield losses in many vegetable, ornamental and fruit crops, in both the field and the greenhouse. Root-knot nematodes have become increasingly important in greenhouse-grown vegetable crops due to the damage they cause and their rapid spread in several countries of the Mediterranean region including Cyprus (Philis, 1990), Morocco (Eddaoudi *et al.*, 1997), Greece (Tzortzakakis, 1997) and Spain (Verdejo-Lucas *et al.*, 1997a). At present, fumigant and non-fumigant nematicides are used for nematode control on vegetable crops.

Cultural methods of control can be used to reduce nematode population densities. Fallowing is a simple method which reduces nematode numbers by starvation, and fallowing coupled with crop destruction generally gives significant and immediate impact on total nematode population densities in soil (Noling and Becker, 1994). Post-harvest reproduction by nematodes can be prevented by mechanically removing root systems from soils and exposing them to drying effects of sun and wind. Nematode densities will continue increasing if roots are not destroyed. However, fallow periods in intensive cropping systems are limited to only a few weeks between subsequent crops. This time coincides with the period before planting when fields are being prepared for the next crop.

There are numerous reports on the overwinter dynamics of *Meloidogyne* spp. in cropping systems involving a single crop per season which is planted in spring and harvested in autumn. In these systems, a negative exponential function between the initial population (post-harvest population) and the overwinter survival has been described (Ferris, 1985; Starr and Jeger, 1985). Nematode survival rate is affected by several factors including size of population, geographical location, previous crop, season and type of soil (De Guiran and Germani, 1980; Ferris, 1985; Windham and Barker, 1988). Soil desiccation and direct heat from the sun may have an immediate impact on population decline, during short-term fallow periods in intensive agriculture.

We are currently evaluating methods of reducing nematode-related problems that are effective and not disruptive to normal farming practices, in order that they be easily accepted by growers and implemented in the field. Previous studies have assessed the use of resistant tomato cultivars and their effect on the following crop (Ornat *et al.*, 1997), the application of the bacterial parasite, *Pasteuria penetrans* Sayre and Starr alone and in combination with non-fumigant nematicides, and the use of solarization (Tzortzakakis and Gowen, 1994). In accordance with these goals, the objectives of this study were first, to study the effect of changes in population densities after short-term fallow periods between subsequent crops in intensive cropping systems, and second, to determine the effect of root destruction in combination with fallow on nematode population densities.

The population densities of microorganisms that parasitize nematodes may fluctuate according to host abundance (Verdejo-Lucas, 1992). Parasitism of *Pratylenchus neglectus* by *Pasteuria* spp. has been found to occur in Germany (Sturhan, 1985), Italy and Croatia (Ciancio *et al.*, 1994), but has not been reported in Spain (Verdejo-Lucas *et al.*, 1997b). However, members of the *Pasteuria* group were recently found parasitizing *P. neglectus* in the greenhouse where the root destruction study was conducted (unpublished data). Therefore, the effect of bean root destruction on incidence of nematodes with spores and on the number of spores attached per nematode was also investigated in this study.

MATERIALS AND METHODS

Fallowing. Changes in population densities after fallow periods ranging from 5-15 weeks were determined in ten commercial vegetable production sites (six greenhouses and four fields) during seven growing seasons in the province of Barcelona, Spain (Table 1). During fallow periods, roots from the previous crop were left undisturbed in the ground after removing the tops manually. Most weed growth was prevented by withholding irrigation during fallowing. If weeds emerged (usually in fields in response to rainfall), they were managed by applying contact herbicides. Usually, pre-emergence herbicides were applied at the end of the fallow period before planting the following crop.

Sites located in El Maresme (sites M-) and Baix Llobregat (sites B-) counties were known to be infested by root-knot nematodes, and the species of *Meloidogyne* present in each site had been previously identified (Sorribas and Verdejo-Lucas, 1994; Ornat and Verdejo-Lucas, 1999). Data were collected in consecutive growing seasons during 1991-1993 and 1994-1997. Sites were sampled during the summer fallow after the spring crop and during the winter fallow after the autumn crop. Composite soil samples were collected from the top 30 cm soil layer with an auger (2.5 cm diam) at the beginning (initial population) and at the end (final population) of the fallow periods. Each sample consisted of 25-30 cores taken at random following a zig-zag pattern. Soil cores were thoroughly mixed and nematodes in two 250 cm^3 soil subsamples were extracted by the centrifugal-flotation method (Jenkins, 1964). Data were subjected to regression analysis to determine the relationship between rate of survival (log Pf/Pi) and initial population densities (log Pi), and rate of survival and length of the fallow periods (weeks). To determine the effects of crops grown under field or greenhouse conditions, and of winter or summer fallow, data on survival rate were transformed to arcsine square root and analyzed by Student's t-Test.

Tillage. The study was carried out in summer 1997 in an unheated plastic greenhouse of 1 300 m^2 located in Argentona, Barcelona, Spain. This commercial greenhouse was selected because it had a history of root-knot nematode problems (Ornat, 1998). The soil was infested by *M. arenaria* race 2, as well as, *Pratylenchus neglectus* (Rensch). Soil texture was a sand (88.3% sand, 6.4% loam, 5.3% clay) with a pH of 7.6. The previous two crop cycles had been tomatoes (*Lycopersicon esculentum* Mill.) from spring to summer and lettuce (*Lactuca sativa* L.) in autumn-winter. French bean (*Phaseolus vulgaris* L.) cv. Iluro was hand-sown before the initiation of this study. After the last harvest, tops were cut manually at ground level following local growers' practices. Plots were established in areas of the greenhouse infested by both nematodes as shown by

Table 1. Population densities and rate of survival of root-knot nematodes at ten sites after short-fallow periods between two subsequent crops in greenhouse and field grown vegetables crops.

Type of cultivation	Fallow season	Fallow period		Soil Texture	Site[w]	Species Meloidogyne	Previous crop[x]	Juveniles/250cm³ soil[y]		Rate of Survival[z]	Average rate of Survival
		Time (wk)	Year					P. initial	P. final		
Greenhouse	Summer fallow	7	1995	Sandy loam	M-27	*M. javanica*	Tomato S	850	452	0.53	
		7	1996	Sandy loam	M-28a	*M. incognita*	Tomato S	39	32	0.82	
		7	1996	Sandy loam	M-28b	*M. incognita*	Tomato S	37	33	0.89	
		8	1994	Sandy loam	M-27	*M. javanica*	Tomato R	720	430	0.60	
		10	1994	Sand	M-04	*M. arenaria*	Tomato S	530	330	0.62	
		10	1995	Sandy loam	M-28	*M. incognita*	Tomato S	659	407	0.62	
		10	1997	Sand	M-04	*M. arenaria*	Tomato R	33	31	0.94	
		10	1997	Sand	M-04	*M. arenaria*	Tomato S	455	219	0.48	
		12	1996	Sandy loam	M-27	*M. javanica*	Tomato S	32	14	0.44	
	Average										0.66
	Winter fallow	4	1995	Sandy loam	M-27	*M. javanica*	Radish	290	276	0.95	
		8	1994	Sand	M-05	*M. javanica*	Lettuce	157	80	0.51	
		8	1994	Sand	M-38	*M. incognita*	Lettuce	475	198	0.42	
		8	1995	Sand	M-04	*M. arenaria*	Lettuce	254	144	0.57	
		8	1995	Sandy loam	M-35	*M. incognita*	Swischard	460	183	0.40	
		9	1995	Sandy loam	M-28	*M. incognita*	Garlic	330	254	0.77	
	Average										0.61

Table 1. (Continued) Population densities and rate of survival of root-knot nematodes at ten sites after short-fallow periods between two subsequent crops in greenhouse and field grown vegetables crops.

Type of cultivation	Fallow season	Fallow period		Soil Texture	Site[w]	Species Meloidogyne	Previous crop[x]	Juveniles/250cm^3 soil[y]		Rate of Survival[z]	Average rate of Survival
		Time (wk)	Year					P. initial	P. final		
Field	Summer fallow	5	1993	Loamy sand	B-26	*M. javanica*	Tomato S	1 482	1 104	0.74	
		6	1995	Loamy sand	M-72	*M. arenaria*	Green pepper	371	270	0.73	
		6	1996	Loamy sand	M-77	*M. javanica*	Green pepper	75	72	0.96	
		7	1995	Loamy sand	M-77	*M. javanica*	Broad bean	3 861	480	0.12	
		7	1996	Loamy sand	M-72	*M. arenaria*	Broad bean	1 927	372	0.19	
		8	1993	Sandy loam	B-32	*M. incognita*	Tomato R	2 790	59	0.02	
		15	1992	Loamy sand	B-26	*M. javanica*	Cauliflower	270	120	0.44	
	Average										0.46
	Winter fallow	5	1991	Loamy sand	B-26	*M. javanica*	Tomato S	748	144	0.19	
		5	1993	Loamy sand	B-26	*M. javanica*	Garlic	136	31	0.23	
		6	1994	Loamy sand	M-72	*M. arenaria*	Tomato S	55	44	0.80	
		6	1995	Loamy sand	M-77	*M. javanica*	Onion	59	37	0.63	
		9	1994	Loamy sand	M-77	*M. javanica*	Tomato S	456	189	0.41	
		9	1995	Loamy sand	M-72	*M. arenaria*	Onion	50	26	0.52	

Table 1. (Continued) Population densities and rate of survival of root-knot nematodes at ten sites after short-fallow periods between two subsequent crops in greenhouse and field grown vegetables crops.

Type of cultivation	Fallow season	Fallow period		Soil Texture	Site[w]	Species Meloidogyne	Previous crop[x]	Juveniles/250cm³ soil[y]		Rate of Survival[z]	Average rate of Survival
		Time (wk)	Year					P. initial	P. final		
		12	1992	Sandy loam	B-32	*M. incognita*	Pumpkin	1 360	783	0.58	
		15	1991	Sandy loam	B-32	*M. incognita*	Tomato R	455	90	0.20	
	Average										0.45
Overall means								647	230	0.54	

[w]Site M-: El Maresme county; site B-: Baix Llobregat county.
[x]Tomato S: Susceptible cultivar; Tomato R: F1 hybrid carrying the Mi resistant gene.
[y]Data are the mean of two subsamples from each site.
[z]Final population/initial population.

nematode analyses, and the experimental design was randomized block with two experimental units (plots) of 12 m^2 (4 × 3 m) per block and four replications. On 17 July 1997, four plots were tilled with a man-driven rototiller to a depth of about 25 cm to destroy bean roots, and another four were left untilled. This implement has an axis with blades that plow the soil and turn it over in the same process. Composite soil samples were collected from each plot just before the tillage treatment and at weekly-intervals during a 2 month fallow period (17 July-12 September, 1997). Each soil sample consisted of 5 soil cores taken at random from the top 30 cm soil layer with an auger (2.5 cm diam). Soil cores were thoroughly mixed and nematodes were extracted from a 500 cm^3 subsample using Baermann trays. Nematodes migrating to the clean water were collected one week later and counted. Initial population densities of each endoparasitic nematode and nematode densities at each sampling date were log transformed (x + 1) and compared by Student's t-Test. To determine the influence of fallow versus fallow + tillage on nematode survival, data were subjected to analysis of covariance in which the initial population was the covariate. The relationship between survival rate and time after harvest in plots receiving fallow and fallow + tillage was determined by regression analysis.

To determine the effect of bean root destruction on incidence of *P. neglectus* with spores of the *Pasteuria* group and the number of spores attached per nematode, a random sample of 20 nematodes from tilled and untilled plots were examined at 400× at each sampling time starting on 31st July 1997. Data were subjected to analysis of covariance in which numbers of *P. neglectus* was the covariate to determine the influence of fallowing versus fallowing + tillage on bacterial parasitism.

RESULTS

Fallowing. The numbers of root-knot nematode juveniles declined in all sites and seasons during the short fallow period between two subsequent crops, and the average survival rate was 0.54 (Table 1). Averages of the initial population densities tended to be higher in fields (940 juveniles/250 cm^3 soil) than in greenhouses (355 juveniles/250 cm^3 soil). Survival rate and initial population densities were correlated negatively (r^2 = 0.3), and there was no significant correlation with the length of fallow periods (5-15 weeks). The species of *Meloidogyne* infesting the sites, previous crop, host resistance, and soil texture had no significant effect on nematode decline. No differences were detected in nematode survival following summer or winter fallow in either greenhouses or fields. However, survival rate tended to be higher ($P = 0.05$) in greenhouses than in fields (64% vs 45%). Nematode decline in fields was more variable than in greenhouses and ranged from 0.02-0.96 and 0.40-0.95, respectively.

Tillage. Survival dynamics of *M. arenaria* race 2 and *P. neglectus* were similar to one another in both tilled or untilled soils after a 2-month-fallow period (Fig. 1 A and B). In tilled soils, initial population densities of *M. arenaria* race 2 and *P. neglectus* were 1 660 ± 747 and 493 ± 84 nematodes/250 cm^3 soil, and final densities were 265 ± 77 and 48 ± 11 nematodes/250 cm^3 soil, respectively. In untilled soils, average initial densities of *M. arenaria* race 2 and *P. neglectus* changed from 1 004 ± 688 and 489 ± 262 nematodes/250 cm^3 soil, to final densities of 307 ± 112 and 158 ± 90 nematodes/250 cm^3 soil, respectively. Fallowing combined with tillage reduced population densities of both endoparasitic nematodes more ($P = 0.05$) than fallowing alone. Significant differences ($P = 0.05$) in survival of

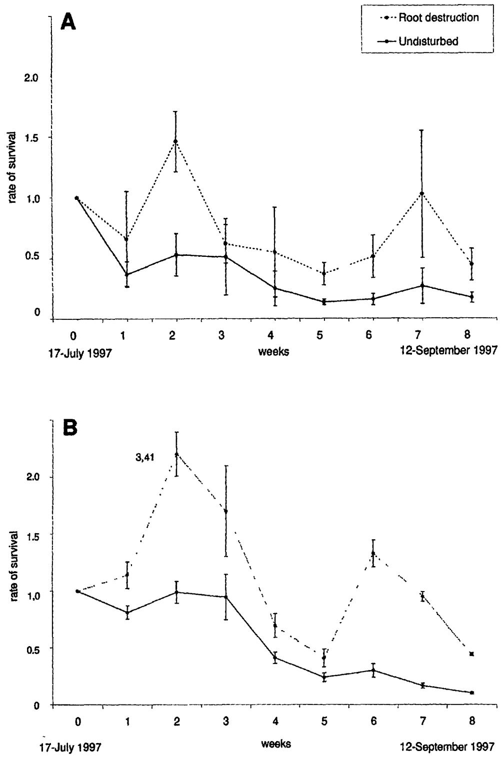

Fig. 1. Rate of survival, compared to initial population densities, of A) *Meloidogyne arenaria* race 2 and B) *Pratylenchus neglectus* during a 2-month-fallow period following a spring crop of French beans. Plots were rototilled to destroy crop roots or were left untilled. Each value is the mean from four replicated plots. Bars represent standard errors of the mean.

M. arenaria race 2 were observed in tilled but not in untilled soils in the 4th and following weeks of fallow. Similarly, the survival rates of *P. neglectus* were lower (P = 0.05) in the 5, 7, and 8th week of fallow in tilled but not in untilled soils with one exception; densities increased (P = 0.05) the 2nd week of fallow in these plots. Correlation analyses showed that the numbers of *M. arenaria* race 2 and *P. neglectus* decreased as the length of time after harvest increased in tilled soils (r^2 = 0.62 and 0.84, respectively, P = 0.01). Population density and time after harvest were not correlated for either nematode in untilled soils.

Spores of the *Pasteuria* group were found on the cuticle of all vermiform stages of *P. neglectus* on all sampling dates. Spores were found filling the body cavity of 4th stage juveniles and females only in the 2nd and 3rd weeks of fallow. More than 75% of the root lesion nematodes with spores had 1 to 2 spores attached. This *Pasteuria* population appeared to be host specific for *P. neglectus* because no second-stage juveniles of *M. arenaria* race 2 with attached spores were observed on any of the sampling dates. The percentage of juveniles with spores was higher (P= 0.05) in untilled soils than in tilled ones regardless of numbers of root-lesion nematodes (Table 2).

DISCUSSION

Overall, population decline of *Meloidogyne* during the short fallow periods studied was ≈ 50%, and was somewhat greater in the field than in greenhouses. Higher average initial population density of nematodes in fields than in greenhouses may have contributed to the difference in survival, because the survival rate was related to the size of the population at the beginning of the fallow period. Density dependent survival of root-knot nematodes has also been reported to occur in infested fields in California (Ferris, 1985) and Texas (Starr and Jeger, 1985). Densities of *Meloidogyne* declined in all sites, but the rate of survival was highly variable as reported by others (Ferris, 1985; Starr and Jeger, 1985; Windham and Barker, 1988; Johnson and Motsinger, 1990). Population densities of plant parasitic nematodes generally exhibit overwinter decline in cropping systems where a plant host is absent for about 6 months (Kinloch, 1982; Ferris, 1985; Goodell *et al.*, 1983; Starr and Jeger, 1985). Although populations declined significantly in our surveys, the population change under fallow in the tillage experiment was

Table 2. Population densities[X] of *Pratylenchus neglectus* and percentage of individuals with spores of members of the *Pasteuria penetrans* group adhering to the nematode cuticle.

	Pratylenchus neglectus			
	Tilled plots[Y]		Untilled plots	
Week[Z]	Nematodes/ 250 cm^3 soil	Percentage with spores	Nematodes/ 250 cm^3 soil	Percentage with spores
1	508 ± 249	6	1 146 ± 247	60
2	452 ± 359	20	485 ± 150	17
3	192 ± 77	42	207 ± 61	20
4	121 ± 66	25	195 ± 316	55
5	151 ± 108	20	316 ± 78	25
6	78 ± 23	15	263 ± 76	50
7	48 ± 11	10	158 ± 90	15
Average	221	19.7	396	34.6

[X]Nematode values are means from four replicated plots. Spores counted on a random sample of 20 nematodes.
[Y]Plots tilled with a rototiller after the last harvest to destroy plant roots on 17 July 1997.
[Z]Beginning 31 July, 1997.

not significant. This was likely due to the short fallow period (two months) and demonstrates that such short intervals may not provide sufficient suppression of root-knot nematodes, which are capable of survival over long periods (Johnson and Motsinger, 1990; Roberts *et al.*, 1981). Because root-knot nematodes are key pests (Thomason and Caswell, 1987) for vegetable production in Spain (Verdejo-Lucas *et al.*, 1997a, Ornat *et al.*, 1998), additional control measures such as the use of nematicides and nematode resistant crop varieties may be required for effective nematode management in heavily infested soils. Population densities after winter fallow periods would be more critical than those after the summer fallow since soil temperatures will increase from spring through summer and will accelerate nematode development and reproduction increasing the damage potential to the crop.

Fallowing is a viable tactic for nematode suppression only if weed-free conditions are maintained during the fallow period. In north-eastern Spain, 46 plant species of weeds commonly associated with vegetable crops have been identified as hosts of root-knot nematodes (Barcelo *et al.*, 1997). Hence, weed management will be necessary to maintain clean fallow conditions in order to prevent root-knot nematode reproduction. Other plant-parasitic nematodes including *P. neglectus* are present in Spanish vegetable fields but their frequency of occurrence and abundance is low (Ornat, 1998). The host status of weeds to these nematodes is unknown.

Fallowing combined with root destruction of French bean was more effective than fallowing alone for population reduction of both endoparasitic nematodes in this study. This effect occurred after the 4th and 5th weeks of fallow on root-knot and root-lesion nematodes, respectively. In practice, this is the minimum time growers need to prepare the soil for next crop. In the absence of a plant host, environmental conditions such as temperature and moisture are the major factors affecting survival rate of nematodes (Bergeson, 1959; Goodell and Ferris, 1989; Roberts *et al.*, 1981; Towson and Apt, 1983). Although environmental data were not collected in this study, changes in soil temperature and moisture conditions associated with soil disturbance was probably responsible for the additional reduction in nematode numbers that occurred in tilled soils. Roots were shredded and exposed to dry conditions and moisture levels appeared to be reduced which likely accelerated the reduction of both nematodes. Soil desiccation effectively reduced numbers of eggs and juveniles of *M. javanica* (Towson and Apt, 1983). Peaks in root-knot and root-lesion nematode densities that occurred in untilled soils could be interpreted as an increase in the number of root-knot nematodes hatching from eggs as reported by Starr and Jeger (1985).

The effect of the *Pasteuria* group on *P. neglectus* was unclear. A density dependent relationship between *Pasteuria* and its nematode host has been reported (Spaull, 1984, Ciancio, 1995), and percent parasitism was correlated with seasonal fluctuations in nematode populations (Verdejo-Lucas, 1992). Environmental conditions suggested above as responsible for the additional population decline of nematodes in tilled soils may also be detrimental to the infection of *P. neglectus* by *Pasteuria* sp. The nematode tended to decline more rapidly in tilled than untilled soils, as did the proportion of the population with attached spores, probably due to changes in soil temperature and moisture conditions. This is the first report of naturally occurring parasitism of *Pratylenchus neglectus* by members of the *Pasteuria* group in Spain.

Although a variety of tactics are available to manage plant-parasitic nematodes (Noling and Becker, 1994, Duncan and Noling, 1998), many are only partially effective, so that a combination of methods are often required to achieve economic control (Roberts, 1993). The results presented here emphasize the importance of an integrated approach to nematode control. Two means of controlling nematodes, fallowing and root destruction, resulted in greater reduction in population densities when used in combination. Fallowing has long been recognized as a means of reducing nematode populations (Noling and Becker, 1994) and, under some conditions, it is also effective in reducing yield losses (Weaver *et al.*, 1995). Root destruction immediately after the last harvest is a practice that can be easily achieved in greenhouse grown vegetable crops.

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