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Compatibility of Soil Amendments with Entomopathogenic Nematodes

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Abstract: The impact of inorganic and organic fertilizers on the infectivity, reproduction, and population dynamics of entomopathogenic nematodes was investigated. Prolonged (10- to 20-day) laboratory exposure to high inorganic fertilizer concentrations inhibited nematode infectivity and reproduction, whereas short (1-day) exposures increased infectivity. *Heterorhabditis bacteriophora* was more sensitive to adverse effects than were two species of *Steinernema.* In field studies, organic manure resulted in increased densities of a native population of *Steinernema feltiae,* whereas NPK fertilizer suppressed nematode densities regardless of manure applications. Inorganic fertilizers are likely to be compatible with nematodes in tank mixes and should not reduce the effectiveness of nematodes used for short-term control as biological insecticides, but may interfere with attempts to use nematodes as inoculative agents for long-term control. Organic manure used as fertilizer may encourage nematode establishment and recycling.

Key words: amendments, biological control, entomopathogenic nematode, fertilizer, *Heterorhabditis bacteriophora,* manure, *Steinernema feltiae, Steinernema anomali.*

Entomopathogenic nematodes are used for the biological control of soil-inhabiting insect pests of turfgrass, ornamentals, mushrooms, artichokes, citrus, cranberries, and apples. Nevertheless, nematodes tend to provide less consistent, reliable control than chemicals (Ehler, 1990; Georgis and Gaugler, 1991). Lack of predictability is partly a function of poor nematode persistence in the soil (Bednarek, 1990). As little as 10% of the initial inoculum may be present in the soil seven days after application (Curran, 1993). Predators (Epsky et al., 1988) and pathogens (Poinar, 1988; Poinar and Jansson, 1986) are known to attack entomopathogenic nematodes, but inadequate persistence is most frequently attributed to abiotic factors (Georgis and Gaugler, 1991; Kaya, 1990).

The impact of many aspects of the abiotic soil environment, particularly soil moisture (Georgis and Gaugler, 1991; Molyneux and Bedding, 1984), temperature (Kung et al., 1991) and structure (Georgis and Poinar, 1983; Molyneux and Bedding, 1984) on entomopathogenic nematode survival and efficacy, has been documented (Kajak et al., 1991). Cultural practices in agriculture other than irrigation (Georgis and Gaugler, 1991) and chemical pesticide incompatibilities (Kamionek, 1991; Rao et al., 1975) have rarely been studied.

The addition of organic and inorganic soil amendments to improve soil fertility and plant growth is among the oldest of agricultural practices. Many of these amendments have nematicidal effects (Muller and Gooch, 1982; Rodríguez-Kábana, 1986). Georgis and Gaugler (1991) recognized the potential of soil amendments to limit the efficacy of entomopathogenic nematodes when they encouraged researchers to record the recent history of fertilizer use in field test plots, but supporting research has not been recorded. Our study assessed the effects of fertilizers on nematode infectivity, reproduction, and native field populations.

MATERIALS AND METHODS

Cultures: All nematode strains used in the laboratory portion of the study were isolated in Poland during the 1980s. The strains were maintained in the laboratory at 25 °C in larvae of the greater wax moth, *Galleria mellonella.* Infective juveniles were less than 2 weeks old when tested.

Infectivity: The influence of NPK (nitrogen, potassium, phosphate) inorganic fertilizer and its mineral components on nematode infectivity was tested using *Steinernema feltiae* (strain Lov/86). Solutions of NPK fer-

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tilizer or its components were prepared in 2.5% agar, poured into 90-mm petri plates, and surface-sterilized with short-wave ultraviolet radiation. The chemicals and concentrations assayed included: calcium nitrate at 500, 1,000, and 1,500 mg/liter of water; acid potassium phosphate at 300, 600, and 900 mg/liter; potassium nitrate at 900, 1,800, and 2,700 mg/liter; and all three components combined at 1,700, 3,400, and 5,100 mg/liter. The lowest NPK dose is equivalent to the standard tank mix prepared for most European horticultural practices. Each treatment was replicated five times; controls were held in agar prepared with tap water. All dishes were sealed tightly with plastic film.

A 1-ml suspension of 1,000 infective juveniles of *S. feltiae* was introduced beneath the agar layer and onto a filter paper. The nematodes were exposed to the chemical treatments at 26 °C for 1, 10, or 20 days except for the calcium nitrate treatment, which was extended to 40 days. At the end of the test period, *G. mellonella* larvae were placed into the dish (10 larvae per dish). All larval mortality occurred within 48 hours. Four days after exposure, cadavers from each treatment were dissected and the number of nematodes established in the hemocoel was counted.

Further infectivity experiments were performed with three steinernematid and one heterorhabditid strains at a high NPK concentration. *Steinernema anomali* (strain Gelan), *S. feltiae* (strains Pol/76 and Lov/86), and *Heterorhabditis bacteriophora* (strain Pol/1981) were treated with 5,100 mg/liter of NPK, equivalent to three times the standard field rate. After 20 days, *G. mellonella* larvae were placed on the agar as described above.

Reproduction: The reproductive capacity of *S. feltiae* (strain Lov/86) treated with NPK fertilizer was determined in *G. mellonella* larvae. Infective juveniles were exposed as previously described for 20 days to the following high-concentration treatments: potassium nitrate (2,700 mg/liter), acid potassium phosphate (900 mg/liter), calcium nitrate (1,500 mg/liter), and NPK (5,100 mg/liter). At the end of the test period, 10 larvae were placed in each dish. After 48 hours, the resulting cadavers were transferred individually to 50-mm petri dish White traps at 26 °C. Infective juveniles emerging from each cadaver were counted over 16 days.

Field populations: The impact of fertilizers on native endemic populations of *S. feltiae* were determined from plots at the Wilanow-Obory experimental farm near Warsaw, Poland. Densities of *S. feltiae* in the light sandy clay loess soil were estimated from plots treated with standard agricultural rates of NPK fertilizer (80 and 160 kg/ha) annually for 20 years and continuing through our study. Crop rotation was potato, barley, wheat, and rape. In addition to NPK, some plots received 20 to 30 tons of animal manure per ha on alternate years. These inorganic and organic fertilizer applications provided the following six treatments: 0, 80, and 160 kg per ha of NPK fertilizer with or without manure.

Each treatment was replicated four times with 4-m^2 plots in a completely randomized design. Soil samples were taken three times (July 1985, April 1987, July 1988). Eight samples were collected from each plot to 25 cm deep with a soil borer (2 cm diam). The samples from each plot were mixed (approximately 800 g) and distributed over four pots. Two *G. mellonella* larvae were added to each pot (8 larvae per sample, 64 larvae per plot), and the pots were held at 26 °C for 16 days. The insects were replaced at four-day intervals, dead larvae were dissected, and infectivity was determined by recording the number of nematodes established in the host. The data were used to estimate *S. feltiae* populations according to the method of Bednarek and Nowicki (1991). Data were subjected to analysis of variance, and a Least Significant Difference test was used for means separation at $P < 0.05$.

Results

The infectivity of *S. feltiae* was significantly influenced by treatment of infective juveniles with NPK fertilizer and its components (Fig. 1). NPK (Fig. 1A), potassium nitrate

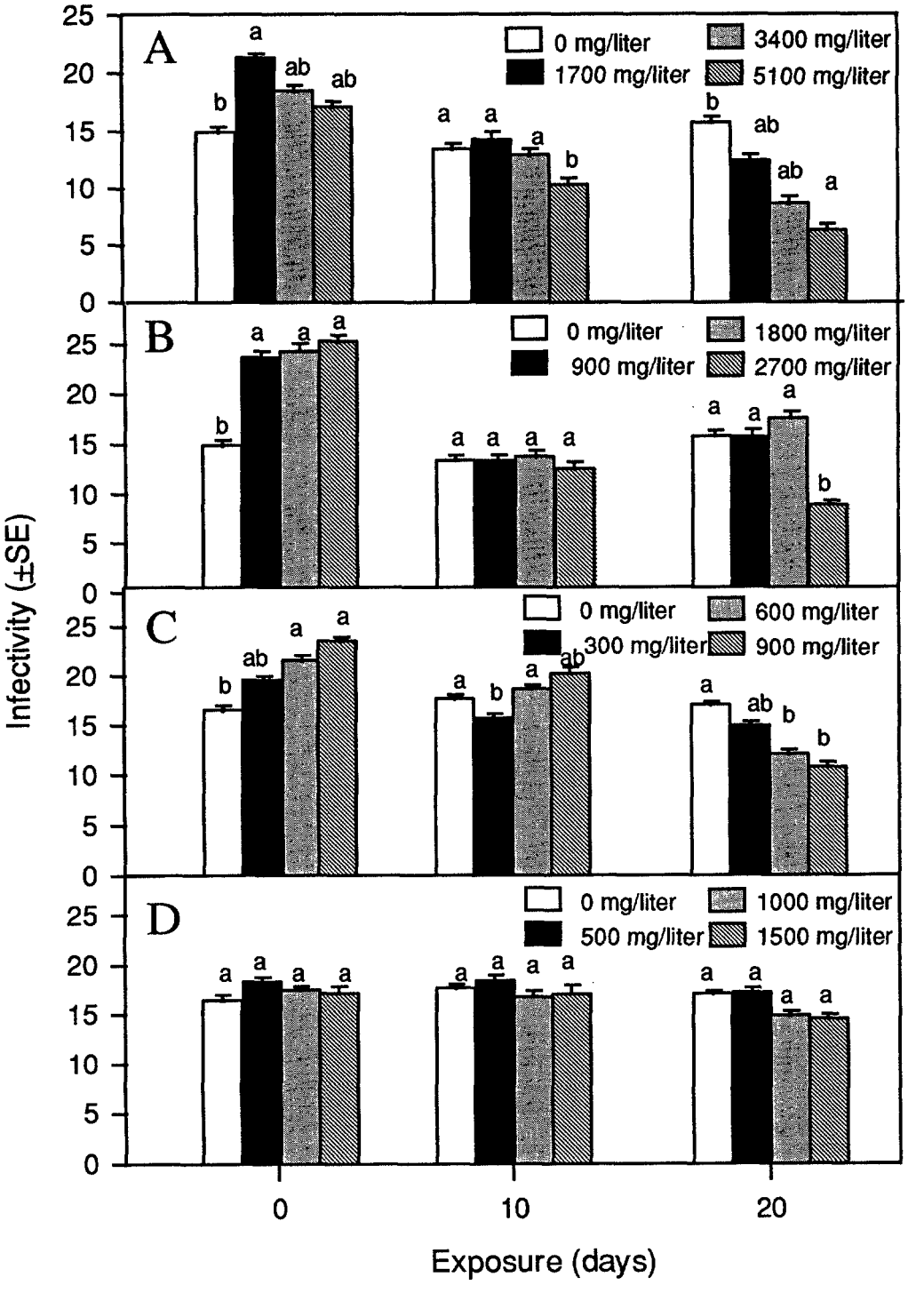

FIG. 1. Effect of NPK fertilizer and its components on the infectivity of *Steinernema feltiae*. A) NPK. B) Potassium nitrate. C) Acid potassium phosphate. D) calcium nitrate. Infectivity measured as mean number of nematodes recovered from *Galleria* larvae 4 days post-exposure. Means within treatments and exposure periods followed by different letters for each exposure differ significantly ($P = 0.05$) based on LSD analysis.

(Fig. 1B), and acid potassium phosphate (Fig. 1C) increased *S. feltiae* infectivity if exposures were of 1 day's duration. This increase was found at all concentrations tested for potassium nitrate. At exposures of moderate duration (10 days) these compounds had little or no effect on infectivity. At an exposure of long duration (20 days) potassium nitrate reduced nematode infectivity at the highest concentration tested, acid potassium phosphate reduced infectivity at the two highest concentrations, and NPK reduced infectivity at the highest concentration. Calcium nitrate did not affect nematode infectivity (Fig. 1D), even at the highest concentration over a 40-day exposure (data not shown).

Decreased infectivity of the nematodes *S. feltiae* and *H. bacteriophora* to *G. mellonella* larvae was noted following a 20-day exposure to a high NPK concentration (Fig. 2). The Pol/76 and Lov/86 strains of *S. feltiae* were equally sensitive, recording a 55% loss in infectivity. *Steinernema anomali* infectivity was not affected by fertilizer. The most sensitive nematode tested was *H. bacteriophora;* this species' infectivity was reduced by more than 70%.

In vivo reproduction of *S. feltiae* was not affected by treatment with calcium nitrate or potassium phosphate, whereas exposure to potassium nitrate resulted in 42.7% fewer progeny being produced (Fig. 3). When nematodes were exposed to all three compounds simultaneously (NPK at 5,100 mg/liter), reproduction declined by 58.2%.

Densities of a native population of *S. feltiae* were positively affected by manure treatment and negatively affected by NPK treatment (Fig. 4). Control plots with manure held nearly three times the nematode density of untreated plots (4,278 and 1,544 nematodes per m^2, respectively). Soil treated with NPK held significantly lower nematode densities at both NPK concentrations tested (58% to 62.7% fewer than the control plots) regardless of manure treatment.

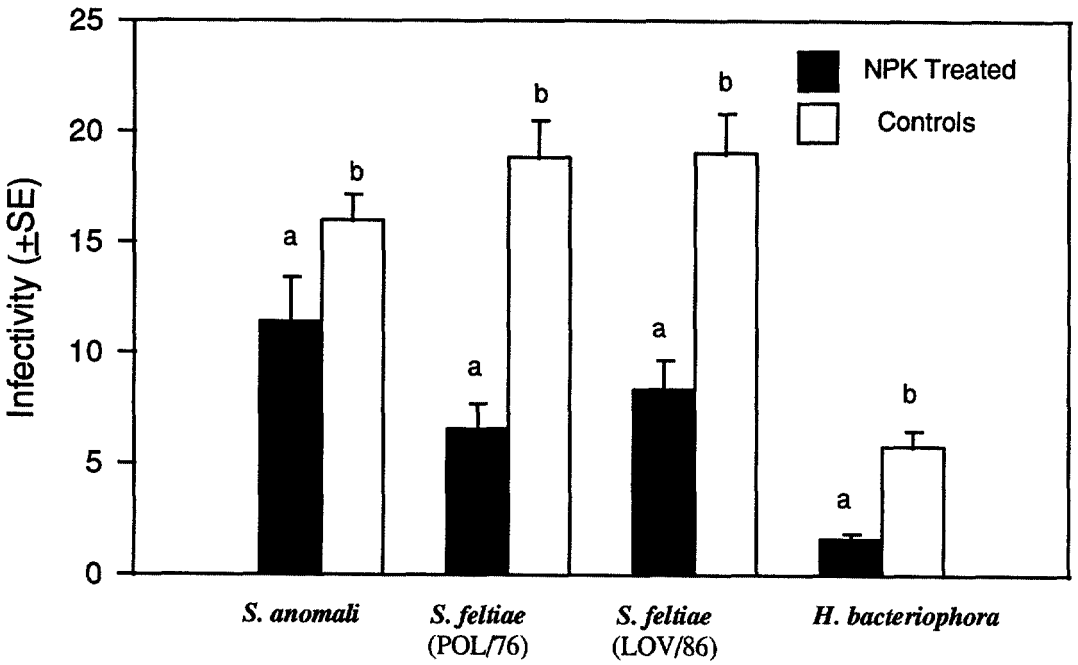

FIG. 2. Effect of NPK fertilizer (20-day exposure at 5,100 mg/liter) on the infectivity of entomopathogenic nematodes. Infectivity measured as mean number of nematodes recovered from *Galleria* larvae 4 days post-exposure. Means within treatments followed by different letters differ significantly ($P = 0.05$) based on LSD analysis.

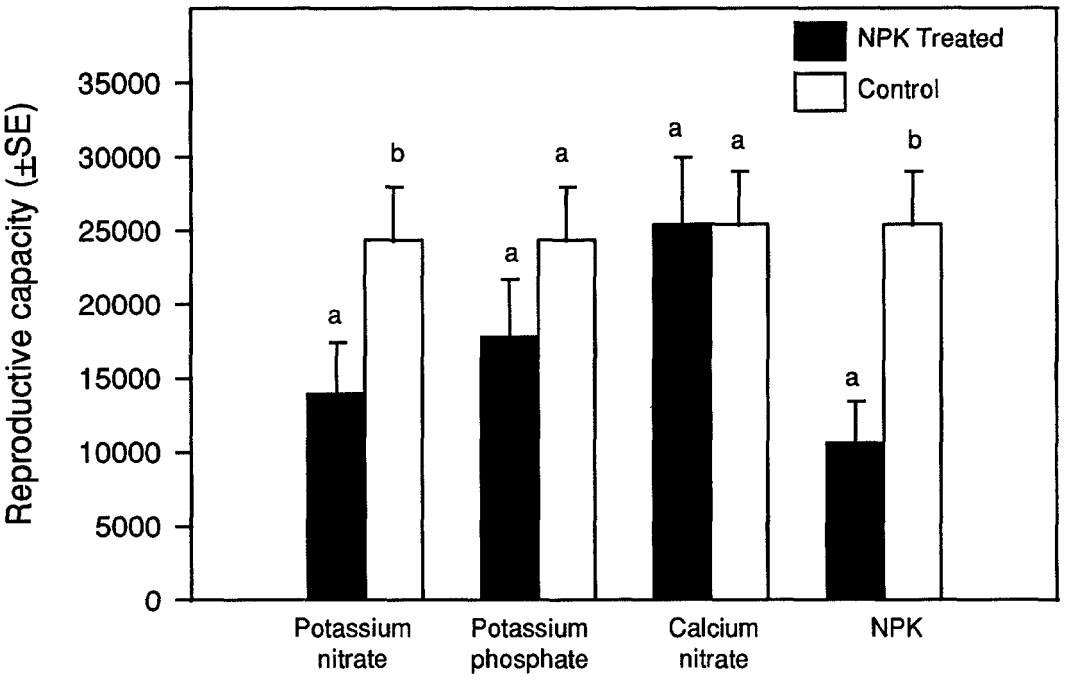

FIG. 3. In vivo reproductive capacity of *Steinernema feltiae* (Lov/86) exposed to NPK fertilizer or its components for 20 days. Reproductive capacity measured as mean number of infective juveniles emerging per *Galleria* cadaver. Means within treatments followed by different letters differ significantly ($P = 0.05$) based on LSD analysis.

DISCUSSION

Our results demonstrate that entomopathogenic nematodes respond to inorganic fertilizer according to the concentration and nature of the compounds and the species of nematode. Short laboratory exposures stimulated nematode infectivity. This result is similar to that of Ishibashi and Kondo (1986), who reported that infective juveniles of *S. carpocapsae* can be chemically activated with low concentrations of chemical insecticides or plant juices from kale or aloe. The enhanced infectivity we noted requires confirmation under field conditions. NPK fertilizer and potassium nitrate suppressed nematode infectivity and reproduction more strongly than potassium phosphate, whereas calcium nitrate had no effect on either infectivity or reproduction. The basis for these different reactions require physiological investigation.

Conversely, long periods of exposure at high fertilizer concentrations reduced both nematode infectivity and reproductive capacity. Other studies also have shown that inorganic fertilizers are detrimental to nematodes. For example, the mosquito-parasitic nematode *Romanomermis culicivorax* is intolerant of inorganic fertilizers (Walker et al., 1985). High rates of inorganic fertilizers adversely affected penetration of *Pratylenchus penetrans* into plant roots (Walker, 1971). Moreover, nematode body size diminished as the concentration of these chemicals in plant tissues increased.

Heterorhabditis bacteriophora was the most sensitive nematode in laboratory studies. This supports the earlier conclusion of Gaugler et al. (1992) that heterorhabditids tend to be less tolerant of environmental stress than steinernematids. The comparative insensitivity of *S. anomali* may have been related to this nematode's large size.

Beneficial effects of inorganic fertilizer treatments noted from short, 1-day laboratory exposures were greatest at the lowest concentration tested—a concentration equivalent to a standard field application. Adverse effects were noted only at concentrations and exposure durations unlikely to be encountered in standard horticultural or

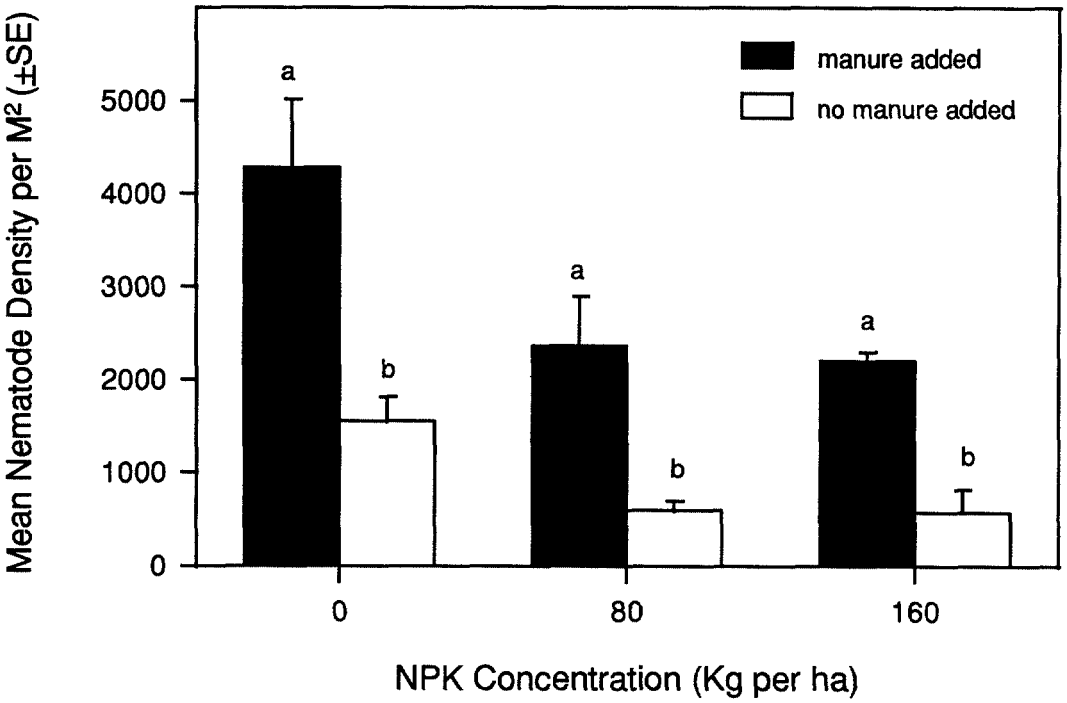

FIG. 4. Mean densities per m^2 of *Steinernema feltiae* in fertilizer-treated soil on the Wilanow-Obory Farm. Means within treatments followed by different letters differ significantly ($P = 0.05$) based on LSD analysis.

agricultural practices. Our data suggest that entomopathogenic nematodes are likely to be compatible with tank mixes of inorganic NPK fertilizers and may be mixed and applied simultaneously to reduce application costs. Rao et al. (1975) similarly reported that *Steinernema carpocapsae* was tolerant to high concentrations of inorganic fertilizer solutions in 1-day exposures. Adverse effects from inorganic amendments may be more likely to occur in the field following application where nitrates can be converted to ammonia (Muller and Gooch, 1982; Rodríguez-Kábana, 1986) and long exposures are the rule.

Our multi-year field study found that higher densities of a native *S. feltiae* population occurred in plots treated with organic manure than in untreated plots. This is in contrast with studies that have shown organic fertilizers, including manure, to have nematicidal properties (Heald and Burton, 1968; Rodríguez-Kábana, 1986). Shapiro et al. (1996) reported that fresh manure reduced *S. carpocapsae* virulence in 60-day field tests, whereas composted manure was not harmful. These authors hypothesized that this detrimental effect may have been attributed to decomposition of fresh manure, leading to reduced oxygen availability. Our multi-year field study would not have detected short-term effects due to decomposition. Rodríguez-Kábana (1986) indicated that decreases in phytoparasitic nematode numbers following organic fertilizer applications tended to be associated with the growth of antagonistic organisms, especially nematophagous fungi. We suggest that populations of *S. feltiae* in our study were influenced by densities of host populations rather than antagonists. The positive response of many populations of soil-inhabiting insects to manure is well established (Kajak et al., 1991), among them potential hosts of entomopathogenic nematodes. Nevertheless, manure applications did not protect *S. feltiae* populations from decline when the applications were accompanied by inorganic fertilizer treatments.

Field populations of *S. feltiae* were nega-

tively affected by standard rates of inorganic fertilizer. The mean density of nematodes was suppressed in plots receiving NPK fertilizer irrespective of manure treatments. The reason for the decline is unclear. Nematicidal effects on populations of other nematode species from mineral fertilizers is well documented (Eguiguren et al., 1979; Heald and Burton, 1968; Rodríguez-Kabána, 1986; Wasilewska, 1986). It is difficult to ascertain whether the observed negative effects are attributable to toxicity, or indirectly by promoting antagonistic soil microflora.

Inorganic fertilizers can reduce the effectiveness of entomopathogenic nematodes as biological control agents in the soil, but this impact will be exposure-dependent. Minimal impact may be expected if nematodes are applied as biological insecticides to achieve short-term results (i.e., inundative biological control) in combating pest outbreaks. The effects of inorganic fertilizer may be more important in the long term when nematodes are used for inoculative biological control, where establishment and recycling are the objectives. By contrast, organic manure used as fertilizer may encourage nematode establishment and recycling, and might be a tool useful for conservation biological control.

Literature Cited

Bednarek, A. 1990. Ecological factors affecting the biological activity of entomogenous nematodes in soil habitats. Warsaw, Poland: Warsaw Agricultural University SGGW Press.

Bednarek, A., and T. Nowicki. 1991. New estimation methods for the density of entomogenous nematodes (Rhabditida: Steinernematidae) in the soil. Révue de Nématologie 14:638–639.

Curran, J. 1993. Post-application biology of entomopathogenic nematodes in soil. Pp. 67–77 *in* R. Bedding, R. Akhurst, and H. K. Kaya, eds. Nematodes and the biological control of insect pests. East Melbourne, Australia: CSIRO Publications.

Eguiguren, R., F. Torres, and G. Robalina. 1979. Influence of NPK on the population dynamics of several nematode genera on potato. Nematropica 9:16–22.

Ehler, L. E. 1990. Some contemporary issues in biological control of insects and their relevance to use of entomopathogenic nematodes. Pp. 1–19 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, Florida: CRC Press.

Epsky, N. D., D. E. Walter, and J. L. Capinera. 1988. Potential role of microarthropods in biotic mortality of entomogenous nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). Journal of Economic Entomology 81:821–825.

Gaugler, R., A. Bednarek, and J. F. Campbell. 1992. Ultraviolet inactivation of heterorhabditid and steinernematid nematodes. Journal of Invertebrate Pathology 59:155–160.

Georgis, R., and R. Gaugler. 1991. Predictability in biological control using entomopathogenic nematodes. Journal of Economic Entomology 84:713–720.

Georgis, R., and G. O. Poinar, Jr. 1983. Effect of soil texture on the distribution and infectivity of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae). Journal of Nematology 15:308–311.

Heald, C. M., and G. W. Burton. 1968. Effect of organic and inorganic nitrogen on nematode populations feeding on turf. Plant Disease Reporter 52:46–48.

Ishibashi, N., and E. Kondo. 1986. *Steinernema feltiae* (DD-136) and *S. glaseri:* Persistence in soil and bark compost and their influence on native nematodes. Journal of Nematology 18:310–316.

Kajak, A., K. Chmielweski, M. Kaczmarek, and E. Rembiakowska. 1991. Experimental studies on the effect of epigenic predators on matter decomposition process in managed peat grasslands. Polish Ecological Studies 17:289–310.

Kamionek, M. 1991. The effect of pesticides on *Steinernema feltiae* (Filipjev) and other entomophilic nematodes. Warsaw, Poland: Warsaw Agricultural University SGGW Press.

Kaya, H. K. 1990. Soil ecology. Pp. 93–115 *in* R. Bedding, R. Akhurst, and H. K. Kaya, eds. Nematodes and the biological control of insect pests. East Melbourne, Australia: CSIRO Publications.

Kung, S.-P., R. Gaugler, and H. K. Kaya. 1991. Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. Journal of Invertebrate Pathology 57:242–249.

Molyneux, A. S., and R. A. Bedding. 1984. Influence of soil texture and moisture on the infectivity of *Heterorhabditis* sp. D1 and *Steinernema glaseri* for larvae of the sheep blowfly *Lucilia cuprina*. Nematologica 30:358.

Muller, R., and P. S. Gooch. 1982. Organic amendments in nematode control: An examination of the literature. Nematropica 12:319–326.

Poinar, G. O., Jr. 1988. A microsporidian parasite of *Neoaplectana glaseri* (Steinernematidae: Rhabditida). Révue de Nématologie 11:359–364.

Poinar, G. O., Jr. and H.-B. Jansson. 1986. Infection of *Neoaplectana* spp. and *Heterorhabditis bacteriophora* by the endoparasitic fungus *Drechmeria coniospora*. Journal of Nematology 18:225.

Rao, P. S. P., P. K. Das, and G. Padhi. 1975. Note on compatibility of DD-136 (*Neoaplectana dutkyi*), an insect-parasitic nematode with some insecticides and fertilisers. Indian Journal of Agricultural Science 45:275–277.

Rodríguez-Kábana, R. 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. Journal of Nematology 18:129–135.

Shapiro, D., G. Tylka, and L. C. Lewis. 1996. Effects of fertilizers on virulence of *Steinernema carpocapsae*. Applied Soil Ecology 3:27–34.

Walker, J. T. 1971. Populations of *Pratylenchus penetrans* relative to decomposing nitrogenous soil amendments. Journal of Nematology 3:43–49.

Walker, T. W., C. L. Meek, V. L. Wright, and J. S. Billodeaux. 1985. Susceptibility of *Romanomermis culicivorax* (Nematoda: Mermithidae) postparasites to agrochemicals used in Louisiana rice production. Journal of the American Mosquito Control Association 1:477–481.

Wasilewska, L. 1986. Wpyw antropopresji na strukturê i funkcjonowanie zespoów nicieni glebowych. Zeszyty Problemowe Postępów Nauk Rolniczych 323: 11–31.