Interaction Between Halofenozide and the Entomopathogenic Nematode *Heterorhabditis marelatus* for Control of Japanese Beetle (Coleoptera: Scarabaeidae) Larvae

C. M. MANNION, H. E. WINKLER,[1] D. I. SHAPIRO,[2] AND T. GIBB[3]

Nursery Crops Research Station, Tennessee State University, 472 Cadillac Lane, McMinnville, TN 37110

J. Econ. Entomol. 93(1): 48–53 (2000)

ABSTRACT Japanese beetle, *Popillia japonica* Newman is a major pest of turf and ornamentals. Laboratory bioassays were conducted to evaluate the potential interactions between a biological control agent, *Heterorhabditis marelatus* (Nematoda: Heterorhabditidae) IN strain and the insecticide halofenozide against both overwintered and nonoverwintered 3rd instars of Japanese beetle. Treatments consisted of all combinations of 2 rates of halofenozid with *H. marelatus* nematodes Imidacloprid was used as a standard. Percentage larval mortality was evaluated at 7, 14, and 21 d after treatment. No deleterious effects were observed. The nematode treatments generally produced significantly greater larval mortality relative to both chemical treatments. Twenty-one days after treatment, both rates of nematodes resulted in 100% mortality, whereas insecticide treatments did not surpass 60% mortality. No synergism was detected in any of the combination treatments. There were no significant differences in nematode reproduction in larvae exposed to halofenozide and nematodes versus larvae exposed to only nematodes.

KEY WORDS *Popillia japonica*, *Heterorhabditis marelatus*, halofenozide, imidacloprid, biological control

THE JAPANESE BEETLE, *Popillia japonica* Newman, is an important pest of turf and ornamentals (Fleming 1972). Japanese beetles feed on >300 species of plants, often causing serious defoliation of fruit and nursery stock. The larvae feed mainly on the roots of grasses and weeds but also may consume the young roots of woody ornamental plants (Smitley 1996). The cost of larval damage on turf alone is estimated to be >$234 million per year (Ahmad et al. 1983). Chemical treatments against these soil-inhabiting pests have not always been successful in part because of development of tolerance or resistance by the host insect and pesticide degradation by soil microorganisms (Ng and Ahmad 1979). Growing concerns of environmental contamination and adverse effects on nontarget organisms necessitate the development of alternative strategies.

Increased efforts in recent years have been focused on biological control using entomopathogenic nematodes in the families Heterorhabditidae and Steinernematidae. These nematodes are capable of parasitizing many economically important pests including the Japanese beetle (Klein 1990). These nematodes are mobile, highly virulent, capable of being cultured in vitro, and have a high reproductive potential. Despite their broad host range and high virulence, these nematodes have shown no mammalian pathogenicity (Gaugler and Boush 1979), and are safe to vertebrates, plants, earthworms, honey bees, and other nontarget organisms (Kaya and Gaugler 1993).

The nematodes *Steinernema glaseri* and *Heterorhabditis* spp. are particularly effective against white grubs such as the Japanese beetle (Klein 1990, Alm et al. 1992, Klein and Georgis 1992). Recently a new species of *Heterorhabditis*, *H. marelatus*, was described (Liu and Berry 1996). This species was found to be highly virulent against Japanese beetle larvae (D.I.S., unpublished data) and is more cold-tolerant than other entomopathogenic nematodes (Berry et al. 1997a). The strain used in these studies was isolated in Indiana (IN strain) and identified as *H. marelatus* (Integrated BioControl Systems, Aurora, IN).

It has been suggested that combining low-impact insecticides or reduced rates of insecticides with biological control could achieve adequate control while reducing the adverse effects of insecticides. Halofenozide is a novel compound in the diacylhydrazine class of insecticides (Rohmid 1997; Cowles et al. 1999). Halofenozide is an ecdysone agonist that accelerates the molting process, and is primarily effective against the larval stages of lepidopteran, coleopteran, and some homopteran species (Rohmid 1997, Cowles et al. 1999). There is little threat of toxicity to predators and other beneficial insects, mammals, birds, and aquatic organisms. The effect of halofenozide on nematodes and their ability to infect and kill Japanese beetle

[1] Entomology and Plant Pathology Division, Department of Natural Resources, 402 West Washington St., W290, Indianapolis, IN 46204.
[2] Citrus Research and Education Center, University of Florida, IFAS, Lake Alfred, FL 33850.
[3] Department of Entomology, Purdue University, 1158 Entomology Hall, West Lafayette, IN 47907–1158.

0022-0493/00/0048–0053$02.00/0

grubs is unknown. Current recommendations for Japanese beetle and other white grubs in turf and ornamentals include Mach2 (halofenozide) at the rate of 2.26 kg (AI)/ha (in turf only) and Marathon (imidacloprid) at the rate of 0.18 kg (AI)/ha (in greenhouse and nursery). Imidacloprid, a chloronicotinyl insecticide, acts on the cholinergic receptors in the postsynaptic membranes and disrupts normal nerve function (Bai et al.).

Numerous studies have demonstrated additive or synergistic relationships between the combined use of low-impact insecticides and biological control agents (Boucias et al. 1996; Kaakeh et al. 1997; Quintela and McCoy 1997, 1998; Koppenhofer and Kaya 1998; Nishimatsu and Jackson 1998). Koppenhofer and Kaya (1998) described a strong synergistic effect on mortality of 2 scarab species, *Cyclocephala hirta* LeConte and *C. pasadenae* Casey with combinations of imidacloprid, another reduced risk insecticide, and entomopathogenic nematodes. The synergistic interaction occurred at recommended and reduced field rates of imidacloprid. The objective of our study was to determine whether halofenozide interacts with the nematode, *H. marelatus* IN strain, when applied to overwintered and nonoverwintered 3rd-instar Japanese beetle.

Materials and Methods

In total, 3 laboratory bioassays were conducted. The interaction between *H. marelatus* and halofenozide on the mortality of Japanese beetle larvae was examined in 2 of the bioassays. One bioassay was conducted in May 1997 on overwintered larvae and the 2nd bioassay was conducted in October 1997 on larvae that had not overwintered. Both bioassays were identical in all experimental parameters except for age of Japanese beetle larvae. The effect of halofenozide on nematode reproduction was tested in the 3rd bioassay conducted in June 1998.

Japanese Beetle Larvae. Third-instar Japanese beetles were collected from the field before each test. In the 1st bioassay, larvae were collected from turf areas in Central Indiana and Ohio. The larvae were collected from 15 to 19 May 1997. In the 2nd bioassay, larvae were collected on 24 October 1997 from turf plots in Indiana. Thus, larvae in the 1st bioassay had overwintered and normally would pupate within a few weeks, whereas those in the 2nd bioassay had not overwintered and normally would not pupate for ≈7 mo. All larvae were held in containers of soil at 10°C until use. Only apparently healthy larvae were used in the bioassays.

Nematodes. *Heterorhabditis marelatus* IN strain were obtained from a commercial source (Integrated BioControl Systems, Lawrenceburg, IN). This particular strain was isolated in Indiana and identified using morphological and molecular methods. The nematodes were reared in *Galleria mellonella* (L.) according to the procedures of Woodring and Kaya (1988). The nematodes were stored on moist sponges under refrigeration (10°C) until use. At the time of experimentation, a sponge containing the nematodes was soaked in water to remove the nematodes from the sponge. A serial dilution of the nematode stock solution was conducted to achieve the necessary concentration of nematodes. The 2 rates of nematodes were 1.25 (half rate) and 2.5 billion (full rate) nematodes per hectare.

Insecticides. The insecticide, halofenozide (Mach2, 60% wettable powder, Rohmid L.L.C., Parsippany, NJ) was delivered at the rate of 1.13 and 2.26 kg (AI)/ha. Imidacloprid (Marathon 1% granular, Olympic Horticultural Products, Bradentown, FL) was chosen as the insecticide standard because it is a commonly used product for grub control. It was applied at the recommended rate of 0.18 kg (AI)/ha.

Bioassay Parameters. *Host Mortality.* Plastic containers (9 cm inside diameter, 4 cm deep) were filled with ≈200 ml of a soil mixture (50:50 sand:/sphagnum peat moss). Ryegrass seed had been mixed previously into the soil mixture. Five 3rd instar Japanese beetles were placed on the soil surface in each container. The larvae were allowed 2 h to dig into the soil mixture. Any larvae remaining on the soil surface were considered unhealthy and replaced. After all larvae moved into the soil mixture, nematode and/or the insecticide treatments were applied. Nematodes were delivered in 2 ml water to each container using a pipette. Approximately 795 and 1,590 nematodes were delivered to each cup for the low and high rate, respectively. Halofenozide also was applied to each container using a pipette (2 ml per container), and imidacloprid was sprinkled on the soil surface and followed by 2 ml water. Each container was capped with a plastic lid with puncture holes for the remainder of the test period. The containers were stored in an incubator at 24 ± 1°C in the dark.

The treatments consisted of 2 rates of *H. marelatus* (1.25 and 2.5 billion nematodes per hectare), 2 rates of halofenozide (1.13 and 2.26 kg (AI)/ha), all combinations of both rates of the nematodes and halofenozide, imidacloprid (0.18 kg (AI)/ha), and an untreated control. Each bioassay was evaluated 7, 14, and 21 d after treatment. Each treatment was replicated 5 times in a completely random design. At the time of evaluation, all larvae were removed from the containers and mortality was recorded. Cadavers were examined for signs of nematode infection (i.e., coloration) (Woodring and Kaya 1988). The stage of the insect (i.e., larva, pupa, or adult) was also recorded.

Nematode Reproduction. In a separate laboratory bioassay, 3rd instar Japanese beetles were exposed to halofenozide and nematodes in the same type of container and soil mix previously described. There were 10 replications per treatment with 2 larvae per replication. The treatments consisted of a combination of halofenozide (1.13 kg [AI]/ha) and *H. marelatus* (2.5 billion/ha), a combination of imidacloprid (0.18 kg [AI]/ha) and *H. marelatus* (2.5 billion/ha), *H. marelatus* (2.5 billion/ha) alone, and a control. The insecticide was applied 24 h after the larvae were place into the containers. The nematodes were applied 72 h after the insecticide application. Seven days after the nem-

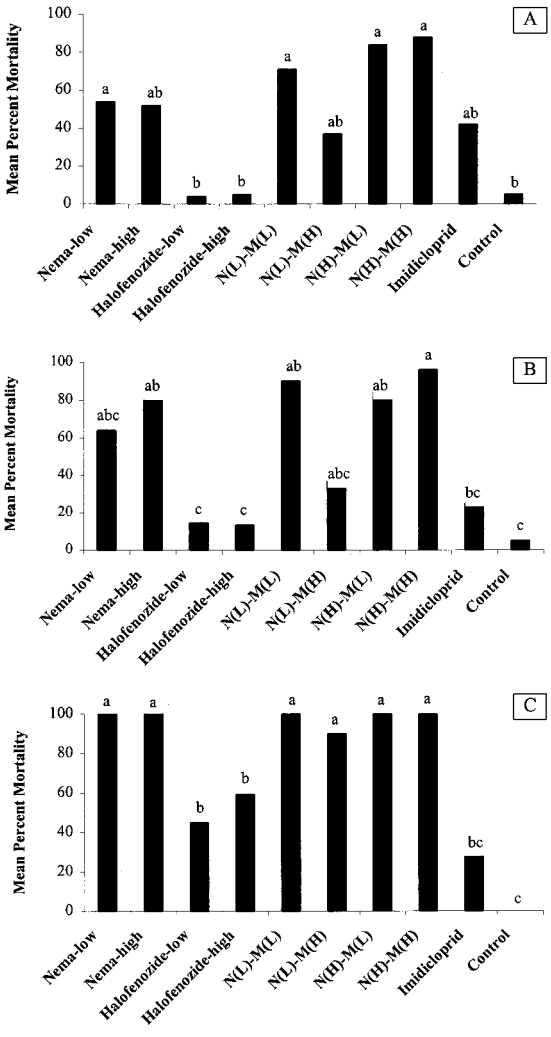

Fig. 1. Mean percentage mortality of overwintered 3rd-instar Japanese beetles exposed to *H. marelatus*, halofenozide, and imidacloprid in a laboratory bioassay. (A) 7 DAT, (B) 14 DAT, (C) 21 DAT. Nema-low = 1.25 billion/ha, nema-high = 2.5 billion/ha, halofenozide-low = 1.13 kg (AI)/ha, halofenozide-high = 2.26 kg (AI)/ha, N(L) = nema-low, N(H) = nema-high, M(L) = (Mach2) halofenozide-low, and M(H) = (Mach2) halofenozide-high.

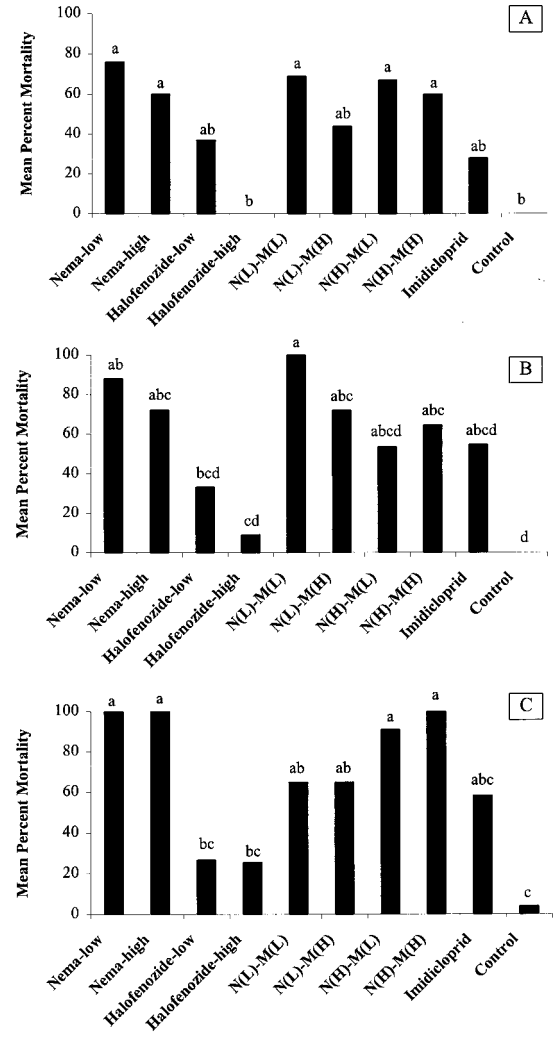

Fig. 2. Mean percent mortality of nonoverwintered 3rd-instar Japanese beetle larvae exposed to *Heterorhabditis marelatus*, halofenozide, and imidacloprid in a laboratory bioassay. (A) 7 DAT, (B) 14 DAT, (C) 21 DAT. Nema-low = 1.25 billion/ha, nema-high = 2.5 billion/ha, halofenozide-low = 1.13 kg (AI)/ha, halofenozide-high = 2.26 kg (AI)/ha, N(L) = nema-low, N(H) = nema-high, M(L) = (Mach2) halofenozide-low, and M(H) = (Mach2) halofenozide-high.

atode application, all larvae were removed. Live larvae were replaced in the containers and re-evaluated 3 d later. Dead larvae from the nematode treatment and the halofenozide plus nematode treatment were placed individually in modified White traps (White 1927) for the collection of nematodes. Nematodes that had moved from the cadavers into the water within the White trap were collected over a 3-wk period and were counted through serial dilution.

Statistics. Percentage host mortality for each evaluation date, infective juvenile nematodes produced, and the percentage of host larvae not pupating were transformed (arcsine square root) and subjected to analysis of variance (ANOVA) (SigmaStat 1995). Significant means were separated with the Tukey Test ($P < 0.05$).

Results

Mortality of Overwintered Larvae. None of the combined effects of nematode and halofenozide treatments differed from applications of nematodes alone. All nematode-halofenozide treatment combinations significantly increased host mortality over halofenozide or imidacloprid alone treatments except one combination treatment (low rate of nematodes and high rate of halofenozide at 7 and 14 days after treatment [DAT]) (Fig. 1) (7 DAT, $F = 8.452$; df = 9, 40; $P <$

Table 1. Mortality of 3rd instar Japanese beetles and number of nematodes produced when exposed to *H. marelatus* alone, halofenozide plus *H. marelatus*, and imidacloprid plus *H. marelatus* in a laboratory bioassay

Treatment	No. dead larvae	% dead larvae	% dead larvae producing nematodes	Total nematodes produced	Mean no. nematodes produced per larva
H. marelatus	11ab	55	100	337,614	30,692a
Halofenozide + *H. marelatus*	17a	85	94	475,551	29,722a
Imidacloprid + *H. marelatus*	14ab	70	100	353,851	25,275a
Control	6b	30	—	—	—

Means within a column followed by the same letter are not significantly different ($P < 0.05$).

0.001; 14 DAT, $F = 6.762$; df = 9, 39; $P < 0.001$; 21 DAT, $F = 28.792$; df = 9, 37; $P < 0.001$). At 14 and 21 DAT, 100% of the cadavers in all the combination treatments exhibited signs consistent with those typical of nematode infection by heterorhabditid nematodes (Woodring and Kaya 1988).

Larval mortality from halofenozide alone was low (<15%) at 7 and 14 DAT and did not differ significantly from the untreated control treatment (Fig. 1 A and B). However, mortality increased to 45 and 59% for the low and high application rates, respectively, by 21 DAT, which was significantly higher than the untreated control ($F = 28.792$; df = 9, 37; $P < 0.001$) (Fig. 1C). Mortality did not differ statistically between the low and high rate of halofenozide on any evaluation date.

The low rate of nematodes (1.25 billion/ha) did not differ significantly in total host mortality from the high rate (2.5 billion/ha) at any evaluation date (Fig. 1). At 21 DAT, both rates of the nematodes caused significantly more mortality than halofenozide, imidacloprid, or the untreated control (Fig. 1C) ($F = 28.792$; df = 9, 37; $P < 0.001$).

Mortality of Nonoverwintered Larvae. The treatment effects on the overwintered larvae were similar to the treatment effects on the nonoverwintered larvae (Fig. 2). Trends were identical, regardless of age of the larva. At 7 DAT, 3 of the 4 combination treatments caused significantly more mortality than the high rate of halofenozide and the untreated control ($F = 6.520$; df = 9, 40; $P < 0.001$) (Fig. 2A). These differences also were demonstrated with the overwintered larvae. At 14 DAT, only the low rate of nematodes combined with the low rate of halofenozide caused significantly more mortality ($F = 6.455$; df = 9, 40; $P < 0.001$) than both rates of halofenozide alone (Fig. 2B). At 21 DAT, the 2 combination treatments with the high rate of nematodes caused significantly more mortality ($F = 9.494$; df = 9, 40; $P < 0.001$) than either rate of halofenozide alone (Fig. 2C). As with the overwintered larvae, none of the combination treatments differed from either rate of nematodes alone at any of the evaluation timings (Fig. 2). With one exception, 100% of all the cadavers in the combination treatments on all the evaluation dates exhibited signs of nematode infection by a heterorhabditid nematode (Woodring and Kaya 1988).

Mortality of larvae exposed to halofenozide alone was relatively low (<40%) throughout the test period (Fig. 2) and was never significantly different from the control. There were no significant differences in mortality between the 2 rates of halofenozide.

As with the overwintered larvae, there were no significant differences in mortality because of the rate of nematodes applied; however, mortality was always significantly greater than in the control treatment (Fig. 2). By 21 DAT, mortality was significantly greater in the nematode treatments compared with both rates of halofenozide used alone and the untreated control.

Host Pupation. Significantly more of the overwintered larvae in the high rate of halofenozide treatment pupated compared with the low rate of halofenozide treatment or the untreated control ($F = 6.403$; df = 2, 12; $P < 0.013$) (Fig. 3). There was no pupation in any other treatment with the overwintered larvae. At the end of the bioassay, all the pupae in the low rate of halofenozide and 57% of the pupae in the high rate of halofenozide were still alive. Halofenozide did not have the same influence on the nonoverwintered larvae as it did on the overwintered larvae. Only 1 individual nonoverwintered larva pupated throughout the test period and it was from the high rate of halofenozide treatment.

Nematode Reproduction. There were no significant differences in number of nematodes produced between larvae exposed to halofenozide and larvae not exposed to halofenozide (Table 1). Host mortality was significantly greater in the halofenozide plus nematode treatment compared with the control treatment ($F = 5.318$; df = 2, 27; $P < 0.011$).

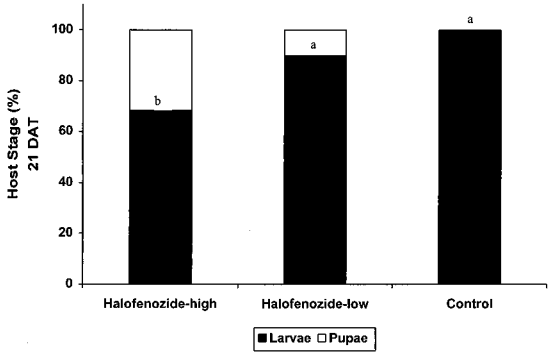

Fig. 3. Developmental stage of Japanese beetle exposed to 2 rates of halofenozide. Halofenozide-low = 1.13 kg (AI)/ha, halofenozide-high = 2.26 kg (AI)/ha at 21 DAT.

Discussion

There was not a positive synergistic relationship between the nematode, *H. marelatus* IN strain, and the turf insecticide, halofenozide, based on these laboratory results. However, there were no deleterious effects to the nematodes when used in combination with the insecticide. The average number of nematodes produced per larva was similar for larvae exposed to halofenozide and larvae not exposed to halofenozide and demonstrates the compatibility of this insecticide and nematode strain.

To achieve maximum efficacy, halofenozide must be ingested; therefore, it is recommended for use against young, actively feeding larvae (Rohmid 1997, Cowles et al. 1999). Although an insect exposed to the insecticide quits feeding shortly after exposure, death of the insect can take numerous days. In these bioassays, halofenozide was not applied at the optimal time, and as expected, larval mortality was relatively low.

Although both bioassays were conducted under similar conditions, the age of the Japanese beetle larvae was different (overwintered larvae versus nonoverwintered larvae). It is apparent from these studies that exposure of overwintered larvae to halofenozide may induce pupation, and if the insect is close enough to the normal pupation time, the insect may survive. Normally, exposure of larvae to halofenozide induces premature molting which ultimately kills the insect (Rohmid 1997, Cowles et al. 1999). Mortality of overwintered larvae was approximately doubled compared with nonoverwintered larvae in the halofenozide treatments on the last evaluation date. It is unknown what effect the age of the 3rd instar has on the interaction between the nematodes and halofenozide. However, at the conclusion of these tests, a greater percentage of the overwintered larvae was killed in the combination treatments with the low rate of nematodes. Mortality of overwintered and nonoverwintered larvae was similar in the combination treatments with the high rate of nematodes.

Combinations of entomopathogenic nematodes and chemical insecticides have been shown to be synergistic in insect suppression (Hatsukade 1990, Ishibashi 1993, Koppenhofer and Kaya 1998, Nishimatsu and Jackson 1998). Researchers have proposed different mechanisms for the increased efficacy such as increased nematode activity and changes in nematode behavior; however, the most common is that the insecticide weakens the host insect, making it more susceptible to nematode attack. Our data are not consistent with past reports of synergism. However, there are differences, such as nematode species and strain, host species, application timing, and type of insecticide, between the studies demonstrating synergism and the current study. These difference likely play a role in the interaction between the nematode and insecticide and will be particular to that situation.

Chemical insecticides are typically recommended for managing Japanese beetle larvae. The tolerance of this pest ranges from moderate (low-value turf) to extremely low (high-value turf and shipping plant material across state lines). There is great concern about movement of infested plant material contributing to the spread of Japanese beetle; therefore, movement of plant material is regulated by many states. In these cases, chemical insecticides are necessary because of the quick action and high level of efficacy. However, combining applications of chemical insecticides and entomopathogenic nematodes may offer numerous benefits. The interaction between the insecticide and the nematodes, although not demonstrated in these tests, may allow for reduced chemical application rates. Additionally, the nematodes may become established and begin to offer a long-term reduction in the larval populations (Klein and Georgis 1992).

The nematode *H. marelatus* IN strain, which has not previously been tested against Japanese beetle, seems to be a highly promising control agent for this pest. *H. marelatus* has been shown to be effective against other insects such as the black vine weevil, *Otiorhynchus sulcatus* (L.), the strawberry root weevil, *O. ovatus* (F.) (Berry et al. 1997a), and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Berry et al. 1997b). *H. marelatus* performed very well in the bioassays mentioned above, ultimately causing 100% mortality of the host insect. The addition of halofenozide did not appear to affect the efficiency, either positively or negatively, of the nematodes. Because host mortality from nematode treatments was so high, any interaction between the nematodes and the insecticide could not be demonstrated in the current study, and evaluation for synergy must be demonstrated under conditions in which the nematodes do not achieve maximum mortality. Additionally, further tests should be undertaken to examine treatment effects on early-instar Japanese beetles.

Acknowledgments

We thank Integrated BioControl Systems for providing the entomopathogenic nematodes, *H. marelatus* IN strain.

References Cited

Ahmad, S., H. T. Streu, and L. M. Vasvary. 1983. The Japanese beetle: a major pest of turfgrass. Am. Lawn Appl. 4: 2.

Alm, S. R., T. Yeh, J. L. Hanula, and R. Georgis. 1992. Biological control of Japanese, oriental, and black turfgrass ataenius beetle (Coleoptera: Scarabaeidae) larvae with entomopathogenic nematodes (Nematoda: Steinernematidae, Heterorhabditidae). J. Econ. Entomol. 85: 1660–1665.

Bai, D., S.C.R. Lummis, W. Leicht, H. Breer, and B. Satelle. 1991. Actions of imidacloprid and related nitromethylene on cholinergic receptors of an identified insect motor neuron. Pestic. Sci. 33: 197–204.

Berry, R. E., J. Liu, and E. Groth. 1997a. Efficacy and persistence of *Heterorhabditis marelatus* (Rhabditida: Heterorhabditidae) against root weevils (Coleoptera: Curculionidae) in strawberry. Environ. Entomol. 26: 465–470.

Berry, R. E., J. Liu, and G. Reed. 1997b. Comparison of endemic and exotic entomopathogenic nematode species

for control of Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 90: 1528–1533.

Boucias, D. G., C. Stokes, G. Storey, and J. Pendland. 1996. Effect of imidacloprid on the termite, *Reticulotermes flavipes* and its interaction with insect pathogens. Pflanzenschutz-Nachr. 49: 103–150.

Cowles, R. S., S. R. Alm, and M. G. Villani. 1999. Selective toxicity of halofenozide to exotic white grubs (Coleoptera: Scarabaeidae). J. Econ. Entomol. 92: 427–434.

Fleming, W. E. 1972. Biology of the Japanese beetle. U.S. Dep. Agric. Agric. Res. Stn. Tech. Bull. 1449.

Gaugler, R., and G. M. Boush. 1979. Nonsusceptibility of rats to the entomogenous nematode, *Neoaplectana carpocapsae.* Environ. Entomol. 8: 658–660.

Hatsukade, M. 1990. Application of entomogenous nematodes for the control of important turfgrass insects, pp. 89–98. *In* N. Ishibashi [ed.], Development of biological integrated control of agricultural pests by beneficial nematodes. Ministry of Education, Saga University, Japan.

Ishibashi, N. 1993. Integrated control of insect pests by *Steinernema carpocapsae*, pp. 105–113. *In* R. Bedding, R. Akhurst, and H. Kaya [eds.], Nematodes and the biological control of insect pests. CSIRO, Canberra, NSW, Australia.

Jandel Scientific. 1995. SigmaStat statistical software user's manual. Jandel Scientific, San Rafael, CA.

Kaakeh, W., B. L. Reid, T. J. Bohnert, and G. W. Bennet. 1997. Toxicity of imidacloprid in the German cockroach (Dictyoptera: Blattellidae), and the synergism between imidacloprid and *Metarhizium anisopliae* (Imperfect fungi: Hyphomycetes). J. Econ. Entomol. 90: 473–482.

Kaya, H. K., and R. Gaugler. 1993. Entomopathogenic nematodes. Annu. Rev. Entomol. 38: 181–206.

Klein, M. G. 1990. Efficacy against soil-inhabiting insect pests, pp. 195–214. *In* R. Gaugler and H. K. Kaya [eds.], Entomopathogenic nematodes in biological control. CRC, Boca Raton, FL.

Klein, M. G., and R. Georgis. 1992. Persistence of control of Japanese beetle (Coleoptera: Scarabaeidae) larvae with steinernematid and heterorhabditid nematodes. J. Econ. Entomol. 85: 727–730.

Koppenhofer, A. M., and H. K. Kaya. 1998. Synergism of imidacloprid and an entomopathogenic nematode: a novel approach to white grub (Coleoptera: Scarabaeidae) control in turfgrass. J. Econ. Entomol. 91: 618–623.

Liu, J., and R. E. Berry. 1996. *Heterorhabditis marelatus* n. sp. (Rhabditida: Heterorhabditidae) from Oregon. J. Invertebr. Pathol. 67: 48–54.

Ng, Y. S., and Ahmad, S. 1979. Resistance to dieldrin and tolerance to chlorpyrifos and bendiocarb in a northern New Jersey population of Japanese beetle. J. Econ. Entomol. 72: 698–700.

Nishimatsu, T., and J. J. Jackson. 1998. Interaction of insecticides, entomopathogenic nematodes, and larvae of the western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 91: 410–418.

Quintela, E. D., and C. W. McCoy. 1997. Pathogenicity enhancement of *Metarhizium anisopliae* and *Beauveria bassiana* to first instars of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) with sublethal doses of imidacloprid. Environ. Entomol. 26: 1173–1182.

Quintela, E. D., and C. W. McCoy. 1998. Synergistic effect of two entomopathogenic fungi and imidacloprid on the behavior and survival of larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in soil. J. Econ. Entomol. 91: 110–122.

Rohmid. 1997. Mach2 turf and ornamental insecticide. Technical information bulletin. Rohmid LLC, Parsippany, NJ.

Smitley, D. R. 1996. Incidence of *Popillia japonica* (Coleoptera: Scarabaeidae) and other scarab larvae in nursery fields. Hortic. Entomol. 89: 1262–1266.

White, G. F. 1927. A method for obtaining infective nematode larvae from cultures. Science (Wash. D.C.) 66: 302–303.

Woodring, J. L., and H. K. Kaya. 1988. Steinernematid and heterorhabditid nematodes: a handbook of biology and techniques. Southern Cooperative Series Bulletin 331, Arkansas Agricultural Experiment Station, Fayetteville, AR.

Received for publication 1 February 1999; accepted 10 August 1999.