Management of Early-Instar Japanese Beetle (Coleoptera: Scarabaeidae) in Field-Grown Nursery Crops

C. M. MANNION, W. MCLANE,[1] M. G. KLEIN,[2] J. MOYSEENKO,[2] J. B. OLIVER,[3] AND D. COWAN[4]

Tropical Research and Education Center, University of Florida, 18905 SW 280th Street, Homestead, FL 33031

J. Econ. Entomol. 94(5): 1151–1161 (2001)

ABSTRACT Numerous field studies were conducted in commercial nurseries in Tennessee from 1996 through 1999 to evaluate chemical and biological treatments, application timing and rates, and method of application for control of early instars of Japanese beetle, *Popillia japonica* Newman. Insecticide treatments included bifenthrin, bendiocarb, chlorpyrifos, carbaryl, fipronil, halofenozide, imidacloprid, permethrin, tefluthrin, thiamethoxam, and trichlorfon. Biological treatments included entomopathogenic nematodes (*Heterorhabditis bacteriophora* HP88 or *H. marelatus*), *Bacillus thuringiensis* Berliner subspecies *japonensis* Buibui strain, and *Beauveria bassiana* (Balsamo) Vuillemin. All treatments were applied on the soil surface or injected into the soil around the base of each tree. Tree type and size varied among and within tests, however, the sampling unit (61-cm-diameter root ball) remained the same throughout all tests. The biological treatments provided poor-to-moderate control (0–75%) of Japanese beetle larvae. Imidacloprid was the most frequently evaluated insecticide and achieved 91–100, 87–100, 83–100, and 41–100% control with applications in May, June, July, and August, respectively. Halofenozide treatments were not significantly different from imidacloprid treatments with one exception. Halofenozide provided 60–87, 85–100, and 82–92 control with applications made in June, July, and August, respectively. Fipronil and thiamethoxam were evaluated to a lesser extent but both performed similarly to imidacloprid. Most other insecticide treatments were less successful in reducing numbers of Japanese beetle larvae and with few exceptions achieved <50% control.

KEY WORDS *Popillia japonica*, scarab, white grubs, nursery crops, ornamentals

THE JAPANESE BEETLE, *Popillia japonica* Newman, was accidentally introduced into the United States in the early 1900s, and has since spread through most states east of the Mississippi River except Florida and Mississippi (Brandenburg and Villani 1995). Distribution appears to be related to the amount of rainfall and to soil temperatures. However, intensive irrigation and agricultural production have expanded suitable habitat for colonization by Japanese beetles. The Japanese beetle is particularly devastating because both the adult and immature stages are pests (Tashiro 1987). Adult beetles feed voraciously on the leaves, flowers, and fruits of >300 species of plants (Fleming 1972a). The adults normally aggregate in large numbers on a single plant, causing severe defoliation and are commonly managed with foliar insecticide. Japanese beetle larvae reside in the soil and feed on the roots of grasses and weeds, but they may also consume the young roots of woody ornamental plants (Fleming 1972a, Vittum et al. 1999). The larvae are considered one of the most serious pests of turf in the northeastern United States and are a major problem in the southeast and parts of the Midwest.

Japanese beetles can be artificially dispersed in several ways, including shipment of nursery stock infested with immature stages. Federal quarantines established in 1920 regulated the interstate movement of many kinds of farm products and plants from Japanese beetle-infested areas (Fleming 1972b). The movement of nursery and greenhouse plants was the most important part of the Japanese beetle quarantine because the immature stages of the beetle are in the soil throughout most of the year. Although the federal quarantine for nursery stock was terminated in 1979, many states maintain quarantines or certification procedures. Shipment of nursery stock containing soil continues to be a primary regulatory concern to growers in infested areas and state regulators throughout the United States.

The U.S. Domestic Japanese Beetle Harmonization Plan (National Plant Board 1998) established in 1992, and last updated in 1998, provides guidelines for quarantine and certification requirements to facilitate the orderly marketing of nursery stock while ensuring that pest risks are acceptably managed. This document, as adopted by the National Plant Board, establishes a framework and reference point for conducting risk

---

[1] Insecticide and Application Technology Section, USDA-APHIS, Building 1398, Otis Ang Base, MA 02542 (cmannion@gnv.ifas.ufl.edu).

[2] USDA, ARS, OARDC, 1680 Madison Avenue, Wooster, OH 44691.

[3] Tennessee State University, Cooperative Agricultural Research Program, Nursery Crop Research Station, 472 Cadillac Lane, McMinnville, TN 37110.

[4] Insecticide and Application Technology Section, USDA-APHIS, Building 1398, Otis Ang Base, MA 02542.

assessments, designing quarantines, and structuring nursery inspection programs. The harmonization plan for Japanese beetle establishes four regulatory strategies based on a state's classification (categories 1–4). Category 1 (primarily the western states) is considered uninfested and beetles are quarantine pests; category 2 (primarily the midwestern states) is uninfested or partially infested and beetles are regulated, nonquarantine pests; category 3 (primarily the eastern states) is partially or generally infested and beetles are of no regulatory significance; and category 4 (Wyoming) is historically not known to be infested and beetles are of no regulatory significance (Blosser 1999). Currently, the only acceptable treatment for field production nursery stock sent from a Japanese beetle infested area to category 1 states is a dip treatment of chlorpyrifos for root balls 30.5 cm or smaller. There are only two acceptable treatments allowed for field nursery stock (81.3 cm or smaller) shipped to category 2 states from infested areas that are either a dip treatment of chlorpyrifos or a soil application of imidacloprid, applied just before oviposition or while the grubs are young and actively feeding (Mannion et al. 2000b).

Due to the pest status of Japanese beetle on turf and in the landscape, there have been tremendous efforts at managing damaging scarab larvae with the use of insecticides and biological control in the turf environment (Klein 1995, Vittum et al. 1999). Although there have been great advances in the management of Japanese beetle larvae in turf and the landscape, there has been considerably less effort at managing Japanese beetle larvae in production nurseries where there is a critical need for consistent and efficacious treatment alternatives to root ball dips with chlorpyrifos. Control efforts in nursery production have generally targeted the adults to protect the foliage of the trees and shrubs. With some exceptions, Japanese beetle larvae are generally not considered a pest in field production nurseries; however, they remain a major regulatory concern because they can be accidentally spread with the movement of soil that surrounds the roots of plants. Additionally, successful treatments for larvae in turf or the landscape may not be as efficacious, economic, or suitable for the production nursery. Several new classes of insecticides have potential for scarab larval control, particularly Japanese beetle larvae (Monthean and Potter 1992, Cowles and Villani 1996, Cowles et al. 1999). These insecticides have low use rates, low mammalian toxicity, and are generally thought less disruptive to the environment and nontarget organisms than traditional insecticides. Additionally, some of these newer insecticides have been shown to be compatible or synergistic with entomopathogenic nematodes and fungi (Koppenhofer and Kaya 1998, Mannion et al. 2000a). These insecticides generally target young, actively feeding larvae, so they must be managed differently. There are minimal data available on the use of any insecticides for use in field production nurseries. Therefore, tests were conducted from 1996 through 1999 in commercial nurseries in middle Tennessee to evaluate chemical and biological treatments, application timing and rates, and method of application for control of early instars of Japanese beetle larvae in field-grown trees.

Materials and Methods

Field tests were conducted for four consecutive years at commercial nurseries comparing treatments of insecticides, entomopathogenic nematodes, *Bacillus thuringiensis* Berliner subspecies *japonensis* Buibui strain (Bt), and *Beauveria bassiana* strain GHA for the reduction of early-instar Japanese beetle. Tests were divided into two types of applications: (1) surface applications of insecticides, entomopathogenic nematodes, and Bt; and (2) injection of insecticides around the base of each tree. All tests were conducted in commercial nurseries in Warren and Grundy counties, TN. Although the type and size of tree varied among and within tests, the sampling unit was a 61-cm-diameter root ball, which remained the same throughout all tests.

Artificial Infestation. Trees in some field tests were artificially infested to increase the number of Japanese beetle larvae in the root ball zone. Approximately 100 adult beetles (50:50 male:female) were confined in a small plastic cage on the soil surface at the base of each tree where they were allowed to oviposit. Each cage was made from a 5.8-liter Rubbermaid storage box (32 by 17 by 11 cm, Wooster, OH) that was inverted on the soil surface without the lid. Two holes (11 cm in diameter) cut in the bottom of the box were covered with a small mesh screen that would prevent beetles from escaping but permit air exchange. A third hole (3.5 cm in diameter) cut in the corner was used to introduce beetles into the cage. The boxes were partially buried with the bottom of the box facing up. The third hole was sealed with a cork after beetles were introduced. Depending on the test, beetles were placed in the cages two to six times during the adult flight period (June through August). All cages were equally infested within a test.

Treatments, Applications, and Evaluation. Not all treatments were included in every test. All treatments were applied to a 0.3-m^2 area around the base of each tree. Granular materials were premeasured and sprinkled around the base of the tree with a small shaker. Liquids and wettable powders were sprayed with a CO_2 backpack sprayer, 25 psi with an 8002 flat fan spray tip. Treatments were applied in 240 ml of water per tree. The nematodes used in 1996 were from a commercial product, Cruiser, obtained from Ecogen, Langhorne, PA. The nematodes used in subsequent years (*Heterorhabditis bacteriophora* HP88 and *H. marelatus*) were obtained from Integrated BioControl Systems, Lawrenceburg, IN. The nematodes were stored on moist sponges under refrigeration (10°C) until use. At the time of application, a sponge containing the nematodes was soaked in water to remove the nematodes. A serial dilution of the nematode stock solution was conducted to achieve the necessary application rate. The nematodes were delivered in 2–4 liters of water per tree by using a watering can. Treat-

Table 1. Effect of insecticides, *B. thuringiensis* subspecies *japonensis* Buibui strain (MYX942-102), and *H. bacteriophora* HP88 applied May through September 1996 on Japanese beetle larvae in a commercial nursery

Treatment	Active ingredient	Rate g (AI)ha (lb [AI]acre)	Mean no. (±SE) Japanese beetle larvae per root ball ($n = 5$)				
			Application timing				
			May	June	July	Aug	Sept
Marathon 1G	Imidacloprid	453.6 (0.4)	0.2bc (±0.20)	0.4ab (±0.40)	0.4ab (±0.26)	0.2b (±0.20)	0.2bc (±0.20)
Marathon 1 G+ Sevin XLR Plus (s)	Imidacloprid+ Carbaryl	453.6 (0.4) 1,134.0 (1.0)	0.2bc (±0.20)	0.0b (±0.00)	0.2ab (±0.20)	0.2b (±0.20)	0.6abc (±0.40)
Marathon 1 G+ Sevin XLR Plus (f)	Imidacloprid+ Carbaryl	453.6 (0.4) 1,134.0 (1.0)	0.0c (±0.00)	0.0b (±0.00)	0.0b (±0.00)	0.2b (±0.20)	0.4abc (±0.25)
Marathon 1 G+ Sevin XLR Plus (s/f)	Imidacloprid+ Carbaryl	453.6 (0.4) 1,134.0 (1.0)	0.6bc (±0.60)	0.0b (±0.00)	0.4ab (±0.26)	0.0b (±0.00)	1.2abc (±0.80)
Merit 75WP	Imidacloprid	453.6 (0.4)	NA	0.0b (±0.00)	0.2ab (±0.20)	1.4ab (±0.93)	0.0c (±0.00)
Merit 75WP+ Sevin XLR Plus (s)	Imidacloprid+ Carbaryl	453.6 (0.4) 1,134.0 (1.0)	NA	0.0b (±0.00)	0.0b (±0.00)	0.4ab (±0.25)	0.0c (±0.00)
Merit 75WP+ Sevin XLR Plus (f)	Imidacloprid+ Carbaryl	453.6 (0.4) 1,134.0 (1.0)	NA	0.4ab (±0.40)	0.0b (±0.00)	0.6ab (±0.60)	0.0c (±0.00)
Merit 75WP+ Sevin XLR Plus (s/f)	Imidacloprid+ Carbaryl	453.6 (0.4) 1,134.0 (1.0)	NA	0.4ab (±0.40)	0.0b (±0.00)	0.0b (±0.00)	0.4bc (±0.40)
Sevin XLR Plus (f)	Carbaryl	1,134.0 (1.0)	4.8ab (±2.40)	4.8a (±2.40)	4.8a (2.40)	4.8ab (2.40)	4.8ab (2.40)
RH-0345 2F	Halofenozide	1,134.0 (1.0)	NA	0.4ab (±0.40)	0.0b (±0.00)	0.4ab (±0.25)	1.4abc (±0.40)
RH-0345 2F	Halofenozide	2,268.0 (2.0)	NA	0.4ab (±0.25)	0.2ab (±0.20)	0.2b (±0.20)	0.4abc (±0.25)
B.t. BuiBui (.5x)	MYX942-102	1,360.8 g/90 sq m (3 lb/1,000 sq ft)	NA	NA	2.6ab (±1.08)	6.2a (±3.11)	2.6abc (±1.29)
B.t. BuiBui (1x)	MYX942-102	2,721.6 g/90 sq m (6 lb/1000 sq ft)	NA	NA	0.6ab (±0.40)	1.0ab (±0.55)	1.8abc (±0.97)
H. bacteriophora HP88		5 bil/h (2 bil/A)	NA	3.0ab (±1.18)	4.2a (±2.20)	2.6ab (±1.69)	1.4abc (±0.68)
Control			6.4a (±2.94)	3.0ab (±1.67)	2.4ab (±0.98)	2.2ab (±1.28)	4.0a (±1.05)

s, soil application; f, foliar application; s/f, soil and foliar application. NA, not applicable (i.e., no treatment was made). Means within a column followed by different letters are significantly different ($P < 0.05$). (Tukey's multiple range test, $P = 0.05$, based on [log(x+1)] transformed data).

ments were not usually irrigated following application, unless noted in the test description. All tests were evaluated in fall or early winter by mechanically digging each tree with a 61-cm-diameter root ball. Each root ball was broken apart and the soil searched for the presence of live scarab larvae. All scarab larvae were counted and identified.

Surface Applications Field Tests. Twelve field tests were conducted during 1996–1999. All applications and evaluations were made as described above.

Year 1996. Four field tests were conducted in 1996. The experimental design in all four tests was completely random with five single-tree replications per treatment-timing combination. Treatments were applied from May through September and evaluated in October. In test 1 the trees were 2–2.5-m linden (*Tilia* sp.) and were artificially infested with adult beetles three times as described above. Treatments, rates, and application timings are listed in Table 1. Nematodes were applied in 3 liters of water. Foliar and soil applications of carbaryl were applied approximately every 2 wk during adult beetle flight (June–August).

The trees in test 2 were 2–2.5-m linden (*Tilia* sp.) and were not artificially infested. Treatments in test 2 consisted of imidacloprid (Marathon 1G) alone and in combination with a soil, a foliar, and a soil plus foliar application of carbaryl (Sevin XLR Plus), a soil application of carbaryl (Sevin XLR Plus), a soil application of imidacloprid (Merit 75 WP), and an untreated control. All treatments were applied in either May, June, July, August, or September except imidacloprid (Merit 75 WP) that was not applied in May. All treatment rates were the same as in test 1 in 1996. Foliar and soil applications of carbaryl (Sevin XLR Plus) were applied approximately at 2-wk intervals during adult beetle flight.

The trees in test 3 were 2–2.5-m linden (*Tilia* sp.) infested by natural populations of Japanese beetle. The treatments in test 3 were imidacloprid (Marathon 1G and Merit 75 WP) and an untreated control. Marathon 1G was applied in May, June, July, August, or September and Merit 75 WP was applied in June, July, August, or September. All treatment rates were the same as in test 1 1996.

The trees in test 4 were 1.2–1.5-m purple plum (*Prunus* sp.) artificially infested twice with adult beetles as described above. The treatments, rates, and application timings are listed in Table 2.

Year 1997. In 1997, three field tests were conducted. Tests one and two included the same treatments but were conducted at different sites. Both tests were artificially infested three times during June and July as described above. The trees used in test 1 were honeylocust (*Gleditsia triacanthos inermis*), ash (*Fraxinus*

Table 2. Effect of insecticides applied to soil in August and September 1996 on Japanese beetle larvae in a commercial nursery

Treatment	Active ingredient	Rate g(AI)ha (lb [AI]acre)	Mean no. (±SE) Japanese beetle grubs per root ball (n = 5)		
			Application timing		
			Mid-August	Late August	Mid-September
Marathon 1G	Imidacloprid	453.6 (0.4)	0.0 ± 0.00a	1.0 ± 0.48ab	1.4 ± 0.87a
Merit 75WP	Imidacloprid	453.6 (0.4)	0.4 ± 0.25ab	0.4 ± 0.25ab	0.4 ± 0.25a
RH-0345 2F (=MACH2)	Halofenozide	2,268.0 (2.0)	0.6 ± 0.40ab	0.2 ± 0.20a	0.6 ± 0.25a
Ambush 2EC	Permethrin	453.6 (0.4)	1.4 ± 0.40bc	2.0 ± 0.84ab	1.4 ± 0.51a
Control			2.6 ± 0.68c	2.6 ± 0.68b	2.6 ± 0.68a

Means within a column followed by different letters are significantly different ($P < 0.05$) (Tukey's multiple range test, $P = 0.05$, based on [log(x + 1)] transformed data).

sp.), and purple plum (*Prunus* sp.) ranging from 2 to 3 m. The trees in test 2 were Kwanzan cherry (*Prunus serrulata* 'Kwanzan') ≈2 m in height. The experimental design for both tests was completely random with nine single-tree replications in test 1 and seven single-tree replications in test 2. Tree species were randomly mixed among treatments in test 1. Treatments, rates, and application timings are listed in Table 3. Both tests were evaluated in October 1997.

The third test in 1997 was conducted in a block of mixed trees (2-m linden, *Tilia* sp., and redbud, *Cercis canadensis*). The trees were artificially infested four times during June and July as described above. Treatments and rates are listed in Table 4. All treatments were randomly assigned in a completely randomized design, applied in September and evaluated 6 mo after treatment. There were nine replications per treatment.

Year 1998. In 1998, two field tests were conducted with the same treatments but with 2-m maple (*Acer* sp.) trees at one site and 2-m cherry (*Prunus* sp.) trees at another site. Trees in both tests were artificially infested twice in July as described above. Treatments, rates, and application timings are listed in Table 5. Both tests used a completely randomized design with 10 single-tree replications per treatment and evaluated in October.

Year 1999. Three field tests were conducted in 1999. The trees in test 1 were 2.1–2.4-m crabapple (*Malus* sp.) and were artificially infested three times June through August as described above. Treatments, rates, and application timings are listed in Table 6.

Table 3. Effect of insecticides and entomopathogenic nematodes applied to soil from June through October 1997 on Japanese beetle larvae in a commercial nursery

Treatment	Active ingredient	Rate g(AI)ha (lb [AI]acre)	Application timing	Mean no. (±SE) live Japanese beetle larvae per root ball (n = 9)
Marathon 1G	Imidacloprid	453.6 (0.4)	June	0.2 ± 0.15h
			July	0.9 ± 0.46defgh
			Aug	0.7 ± 0.37fgh
			Sept	0.6 ± 0.38gh
			Oct	5.2 ± 1.13abc
Marathon 60WSP	Imidacloprid	453.6 (0.4)	June	0.4 ± 0.24gh
			July	0.4g ± 0.24h
			Aug	1.3 ± 0.97cdefgh
			Sept	2.1 ± 0.79bcdefgh
Fipronil 0.1G	Fipronil	187.0 (0.165)	June	0.8 ± 0.47efgh
		340.3 (0.30)	June	8.7 ± 2.90ab
		567.0 (0.50)	June	0.2 ± 0.15h
Mach2 2F	Halofenozide	2,268.0 (2.0)	June	1.2 ± 0.55cdefgh
			July	1.4 ± 0.67cdefgh
			Sept	4.1 ± 0.82abcdef
Fireban 1.5G	Tefluthrin	793.8 (0.7)	July	5.9 ± 2.07abcde
			Sept	4.6 ± 1.25abcdefg
Sevin 6.3G	Carbaryl	9,298.8 (8.2)	Aug	6.8 ± 2.53abcd
Dylox 80 T&O	Trichlorfon	9,298.8 (8.2)	Sept	6.7 ± 2.36ab
Marathon 1G + Dylox 80 T&O	Imidacloprid + Trichlorfon	453.6 (0.4) + 9,298.8 (8.2)	June and Sept	0.8 ± 0.28cdefgh
H. bacteriophora HP88		5 billion/h (2 billion/acre)	Aug	4.3 ± 1.43abcdefg
H. bacteriophora HP88			Sept	4.6 ± 1.43abcdefg
Control				9.1 ± 2.55a

Means within a column followed by different letters are significantly different ($P < 0.05$). (Tukey's multiple range test, $P = 0.05$, based on [log(x + 1)] transformed data).

Table 4. Effect of insecticides and entomopathogenic nematodes applied to soil in a commercial nursery in September 1997 for Japanese beetle larvae and evaluated in March 1998

Treatment	Active ingredient	Rate g(AI)ha (lb [AI]acre)	Mean no. (±SE) live Japanese beetle larvae per root ball ($n = 9$)
Marathon 1G	Imidacloprid	453.6 (0.4)	0.6 ± 0.24b
Mach2 2F	Halofenozide	2,268.0 (2.0)	1.6 ± 1.19ab
H. bacteriophora HP88		5 billion/h (2 billion/acre)	5.1 ± 1.45a
Control			5.2 ± 1.10a

Means within a column followed by different letters are significantly different ($P < 0.05$). (Tukey's multiple range test, $P = 0.05$, based on [log(x + 1)] transformed data).

Test 2 was conducted at the same site as test 1 with 2.1–2.4-m crabapple (*Malus* sp.). The trees were artificially infested with Japanese beetle three times during June through August as described above. Treatments included four rates of thiamethoxam (CGA-293343 25 WG) (142, 225, 300, and 600 g [AI]/ha [0.13, 0.20, 0.26 and 0.53 lb [AI]/acre]), one rate of thiamethoxam (CGA-293343 0.22G) (150 g [AI]/ha [0.13 lb [AI]/acre]), three rates of imidacloprid (Marathon 60 WP) (226.8, 340.2, and 453.6 g [AI]/ha [0.2, 0.3, and 0.4 lb [AI]/acre]), three rates of both formulations of halofenozide (Mach2 2 F and Mach2 1.5G) (1,134.0, 2,268.0, and 3,402.0 g [AI]/ha [1.0, 2.0, and 3.0 lb [AI]/acre]) and an untreated control. All applications were made in June and evaluated in October.

The trees in test 3 were approximately 3-m maple (*Acer* sp.) and were artificially infested twice in July as described above. Treatments included four rates of thiamethoxam (Flagship, CGA-293343 25 WG), one rate of thiamethoxam (CGA-293343 0.22G), three rates of imidacloprid (Marathon 60 WP), three rates of both formulations of halofenozide (Mach2 2 F and Mach2 1.5 G), and an untreated control. All application rates were the same as in test 2 in 1999. Thiamethoxam treatments were applied in June. All other treatments were applied in June and July. All treatments were evaluated in October.

Field Injection Tests. One field injection test was conducted in 1997 and two field injection tests were conducted in 1998. In 1997, 4.5-m honeylocust (*Gleditsia triacanthos inermis*) were artificially infested with Japanese beetle four times as described above. The 1997 treatments, rates, and application timings are listed in Table 7. All treatments were injected using a modified CO_2 backpack sprayer (25 psi). A probe was inserted eight times around the base of the tree at a depth of 7.6 cm. The experimental design was completely random with 10 single-tree replications per treatment. The test was evaluated in October.

Two field injection tests were conducted in 1998 using the same treatments but were conducted at different sites. In both tests, maple (*Acer* sp.) trees were artificially infested with Japanese beetle three times as described above. Both tests were evaluated in October. All treatments were injected as described in the 1997 test. The experimental designs were completely random with 10 single-tree replications. Treatments, rates, and application timings are listed in Table 8.

Statistics. All data were transformed [log(x + 1)] and subjected to analysis of variance (ANOVA) or Student's t-test (SigmaStat 1995). Means were separated with Tukey test ($P < 0.05$).

Results

Year 1996. In test 1, Japanese beetle was the dominant scarab grub (80.9%), followed by *Phyllophaga* spp. (11.3%), *Cotinus nitida* (L.) (5.0%), and unidentified scarab larvae (2.8%). The mean number of scarab larvae per root ball other than Japanese beetle in the control treatment was never >1. The mean number of Japanese beetle larvae in the untreated controls ranged from 2.2 to 6.4 and the mean number

Table 5. Effect of insecticides applied to soil in July and August 1998 on Japanese beetle larvae in a commercial nursery

Treatment	Active ingredient	Rate g (AI)hectare (lb[AI]acre)	Application timing	Mean no. (±SE) live Japanese beetle larvae per root ball ($n = 10$, both tests) Test 1 Maple trees	Test 2 Cherry trees
CGA-293343 25WG	Thiamethoxam	225.0 (0.20)	July	0.5 ± 0.34c	0.0 ± 0.00c
			Aug	2.0 ± 0.86bc	0.4 ± 0.22bc
Marathon 60WSP	Imidacloprid	453.6 (0.4)	July	0.0 ± 0.00c	0.1 ± 0.10c
			Aug	4.4 ± 1.36ab	0.3 ± 0.15bc
Turcam 2.5G	Bendiocarb	3,402.0 (3.0)	July	0.8 ± 0.34c	2.2 ± 0.57ab
			Aug	1.4 ± 0.54bc	0.6 ± 0.50bc
Fipronil 0.1G	Fipronil	340.3 (0.3)	July	0.1 ± 0.10c	0.1 ± 0.10c
Control				7.5 ± 1.08a	3.4 ± 0.96a

Means within a column followed by different letters are significantly different ($P < 0.05$) (Tukey's multiple range test, $P = 0.05$, based on [log(x + 1)] transformed data).

Table 6. Effect of insecticides, entomopathogenic nematodes, and *B. bassiana* strain GHA applied in June, July, or September 1999 on Japanese beetle larvae in a commercial nursery

Treatment	Application	Timing	Mean no. (±SE) Japanese beetle larvae per root ball (n = 10)
B. bassiana-debris removed and fungus raked into the soil	2.5×10^9 cfu/m^2	July	1.5 ± 0.76abc
B. bassiana-debris removed and fungus distributed on soil surface	2.5×10^9 cfu/m^2	July	0.5 ± 0.22bcd
B. bassiana-debris not removed and fungus distributed on soil surface	2.5×10^9 cfu/m^2	July	3.7 ± 2.12a
B. bassiana-debris removed and fungus placed 5 cm below soil surface	2.5×10^9 cfu/m^2	July	1.0 ± 0.33abcd
B. bassiana-debris removed and rehydrated fungus placed 5 cm below soil surface	2.5×10^9 cfu/m^2	July	2.2 ± 1.08abc
B. bassiana-debris removed and fungus distributed on soil surface - repeat application	2.5×10^9 cfu/m^2	July and Sept	0.3 ± 0.21cd
Heterorhabditis marelatus	2.5 billion/h (1 billion/acre)	Sept	1.2 ± 0.55abcd
Heterorhabditis marelatus	5.0 billion/h (2 billion/acre)	Sept	0.7 ± 0.37bcd
Marathon 60WP (imidacloprid)	453.6 g (AI)/h	June	0.1 ± 0.10d
Mach2 2F (halofenozide)	2,268.0 g (AI)/h	June	0.6 ± 0.50bcd
CGA-293343 25 WG (thiamethoxam)	225 g (AI)/h	June	0.0 ± 0.00d
CGA-293343.22G (thiamethoxam)	150 g (AI)/h	June	2.0 ± 0.70ab
Control			1.5 ± 0.60abc

Means followed by different letters are significantly different (Tukey's multiple range test, $P = 0.05$, based on [log(x + 1)] transformed data, n = 10). *Beauveria bassiana* obtained from USDA-APHIS (Weslaco, TX). *Heterorhabditis marelatus* obtained from Integrated BioControl Systems, Aurora, IN.

of Japanese beetle larvae per root ball treated with imidacloprid (either formulation or in combination with carbaryl) remained below one for all application timings with two exceptions (Table 1). The number of Japanese beetle larvae in the imidacloprid granular treatments applied in May was significantly lower than in the control ($F = 7.419$; df = 5, 24; $P < 0.001$) (Table 1). The number of Japanese beetle larvae was not significantly reduced by any treatment made in June, July, or August compared with the control. However, out of the 30 imidacloprid and halofenozide treatments made during that period, 12 treatments had no Japanese beetle larvae and 16 of the treatments had a mean equal to or <0.4 Japanese beetle larvae per root ball (Table 1). The average number of Japanese beetle larvae in the control treatments for June, July, and August were 3.0, 2.4, and 2.2, respectively. In September, imidacloprid alone (both formulations) and imidacloprid (75 WP) in combination with carbaryl significantly reduced the number of Japanese beetle larvae ($F = 3.703$; df = 14, 60; $P = 0.002$) (Table 1). Combining imidacloprid with carbaryl as a soil, foliar, or both soil and foliar application did not improve the efficacy of imidacloprid alone. The mean number of Japanese beetle larvae per root ball in the biological treatments was generally greater than in the insecticide treatments and ranged from 0.6 to 6.2 larvae per root ball.

Table 7. Effect of insecticides injected around the base of a tree in June, August, or September 1997 on Japanese beetle larvae in a commercial nursery

Treatment	Active ingredient	Rate g (AI)hectare (lb [AI]acre)	Application timing	Mean no. (±SE) live Japanese beetle larvae per root ball (n = 10)
Fipronil 1.67 SC	Fipronil	567.0 (0.5)	June	0.1 ± 0.10ab
			Aug	0.3 ± 0.21ab
Marathon 60 WSP	Imidacloprid	453.6 (0.4)	June	0.2 ± 0.20ab
			Aug	0.0 ± 0.00b
			Sept	0.2 ± 0.20ab
Mach2 2F	Halofenozide	2,268.0 (2.0)	June	0.0 ± 0.00b
			Aug	0.0 ± 0.00b
			Sept	0.0 ± 0.00b
Sevin 4F	Carbaryl	1,134.0 (1.0)	June	1.0 ± 0.40a
			Aug	0.5 ± 0.27ab
Dylox 80 T&O	Trichlorfon	9,298.8 (8.2)	Sept	0.1 ± 0.10ab
Dursban 4E	Chlorpyrifos	1,234.0 (1.0)	June	0.5 ± 0.27ab
			Aug	0.1 ± 0.10ab
Control				0.9 ± 0.46ab

Means within a column followed by different letters are significantly different ($P < 0.05$). (Tukey's multiple range test, $P = 0.05$, based on [log(x + 1)] transformed data).

Table 8. Effect of insecticides injected around the base of a tree in July or August 1998 on Japanese beetle larvae in a commercial nursery

Treatment	Active ingredient	Rate g(AI)ha (lbs [AI]/acre)	Application timing	Mean no. (±SE) live Japanese beetle larvae per root ball: Test 1 Maple trees ($n = 12$)	Mean no. (±SE) live Japanese beetle larvae per root ball: Test 2 Maple trees ($n = 10$)
Marathon 60WSP	Imidacloprid	453.6 (0.4)	July	0.6 ± 0.23b	1.2 ± 0.39c
			Aug	1.6 ± 0.47ab	5.0 ± 1.37abc
Dursban 4E	Chlorpyrifos	1,234.0 (1.0)	July	1.4 ± 0.65ab	2.1 ± 0.92c
			Aug	4.3 ± 0.97a	6.0 ± 0.93ab
Mach2 2F	Halofenozide	2,268.0 (2.0)	July	1.4 ± 0.43ab	2.3 ± 0.42bc
Fipronil 1.67SC	Fipronil	567.0 (0.5)	July	1.1 ± 0.40b	0.9 ± 0.59c
Talstar 7.9%	Bifenthrin	226.8 (0.2)	July	0.7 ± 0.28b	1.9 ± 0.61bc
Dylox 80 T&O	Trichlorfon	9,298.8 (8.2)	Aug	3.5 ± 0.97ab	10.8 ± 1.98a
Control				4.1 ± 0.82a	6.3 ± 1.20ab

Means within a column followed by different letters are significantly different ($P < 0.05$). (Tukey's multiple range test, $P = 0.05$, based on $[\log(x + 1)]$ transformed data).

In test 2, 1996, the average number of Japanese beetle larvae per root ball was low (<1) and represented only 27.1% of the scarab larvae recovered from the control treatment. The percentage of remaining scarab larvae were *Phyllophaga* spp. and unidentified scarab larvae at 25.4 and 47.5%, respectively. Treatment applications did not significantly reduce the number of Japanese beetle larvae per root ball (May: $F = 1.010$; df = 5, 24; $P = 0.433$; June: $F = 1.457$; df = 6, 28; $P = 0.229$; July: $F = 0.844$; df = 6, 28; $P = 0.547$; August: $F = 1.875$; df = 6, 28; $P = 0.121$; September: $F = 1.698$; df = 6, 28; $P = 0.160$). The average number of Japanese beetle larvae in the insecticide treatments ranged from 0.0 to 1.4 with the majority of the treatments containing <0.2 larvae per root ball. The range of Japanese beetle larvae in the control treatments was 0.6–1.5. The combination of imidacloprid and carbaryl did not improve the efficacy of imidacloprid alone; however, due to the low numbers of larvae, differences among treatments would be difficult to detect.

In test 3, 1996, the mean number of Japanese beetle larvae in the control treatments ranged between 1.6 and 2.8 per root ball. Japanese beetle larvae made up 46.4% of the scarab larvae recovered in the control treatment followed by 30.9% *Phyllophaga* spp., 16.4% *C. nitida,* and 6.4% unidentified scarab larvae. There were no treatment differences for the *Phyllophaga* spp. The mean number of Japanese beetle larvae in the insecticide treatments ranged from 0.0 to 1.0 with seven of the nine treatment-application timings with less than a mean of 0.4 larvae per root ball. Neither formulation of imidacloprid significantly reduced the number of Japanese beetle larvae (May: $t = 1.033$; df = 8; $P = 0.332$; June: $F = 2.980$; df = 2, 12; $P = 0.089$; July: $F = 1.814$; df = 2, 12; $P = 0.205$; August: $F = 1.987$; df = 2, 12; $P = 0.180$; September: $F = 1.701$; df = 2, 12; $P = 0.224$). (Note: There were only two treatments [granular formulation of imidacloprid and the untreated control] in May.)

Japanese beetle larvae were the dominant scarab species (98.4%) recovered in test 4, 1996. The mid-August application of both formulations of imidacloprid and halofenozide significantly reduced the number of larvae ($F = 6.310$; df = 4, 20; $P = 0.002$) (Table 2). Only halofenozide significantly reduced the number of larvae in the late August application ($F = 3.658$; df = 4, 20; $P = 0.022$). There were no significant treatment differences in the mid-September application ($F = 2.350$; df = 4, 20; $P = 0.089$). The average number of Japanese beetle larvae in the control treatment was 2.6.

Year 1997. In test 1, Japanese beetle larvae recovered from the control treatment made up 83.7% of the scarab larvae followed by 15.3% *Phyllophaga* spp., and 1.0% *C. nitida.* Both formulations of imidacloprid applied from June through September, halofenozide applied in June and July, and two of the three rates of fipronil applied in June significantly reduced the number of Japanese beetle larvae ($F = 7.721$; df = 22, 184; $P < 0.001$); however, none of these treatments was different from each other (Table 3). A June application of imidacloprid followed by a September application of trichlorfon was not significantly different from imidacloprid alone.

In test 2 of 1997, the number of scarab larvae recovered from the control treatment was very low with 50% Japanese beetle and 50% *Phyllophaga* spp. The mean number of Japanese beetle larvae per root ball in the control was 0.6 and there were no significant differences among treatments ($F = 1.425$; df = 22, 138; $P = 0.113$). The mean number of Japanese beetle larvae per root ball in all but two of the treatments (nematodes applied in September and tefluthrin applied in July) was 0.1 or less.

In test 3 of 1997, the number of Japanese beetle larvae was significantly reduced in the imidacloprid treatment compared with the control and the nematode treatments ($F = 4.845$; df = 3, 32; $P = 0.007$) (Table 4). Japanese beetles represented 88.7% of the scarab larvae recovered in the control, followed by 7.5% *Phyllophaga* spp. and 3.8% *C. nitida.*

Year 1998. All treatments except one in each test (imidacloprid applied in August in test 1 and bendiocarb applied in July in test 2) significantly reduced the number of Japanese beetle larvae compared with the control (test 1: $F = 12.611$; df = 7, 72; $P < 0.001$; test 2: $F = 7.733$; df = 7, 72; $P < 0.001$) (Table 5). Imidacloprid (test 1) and thiamethoxam (test 2) both applied in July provided 100% control of Japanese beetle larvae. Japanese beetle represented 93.7 and 97.1% of the scarab larvae recovered in the control treatment from test 1 and 2, respectively.

Year 1999. In all three tests conducted in 1999 the mean number of Japanese beetle larvae per root ball was low. In test 1, Japanese beetle larvae comprised 53.6% of the scarab larvae in the control treatment followed by 46.4% *Phyllophaga* spp. Although none of the biological treatments significantly reduced the number of Japanese beetle larvae (Table 6), of the six *B. bassiana* treatments, an application in July and repeated in September showed the highest reduction in larval numbers, followed by the treatment where debris was removed and the fungal spores were distributed on the soil surface. The highest mean number of Japanese beetle larvae in the entire test was found where *B. bassiana* was applied over uncleared soil surface. Only imidacloprid and thiamethoxam (25 WG) significantly reduced the number of larvae ($F = 2.176$; df = 14, 134; $P < 0.012$) compared with the controls.

There were no significant differences among treatments in tests 2 and 3. The average number of larvae in the control in test 2 was 0.6 and 0.3 in test 3. In test 2, Japanese beetle larvae represented 54.5% of the scarab larvae recovered from the control treatment and *Phyllophaga* spp. represented 45.5%. In test 3, Japanese beetle made up 60% of the scarab larvae recovered from the control treatment and 40.0% *Phyllophaga* spp. The mean number of Japanese beetle larvae in the treatments in test 2 was 0.1 or less with two exceptions (the low and high rates of the granular formulation of halofenozide). No larvae were found where trees were treated with the highest three rates of thiamethoxam 25 WG, all rates of imidacloprid, or the highest rate of halofenozide 60 WP. In test 3, larvae were found only in the controls and in root balls treated with the lowest rates of thiamethoxam, imidacloprid, and halofenozide.

Field Injection. In 1997, the number of Japanese beetle larvae was low (less than a mean of 1.0 per root ball) but comprised 75% of the scarab larvae in the control treatment. None of the treatments significantly reduced the number of larvae compared with the control, although imidacloprid injected in August and all applications of halofenozide significantly reduced the number of larvae compared with the carbaryl treatment injected in July ($F = 2.505$; df = 13, 126; $P = 0.004$) (Table 7). No larvae were found in any root ball treated with halofenozide. The mean number of Japanese beetle larvae in untreated root balls was 0.9.

In 1998, imidacloprid and fipronil injected in July significantly reduced the number of Japanese beetle larvae in both tests (test 1: $F = 4.578$; df = 8, 99; $P < 0.001$; test 2: $F = 9.094$; df = 8, 82; $P < 0.001$) (Table 8). Japanese beetle larvae represented 60.5 and 86.3% of the total scarab larvae in the control in tests 1 and 2, respectively.

Discussion

Treatment options for control of Japanese beetle larvae, as well as other pest scarab larvae, have been extremely limited for production field nurseries and there is a critical need for consistent, efficacious treatments. Growers of field production nursery stock would prefer treatment options that allow them the freedom to treat, and then ship their nursery stock relatively soon after treatment. However, this approach is difficult for several reasons. Many of the insecticides currently available for Japanese beetle control in a production nursery target the young actively feeding larvae. Digging and shipping in a field nursery commonly occurs during the fall, winter, and early spring when the larvae are generally third instars, soil temperatures are cold, and the larvae are deep in the soil and are not actively moving or feeding (or accessible to insecticides). Therefore, contact between the insecticide and the larvae is difficult (Mannion et al. 2000b). Some biological controls such as nematodes generally require soil temperatures above 15°C to be effective.

The overall goal of these tests was to determine which treatments were consistent and efficacious and how to best apply them. Most of the insecticides evaluated in these tests require an early application (i.e., when larvae are young and actively feeding). Imidacloprid was the most tested active ingredient in the trials conducted during the past 4 yr. Imidacloprid is labeled for use in turf and commercial nurseries and has been shown to be an excellent treatment for Japanese beetle larvae. Imidacloprid is a chloronicotinyl insecticide that acts on the cholinergic receptors in the postsynaptic membranes and disrupts normal nerve function (Bai et al. 1991). The major advantages of imidacloprid are the low application rates and the extremely low vertebrate toxicity. Koppenhofer and Kaya (1998) found that combinations of imidacloprid and entomopathogenic nematodes were synergistic against a white grub species and there was no negative effect on survival or infectivity when nematodes were agitated in solutions of imidacloprid. Quintela and McCoy (1997, 1998) found a similar synergistic relationship and pathogenicity enhancement between imidacloprid and entomopathogenic fungi used against larvae of *Diaprepes abbreviatus* (L.). A possible practical disadvantage associated with imidacloprid is that it is generally most effective applied when the larvae are young and actively feeding. In typical nursery production, trees are not treated well in advance of digging and shipping, so extra planning and management is required to ensure only treated trees are shipped to noninfested areas. Growers must also be selective in their use of this insecticide because of the expense.

Overall, imidacloprid performed very well when applied in May, June, or July. August and September applications provided less consistent results. There were no differences in control between the granular and wettable powder formulations. The range of control widened with the month of application. For example, 91–100% control was achieved with a May application, 87–100% control with a June application, 83–100% control with a July application, 41–100% control with an August application, and a 46–100% control with a September application. Approximately 60% of all imidacloprid treatments applied in May, June, or July provided 95% or better control. In contrast, ≈25% of all imidacloprid treatments applied in August or September provided 95% or better control.

In 1996, imidacloprid treatments were combined with foliar or soil treatments of carbaryl. Carbaryl is commonly used as a foliar spray to kill feeding beetles. It was hypothesized that the carbaryl would reduce the number of beetles laying eggs as well as potentially create a barrier on the soil surface that the beetles would contact when seeking an oviposition site. The addition of carbaryl as a soil or foliar application to imidacloprid, however, did not improve the control over imidacloprid alone. A foliar spray of carbaryl alone did not significantly reduce the number of Japanese beetle larvae.

Imidacloprid was injected into the soil around the base of each tree in tests conducted in 1997 and 1998. Injection is a labor-intensive procedure but may potentially increase the efficacy of a treatment by reducing exposure to weathering as well as by placing the chemical in closer proximity to the larvae. However, this method of application did not improve the efficacy of imidacloprid in these trials.

In 1997, a June treatment of imidacloprid was followed with a fall application of trichlorfon. It was assumed that any larvae remaining after the imidacloprid treatment would be controlled with the trichlorfon. However, in that particular test, the trichlorfon performed poorly when used alone or in conjunction with imidacloprid and therefore did not improve the efficacy of imidacloprid alone.

Halofenozide was field tested in three of the past 4 yr (1996, 1997, and 1999) and provided control of Japanese beetle larvae similar to imidacloprid. Halofenozide is in the diacylhydrazine class of insecticides, which are ecdysone agonists (RohMid 1997). Once fed upon, this compound accelerates the molting process, causing premature death with little threat of toxicity to nontarget organisms. Mannion et al. (2000a) found that halofenozide did not have a detrimental effect on survival or infectivity of entomopathogenic nematodes. Halofenozide has been shown to be toxic against Japanese beetle larvae (Monthean and Potter 1992, Cowles and Villani 1996, Cowles et al. 1999) and is currently labeled for control of this pest as well as immature stages of certain insects in turfgrass.

Although the numbers of Japanese beetle larvae observed in root balls after surface application with halofenozide never significantly differed from surface treatment with imidacloprid, with the exception of one application in September in one test, the range of control was slightly narrower. Larval control ranged from 60 to 87% for June applications, 85 to 100% for July applications, 82 to 92% for August applications, and 55 to 90% for September applications. When halofenozide was injected into the soil around the base of the tree in June, August, and September 1997, no larvae were found in root balls (although infestations were very low to begin with). However, in 1998 it provided 63 and 66% control when injected in July.

Fipronil and thiamethoxam are two other compounds that demonstrated some potential for Japanese beetle larval control in nursery field production. Fipronil, a phenylpyrazole insecticide, disrupts central nervous system activity. Studies have demonstrated that fipronil displays higher potency for insects compared with vertebrates due to the target specificity (Mudge et al. 2000). Fipronil was applied at three rates in a test in June 1997 achieving 91% control with the low rate and 98% control with the high rate. The middle rate performed poorly. In 1998, fipronil was applied in two tests in July and showed 99 and 97% control in those tests. When fipronil was injected into the soil around the base of each tree in June and August 1997, numbers of Japanese beetle larvae were reduced by 89 and 67%, respectively, although there were no statistical differences compared with untreated plants, probably due to the low number of larvae found. Fipronil injected in July 1998 in two tests significantly reduced Japanese beetle infestations by 73 and 86%. The method of injection did not appear to improve the efficacy of fipronil compared with a surface application as was the case with imidacloprid. Although fipronil is currently not labeled for Japanese beetle larvae, several labels are expected in the year 2000 for imported fire ants and other pests. Both Japanese beetle and imported fire ants are regulated pests that can be spread through the movement of infested nursery stock. Therefore, similarities in the requirements for use against both pests suggest that insecticides found to be effective against one pest could be considered for use against the other. However, the rates currently shown to be effective against imported fire ant are lower than the rates tested against Japanese beetle and therefore, may not be effective. More research is necessary to determine whether fipronil will provide consistent and efficacious control of Japanese beetle larvae.

Thiamethoxam is a new insecticide in the neonicotinoid class (subclass thianicotinyl) that has similar attributes to imidacloprid (subclass chloronicotinyl). It is effective against many sucking insects and some Coleoptera at low use rates. Thiamethoxam was first tested in these trials in 1998. Japanese beetle larvae were reduced 93 and 100% in two tests with July applications and 73 and 88% with August applications. In a June application in 1999, no larvae were found in root balls following treatment. The granular formulation, however, performed poorly, perhaps due to poor distribution through the soil profile.

A series of other insecticide treatments (permethrin, tefluthrin, trichlorfon, and bendiocarb) applied during the summer months as a surface application were generally not successful in reducing the numbers of Japanese beetle larvae in field-grown nursery. Most of these treatments achieved <50% reduction in the number of larvae. The exception was bendiocarb, which provided 81–89% control in three of four treatments applied in July and August in two tests conducted in 1998. In 1997, trichlorfon, chlorpyrifos, and carbaryl injected into the soil provided poor-to-moderate control; however, the Japanese beetle larvae infestations were low and none of the insecticide treatments was significantly different from the control. In both injection tests in 1998, chlorpyrifos and trichlorfon resulted in <67% control of Japanese beetle larvae; in most cases there was no difference from untreated trees. In the same tests in 1998, bifenthrin injected into the soil provided slightly better control (70 and 83%), but the effect was only significant in one test.

Generally, the biological control treatments provided poor-to-moderate control of Japanese beetle larvae in these field tests. In 1996, applications of the nematode, *H. bacteriophora* HP88, provided no reduction in Japanese beetle larvae when applied in June, July, or August, and only 65% reduction when applied in September 1996. Similar results were achieved in a test conducted in 1997 in which 52 and 49% control was achieved from August and September applications. In another test conducted in 1997, no reduction was achieved from an application of this nematode in September. In 1999, *H. marelatus,* applied in September performed poorly. The low rate of the nematodes (2.5 billion/ha) provided 20% control and the high rate (5 billion/ha) provided 53% control. Although some researchers have found entomopathogenic nematodes to provide moderate-to-excellent control of Japanese beetle larvae in a turf environment (Klein and Georgis 1992; Selvan et al. 1993, 1994; Yeh and Alm 1995; Klein and Moyseenko 1997) results are not consistent and depend on numerous factors (i.e., favorable temperature and moisture, soil type, application process, nematode storage and handling, nematode species/strain, nematode quality, host insect) (Georgis and Gaugler 1991, Georgis and Poinar 1994). An understanding of these factors and how to appropriately use the nematodes is imperative to improve chances of success. Although entomopathogenic nematodes alone are unlikely to achieve the level of control required for regulatory purposes, incorporation into a long-term management program to reduce Japanese beetle populations may be a possibility. Additionally, the efficacy of nematodes may be enhanced in combination with other control agents or insecticides.

Bacillus thuringiensis subspecies *japonensis* Buibui strain is a facultative pathogen that is easily mass-produced and is scarab species-specific (Suzuki et al. 1992). This product is efficacious against the early instars of scarabaeid pests in Japan (Suzuki et al. 1994a) and retains a high activity for several months against scarab larvae in natural soil (Suzuki et al. 1994b). Currently, however, this product is not available. *B. thuringiensis* subspecies *japonensis* Buibui strain was evaluated in 1996 only and provided inconsistent control of Japanese beetle larvae (0–75%). This is in contrast to the results found in a turf environment (Alm et al. 1997, Michaels 2000), which showed generally higher levels of control.

Several application methods of *B. bassiana* were compared for control of Japanese beetle larvae in 1999. Application parameters included removing or not removing the surface debris and the placement (i.e., broadcast on soil surface or incorporated into the soil) of the fungal spores. Four of the six treatments provided poor control. There did appear to be some reduction when surface debris was removed and the fungal spores were distributed on the surface in July (70% reduction) or in July with a repeat application in September (80%), although neither effect was statistically significant.

It is very difficult, if not impossible, to ensure complete control of Japanese beetle larvae in field-grown plants from infested areas. Even the best treatments did not provide 100% control all of the time or reach levels acceptable under regulatory guidelines and none of the field-applied treatments provided improved efficacy over dipping root balls in chlorpyrifos (Mannion et al. 2000b). This suggests two things. First, can an acceptable risk of movement of Japanese beetle be determined and thereby (based on that), choose an acceptable treatment. Second, at the present state of art, neither chemical nor biological treatments can guarantee 100% control, indicating that more than one control tactic will be necessary. In addition, long range plans to reduce the local population of Japanese beetle may help in reducing the risk of unintentionally spreading Japanese beetle. Several of the insecticides, i.e., imidacloprid, halofenozide, and thiamethoxam, provided good to excellent control when used at the appropriate timing. These data are consistent with data compiled from 1977 to 1999 *Insecticide and Acaracide Tests and Arthropod Management Tests,* in which thiomethoxam, imidacloprid, and halofenozide provided an average of 99.8, 93.2, and 93.1% control, respectively, of masked chafer and Japanese beetle larvae in turf (Shetlar 1999). These products have numerous advantages such as low use rates, low toxicity to many nontarget organisms and compatibility (sometimes synergy) with some biological controls. The disadvantages are expense and a limited window of application. Currently, only imidacloprid is registered for use in commercial nurseries with expected labels for thiamethoxam in 2001. Therefore, treatment options for nursery growers continue to be limited and none are expected to provide levels of control that meet regulatory standards. There remains a tremendous need for reliable treatment methods and management programs that will allow nursery growers in areas infested with Japanese beetle to continue to ship to uninfested areas.

Acknowledgments

We thank the inspectors of the Tennessee Department of Agriculture for their help in evaluating some of the field tests. We also thank all the companies and agencies that provided insecticide or biological products for testing. Florida Agricultural Experiment Station Journal Series No. R-07887.

References Cited

Alm, S. R., M. G. Villani, T. Yeh, and R. Shutter. 1997. *Bacillus thuringiensis* serovar *japonensis* strain buibui for control of Japanese and oriental beetle larvae (Coleoptera: Scarabaeidae). Appl. Entomol. Zool. 32: 477–484.

Bai, D., S.C.R. Lummia, 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.

Blosser, W. 1999. Japanese beetle quarantines–where are we now? Reg. Hortic. 25: 4–6.

Brandenburg, R. L., and M. G. Villani. 1995. Handbook of turfgrass insect pests. Entomological Society of America, Lanham, MD.

Cowles, R. S., and M. G. Villani. 1996. Susceptibility of Japanese beetle, oriental beetle, and European chafer (Coleoptera: Scarabaeidae) to halofenozide, an insect growth regulator. J. Econ. Entomol. 89: 1556–1565.

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. 1972a. Biology of the Japanese beetle. U.S. Dep. Agric. Agric. Res. Ser. Tech. Bull. 1449.

Fleming, W. E. 1972b. Preventing Japanese beetle dispersion by farm products and nursery stock. U.S. Dep. Agric. Agric. Res. Ser. Tech. Bull. 1441.

Georgis, R., and R. Gaugler. 1991. Predictability in biological control using entomopathogenic nematodes. J. Econ. Entomol. 84: 713–720.

Georgis, R., and G. O. Poinar Jr. 1994. Nematodes as bioinsecticides in turf and ornamentals, pp. 477–489. *In* A. R. Leslie [ed.], Integrated pest management for turf and ornamentals. Lewis, Boca Raton, FL.

Klein, M. G. 1995. Microbial control of turfgrass insects, pp. 95–100. *In* R. L. Brandenburg and M. G. Villani [eds.], ESA handbook of turfgrass insect pests. Entomological Society of America, Lanham, MC.

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.

Klein, M. G., and J. J. Moyseenko. 1997. Isolation and infectivity of entomopathogenic nematodes from Ohio. J. Nematol. 29: 590.

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

Mannion, C. M., H. E. Winkler, D. I. Shapiro, and T. Gibb. 2000a. Interaction between halofenozide and the entomopathogenic nematode *Heterorhabditis marelatus* for control of Japanese beetle (Coleoptera: Scarabaeidae) larvae. J. Econ. Entomol. 93: 48–53.

Mannion, C. M., W. McLane, M. G. Klein, D. G. Nielsen, and D. A. Herms. 2000b. Insecticide dips for control of Japanese beetle and other soil-infesting white grubs in B&B nursery stock. J. Environ. Hortic. 18: 89–93.

Michaels, T. E. 2000. Use of insect pathogen *Bacillus thuringiensis* for control of grubs (Coleoptera: Scarabaeidae) Ph.D. dissertation, Iowa State University, Ames.

Monthean, C., and D. A. Potter. 1992. Effects of RH 5849, a novel insect growth regulator, on Japanese beetle (Coleoptera: Scarabaeidae) and fall armyworm (Lepidoptera: Noctuidae) in turfgrass. J. Econ. Entomol. 85: 507–513.

Mudge, M., K. Lewis, K. Kukorowski, G. Werner, and B. Chism. 2000. Fipronil–a summary of three years of field testing on red imported fire ants. *In* J. L. Croker, K. M. Vail, and R. M. Pereira [eds.], Proceedings of the 2000 Imported Fire Ant Conference, 5–7 April 2000. University of Tennessee, Knoxville, TN.

National Plant Board. 1998. U.S. Domestic Japanese Beetle Harmonization Plan (http://www.aphis.usda.gov/npb/jbplan98.html).

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

Quintela, E., and C. W. McCoy. 1998. Synergistic effect of imidacloprid and two entomopathogenic fungi 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.

Selvan, S., R. Gaugler, and J. F. Campbell. 1993. Efficacy of entomopathogenic nematode strains against *Popillia japonica* (Coleoptera: Scarabaeidae) larvae. J. Econ. Entomol. 86: 353–359.

Selvan, S., P. S. Grewal, R. Gaugler, and M. Tomalak. 1994. Evaluation of steinernematid nematodes against *Popillia japonica* (Coleoptera: Scarabaeidae) larvae: species, strains, and rinse after application. J. Econ. Entomol. 87: 605–609.

Shetlar, D. 1999. White grubs "tough to kill or not?" Ohio Turfgrass Foundation Turfnews 61: 6–7.

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

Suzuki, N., H. Hori, K. Ogiwara, S. Asano, R. Sato, M. Ohba, and H. Iwahana. 1992. Insecticide spectrum of a novel isolate of *Bacillus thuringiensis* serovar *japonensis.* Biol. Control 2: 138–142.

Suzuki, N., H. Hori, M. Tachibana, and S. Asano. 1994a. *Bacillus thuringiensis* strain Buibui for control of cupreous chafer, *Anomala cuprea* (Coleoptera: Scarabaeidae), in turfgrass and sweet potato. Biol. Control 4: 361–365.

Suzuki, N., H. Hori, M. Tachibana, L. S. Indrasith, and A. Fujiie. 1994b. Residual effects of the toxin from *Bacillus thuringiensis* serovar *japonensis* strain Buibui in the soil. Appl. Entomol. Zool. 29: 610–613.

Tashiro, H. 1987. Turfgrass insects of the United States and Canada. Cornell University Press, Ithaca, NY.

Vittum, P., M. G. Villani, and H. Tashiro. 1999. Turfgrass Insects of the United States and Canada. Cornell University Press, Ithaca, NY.

Yeh, T., and S. Alm. 1995. Evaluation of *Steinernema glaseri* (Nematoda: Steinernematidae) for biological control of Japanese and oriental beetles (Coleoptera: Scarabaeidae). J. Econ. Entomol. 88: 1251–1255.

Received for publication 22 November 2000; accepted 22 April 2001.