N O T E

Biological Control of Grape Root Borer (Lepidoptera: Sesiidae) with Commercially Available Entomopathogenic Nematodes in Florida Muscadine and 'Cynthiana' Grapes[1]

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The grape root borer, *Vitacea polistiformis* (Harris) (Lepidoptera: Sesiidae), is a clearwing moth species native to the eastern United States. Its range extends north to Michigan, west to the Mississippi Valley, and south to Florida. It is a major pest of grapes in the eastern United States, damaging vines by girdling the roots, thus cutting off nutrients and water transfer from the roots to the remainder of the plant. A single larva feeding on the root system is able to reduce a vine's yield by 50%, and two or three larvae within a root system can reduce winter hardiness, fruit quality, and yield and can ultimately destroy an entire vine (Dutcher and All 1979, J. Econ. Entomol. 72: 159–161).

Adult grape root borers do not feed and they live for about 8 d (Dutcher and All 1978, J. Georgia Entomol. Soc. 13: 59–63). Females lay their eggs on or near grape vines and nearby weeds, and the eggs fall to the soil surface where they hatch approximately 18 d later. Younger larvae feed throughout the entire root zone while older larvae are concentrated at the trunk. Most researchers agree that the borer's life cycle takes 2 yr to complete and that it overwinters in the larval stage, which lasts for up to 22 mo. Pupation occurs near the soil surface in June and July (Dutcher and All 1979). Weihman and Liburd (2007, Florida Entomol. 89: 245–250), however, postulate that there is a 2-yr life cycle in the East but that populations in Florida may have a 1-yr cycle, with adults emerging over a period of 6 mo compared to the 2-mo period farther north.

Natural enemies, resistant root stocks, and insecticides offer some management of the grape root borer. Other pest-management strategies include mating disruption (Johnson et al. 1981, Misc. Publ. Entomol. Soc. Ark. 12(2):1–7; Johnson

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et al. 1986, J. Entomol. Sci. 21: 231–236; Johnson et al. 1991, Environ. Entomol. 20: 931–934; Weihman and Liburd 2007), soil mounding, and use of row covers. Historically, some control has been achieved by a single application of chlorpyrifos targeted at egg-laying adults emerging from pupae, and adult populations which fly onto plants in early July can be reduced with one insecticide application. However, chlorpyrifos can only be applied once during a growing season and has a 35-d postharvest interval. Effective control of larvae is more difficult and, once the larvae hatch and reach the root system, insecticidal control is ineffective. Florida grape growers have no control methods for the larvae, which are the most-destructive stage of the insect.

A number of natural enemies or biocontrol approaches have been explored including predators and parasitoids of eggs and larvae near the soil surface (Dutcher and All 1978, Environ. Entomol. 7: 456–460) and the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Sorokin) Metchnikoff (Clark and Enns, 1964, J. Kansas Entomol. Soc. 37: 57–63; Dutcher and All 1978). However, entomopathogenic nematodes appear to have the greatest potential for biological control of the larval stages (All et al. 1981, Misc. Publ. Entomol. Soc. Am. 12: 9–14; Saunders and All 1985, J. Invert. Path. 45: 147–151; Williams et al. 2002, Biocontr. Sci. Tech. 12: 35–42). Florida citrus growers are already applying nematodes to over 20,000 ha of oranges each year to control the citrus root weevil, *Diaprepes abbreviatus* (L.). A similar approach might be taken for control of the grape root borer in grapes.

The entomopathogenic nematodes in the genera *Heterorhabditis* and *Steinernema*, together with their respective bacterial symbionts *Photorhabdus* and *Xenorhabdus*, are obligate parasites of insects. Mass-produced nematodes can provide effective biological control of some lepidopteran, dipteran, and coleopteran pests of commercial crops. Genera in the family Heterorhabditidae are unique in that each has a symbiotic relationship with a bacterium species. This relationship is necessary in order to kill the insect host and digest the host tissues so as to provide suitable nutrient conditions for nematodes to grow and develop. The foraging strategies of nematodes are influenced by soil depth and host preferences. An infective juvenile stage larva, which is the only stage of the nematode capable of transporting and infecting a host, uses strategies to find hosts that vary from ambush to cruise foraging (Campbell and Gaugler 1993, Behavior 126: 155–169). For instance, some *Steinernema* species nictate (raise their bodies above the soil surface) in order to ambush prey (Campbell and Gaugler 1993) or jump by forming a loop with their bodies to attack prey (Campbell and Kaya 2000, Behavior 137: 591–609 Part 5). The behavior depends on environmental conditions. Other species adopt a cruising strategy and tunnel the soil searching for potential hosts. Ambush predators such as *Steinernema carpocapsae* (Weiser) mostly infect insects on the soil surface, while cruising predators like *Heterorhabditis bacteriophora* (Poinar) infect insects such as grape root borer larvae that live deep in the soil or in roots (Campbell and Gaugler 1993).

Research conducted by Williams et al. (2002) evaluated 10 nematode species representing 17 nematodes strains of *Heterorhabditis* and *Steinernema* and found *H. bacteriophora* and the New Zealand species *Heterorhabditis zealandica* Poinar produced 92% and 86% control of grape root borer, respectively, in lab studies that duplicated field conditions. The New Zealand species *H. zealandica* has been

naturalized and is considered endemic to Florida. Although *H. zealandica* may eventually be commercialized, it is not available at present.

Two nematode species—*Heterorhabditis bacteriophora* (Poinar) and *Heterorhabditis megidis* Poinar, Jackson & Klein—were selected to assess their efficacy against grape root borer larvae based on their commercial availability and demonstrated effectiveness. *Heterorhabditis bacteriophora* is mobile and roams deeply through the soil searching for potential insect hosts, while *H. megidis* is larger in size and possesses the ability to withstand longer dry periods in the soil column without a host (Poinar et al. 1987, Proc. Helminthological Soc. Wash. 54: 53–59).

The study was conducted at Florida A&M University Center for Viticulture and Small Fruit Research where an 18.2-ha vineyard is planted with multiple varieties of grapes. This vineyard has a loam soil which is periodically checked for pH and nutrients. The row orientation is north–south with a row length of 68.4 m and a row spacing of 3 m. The vineyard uses several trellis systems (single wire, T-trellis, and Geneva double curtain) as part of other studies. All plants are irrigated by a drip irrigation system (turbo T-tape model 40, 5.0 L/m/h).

Adult male grape root borer activity was monitored throughout the study using pheromone bucket traps (Great Lakes IPM, Vestaburg, MI). Each bucket trap contained a septum baited with female grape root borer pheromone (99% (*E,Z*)-2,13-octadecadienylacetate, 1% (*Z,Z*)-3,13-ctadecadienylacetate), 1 mg per septum, and an insecticide strip (Great Lakes IPM) which lasted all season. Five traps were placed 200 m apart in the vineyard and checked biweekly in 2008, 2009, and 2010. In the summer of 2008, traps were placed on 15 May and removed on 10 October.

The 2008 trial consisted of five treatments replicated five times in a completely randomized design with each treatment replicate (plot) consisting of three 'Cynthiana' grape vines of *Vitis aestivalis* Michx. All plots were confirmed to have grape root borer activity. Treatments were to apply *H. bacteriophora* once (21 May 2008), *H. bacteriophora* twice (21 May and 21 June 2008), *H. megidis* once (21 May 2008), the chemical standard Lorsban 4E once (4 September 2007), and the control. The nematodes *H. bacteriophora* or *H. megidis* were applied at a rate equivalent to 9 billion/ha, and Lorsban 4E (Dow Agro Science) was applied following label instructions. The nematodes were applied in water using a standard 10-L watering can. An equal amount of water was applied around the vines in the control using the watering can. Nematodes were applied to the soil surface immediately under the grape trellis within a 0.5-m radius around the vine trunk, a method modified from that of Miller and Bedding (1982, Entomophaga 27: 109–114). Applications were timed to target larvae embedded in the roots as well as those moving toward the surface for pupation based on the record of grape root borer flight activity in the field.

Efficacy was determined by monitoring for pupal cases from emerging adults. Sampling was conducted on a weekly basis from 1 August through 30 September by searching an area within a 0.5-m radius of the vine for the cast pupal exuviate as per Johnson et al. (1991). The number of pupal skins was tabulated for each treatment and subjected to statistical analyses using analysis of variance (SAS Institute 2005, Cary, NC). Treatment means were separated using Tukey's honest significant difference ($\alpha = 0.05$).

The 2009 efficacy trial used a randomized complete block design with 5 treatments and 4 replicates. Each replicate consisted of a trellis with eight *Vitis rotundifolia* Michx 'Fry' cultivar vines planted at a density of 716 plants/ha. Applications of nematodes were made by drip irrigation, a method more convenient to the grower, using a mixer-proportional (Young Products, Concord, CA) at a pressure of 5.17 bar for a 3-h period early morning, at a proportion of 30 parts water to 1 part solution, at a rate equivalent to 9 billion nematodes/ha. Each vine had a macro dripper which runs at 1.9 L of water per vine. Irrigation continued for 2 h following each nematode treatment. The first treatments began on 25 May 2009 and the second treatment with *H. bacteriophora* occurred on 22 June 2009. Lorsban 4E was applied on 3 September 2008. Sampling and statistical methods were the same as employed in 2008.

Soil samples were taken from each treatment plot 1, 4, and 12 weeks posttreatment and before nematode applications the following season. A core sampler was used to extract each soil core (2-cm diameter by 15.24 cm depth). Samples were placed individually in plastic containers and 10 *Galleria mellonella* L. larvae were added to each container. The containers with lids were maintained in a laboratory growth chamber with conditions equivalent to those in the field (temperature, moisture) for 1 week, after which the larvae were removed and checked for mortality and nematode infection. Larvae that died from nematode infection were easily recognized because of the red body color produced by the symbiotic bacteria; larvae dying from other causes were black and putrefied (Grewal et al. 1994, Parasitol. 108: 207–215).

Level of nematode infectivity was determined for each nematode preparation when each test was initiated. Briefly, 1 g of the nematode preparation was suspended in 10 ml of water. One milliliter of the solution was placed in a glass Petri dish, and three *G. mellonella* larvae and their food (oatmeal and honey mixture) were placed in a smaller, inverted glass Petri dish over the droplet. Each container was covered with the glass Petri dish lid and each dish was maintained in the lab for 1 week, then larvae were removed and checked for mortality and nematode infection.

The mean number of grape root borer pupal cases observed in the grapes treated with *H. bacteriophora*, *H. megidis*, and two applications of *H. bacteriophora* were not significantly different from each other, but the three nematode treatments and the one Lorsban treatment were significantly different from the control ($F = 29.037$; df = 4, 20; $P < 0.001$) (Fig. 1a). Pupal counts for *H. bacteriophora* were not significantly lower than the Lorsban 4E, ($P = 0.574$). Similar results were found for *H. megidis* ($P = 0.302$).

The mean of pupal cases observed in the grapes treated with one application of *H. bacteriophora* and two applications of *H. bacteriophora* were not significantly different from each other; however, the two applications of *H. bacteriophora* and one application of *H. megidis* significantly differed, while the three nematode treatments and the Lorsban treatment were significantly different from the control ($F = 72.420$; df = 4, 12; $P < 0.001$) (Fig. 1b). Pupal counts for *H. bacteriophora* were significantly lower than with the Lorsban 4E while pupal counts for *H. megidis* were not ($P = 0.095$). The vines with the double treatments of *H. bacteriophora* had similar pupal case counts as the single treatment of *H. bacteriophora*, ($P = 0.981$) and were significantly lower than vines with one application of *H. megidis* ($P = 0.012$).

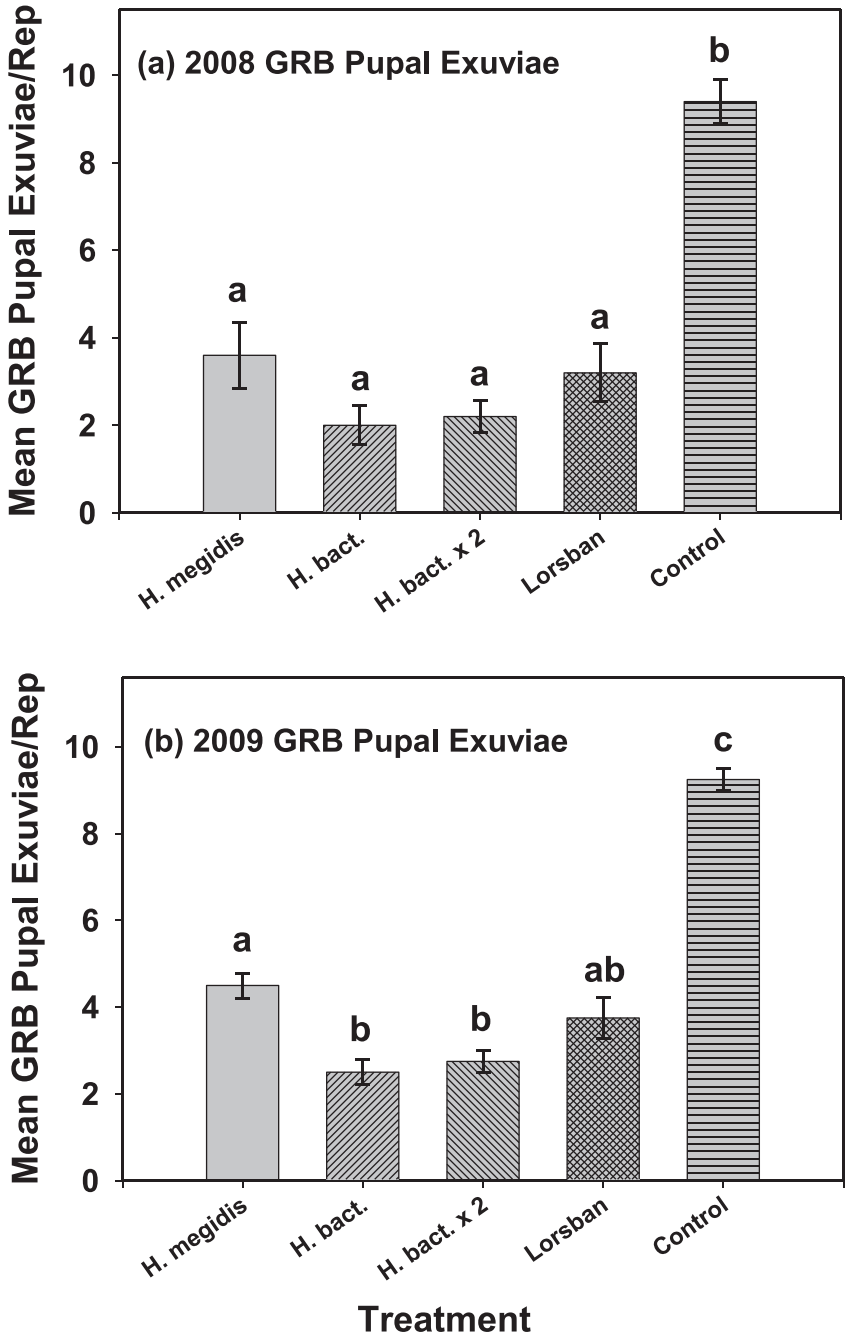

Fig. 1. Mean numbers of cast grape root borer (GRB) pupal skins of the five treatments in 2008 (a) and 2009 (b). Different letters above bars indicate significant treatment differences ($P < 0.05$ Tukey's test).

Table 1. Soil sampling to determine the presence or absence of nematodes. A (+) is entered for each plot that had wax moth larvae positive for infection and (–) is entered for negative results. No nematodes were recovered prior to treatment.

	2008			2009
Treatment	01 June	22 June	17 August	01 May 1
H. bacteriophora	+++++	+++	+	–
H. megidis	+++++	+++	++	–
H. bacteriophora × 2	+++++	++++	++	–
Lorsban	–	–	–	–
Control (water only)	–	–	–	–

Nematodes were successfully recovered from the nematode treatment plots but not from the Lorsban 4E treatment or the control (Table 1). *Heterorhabditis bacteriophora* nematodes from single treatments were recovered on all five replications on 1 June 2008, three replications on 22 June 2008, and only one replication on 17 August 2008. A similar pattern was observed for the treatments that received two applications of *H. bacteriophora*, with nematodes being recovered in all replications on 1 June 2008, four replications on 22 June 2008, and two replications on 17 August 2008. No *H. bacteriophora* were recovered 11 mo later on 1 May 2009. *Heterorhabditis megidis* nematodes were recovered from all five replications on 1 June 2008, three replications on 22 June 2008, and two replications on 17 August 2008. No *H. megidis* were recovered 11 mo later on 1 May 2009.

In 2008, 2009, and 2010 the first male grape root borers were captured around 7 July and the last captured around 13 October (Fig. 2). The peak was in late August until the end of September for 2008 and 2009, but the peak started earlier in August for 2010. The male trap catches were consistent with the results of Weihman and Liburd (2007).

This study shows that both species of *Heterorhabditis* can control grape root weevil and that effective inundation biological control with nematodes can be achieved by drip irrigation. Because nematodes do not appear to overwinter well in the soil, yearly applications may be needed. Two nematode treatments could be made in a growing season even at or near harvest and would integrate into other strategies being developed by others.

The rate of 9 billion nematodes/ha was chosen based on the studies of citrus root weevil biological control. Although this rate provided significant control of grape root borer, a higher level of control is desirable; this may require either changes in the rate of application or a second application each year, or both. Unlike the standard chemical tactic, these entomopathogenic nematodes can be applied more than once per season, have no preharvest or postharvest interval restrictions, and are acceptable in organic production systems.

The increasing incidence of insect resistance to insecticides, and proposed governmental restrictions of pesticides on food crops, have led many fruit growers

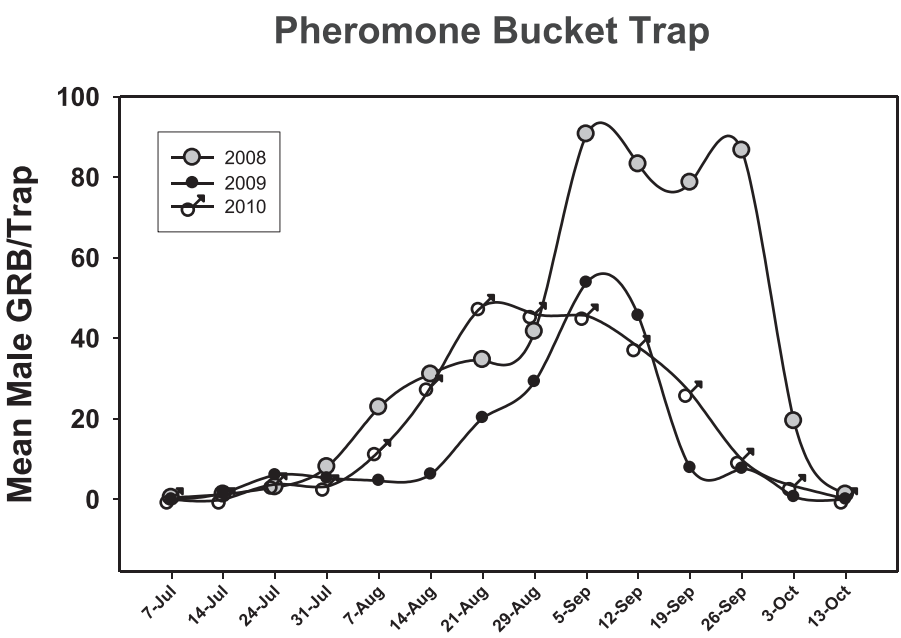

Fig. 2. Mean number of male grape root borer (GRB) adults trapped weekly in bucket trap baited with grape root borer pheromone.

to consider the use of synthetic pheromones and entomopathogenic nematodes as methods of managing lepidopteran pests of fruit crops. This strategy would be much like a biorational insecticide application, which is preferable to a chemical insecticide application and equally efficacious.

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