Evaluation of Application Technologies of Entomopathogenic Nematodes for Control of the Black Vine Weevil

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ABSTRACT Black vine weevil, *Otiorhynchus sulcatus* (F.), is a severe pest of small fruit and nursery crops around the world. These studies were conducted to determine the efficacy of three species of entomopathogenic nematodes (*Heterorhabditis marelatus*, *Heterorhabditis bacteriophora*, and *Steinernema riobrave*) applied in infected host cadavers or as aqueous applications for black vine weevil larval control. Experiments were conducted in the greenhouse and outdoors. Application of three infected host cadavers or 40 infective juvenile nematodes (IJs)/cm^2 were made to pots of *Impatiens walleriana* 5–7 d after larval infestation. Efficacy was assessed at 14 d in the greenhouse and at 14 and 28 d after nematode application in outdoor trials. In the greenhouse, all treatments with the exception the *S. riobrave* (cadaver and aqueous applications) provided nearly 100% efficacy after 14 d. The *S. riobrave* applications, although significantly better than the control, only provided 40–70% control and were not included in the outdoor trials. Nematode efficacy was slowed in the outdoor trials particularly in the cadaver applications. In the initial outdoor trial (soil temperatures <12°C), there were no significant differences between any nematode treatment and the control after 14 d. The nematode efficacy in the initial outdoor trial after 28 d was improved from the 14-d evaluation but not to the level seen in the second trial. In the second outdoor trial, in which soil temperatures were higher (>12°C), the aqueous applications of *H. marelatus* and *H. bacteriophora* provided nearly complete control after 14 d. The cadaver applications also provided nearly complete control in the second outdoor trial after 28 d. Even though the potential total number of IJs estimated per pot was higher in the cadaver-applied treatments, cool soil temperatures apparently delayed or potentially reduced IJ emergence from cadavers resulting in delayed control.

KEY WORDS host cadavers, ornamentals, black vine weevil, *Heterorhabditis*, *Steinernema*

BLACK VINE WEEVIL, *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae), is a severe pest of field and container-grown ornamental and small fruit crops worldwide (Moorhouse et al. 1992). Black vine weevil is a polyphagous (>140 species) and univoltine insect. Adults reproduce by thelytokous parthenogenesis, so a single individual left unchecked can infest an entire nursery. Adults are nocturnal feeders and cause mainly cosmetic damage to plants by notching leaves as a result of their feeding. Eggs are deposited on the soil surface, starting in the spring, and the resulting larvae cause severe damage by feeding on plant roots (Smith 1932).

Current black vine weevil management programs rely on the use of broad-spectrum insecticides that target adults in the spring in an attempt to prevent oviposition. However, even when implementing an extensive insecticidal spray program against adults, nursery growers often discover plant material infested with last instars in the winter or in the spring before shipping. There is a zero tolerance for black vine weevil in nursery stock, so growers rely on curative treatments to market infested plants. Infested plants cannot be sold and if infested plants are shipped, the grower risks refusal of the plants by the buyer and will incur the additional return shipping costs and potential loss of future sales.

Entomopathogenic nematodes (*Heterorhabditis* spp. and *Steinernema* spp.) are obligate pathogens of insects (Poinar 1979). Both genera can be massed reared in vitro, are commercially available from a number of suppliers, and have been used extensively for the biological control of a wide range of insect pests, including black vine weevil (Georgis and Poinar 1984, Kaya and Gaugler 1993, Berry et el. 1997, Booth et al. 2002, Gaugler 2002, Willmott et al. 2002). Nematode infective juveniles (IJs) are provided in a variety of commercially available formulations and generally are applied through agricultural sprayers or irrigation systems (Grewal 2002). However, nematodes also can be

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applied via infected host cadavers (Creighton and Fassuliotis 1985, Jansson et al. 1993, Jansson and Lecrone 1994, Shapiro-Ilan et al. 2003). Laboratory studies indicate that nematodes applied while still inside infected cadavers have superior dispersal (Shapiro and Glazer 1996), infectivity (Shapiro and Lewis 1999), and survival (Perez et al. 2003) compared with nematodes applied in aqueous solution. Greenhouse studies directly comparing aqueous and cadaver application of several species of nematodes against the diaprepes root weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae), and black vine weevil found the cadaver application provided superior efficacy (Shapiro-Ilan et al. 2003). These experiments focused on testing the relative virulence of nematodes applied as either infected cadavers or aqueous suspensions. Applications were timed so that infective juveniles were available in the soil at approximately the same time. However, tests have not been performed to directly assess cadaver and aqueous applications of entomopathogenic nematodes as a curative approach such as they would be used by ornamental growers. In such cases, achieving control in a timely manner is critical and thus timing of nematode availability is an issue.

The objectives of these studies were to directly compare cadaver and aqueous applications of three species of nematodes [*Heterorhabditis bacteriophora* Poinar (Oswego), *Heterorhabditis marelatus* Liu & Berry (Point Reyes), and *Steinernema riobrave* Cabanillas, Poinar & Raulston (355)] in container-grown ornamentals for control of black vine weevil larvae. Experiments were performed in the greenhouse and at ambient outdoor temperatures. Nematode applications were made to simulate grower application methods as closely as possible.

Materials and Methods

Black vine weevil larvae were obtained from a colony maintained at the USDA–ARS Horticultural Crops Research Laboratory (Fisher and Bruck 2004). All nematodes were reared in vivo by using *Galleria mellonella* (L.) as a host (Kaya and Stock 1997). Nematodes from these cultures were used to inoculate the host insect *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) for cadaver applications. *T. molitor* larvae were exposed, 10 at a time, in petri dishes (90 mm), to 600 IJs of either *H. bacteriophora* (Oswego), *H. marelatus* (Point Reyes), or *S. riobrave* (355) per *T. molitor* larva 7 d before cadaver application. Infective juveniles for the aqueous applications also were produced as described above, except the *T. molitor* larvae were infected 14 d before application, placed on a White trap (White 1927) at 24°C, and the IJs were collected. The collected IJs were maintained at 8°C in culture flasks until use. The number of IJs produced per cadaver was estimated for each trial. Ten cadavers infected with each species of nematode were maintained individually on White traps at 24°C, and the number of IJs produced per cadaver during the period of nematode emergence was counted.

A soilless potting medium was used in all experiments that consisted of a 2:1 mixture of peat moss (Sunshine Mix #3, Sun Gro Horticulture, Bellevue, WA) and turkey grit (Cherry Stone Grit #3, New Ulm, MN). The turkey grit was used in place of perlite to provide drainage and aeration while not interfering with locating larvae at the end of the trials. Rooted cuttings of *Impatiens walleriana* Hook. f. (Ericales: Balsaminaceae) were planted in 3.8-liter pots and allowed to grow for ≈1 mo before infestation. Each pot was infested with 10 fifth to sixth instar black vine weevils. Five to 7 d later, pots were treated with either infected cadavers or an aqueous suspension of IJs. Three *T. molitor*-infected cadavers were evenly spaced around the surface of each pot and buried to a depth of 1 to 2 cm. Aqueous applications were made by applying 40 IJs/cm^2 (8,000 IJs/pot) in 200 ml of water with a CO_2 sprayer at 1.05 kg/cm^2. All pots were watered with ≈1 cm depth of water before and after cadaver or aqueous application. Thereafter, soil moisture was maintained at a level similar to that provided by a grower.

The experiments were performed as full factorials and completely randomized with 10 replications. The number of live black vine weevil larvae remaining after 14 d was determined in greenhouse trials and after 14 and 28 d in outdoor trials. Trials were performed twice in the greenhouse (18–21°C) as well as outdoors at ambient temperatures under an open sided plastic "hoop" structure. All trials were performed on separate dates. Soil temperatures in the second greenhouse trial and both outdoor trials were monitored using a Hobo U12 data logger (Onset Computer Corporation Bourne, MA). Greenhouse trials were performed first and because of the 100% control provided by both applications of *H. bacteriophora* and *H. marelatus* at 14 d in the first trial; the 28-d evaluation was not performed nor included in the second trial. Also, because of the poor performance of *S. riobrave* compared with the other nematode species in the greenhouse trials, it was not included in the outdoor experiments where conditions were expected to be less than optimal. Data were analyzed using analysis of variance followed by Tukey's multiple range test to separate means (SAS Institute 1999).

Results and Discussion

Under near ideal conditions in the greenhouse, both *H. marelatus* and *H. bacteriophora* provided nearly 100% control when applied as infected cadavers and complete control when applied in an aqueous suspension after 14 d (Table 1). These species also have been shown by others to be effective biological control agents against black vine weevil in the greenhouse and field (Georgis and Poinar 1984, Hanula 1993, Berry et al. 1997, Wilson et al. 1999, Booth et al. 2002, Shapiro-Ilan et al. 2003). Our greenhouse results with *H. marelatus* and *H. bacteriophora* 14 d postapplication were similar to those of Shapiro-Ilan et al. (2003); they also observed significant reductions in the percentage of live black vine weevil larvae at 14 d with both cadaver

Table 1. Mean (± SD) number of live *O. sulcatus* larvae per pot 14 d after application of entomopathogenic nematodes in the greenhouse

Trial	Cadaver[a]			Aqueous[b]			
	H.m.	H.b.	S.r.	H.m.	H.b.	S.r.	Control
1	0 (0)c	0 (0)c	4 (2.2)b	0 (0)c	0 (0)c	2.5 (1.8)b	8.9 (1)a
2	0.7 (1.9)c	0.8 (2.5)c	6.1 (1.1)a	0 (0)c	0 (0)c	3 (2)b	7.7 (1.2)a

Means followed by the same letter within a row are not significantly different ($P < 0.05$) (SAS Institute 1999). H.m., *H. marelatus* (Point Reyes); H.b., *H. bacteriophora* (Oswego); S.r., *S. riobrave* (355).

[a] *T. molitor* larvae 7 d postinfection applied at the rate of three cadavers per 3.8-liter pot ($n = 10$).

[b] An aqueous application of 40 IJs/cm^2 per 3.80liter pot ($n = 10$).

and aqueous applications. These are the first data addressing the efficacy of *S. riobrave* against black vine weevil. *S. riobrave* applied as infected cadavers or an aqueous suspension caused greater black vine weevil mortality than observed in the control but did not eliminate black vine weevil larvae (Table 1). The aqueous application of *S. riobrave* performed significantly better than the cadaver application in the second greenhouse trial but not to the level of either application of the other two species. Had the pots been evaluated at 28 d postnematode application, *S. riobrave* treatments may have provided control similar the *Heterorhabditis* spp. at 14 d. However, considering the additional time necessary and the efficacy of the other two species, *S. riobrave* is not an attractive candidate for black vine weevil control. It is possible that black vine weevil is not attractive to *S. riobrave*, IJ penetration of black vine weevil larvae is low or the innate virulence of *S. riobrave* once inside black vine weevil is low. Other species of *Steinernema*, including *S. carpocapsae* and *S. glaseri* have been shown to reduce black vine weevil larval infestations in cranberry by 96 and 100%, respectively (Booth et al. 2002).

Efficacy in the outdoor trials was lower than the greenhouse trials, particularly after 14 d. In the first outdoor trial, there were no significant differences between any treatment and the control after 14 d (Table 2). In the second outdoor trial after 14 d, there was a significant reduction in the number of live larvae in the pots from all treatments compared with the control, and those receiving an aqueous application of *H. marelatus* or *H. bacteriophora* had fewer larvae than those treated with infected cadavers. All nematode applications in the first outdoor trial after 28 d, with the exception of the *H. bacteriophora*-infected cadavers, contained significantly fewer live larvae than the control. In the second outdoor trial after 28 d, nearly complete control was provided by both application techniques with both species (Table 2).

The differences in efficacy observed between the greenhouse and outdoor trials were likely due to the significantly lower temperatures in the outdoor tests (Fig. 1). Soil temperatures were not available from the initial greenhouse trial. However, the daily mean soil temperature in the second greenhouse trial (20.0°C) was significantly higher than soil temperatures during the initial 14 d of the first (11.5°C) and second (14.2°C) outdoor trials ($F = 304.79$; df = 2, 39; $P < 0.0001$). The virulence of *H. bacteriophora* drops at temperatures below 25°C and has an optimum temperature for reproduction of 21.5°C (Grewal et al. 1994). *H. marelatus* is considered to be a cool-adapted nematode with efficacy against black vine weevil down to 14°C (Berry et al. 1997). Treatments applied in the outdoor trials were adversely affected by the low soil temperatures, particularly the cadaver treatments, in which IJ emergence was reduced or potentially delayed. The mean daily soil temperature during the second outdoor trial (14.2°C) was significantly greater than during the first outdoor trial (11.5°C) ($F = 75.94$; df = 1, 54; $P < 0.0001$), particularly from day 14 to 28 (Fig. 1). There was no significant difference in the number of live black vine weevil larvae in cadaver applications of *H. marelatus* and *H. bacteriophora* at 14 d between outdoor trials ($F = 1.37$; df = 1, 36; $P = 0.25$), but the warmer soil temperatures allowed for increased efficacy of the cadaver applications in the second trail compared with the first when evaluated at 28 d ($F = 138.74$; df = 1, 36; $P < 0.0001$) (Table 2). The cooler soil temperatures, 12°C or less

Table 2. Mean (± SD) number of live *O. sulcatus* larvae per pot 14 and 28 d after application of entomopathogenic nematodes outdoors

Trial	Cadaver[a]		Aqueous[b]		
	H.m.	H.b.	H.m.	H.b.	Control
1					
14 d	7.7 (1.6)a	7.3 (1.4)a	7.1 (1.9)a	6.2 (1.4)a	8.1 (1.3)a
28 d	5.9 (2.3)b	7.6 (1.4)ab	3 (1.7)c	1.3 (1.6)c	8.4 (1.2)a
2					
14 d	6.5 (2.8)b	7.4 (2.6)b	0.1 (0.3)c	0.1 (0.3)c	10 (0)a
28 d	0 (0)b	0.6 (1.9)b	0 (0)b	0 (0)b	9.2 (0.8)a

Means followed by the same letter within a row are not significantly different ($P < 0.05$) (SAS Institute 1999). H.m., *H. marelatus* (Point Reyes); H.b., *H. bacteriophora* (Oswego).

[a] *T. molitor* larvae 7 d postinfection applied at the rate of three cadavers per 3.76-liter pot ($n = 10$).

[b] An aqueous application of 40 IJs/cm^2 per 3.8-liter pot ($n = 10$).

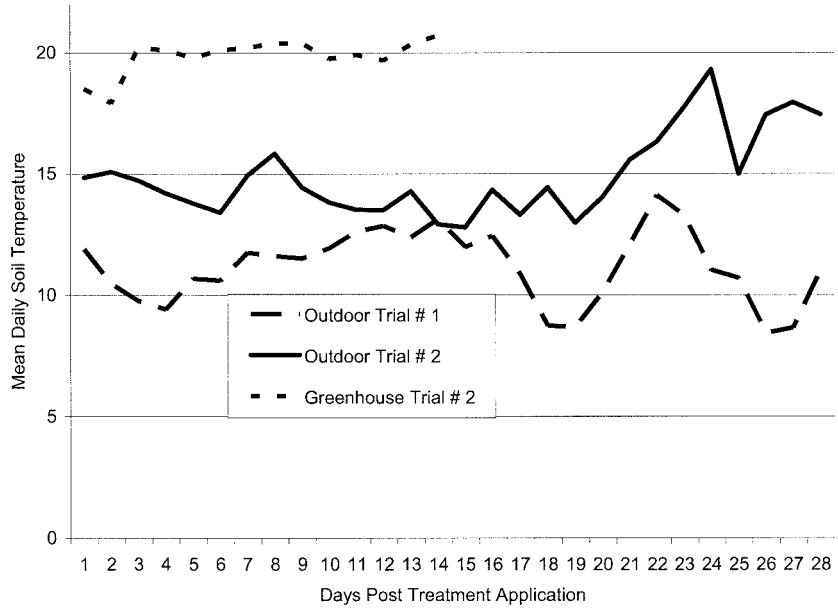

Fig. 1. Mean daily soil temperatures (°C) in the second greenhouse trial and two outdoor trials evaluating the efficacy of infected cadaver and aqueous applications of entomopathogenic nematodes.

the first 10 d of the experiment, observed in first outdoor trial also impeded the infection process of aqueous nematode applications at 14 d. In the second outdoor trial where the soil temperature never fell below 12°C, the aqueous applications provided nearly complete control after 14 d. The outdoor temperatures in both trials were below optimum for *H. bacteriophora* and we would have expected *H. marelatus* to perform better at these temperatures. The infected cadavers were applied to the pots 7 d postinfection. Even under ideal conditions in the laboratory, IJs were not collected from cadavers until 9 and 11 d after infection from the two *Heterorhabditis* sp. and *S. riobrave*, respectively. Grewal et al. (1994) found that it took an average of 4 and 7 days longer for the first *S. riobrave* and *H. bacteriophora* IJ to emerge at 20 than at 25°C, respectively. Even the potential for a vastly elevated number of IJs present in pots receiving the cadaver application (Table 3) was not enough to offset the delay in IJ emergence. Whereas we expect that the number of IJs that actually emerged from the cadavers in the outdoor trials was less than what was observed in the laboratory; we have no estimate of this.

Shapiro-Ilan et al. (2003) found application of infected cadavers to be as or more effective than aqueous applications of *H. bacteriophora* for black vine weevil control in the greenhouse at 7, 14, and 21 d after nematode emergence. The cadaver treatment resulted in lower black vine weevil larval survival than the aqueous treatment on the first sample date (7 d) and was the only treatment causing significantly lower survival than the control on all sample dates. Survival of *D. abbreviatus* larvae was significantly lower when treated with infected cadavers than an aqueous application of *H. indica* on all sample dates (7, 14, 21, and 28 d) (Shapiro-Ilan et al. 2003). It seems that under near optimal conditions in the greenhouse the application of infected cadavers can be as efficacious as aqueous applications and these results were confirmed in the current study. Shapiro-Ilan et al. (2003) found application of infected cadavers to provide superior control when directly comparing virulence of nematodes in the two application methods (either as infected cadavers or daily white trap captures poured onto the pot surface). The former study made the nematodes available simultaneously; time of application was not a factor.

This current study focused on a curative approach of nematode application comparing infected cadavers and a traditional aqueous suspension of IJs with an emphasis on control of overwintering black vine weevil populations. The efficacy of each application technique in an applied setting must be assessed to determine which is going to provide growers the greatest prospect for control. Growers targeting a black vine weevil larval infestation in the greenhouse could use either application technique. However, if soil temperatures are below optimum for IJ emergence from infected cadavers, but above the temperature for infection to take place, aqueous suspensions would likely provide more rapid control. Infected cadavers may

Table 3. Mean (± SD) number of infective juveniles emerging per cadaver in each experiment at 24°C

Exp	*S. riobrave*	*H. marelatus*	*H. bacteriophora*
Greenhouse trial 1	22,871 (19,836)	46,315 (18,416)	59,797 (27,333)
Greenhouse trial 2	4,615 (7,870)	21,021 (14,757)	31,324 (21,247)
Outdoor trial 1		20,485 (17,026)	30,122 (11,505)
Outdoor trial 2		25,891 (19,405)	31,209 (20,471)

prove useful when longer term control is needed, i.e., when the pest is present or reoccurring in the soil over an extended period. We would expect IJs to emerge from infected cadavers and become available over and extended period. Furthermore, nematodes emerging from infected cadavers also persist in the soil for longer periods of time than those applied in aqueous suspensions (Perez et al. 2003). The possibility of long-term control, although important for certain pests in various cropping systems, is of little practical importance for black vine weevil control when immediate curative applications are necessary.

Control of black vine weevil with entomopathogenic nematodes has been historically limited by low soil temperatures experienced when late instars are present in the field in north temperate regions such as the Pacific Northwest. Many nematode isolates have been screened for their activity in cold conditions (Griffin and Downes 1994, Long et al. 2000). The cold-active nematode *S. kraussei* is effective at controlling overwintering black vine weevil in outdoor strawberry plants (Willmott et al. 2002), but this isolate is not available for commercial use in the United States. Berry et al. (1997) observed that *H. marelatus* provided significantly greater black vine weevil control (82.5% control) than *H bacteriophora* (44.1% control) at low (14°C) soil temperatures 12 d after application. Therefore, it was somewhat surprising to see efficacy near 100% after as few as 14 d with both *H. marelatus* and *H. bacteriophora* in the second outdoor trial where soil temperatures ranged between 13 and 16°C. Until a cold-active entomopathogenic nematode is identified for use in the United States, growers will have to rely on the strains currently available. Although the use of infected host cadavers can be as effective as an aqueous application of nematodes for various soil insects, including black vine weevil when soil temperatures are near or slightly above optimum, their efficacy is delayed when soil temperatures are low. However, if growers in the Pacific Northwest could be convinced to adjust their scouting program forward to identify black vine weevil-infested plants in September or October; the larvae would be large enough to be located and soil temperatures would likely be appropriate for either type of nematode application to provide adequate control.

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