APPLICATION METHODS FOR ENTOMOPATHOGENIC NEMATODES (RHABDITIDA: HETERORHABDITIDAE): AQUEOUS SUSPENSIONS VERSUS INFECTED CADAVERS

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Entomopathogenic nematodes have potential as biological control agents of many soil insect pests. These nematodes have a broad host range, and they are highly virulent with high reproductive rates. They have the ability to seek out and quickly kill hosts (even cryptic hosts) within 24-48 h, and they are safe to vertebrate and other nontarget organisms (Gaugler 1981, Kaya 1985). In addition, these nematodes are easy to apply using a variety of methods ranging from aqueous suspensions applied by hand to those made with standard sprayers (up to 70.4 kg/cm^2) and irrigation systems (Kaya 1985, Georgis 1990).

Entomopathogenic nematodes have also been shown to have potential for controlling field populations of the sweetpotato weevil, *Cylas formicarius* (Fabricius) (Jansson et al. 1990, 1991, 1993). Heterorhabditid nematodes were superior to steinernematids at reducing weevil populations and their concomitant damage to storage roots (Jansson et al. 1990, 1993). Welch & Briand (1960) found that aqueous applications of *Steinernema carpocapsae* (Weiser) DD136 strain, and those made using infected greater wax moth, *Galleria mellonella* (L.), cadavers, were equally effective at controlling cabbage root maggot, *Hylemya brassicae* (Bouché). More recently, Jansson et al. (1993) showed that applications of *G. mellonella* cadavers infected with heterorhabditid nematodes were efficacious for controlling field populations of *C. formicarius* and their concomitant damage; however, nematode applications via infected cadavers were not compared with those made via aqueous suspensions. The present study compared efficacy and persistence of a heterorhabditid nematode against *C. formicarius* when applied via aqueous suspensions with those made using infected *G. mellonella* cadavers.

The experiment was conducted in a Krome, very gravelly loam soil at the Tropical Research and Education Center in Homestead. Sweet potato, *Ipomoea batatas* (L.) Lam. cv. Jewel, transplants were hand planted 20 cm apart on raised beds with centers spaced 1.9 m apart on 5 June 1991. All production practices were similar to those described previously (Jansson et al. 1990, 1991, 1993). No herbicides or fungicides were applied to the experimental plot. Plants were drip irrigated 4 h per day using a drip turbo T-tape-irrigation system (model 40) (5.0 liter per m per h) from shortly before planting until the experiment terminated.

A research plot (0.2 ha) was subdivided and arranged into a randomized complete block design with four replications. Treatment plots were three beds by 15.2 m long. A 3-m buffer of nontreated plants separated replicates. Treatments evaluated were: a single aqueous application of *Heterorhabditis bacteriophora* Poinar HP88 strain (4.9 billion infective juveniles per ha) made on 23 July 1991, a single application of 5- and 10-day-old *G. mellonella* cadavers (83,700 per ha) infected with *H. bacteriophora* HP88 strain made on 26 July 1991, and nontreated plants. Jansson et al. (1991) showed that a single application of *H. bacteriophora* HP88 strain was efficacious for controlling populations of *C. formicarius*. Potential rates of infective juveniles applied in the cadaver treatments were 6.4 to 18.3 billion infective juveniles per ha based on mean numbers of infective juveniles produced per cadaver of between

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76,260 and 219,181 (Jansson et al. 1993). Inoculation procedures were similar to those described previously (Jansson et al. 1993).

Two 3-m sections were dug from the middle bed of each treatment plot on 19-20 December 1991. Storage roots were divided into three size categories (small, medium, and jumbo) and each individual root was then visually inspected for weevil damage and rated on a scale from 1 to 6 (Jansson et al. 1990): 1, no weevil damage, no feeding or oviposition scars, no adult exit holes; 6, severe weevil damage, > 6 adult exit holes per root. The percentage of root biomass in each damage category and the mean damage index were determined. The percentage of marketable roots was determined by calculating the percentage of medium-sized roots with a rating ≤ 2 (Jansson et al. 1990).

Soil was assayed for native nematode populations once [19 July 1991, 12 soil samples (720-1045 g each) per plot] before applications were made. Levels of recovery of HP88 nematodes were determined on three dates after application (19 September, 5 December 1991 and 23 July 1992; 3 soil samples per treatment plot) using methods described previously (Jansson et al. 1991, 1993). Soil was transferred to separate plastic cups (1 liter) and ten late-instar *G. mellonella* were placed in the bottom of each cup, covered with soil (Bedding & Akhurst 1975) and stored for 10-14 days in the dark at 24±3°C after which cadavers were examined for nematode infection (Jansson et al. 1991, 1993).

Percentages of root weight in various damage categories were transformed to the arcsine of the square root and analyzed by least squares analysis of variance (Zar 1984). Total numbers of infected *G. mellonella* larvae were compared among treatments by X^2 analysis (Steel & Torrie 1980).

Percentages of roots that had no weevil damage (rating = 1), slight weevil damage (rating = 2), or little or no damage (rating = 1 or 2) did not differ ($F \geq 0.9$, df = 3,9, $P > 0.05$) among treatments (Table 1). Percentages of root weight in these three damage categories tended to be higher on nematode-treated plants than on nontreated plants, although not significantly. These data suggest that aqueous applications of nematodes were comparable in efficacy to those made using infected cadavers of *G. mellonella* that were either 5 or 10 days old.

Few soil samples (0.7%, n = 388) and fewer *G. mellonella* larvae (0.1%, n = 3,880) were positive for entomopathogenic nematodes before applications were made. After

TABLE 1. PERCENTAGES OF STORAGE ROOT WEIGHT WITH NO WEEVIL DAMAGE, SLIGHT WEEVIL DAMAGE, AND NONE OR SLIGHT DAMAGE, ON SWEET POTATOES TREATED WITH AQUEOUS OR CADAVER APPLICATIONS OF *H. BACTERIOPHORA* HP88 STRAIN AND ON NONTREATED PLANTS.

Treatment Rate/ha[2]	% Root Weight [1]		
	No Damage, Rating = 1	Slight Damage, Rating = 2	Little/No Damage, Rating = 1 or 2
Aqueous, 4.9 B/ha	50.6±3.6	28.3±1.8	79.0±3.1
Cadaver, 5-day-old, 83,700/ha	47.9±6.9	31.1±3.6	79.0±3.7
Cadaver, 10-day-old, 83,700/ha	51.5±3.7	28.8±1.6	80.4±3.6
Nontreated	34.6±4.8	32.1±5.4	66.7±4.2

[1]Means within a column did not differ ($P > 0.05$) by least squares analysis of variance (Zar 1984).
[2]Aqueous applications are in terms of infective juveniles per ha; cadaver treatments are numbers of cadavers per ha.

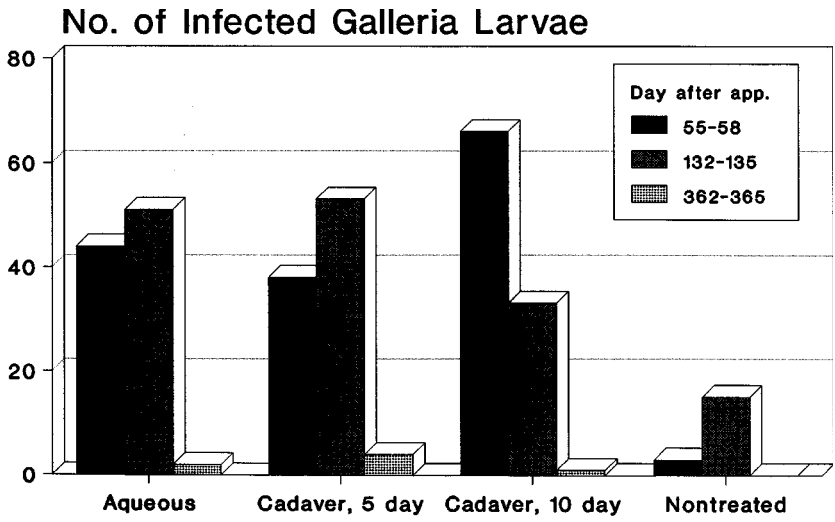

Fig. 1. Total numbers of *G. mellonella* larvae infected with *H. bacteriophora* HP88 strain in sweet potato plots treated with aqueous suspensions of HP88 or cadavers of *G. mellonella* infected with HP88 and in nontreated plots (n= 120 larvae per treatment).

applications, high levels of recovery of nematodes were found on the first two sample dates (55-58 and 132-135 days after application) (Fig. 1). Little recovery was found on the last sample date (362-365 days after application) approximately 7 months after the experiment terminated. Recovery of nematodes did not differ ($X^2 \leq 8.8$, df = 2, $P > 0.05$) among the three nematode treatments, but was considerably higher in nematode-treated plots than in nontreated plots (Fig. 1). Levels of recovery concur with our previous experiments in which either aqueous suspensions or cadaver applications were used (Jansson et al. 1991, 1993).

The data show that aqueous suspensions of HP88 nematodes were as effective as applications of *G. mellonella* cadavers infected with HP88 for controlling damage by sweetpotato weevil to storage roots. Also, we found that persistence of nematodes was similar for the two of methods of application. Application of infected cadavers for introducing nematodes was shown to have merit in a previous report (Jansson et al. 1993). Applications of nematodes via infected insect hosts may have potential for integrating into developing countries because its simple and requires no special equipment and no water at the time of application. However, it does require a constant and large supply of susceptible hosts and considerable space and labor. We encourage researchers to explore the possibility of using this approach in a variety of insect host/crop systems.

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SUMMARY

Aqueous suspensions of HP88 nematodes were as effective as applications of *Galleria mellonella* cadavers infected with HP88 for controlling damage by sweetpotato weevil to storage roots. Persistence of nematodes was similar for the two types of application.

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