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Efficiency of steinernematid nematodes (Nematoda: Steinernematidae) in controlling larvae of the black vine weevil, *Otiorrhynchus sulcatus* (Coleoptera: Curculionidae) in laboratory and field experiments

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Abstract. Laboratory and field experiments were performed to test the ability of steinernematid nematodes to control larvae of the black vine weevil, *Otiorrhynchus sulcatus*, which infests rhododendrons and azaleas in Czech Republic. Of five strains tested in the laboratory the best results were achieved with the *Steinernema feltiae* strain Hyl isolated in Czech Republic, which provided a 100% elimination of the weevil larvae on azaleas in laboratory tests. Field tests were made in a nursery of ornamentals at Plzeň-Křimice where an outbreak of the pest had lasted a long time. Preventative treatment, three releases of the nematode, was performed in three beds in successive seasons 1989–90, resulting in a 72% to 88% protection of plants in the beds, including a 52% to 77% protection in adjacent plots due to migration of nematodes from the treated areas. Only 30% of plants survived on more distant control plots. This system of control of *O. sulcatus* with steinernematids is a promising method of biological protection in ornamental nurseries and gardens.

INTRODUCTION

Otiorrhynchus sulcatus (F.) (Coleoptera: Curculionidae) is a serious pest of many ornamentals and field crops in Europe, North America, Australia and New Zealand. The list of host plants includes over 140 species (Warner & Hegley, 1976). Damage is caused by the insect larvae, which can destroy or reduce the root system of the host plants causing death or impairing development.

Entomopathogenic nematodes of the family Steinernematidae and Heterorhabditidae are important non-selective parasitoids of most groups of insects and they can be used for the biological control of many insect pests, especially of species developing in soil. They search for hosts and enter their bodies in the larval, pupal and adult stages, which is an advantage over other biological agents (Weiser & Mráček, 1988).

Simons (1981) reported the successful control of *O. sulcatus* larvae by *Heterorhabditis* sp. (Nematoda: Heterorhabditidae) in glasshouses. When 100 nematodes were applied to 1 cm^2 soil at a time when eggs were hatching, larval mortality in strawberry cultures was 90–97% and the plants were undamaged. Half the dosage brought a similar mortality, but the plants were evidently damaged. Bedding & Miller (1981) obtained good results with the nematode *H. heliothidis* in raspberry plantations and grapefruit nurseries. Nematode application to soil in pots resulted in a 100% infestation of *O. sulcatus*.

Rutherford et al. (1987) performed experimental applications of two heterorhabditid strains and *S. feltiae* to control *O. sulcatus* and *O. ovatus* in British Columbia. Field application of *H. heliothidis* at dosages of 500–5,000 nematodes per litre of soil were effective and gave significantly better control of black vine weevil larvae on potted lodgepole pine trees than did a diazinon drench. *S. feltiae* was evidently less effective than heterorhabditids. Further reports of the beneficial effects of different nematodes in the control of *O. sulcatus* were published by Georgis & Poinar (1984a). Differences in the effects of various isolates were reported by Bedding et al. (1983).

Klingler (1988) used *Heterorhabditis* sp. for controlling some developmental stages of *O. sulcatus* and *O. salicicola*. Laurel seedlings were dipped in suspensions of nematodes with 56,000 to 62,000 infective juveniles per litre, which caused a 100% mortality of larvae, pupae and adults. Treatment of pots of *Ligustrum* sp. (Oleales: Oleaceae) initially inoculated with eggs of *O. salicicola* with nematodes at a concentration of 20,000 larvae per litre of soil caused 78% mortality. When larvae of the pest were used instead of eggs, the resulting mortality was 76 to 100%. In general, steinernematids are considered to be less effective for control of weevils than are heterorhabditids, which were more efficient than *Steinernema* in the experiments of Bedding & Miller (1981) and Rutherford et al. (1987), but regional and climatic influences may cause variations, as well as the nematode strain. Our main objectives were to select in laboratory experiments from our collection the most effective steinernematid species or strain, for nursery treatments, as well as repeated preventative control of the pest by nematodes, in two seasons.

MATERIAL AND METHODS

NEMATODES. Nematodes used for the laboratory selection treatment were these species and strains: *Steinernema feltiae* (= *S. bibionis*) strain Hyl (isolated from larvae of *Hylobius abietis* from Czech Republic); *Steinernema rara* (isolated from soil samples in Argentina); *Steinernema* sp. strain Tlein (isolated from soil samples in Poland); *Steinernema* sp. strain Päc (isolated recently in Finland); and *Steinernema* sp. strain Cuban (isolated from Cuban soils).

All species and strains were reared on the larvae of *Galleria mellonella* L. (Lepidoptera: Galleriidae) by the method of Dutky et al. (1964). To minimize a loss of infectivity, the nematodes were stored in the dark on dampened polyurethane sponge in plastic bags at 4°C.

LABORATORY EXPERIMENTS. The black vine weevil larvae were collected for laboratory experiments in the Plzeň-Křimice nursery by digging in infested beds of rhododendrons. The larvae were transported in soil and added to the experimental plants three days before treatment with nematodes, in order to have time to find their favoured sites in the host-plant root system.

The host plants were 3-year-old azaleas, Knap Hill hybrid, planted in a bark and peat substrate in pots 13 cm in diameter. They were covered with nylon cylinders. The nematode suspension was poured around the plants or injected to their root systems.

An assessment of infectivity of steinernematid species and strains was performed in two experiments. In Experiment I, from Sept. 24 to Dec. 21, 1987 (Table 1), 10 pots containing 10 *O. sulcatus* larvae and one host plant each were treated by all nematode species and strains. The dosages of infectives per pot were 15,000 to 30,000 in 20–30 ml of the water solution. In Experiment II, from January 19 to March 4, 1988 (Table 2), performed under the same conditions, 8 pots were treated by infectives of *S. feltiae* strain Hyl at the concentrations between 15,000 to 30,000 per pot. In this experiment, another 10 larvae of the pest were added into some pots (Table 2) on March 4 and evaluated on April 26. For evaluation of the effects of the treatment we inspected the soil in the pots and counted survivors including adults that had emerged. Another criterion of the effects of treatment was the degree of damage to the plant. At the end of the experiment the degree of infestation of the soil with nematode infectives was evaluated in modified *Galleria* traps (Mráček, 1980).

NURSERY EXPERIMENTS. We used *Steinernema feltiae* strain Hyl which had been the most efficient in the laboratory experiments. Treatments were performed in the nursery of the Plzeň-Křimice Gardeners Centre, in an area severely infested with weevils, without a preliminary natural occurrence of steinernematid nematodes. There were 1–4 larvae of *O. sulcatus* per plant. We treated 3 beds, each 30 m long by 1 m wide (415 plants), of 3-year-old Cunningham's white rhododendrons. Each bed was divided into two parts. The former (273 plants) was sprinkled with the nematode suspension (30 × 10,000 infectives/plant) and the latter (142 plants) was not treated, so that the effect of migration of the nematodes in soil could be observed. The separated control bed contained 540 plants. Timing: Beds were prepared in the beginning of May, 1989 and treated again at the end of October, 1989 and at the beginning of April, 1990. The results were evaluated in June, 1990. The G-test (Sokal & Rohlf, 1969) for testing the equality of two percentages (COSTAT) was used for the determination of differences between the percentages of surviving plants in the plot containing nematodes, in contrast with the plots without nematodes (the zone of migration and the control bed).

TABLE 1. Efficiency of *Steinernema* spp. against the larvae of *Otiorrhynchus sulcatus* on potted plants (10 pest larvae per pot).

Trial (pot)	*Steinernema* sp. strain	Application dose/pot	Pest mortality (%)	Degree of damage	*G. mellonella* trap test
1	*S. feltiae* Hyl	15×10^3	100	zero	+
2	*S. feltiae* Hyl	30×10^3	100	zero	+
3	*S.* sp. Tlein	15×10^3	70	slight	+
4	*S.* sp. Tlein	30×10^3	100	zero	+
5	*S. rara*	15×10^3	70	slight	+
6	*S. rara*	30×10^3	80	slight	+
7	*S.* sp. Päc	15×10^3	20	heavy	–
8	*S.* sp. Päc	30×10^3	100	zero	+
9	*S.* sp. Cuban	15×10^3	30	zero	+
10	*S.* sp. Cuban	30×10^3	50	heavy	+
11	Control	H_2O	10	heavy	–

Dates: Sept. 24, 1987 (treatment); Nov. 21, 1987 (evaluation)
Plants: "Knap Hill" azaleas, 3 years old, in pots (13 cm)
Location: Laboratory experiment
Aplication method: Injection of nematode suspension

RESULTS

LABORATORY EXPERIMENTS. The laboratory experiments with different strains and applications of dosages proved that infective juveniles of steinernematids have a high affinity for the larvae of *O. sulcatus* and search for them. The efficiency of killing weevil larvae was higest in *S. feltiae* strain Hyl and *Steinernema* sp. strain Tlein; *S. rara*, *Steinernema* sp. strain Päc and *Steinernema* sp. strain Cuban were less effective in Experiment I (Table 1) where damage to treated plants was rather higher. The final evaluation of infective juveniles presence in "*Galleria*" traps after experiments was negative with *Steinernema* sp. strain Päc only.

In Experiment II (Table 2), tests of efficiency of dosages between 15,000 and 30,000 infectives/pot of *S. feltiae* strain Hyl did not produce noticeable differences, so that the dose of 15,000 may be regarded as more than adequate to ensure positive results with 100% mortality. In these tests a preventative and protective value of the nematodes was tested on weevil larvae which were added during the experiment. The nematodes proved their effectiveness, as the plants were moderately damaged in two cases only. There was no significant difference between the modes of application (injection and surface-sprinkling). The destruction of weevil larvae was so rapid that no remains of their dead bodies were found, and evidence was merely in the degree of damage to the experimental plants.

TABLE 2. The effect of treatment with *S. feltiae* strain Hyl on survival of azaleas in pots.

Trial (pot)	Application mode, dose/pot	*O. sulcatus* larvae (no.)	Plant damage	*G. mellonella* trap test
1	inj. 15×10^3	5	zero	+
2	inj. 30×10^3	$5+10^d$	medium	+
3	inj. 15×10^3	5	zero	+
4	inj. 30×10^3	$5+10^d$	medium	+
5	surf. 15×10^3	5	zero	+
6	surf. 30×10^3	$5+10^d$	zero	+
7	surf. 15×10^3	5	zero	+
8	surf. 30×10^3	$5+10^d$	zero	+
Control 1	inj.	5	heavy	–
Control 2	surf.	5	slight	–

Dates: Jan. 19, 1988
Plants: 3-years-old azaleas
Location: Laboratory experiment
d – 10 larvae of *O. sulcatus* added March 4, 1988
inj. – injection
surf. – surface application

FIELD EXPERIMENTS. In field experiments (Table 3) in flower beds with rhododendrons treated with injections of infective juveniles to the roots the results were more variable. In these experiments the average result was 77.6% protection from damage, but with variation from 72% to 88%. In the untreated parts of the beds the damage to shrubs was reduced to an average of 64.1% (52.1–77.4%) due to immigration of nematodes from the treated plots. This interference was most evident in comparison with the damage in untreated-control beds nearby, where only 30% of the plants survived the attacks of weevils. While all *Galleria* tests before the treatment were negative, those after the experiment were positive, including tests of untreated experimental parts. The control beds were nematode negative.

TABLE 3. Effect treatement with *Steinernema feltiae* strain Hyl to protect rhododendrons in the nursery.

Bed	Number of plants in the experiment								
	T			UT -ZD			Control bed		
	before	after	in %	before	after	in %	before	after	in %
1	93	67	72.0	46	24	52.1			
2	88	64	72.7	53	41	77.4			
3	92	81	88.0	43	27	62.8	540	162	30.0

T – part treated by nematodes
UT–ZD – untreated part, zone of dispersal of nematodes

Data analyses

Equality of two percentages (the control plot against the plot treated by nematodes),

G = 171.6420709

df = 1

P = 6.5960539E-06 ***

Equality of two percentages (within the plot treated with nematodes; plot where nematodes were released against that to which they migrated)

G = 7.621853914

df = 1

P = 5.7676238E-03 **

The value of P in each case indicates the significance level of the test. A highly significant difference was found between the resulting percentages of surviving plants both between the treated and untreated plots and between the treated plot and that to which the nematodes migrated.

DISCUSSION

The damage caused by the black vine weevil, *O. sulcatus* to the control plants indicates the extent of the potential damage to ornamental plants in Czech Republic. Our previous results and reports of others show that it is a difficult pest to control since it is resistant to insecticides. The present results as well as those of Simons (1981) and Bedding & Miller (1981) indicate the important role of biological control agents, such as steinernematid and heterorhabditid nematodes in its control of natural enemies. The lethal dose of weevil larvae for an azalea or a rhododendron is less than 5 and, when the infested plant has been destroyed, they migrate to the next. In this study we have concentrated on steinernematids which are easier to rear and store compared to heterorhabditids, not only for their efficiency in killing weevil larvae, but also their ability to prevent infestation, as indicated by the repeated treatment of rhododendron plantations. Interestingly the species isolated in Central Europe (*S. feltiae* strain Hyl and *Steinernema* sp. strain Tlein) were more efficient than species from other regions (Argentina and Cuba), whose ability to infect was very low. For this reason (and also for reasons of quarantine) *S. feltiae* strain Hyl was the only one used for release in the field.

It was evident in laboratory tests that a dose of 15,000 – 30,000 larvae/plant was sufficient, similarly to a previous study using *Heterorhabditis* (Bedding & Miller,1981). Also the preventative and protective function of the nematodes in pots was proven positive in experiments where new weevil larvae were added 6 weeks after. The resulting damage was slight or nil at the moment when the number of weevils per plant considerably exceeded the critical number of 5–10/plant. It is unusual to find such a high concentration of pest larvae on plants in nature.

In field trials it was difficult to keep conditions of experiment as precise as those in the laboratory. It was impossible to follow the fate of larvae and adults by counting the dead and living specimens and, therefore, the field experiment was aimed at a long period of preventive control. The main criterion was the survival of the plants and estimation of the degree of damage which also eliminated interference by migrations of the weevils and infective juveniles of the nematodes. Tests with *Galleria* traps have proven that nematode juveniles have a tendency to migrate from areas with a high juvenile density to free territories with uninfested hosts. Horizontal movement of steinernematids in soil is slow. Moyle & Kaya (1981) noted that they moved up to 7 cm in two days, and Poinar & Hom (1986) observed 4.35 cm per day. In our field experiment, *S. feltiae* strain Hyl moved at least 5 m in 6 weeks (first positive *Galleria* trap from the central part of the zone of dispersal). This migration was very efficient, the survival of plants in the zone of dispersal (where weevils are evenly distributed) was in an average 64%, compared with an optimum survival of 30% of plants in the separated and untreated control zone. The nematode dispersal to adjacent parts was only 13% less effective for the weevil control than the mass treatment with larvae injected into the root system.

Heterorhabditis nematodes were prefered to *Steinernema* in recent projects, as they have proven to be more infective for this type of pest. For biofactory industry, the rearing of *Steinernema* nematodes and their storage is easier, which is a compensation for their less efficient infectivity. However, Georgis & Poinar (1984b) reported that both genera are equally effective in black vine weevil larvae control. The damage to ornamentals of the azalea-rhododendrons group in nurseries and home gardens is so serious that treatment with nematodes in a single dose or repeatedly is essential for the satisfactory marketing of plants. The results of migration of the Steinernematidae out of the treated area indicate another positive factor in the use of entomopathogenic nematodes in the field.

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