A PRELIMINARY EVALUATION OF ENTOMOGENOUS NEMATODES TO CONTROL THE AFRICAN SUGARCANE STALK BORER *ELDANA SACCHARINA* (LEPIDOPTERA: PYRALIDAE)

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Abstract

A species of *Heterorhabditis* and two species of *Steinernema* were isolated from soil in Natal. A third species of *Steinernema* was obtained from a dead beetle grub. Larvae of *Eldana saccharina* Walker were more susceptible to the *Heterorhabditis* and, to a lesser extent, one of the *Steinernema* species than to the other species. Pupae of eldana were less susceptible than larvae to the *Heterorhabditis* and this *Steinernema* species. The *Heterorhabditis* sp. was tested against eldana in the field by spraying infested sugarcane with various concentrations and volumes of an aqueous suspension of the infective stage of the nematode. These ranged from 50 to 8 000 infectives ml^{-1} and 50 to 200 ml $stalk^{-1}$. Large volumes of water and very large numbers of nematodes were required to achieve moderate control of eldana.

Introduction

Eldana saccharina Walker is the most serious insect pest of sugarcane in South Africa. Larvae bore into cane stalks causing not only physical damage within the stalk but also, in heavy infestations, a reduction in the sucrose content (Anon[1]). It is estimated that there is approximately 0,5% loss in cane yield per eldana larva per 100 stalks (Anon[2]).

Eldana populations may be reduced by cutting cane at a younger age than was normal in the past (Atkinson[7]; Carnegie & Smaill[13]) and by pretrashing, ie removing the lower dead leaves from maturing sugarcane (Atkinson[7]; Carnegie & Smaill[14]). Attempts to control the borer with insecticides have not been successful (Carnegie[12]) but some success has been achieved with selecting resistant sugarcane varieties (Anon[5]). The possibility of using biological control against the insect is receiving much attention (Anon[3]; Anon[4]; Carnegie *et al.*[15]; Conlong & Hastings[16]; Conlong *et al.*[17]). This paper reports preliminary work carried out to assess the potential of entomogenous nematodes to control eldana. The nematodes selected for this study were species of two genera, *Heterorhabditis* and *Steinernema*. The infective juveniles of these nematodes carry in their intestines a symbiotic bacterium that is pathogenic to insects. The infective juveniles gain access to the host insect via the mouth, anus or spiracles and then move to the haemocoel. There they release the cells of their bacterial associate which multiply rapidly and kill the host. The nematodes develop to maturity as they feed on the bacteria and the decomposed tissues of the host. Usually two generations of the nematode are produced in one host before infective juveniles leave the cadaver with their bacterium in search of new hosts (Wouts[24]).

Materials and methods

A species of *Heterorhabditis* and two species of *Steinernema*, referred to as *Steinernema* Sg and *Steinernema* CFS3, were isolated from soil samples collected in Natal by means of the technique of Bedding and Akhurst.[10] A third species of *Steinernema*, designated WG, was obtained from the dead grub of a lamellicorn beetle. The nematodes were reared in larvae of eldana from a laboratory culture by means of the method of Dutky *et al.*[18] and the infective juveniles were stored in 5% Ringer's solution at 12°C until required.

To assess the susceptibility of eldana larvae to these nematodes, 5 small, medium or large larvae were placed in 90 mm diameter petri dishes lined with two filter papers. Two ml of 5% Ringer's solution containing 1 000 infective juveniles were added to each petri dish. Controls received only 2 ml of the Ringer's solution. The petri dishes were stored in the dark at a temperature that ranged between 25 and 28°C; treatments were replicated 10 times. After 48 h the dead larvae were placed on traps (Wouts[24]) where they were subsequently examined for the presence of nematodes. At the time that the eldana larvae were exposed to the nematodes, the width of the head capsule of a sample of each of the 3 sizes of larvae was measured and individuals were assigned to instars according to Atkinson's[6] frequency distribution.

A comparison was made between the susceptibility of eldana larvae and pupae exposed to the *Heterorhabditis* species and *Steinernema* Sg. Larvae and naked and cocooned pupae were placed in separate 90 mm diameter petri dishes lined with two filter papers. Two ml of 5% Ringer's solution containing 1 000 infective juveniles of *Heterorhabditis* or *Steinernema* Sg were added to each petri dish. Controls received only 2 ml of the Ringer's solution. There were 5 insects per dish with 18 replicates for the naked pupae, 11 for the cocooned pupae, and 10 for the larvae. The petri dishes were kept in the dark at approximately 20°C. After 48 h exposure the cocoons were removed and the pupae and larvae washed in tap water for 1 min. They were then placed on moist vermiculite in petri dishes and examined after a further 6 or 7 days. The few pupae that became moths within the 48 h exposure period were not included in subsequent calculations.

A series of 4 tests were conducted in the field to investigate the effects of different volumes and concentrations of suspensions of the *Heterorhabditis* species on eldana in sugarcane. In the third test, treatments were included to (i) determine the best time to sample the cane after applying the nematodes, and (ii) assess the effect of pretrashing the cane prior to application. In each test, plots of a single row of 50 stalks were marked out in mature cane heavily infested with eldana. A concentrated suspension of infective juveniles was taken to the field and diluted to the required concentration and volume. These ranged from 50 to 8 000 infectives ml^{-1} and 50 to 200 ml $stalk^{-1}$ (see Tables of results). In the first two tests, the infectives were sprayed onto the cane stalks by means of a CP3 Mk 2 manual knapsack sprayer with a Spraying System Co TK2,5 spray nozzle at 150 to

200 kPa; at this pressure 10 ℓ of the suspension were delivered in approximately 14 min. In the other two tests a motorised Hatsuta Blowmic mist-blower was used. The normal short discharge tubes of the mist-blower were extended by 8 m using flexible 63 mm diameter hose for the air and 10 mm diameter polypropylene tubing for the nematode suspension. This extension facilitated the spraying operation as it was then necessary to move only the tubes and not the mist-blower up and down the cane rows. The throttle was set so that 10 ℓ of the nematode suspension were delivered in approximately 6,5 min. With both the knapsack sprayer and the mist-blower the nozzle was held approximately 200 to 600 mm from the stalks. Treatments were replicated twice in the first test and 3 times in the other tests.

To check the infectivity of the nematodes used in each of the tests, excess individuals were returned to the laboratory and after overnight storage at 12°C, a 2 ml suspension containing 1 000 infectives was added to 5 eldana larvae in a petri dish lined with two filter papers. This infectivity check was replicated 5 to 20 times. After 48 and 72 h the number of larvae killed by the *Heterorhabditis* was recorded.

Three to 21 days after treating the cane (see Tables of results) all the stalks in each of the plots were cut and split open and examined for eldana larvae and pupae. Fifty to 150 untreated stalks were also examined in the third and fourth tests. All the eldana were taken to the laboratory where the dead larvae and pupae were examined later. Larvae and pupae killed by *Heterorhabditis* and its bacterial associate were usually readily identified by their red-brown colouration. The larvae were also characteristically flaccid and upon dissection revealed either various stages of the nematode or, when no nematodes were found, typical symptoms associated with the presence of the bacterium, ie the contents of the body had a yellowish-red colouration and a mucoid consistency.

Results

Eldana larvae were much more susceptible to the *Heterorhabditis* species and, to a lesser extent, *Steinernema* Sg than to the other two species of *Steinernema* (Table 1). Head capsule measurements showed that the size categories, small, medium and large, each included 2 or 3 instars (Table 2). Nevertheless the data suggest that the earlier instars of eldana are less susceptible to *Steinernema* species Sg and WG than the later instars.

Eldana pupae, whether naked or enclosed in a cocoon, were equally susceptible to the *Heterorhabditis* species and *Steinernema* Sg (Table 3). They were far less susceptible than were the larvae.

In the 4 field tests the effectiveness of the *Heterorhabditis* against eldana larvae was greatest when a suspension of 1 000 000 infectives in 200 ml of water was applied per stalk (Tables 4 to 7). Data from the first 3 tests, with one exception, showed that at any given number of infectives, the *Heterorhabditis* was about twice as effective when the suspension of infectives was applied at a rate of 200 ml per stalk than at a rate of 100 ml per stalk (Tables 4, 5 & 6). At a rate of 50 ml per stalk and 8 000 infectives ml^{-1} none of

Table 1

Mortality of eldana larvae exposed to infective stage juveniles of *Heterorhabditis* species and 3 species of *Steinernema* (means of 10 replicates each with 5 larvae)

Species of nematode	Size of larvae	% mortality of eldana larvae after 48 h exposure: Without nematodes ± SE	With Nematodes ± SE
Heterorhabditis sp.	Small	4,0 ± 2,7	96,0 ± 2,7
	Medium	2,0 ± 2,0	98,0 ± 2,0
	Large	0	94,0 ± 3,1
	Total		96,0 + 1,6
Steinernema Sg	Small	10,0 ± 5,4	68,0 ± 7,4
	Medium	10,0 ± 4,5	74,0 ± 9,0
	Large	2,0 ± 2,0	84,0 ± 5,0
	Total		75,3 ± 4,3
Steinernema CFS3	Small	18,0 ± 7,6	26,0 ± 7,3
	Medium	8,0 ± 3,3	32,0 ± 6,1
	Large	0	30,0 ± 8,0
	Total		29,3 ± 4,0
Steinernema WG	Small	6,0 ± 3,0	4,0 ± 2,7
	Medium	4,0 ± 2,7	30,0 ± 8,0
	Large	2,0 ± 2,0	38,0 ± 7,6
	Total		24,0 ± 4,5
Control	Small	14,0 ± 5,2	0
	Medium	14,0 ± 6,7	0
	Large	4,0 ± 2,7	0

Table 2

Proportion of different instars of eldana larvae in 3 arbitrary size classes

Size class	Number of head capsules measured	% No of individuals — Instar: 3rd	4th	5th	6th
Small	54	13	87	0	0
Medium	52	0	65	27	8
Large	44	0	4	71	25

Table 3

Mortality of eldana pupae and larvae exposed to infective juveniles of *Heterorhabditis* species and *Steinernema* Sg

Stage of insect	Number of replicates (5 insects replicate^{-1})	Eldana exposed to *Heterorhabditis*: n*	With nematodes ± SE	Without nematodes ± SE	Eldana exposed to *Steinernema* Sg: n	With nematodes ± SE	Without nematodes ± SE	Control: n	Without nematodes ± SE
Naked pupae	18	86	22,2 ± 4,9	16,4 ± 4,1	80	24,0 ± 5,1	18,1 ± 4,9	80	26,8 ± 5,7
cocooned pupae	11	42	26,8 ± 9,5	16,5 ± 5,3	46	35,1 ± 7,6	1,8 ± 1,8	43	9,5 ± 4,3
Larvae	10		96,0 ± 2,7	4,0 ± 2,7		90,0 ± 3,3	0		—

* n = actual number of pupae exposed for 48 h period

the eldana larvae was killed (Table 4). Also the effectiveness of treatment with *Heterorhabditis* declined markedly when fewer than 100 000 infective juveniles were applied per stalk (Table 5). Pretrashing the cane stalks before spraying the infectives did not improve the effectiveness of treatment with the *Heterorhabditis* (Table 6).

Table 4

Test No 1 - Effect of different concentrations and volumes of suspensions of *Heterorhabditis* sp. on eldana larvae in field cane. Treatments assessed 3 to 4 days after spraying*

Treatment (18.5.87)		Mean total number of eldana larvae 100^{-1} stalks	% Eldana larvae killed by *Heterorhabditis* ± SE
Volume of suspension $stalk^{-1}$ (ml)	No of infectives $stalk^{-1}$		
100	100 000	118,0	27,7 ± 8,7
100	200 000	78,0	20,9 ± 4,1
100	400 000	117,5	16,9 ± 8,1
200	400 000	115,0	40,7 ± 6,8
50	400 000	175,5	0

* Pupae not recorded in this test

Table 5

Test No 2 - Effect of different concentrations and volumes of suspensions of *Heterorhabditis* sp. on eldana larvae and pupae in field cane. Treatments assessed 6 to 8 days after spraying

Treatment (18.8.87)		Mean total no eldana 100^{-1} stalks		% Eldana killed by *Heterorhabditis* ± SE	
Volume of suspension $stalk^{-1}$ (ml)	No of infectives $stalk^{-1}$ 100^{-1} stalks	Larvae	Pupae	Larvae	Pupae
100	10 000	56,7	24,0	2,1 ± 2,1	0
200	10 000	54,7	26,0	3,5 ± 1,8	0
100	50 000	85,3	37,3	4,7 ± 0,9	0
200	50 000	62,0	25,3	9,3 ± 5,4	1,4 ± 1,4
100	100 000	91,3	18,7	12,5 ± 1,4	5,1 ± 3,0
200	100 000	93,3	40,7	23,8 ± 0,9	5,4 ± 2,9

Table 6

Test No 3 - Effect of pretrashing sugarcane on the efficacy of *Heterorhabditis* species against eldana and the influence of time after spraying when measuring the effect of treatment

Treatment (19.11.87)		Mean total no eldana 100^{-1} stalks		% Eldana killed by *Heterorhabditis* ± SE	
Time of assessing effect of treatment after spraying (d)	Volume of suspension containing 100 000 infectives $stalk^{-1}$ (ml)	Larvae	Pupae	Larvae	Pupae
7	200 p-t*	140,0	9,3	13,3 ± 4,8	11,1 ± 11,1
	200	104,7	3,3	18,0 ± 1,6	0
	100	144,7	3,3	18,0 ± 2,5	33,3 ± 33,3
14	200	134,0	16,7	21,5 ± 0,4	19,0 ± 15,6
	100	141,3	8,0	10,8 ± 1,4	0
21	200	101,3	14,0	27,9 ± 5,7	8,9 ± 4,5

* Stalks pretrashed

Table 7

Test No 4 - Effect of 2 concentrations of a suspension of *Heterorhabditis* on eldana larvae and pupae in field cane. Treatments assessed 7 days after spraying.

Treatment (8.12.87)		Mean total no eldana 100^{-1} stalks		% Eldana killed by *Heterorhabditis* ± SE	
Volume of suspension $stalk^{-1}$ (ml)	No of infectives $stalk^{-1}$	Larvae	Pupae	Larvae	Pupae
200	100 000	142,7	5,7	27,0 ± 8,0	20,6 ± 10,4
200	1 000 000	98,7	6,7	56,2 ± 3,9	16,7 ± 8,3

Mortality of eldana larvae in sugarcane sprayed with 100 000 infectives in 200 ml of water was greater when the cane was examined 3 weeks after treatment than when it was examined after one or possibly 2 weeks (Table 6), but where 100 ml was used, observed mortality was less after 2 weeks that after 1 week. Infectivity of the excess nematodes from the second and third field tests was much lower than expected (Table 8).

Table 8

Mortality of eldana larvae exposed in the laboratory to excess infectives from the field tests (5 larvae plus 1 000 infectives per replicate)

Field test	No of repli-cates	% dead eldana larvae	
		48 h exposure	72 h exposure
1	5	96	—
2	13	56	69
3	14	54	88
4	20	92	100

In the third and fourth tests the mist-blower was used, instead of the hand-operated knapsack sprayer, to facilitate spraying large volumes of nematode suspension onto the cane. No direct comparison was made between the two types of sprayer, but mortality of eldana from 100 000 infectives in 200 ml of water applied by the knapsack sprayer in the second test (24%) was similar to that from the same treatment applied by the mist-blower in the third and fourth field tests (18 and 27%) (Tables 5, 6 & 7).

Except in the second field test, few pupae were recovered from the cane stalks and mortality due to *Heterorhabditis* was generally erratic (Tables 5, 6 & 7). Between 3 and 7% of the larvae recovered from the cane in the 4 field tests were infected with and possibly killed by unidentified bacteria and fungi. The number of dead eldana associated with these organisms was no greater in cane sprayed with nematodes than in untreated cane. None of the larvae or pupae from untreated stalks contained nematodes.

Discussion

The work described in this paper is part of a continuing study to assess the potential of species of *Heterorhabditis* and *Steinernema* as control agents of eldana. In the laboratory, 2 species were found to be highly pathogenic to eldana larvae (Tables 1 & 3). One of these, the *Heterorhabditis*, was tested in the field and found to provide moderate control when applied at a rate of 1 000 000 infectives in 200 ml of water per stalk (Table 7). At this rate, with approximately 130 000 stalks of sugarcane per ha, and assuming the cost of mass producing the nematodes is 1 cent per million infectives (Bedding[9]), the cost of rearing sufficient nematodes to treat a hectare would be approximately R1 300. The cost of transporting and spraying 200 ml of water per stalk (equivalent to approximately 26 000 ℓ ha^{-1}) would be an additional cost. Production costs alone indicate that it would be uneconomical to use *Heterorhabditis* to control eldana in sugarcane.

However there is some evidence that measuring the effect of treatment after only 7 days underestimates mortality (Table 6). Thus *Heterorhabditis* may be more effective than was indicated in the first, second and fourth tests. In addition, the infectivity of the excess nematodes from the second and third tests was much lower than expected (Table 8). The reason for this could not be ascertained, but if it occurred before the cane was treated, the results from these 2 tests would further underestimate the potential of *Heterorhabditis*.

A major constraint in the use of *Heterorhabditis* and *Steinernema* to control insects is their susceptibility to desiccation (Gaugler[19]; Kaya[20]). Although the tunnels made by eldana are usually moist, the ejected frass at the entrance to the borings is usually dry. The addition of an antidesiccant and a surfactant to the nematode suspension might increase the survival of the infectives outside the borings and facilitate wetting of the frass (MacVean *et al.*[21]; Shapiro *et al.*[22]; Webster and Bronskill[23]). Selecting overcast or rainy days on which to spray the nematodes is not practical in South Africa, but spraying in the evening instead of during the day, as was done in the present study, might increase survival and thus effectiveness of the nematodes (MacVean *et al.*[21]). By increasing survival it may be possible to achieve moderate to good control of eldana with far fewer nematodes. Moreover, other isolates of *Heterorhabditis* and *Steinernema* should be tested, because different populations vary in their behaviour and their ability to withstand adverse environmental conditions (Bedding[8]; Bedding & Miller[11]).

Another constraint that must be overcome is the need to use large volumes of water to obtain adequate spray cover of the cane. The borings of eldana are usually located in the lower two-thirds of the stalk. In mature cane the leaves associated with this part of the stalk are dead and the leaf blades hang down from their point of attachment to the sheaths. In the field tests it was observed that a proportion of the aqueous suspension directed at the cane stalks was lost when it passed through the cane row and, in particular, when it was intercepted by the pendant blades of the numerous dead leaves, and dripped onto the soil. Preliminary observations indicate that as much as 40% of the nematode suspension sprayed onto the cane may be lost as runoff from the leaves.

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