

FIELD TESTS TO CONTROL THE PYRALID, *ELDANA SACCHARINA*, WITH AN ENTOMOGENOUS NEMATODE, *HETERORHABDITIS SP*

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Abstract

A series of field tests were conducted to assess the performance of a *Heterorhabditis* sp against the sugarcane stalk borer, *Eldana saccharina* Walker. More eldana larvae were killed when the nematodes were applied to the cane in the late afternoon than when applied just before sunrise or at midday. Removing the pendant blades of the leaves from the cane stalks prior to spraying had little effect on the efficacy of the nematodes but removing the leaf blades and leaf sheaths was detrimental. The addition of various chemicals to thicken the water, with or without a spreader-sticker or a surfactant, did not improve the performance of the nematodes. Up to 40% mortality of eldana larvae was achieved when spraying at a rate of approximately 11 000 million infective stage nematodes in 7 400 litres of water per hectare, although the results were erratic. Using half this volume, even under apparently ideal conditions, halved the effectiveness of the nematodes.

Introduction

It has been reported previously that the larvae of the stalk borer *Eldana saccharina* infesting sugarcane could be killed by an entomogenous nematode, *Heterorhabditis* isolate Hsp1, sprayed onto the surface of the cane (Spaull⁴). However, very large numbers of infectives (c. 130 billion/ha) and large volumes of water (c. 26 000 l/ha) were required to achieve even moderate (56%) control of the borer. An improvement in the method of applying the nematodes to the cane should enhance their performance against eldana. Accordingly a number of field tests (FTs) were conducted with this as the principal objective.

Methods

Twelve tests were conducted to assess the effect of the following procedures on the efficacy of *Heterorhabditis* Hsp1:

1. removing the leaf blades and leaf sheaths from the cane stalks before applying the nematodes (FT 1)
2. varying the time of application (FT 2-5)
3. prewetting the cane prior to applying the nematodes (FT 8 and 10)
4. adding water thickeners, (glycerol, Reverseal 3 and carboxymethylcellulose) with and without a spreader-sticker, (Reverseal 10) or a surfactant, (Triton X100) (FT 4 and 6)
5. applying the suspension in a foam of household detergent (FT 9 and 10).

In addition, relatively low volumes of approximately 3 000 l/ha were tested (FT 11 and 12) and the best time to sample the cane was investigated (FT 7). Also the performance of a related nematode, *Steinernema* isolate Sg, was compared with that of the *Heterorhabditis* isolate (FT 8).

The methods used in the tests were similar to those described previously (Spaull⁴). Plots of cane comprising approximately equal numbers of stalks per field test, with up to 69 stalks/plot, were marked out in mature cane infested with eldana. Aqueous suspensions of a known number of infectives of the *Heterorhabditis* sp, with or without Triton X100 or other additives, were prepared and carried to the field in polythene bags. These were sprayed onto the lower two-thirds of the cane stalks, i.e. the region where most of the borings of eldana larvae occur. Details of the treatments in each field test are given in the relevant table of results (Tables 1-7).

Spraying was performed initially with a motorized Hatusa Blowmic mistblower with extended discharge tubes (FT 1-4). In the remaining field tests (FT 5-12) this somewhat cumbersome apparatus was replaced with a knapsack sprayer fitted with a self priming Flowjet pump driven by a 12v, 14A motor-cycle battery. A Spraying Systems Co T4006 spray nozzle delivering 2 l/min at 240 kPa was fitted to the knapsack sprayer in FT 6-8, and a T9520 nozzle delivering 5 l/min at 83 kPa was used in FT 9-12. In FT 9 and 10 the nematodes were also applied to the cane in a foam produced by spraying a 0,2% solution of 'Omo' washing powder through a bathroom shower nozzle at 500 kPa.

Treatments were replicated five times in all the tests except FT 7 and 11 where there were six replicates. Where the size of the field test necessitated spraying on two or three consecutive days, an equal number of replicates from each treatment were treated on each occasion.

The time interval between spraying and assessing the performance of the nematodes varied as follows: between 7 and 12 days in FT 3 and 4; between 10 and 14 days in FT 1, 2, 5 and 6 and 8-12; 14 to 56 days in FT 7. The methods of checking the pathogenicity of the nematodes and of assessing their performance against eldana larvae in the field tests were the same as those used previously (Spaull⁴).

Table 1

Effect of removing all or part of the dead leaves from the cane stalks on the efficacy of *Heterorhabditis* Hsp1 against eldana larvae. Treatments applied during the afternoon when humidity increased from 77 to 95%

Field Test No.	No. infectives and volume per stalk	Treatment	No. eldana larvae per 100 stalks	% eldana killed by <i>Heterorhabditis</i> ± SE
1	72 000 in 145 ml	Control (dead leaves intact)	120	37,0 ± 2,4
		Dead leaf blades removed	138	41,0 ± 2,9
		Dead leaf blades and sheaths removed	97	20,9 ± 3,0

Results and Discussion

The results of the 12 field tests are summarized in Tables 1-7. As found previously (Spaull⁴), removing both the leaf blades and leaf sheaths from the stalks prior to spraying the nematodes reduced their efficacy (Table 1). This may have been partly due to increased evaporation of water from the nematode suspension on the exposed surface of the stalks. Removing only the leaf blades, which to some extent deflect sprayed suspension from the stalk, did not improve the performance of the nematodes (Table 1).

The efficacy of the nematodes was generally greater when applied in the late afternoon than when applied at about midday (Table 2). The most probable reason for this is the greater humidity at the time when the late afternoon treatments were applied. In FT 4, where late afternoon treatments were applied on the day following the midday treatments, humidity was greater at the time of applying the latter; mortality of eldana was also apparently greater. However the results from FT 5 (Table 2) show that humidity *per se* is not the only criterion. In this test the mortality of eldana was much greater when the nematodes were applied to the cane in the late afternoon than when applied in the early morning just before sunrise, and yet the humidity at the time

of spraying (and during the preceding 12 hours) was greater in the latter treatment. It seems likely that the difference in the performance of the *Heterorhabditis* resulted from the difference in the humidity *after* treatment. Humidity following the late afternoon treatment rose from 86% to 95% within an hour of spraying and remained above 95% for a further 14 hours. Following the early morning treatment, however, the humidity remained above 95% for only 2 hours before dropping to 40% in the succeeding 2 hours. The greater efficacy of the *Heterorhabditis* when applied in the late afternoon supports previous observations made with *Steinernema* (= *Neoaplectana*) *carpocapsae* against the Colorado potato beetle (MacVean *et al*⁵).

The influence of solutions of glycerol, carboxymethylcellulose and Reverseal 3 on the performance of the *Heterorhabditis* infectives against eldana was tested in FT 5 and FT 6 (Tables 2 and 3). All three chemicals increase the viscosity of water and although only glycerol retarded evaporation of water in laboratory tests, it was presumed that an infective stage larva of *Heterorhabditis* would survive longer in a thick drop of water than in a thin drop. However the addition of carboxymethylcellulose and Reverseal 3, with or without the spreader-sticker, Reverseal 10, or a surfactant, Triton X100, did not improve the performance of the nematodes (Table 3). The addition of glycerol was detrimental (Table 2). Triton X100 had no apparent influence on the performance of the infectives in FT 4 (Table 2). However it was included in some of the subsequent tests (FT 5 and 9-12) since it had been observed that the wetting of eldana frass was considerably improved when the surfactant was present.

Table 2

Effect of time of application of the infective stage juveniles of *Heterorhabditis* Hsp1 on eldana larvae. In FT 4 the afternoon treatment was applied on the day following the midday treatment

Field Test No.	Treatment		No. eldana larvae per 100 stalks	% eldana killed by <i>Heterorhabditis</i> ± SE
	No. infectives and volume/stalk	Time of application & humidity		
2	82 000 in 82 ml	Midday 12h30-13h15 64-68%	98	4,4 ± 0,9
		pm 17h00-17h30 85-87%	111	7,7 ± 2,5
3	98 000 in 98 ml	Midday 11h40-13h20 65-75%	68	14,2 ± 4,2
		pm 17h00-17h30 87-93%	62	23,0 ± 2,0
	98 000 in 196 ml	Midday as above	100	18,8 ± 0,7
		pm as above	89	29,5 ± 4,7
4	96 000 in 192 ml	Midday 12h30-15h30 55-70%	160	22,0 ± 3,6
		Midday (as above)*	145	16,2 ± 2,7
		pm 15h20-17h15 32-60%	175	18,7 ± 2,6
	As above plus 10% glycerol	Midday (as above)	136	10,5 ± 1,9
		pm (as above)	182	6,9 ± 1,4
5	96 000 in 62 ml 0,1% Triton X100	pm 17h10-17h40 74-86%	24	21,8 ± 6,6
		am 06h15-06h40 97-97%	25	5,3 ± 3,9

* with 0,01 Triton X100

Table 3

The effects of various additives on the performance of *Heterorhabditis* against eldana. Treatments applied on two consecutive afternoons when humidity increased from 34 to 56% on day 1 and from 53 to 75% on day 2

Field Test No.	Treatment per stalk	No. eldana larvae per 100 stalks	% eldana killed by <i>Heterorhabditis</i> ± SE
6	74 000 infectives in 148 ml of:		
	Water	127	23,4 ± 5,5
	1% Reverseal 3	129	13,9 ± 3,0
	1% Reverseal 3 plus 0,01% Reverseal 10	114	13,1 ± 3,9
	1% Reverseal 3 plus 0,1% Triton X100	109	14,0 ± 2,7
	0,1% Carboxymethylcellulose	107	14,2 ± 3,7
	0,1% Carboxymethylcellulose plus 0,01% Reverseal 10 plus 0,1% Carboxymethylcellulose plus 0,1% Triton X100	119	18,7 ± 4,2
		114	21,6 ± 6,1

The results of FT 7 and of an earlier test (Spaull⁴) showed that the best time to assess larval mortality was two to three weeks after applying the treatment (Table 4). The small number of eldana larvae killed by nematodes in the control plots in FT 7 was almost certainly the result of spray drift passing through adjacent treated cane. Spray drift was largely avoided in subsequent field tests by applying the nematode suspension to the cane row at an oblique angle. The relatively large proportion of eldana larvae killed by *Heterorhabditis* that was recorded when the cane was sampled after 2 weeks, was not apparent when samples were taken after 4 and 8 weeks (Table 4). Water alone had no apparent influence on the eldana larvae.

Table 4

Effect of *Heterorhabditis* Hsp1, water and time of sampling on eldana. Spraying was performed on 3 consecutive afternoons; two replicates being treated on each occasion. Humidity ranged from 79 to 84% on the first 2 days and from 33 to 37% on the third day

Field Test No.	Time of assessing effect of treatment after spraying	Treatment	No. eldana larvae per 100 stalks ± SE	% eldana killed by <i>Heterorhabditis</i> ± SE
7	2 weeks	106 000 Hsp1 infectives in 212 ml/stalk	59 ± 14,0	38,9 ± 12,0
		Control	63 ± 7,1	1,0 ± 1,0
		212 ml water/stalk	77 ± 10,7	0
	4 weeks	106 000 Hsp1 infectives in 212 ml/stalk	39 ± 7,1	4,5 ± 2,0
		Control	49 ± 6,3	0
		212 ml water/stalk	48 ± 6,4	0
	8 weeks	106 000 Hsp1 infectives in 212 ml/stalk	60 ± 10,4	0
		Control	81 ± 9,6	0
		212 ml water/stalk	66 ± 14,1	0

A species of *Steinernema*, isolate Sg, which was almost as pathogenic as *Heterorhabditis* Hsp1 against eldana in the laboratory (Spaull⁴), was ineffective in the field (Table 5).

Table 5

Effect of *Heterorhabditis* Hsp1 and *Steinernema* Sg on eldana with and without prior wetting of the cane. Treatments applied in the late afternoon on two consecutive days when humidity increased from 63% to a maximum of 80%

Field Test No.	No. infectives and volume per stalk	Stalks prewetted (pw)	No. eldana larvae per 100 stalks	% eldana killed by <i>Heterorhabditis</i> ± SE
8	76 000 Hsp1 in 76 ml	pw*	90	9,2 ± 3,0
	76 000 Hsp1 in 152 ml	-	98	13,5 ± 2,7
		pw	100	13,0 ± 3,0
	76 000 Hsp1 in 304 ml	-	105	24,8 ± 2,1
	76 000 Sg in 152 ml	-	107	4,3 ± 1,4
		pw	100	4,8 ± 1,8

*Cane prewetted with water ≡ 4 mm rainfall just before spraying the nematodes.

The mean diameter of the droplets delivered by the Spraying System T9520 nozzle used in FT 9-12 was above 200 μm (Anon¹). Such droplets shatter on impact (Matthews³). Thus some of the nematode suspension may have been lost when the droplets splashed off the cane. It was thought that by applying the nematodes in a foam of washing powder splashing would be avoided; also that as the bubbles in the foam burst, the soapy suspension would coalesce and carry the

nematodes down the stalk to the site of the borings. However this assumption was not supported by the results of FT 9 and FT 10 (Table 6).

Table 6

The effects of applying the infectives of *Heterorhabditis* Hsp1 in a foam containing 0,2% Omo on their performance against eldana. Treatments applied in the late afternoon when humidity in the 9th test increased from 58 to 72% and from 70 to 80% in the 10th test.

Field Test No.	No. infectives and volume per stalk	Treatment	eldana larvae per 100 stalks	% eldana killed by <i>Heterorhabditis</i>
9	87 000 in 57 ml	0,1% Triton X100	31	39,7 ± 4,6
		0,2% Omo	29	32,7 ± 10,3
10	86 000 in 56 ml	0,1% Triton X100	76	37,4 ± 5,9
		0,1% Triton X100 (pw*)	74	44,7 ± 2,4
		0,2% Omo	57	25,3 ± 4,1
		0,2% Omo (pw)	89	28,9 ± 3,2

* Cane prewetted with water ≡ 9 mm rainfall three days before spraying the nematodes

The performance of the infectives applied in a relatively low volume of a solution of Triton X100 in FT 9 was as good as the best obtained with approximately three times the volume in previous tests (see Tables 1 and 4). It may be significant that four to five days before the 9th FT the cane had been soaked with 87 mm of rain. This apparent association prompted a re-examination of the performance of the *Heterorhabditis* applied after rainfall. Previously, in FT 8, prewetting the cane with a simulated rainfall of 4 mm had no effect on the efficacy of the nematodes (Table 5). However, in FT 10 there was some indication that simulated rainfall of 9 mm, applied 3 days before the nematodes, did improve their performance (Table 6). In this field test, 14 mm of rain fell just after the simulated rainfall treatment had been applied. From these observations and from the results of FT 3, which indicated that high humidity is important, it seemed that rainfall at the time of spraying could provide optimum conditions for the nematodes. However this is not borne out by the results of FT 12, where the treatments were applied while it was raining. The proportion of eldana larvae killed by the nematodes was much lower in this field test than in FT 9 and 10 (cf Tables 6 and 7). This may be related to the infectivity of the nematodes which, in the post treatment pathogenicity check, after 48 hours exposure, was much less than expected (Table 8).

Table 7

Effect of low volumes on the performance of *Heterorhabditis* Hsp1 against eldana. Treatments applied in late afternoon when humidity in FT 11 increased from 80 to 91%. In FT 12 it was raining at the time the treatments were applied. All suspensions contained 0,1% Triton X100

Field Test No.	No. infectives and volume per stalk	No. eldana larvae per 100 stalks	% eldana killed by <i>Heterorhabditis</i> ± SE
11	92 000 in 28 ml	23	15,8 ± 5,5
	92 000 in 56 ml	18	12,5 ± 6,2
12	85 000 in 26 ml	33	20,1 ± 2,3
	85 000 in 52 ml	26	21,6 ± 6,6

Table 8

Pathogenicity check for the *Heterorhabditis* and *Steinernema* infectives used in the field tests

Field Test No.	Time/genus	% eldana killed by nematodes. (Mean of 10 replicates with five eldana larvae and 1 000 infectives per replicate)	
		48 hr exposure	72 hr exposure
1		32	100
2	Midday Afternoon	96 100	- -
3	Midday Afternoon	100 98	- -
4	Midday Afternoon	- -	100 98
5	Afternoon Morning	82 74	96 94
6		90	100
7		100	-
8	<i>Heterorhabditis</i> <i>Steinernema</i>	98 93	100 95
9		46	94
10		52	72
11		67	92
12		13	87

In previous tests the *Heterorhabditis* infectives were about twice as effective when the suspension of nematodes was applied at a rate of 200 ml/stalk than at a rate of 100 ml/stalk. At 50 ml/stalk the nematodes were ineffective (Spaull⁴).

In the current series of field tests the relationship between the volume of suspension and effectiveness of the nematodes was much more variable. Thus while in FT 8 and, to a lesser extent in FT 3, the larger volumes were more effective (Tables 2 and 5), in FT 11 and 12 there was no difference between volumes (Table 7).

Forty to 45% mortality of eldana larvae was achieved by spraying eldana-infested cane with a suspension of 87 000 *Heterorhabditis* infectives in 57 ml per stalk (Table 6). This is a considerable reduction in the volume and concentration of the nematode suspension necessary to achieve a moderate kill, when compared with the suspensions used in the earlier field tests (Spaull⁴). The improvement appears to have been due mainly to the change from spraying at about midday, as in the original field tests, to spraying in the late afternoon when the humidity was normally higher. With approximately 130 000 stalks/ha, 57 ml/stalk is equivalent to approximately 7 400 l/ha. To be of use as a practical control measure it will be necessary to reduce the volume further. However, in the two field tests where the lowest volumes were tested, viz 26-28 ml/stalk (c 3000 l/ha; Table 7), only 16-20% mortality of eldana larvae was achieved. Thus the prospect of attaining a reasonable kill using lower volumes seems unlikely.

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