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On the use of a methylcellulose polymer to increase the effectiveness of a *Heterorhabditis* species against the sugarcane stalk borer, *Eldana saccharina*

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Summary — *Eldana saccharina* Walker (Lepidoptera : Pyralidae) is the most important insect pest of sugarcane in South Africa. Previous field tests had shown that, at best, only moderate control of this stalk borer could be achieved by spraying infested cane with an aqueous suspension of the infective stage juveniles of a species of *Heterorhabditis*, designated Hsp1. A description is given of six field tests to assess the effect of adding a water thickener, Methocel J75, on the performance of the nematodes. A mean of 20 % mortality of *E. saccharina* larvae was achieved in four tests when the nematodes were applied at a rate of 11-13 × 10^9 infectives in 3380-7800 l 0.1 % Triton X100/ha. With the addition of 0.5 % Methocel the mortality of *E. saccharina* was increased to a mean of 33 %. In one test increasing the rate at which the nematodes were applied from 13 × 10^9 to 65 × 10^9 infectives in 3900 l 0.1 % Triton X100/ha almost doubled the effectiveness of the nematodes, from 26 % to 49 % mortality of the larvae. The addition of 0.5 % Methocel to a suspension of the higher concentration of nematodes increased mortality of *E. saccharina* from 39 % to 75 %. Additional observations indicate that the Methocel did not increase the survival of the nematodes nor their retention on the cane stalks.

Résumé — ***Emploi d'un polymère de méthylcellulose pour augmenter l'efficacité d'une espèce d'*Heterorhabditis *contre le foreur des tiges de la canne à sucre,* Eldana saccharina** — *Eldana saccharina* Walker (Lepidoptera : Pyralidae) est l'insecte nuisible le plus important de la canne à sucre en Afrique du Sud ; les essais au champ ont antérieurement démontré, qu'au mieux, un contrôle modéré de ce foreur de la tige des cannes peut être obtenu en appliquant par vaporisation une suspension aqueuse des stades juvéniles infestants d' *Heterorhabditis*. Six essais aux champs sont décrits ayant eu pour but de déterminer l'effet d'un additif épaississant, le Methocel J75, sur l'efficacité du nématode. Une mortalité moyenne de 20 % des larves de *E. saccharina* est obtenue dans quatre essais où les nématodes sont appliqués à la dose de 11-13 × 10^9 dans 3380-7800 l 0.1 % Triton X100/ha. En ajoutant 0,5 % de Methocel, la mortalité d'*E. saccharina* augmente en moyenne de 33 %. Dans un essai, en portant le taux de nématodes à 13 × 10^9 à 65 × 10^9 dans 3900 l 0.1 % Triton X100/ha, l'efficacité du contrôle augmente presque du double, la mortalité des larves d'insectes passant de 26 % à 49 %. L'addition de 0.5 % de Methocel à une suspension plus concentrée de nématodes augmente la mortalité d'*E. saccharina* de 39 % à 75 %. Des observations supplémentaires montrent que le Methocel n'accroît pas la longévité du nématode, ni sa rétention sur les tiges de la canne.

Key-words : Nematodes, *Heterorhabditis*, stalk borer, sugarcane, biological control.

Lepidopteran stalk borers are among the most important insect pests of sugarcane (Williams *et al.*, 1969; Hawkins & Smith, 1986; Atkinson & Carnegie, 1989). Their control by means of insecticides is difficult because, for a large proportion of their life cycle, they are concealed within the borings in the sugarcane stalks. However, in this protected habitat they are potential candidates for control by entomogenous nematodes of the genera *Heterorhabditis* and *Steinernema* (Gaugler, 1981; Kaya, 1985). Such control was attempted with *Steinernema feltiae* against *Diatraea saccharalis* in sugarcane in Louisiana and with *S. carpocapsae* against *Eoreuma loftini* in cane in Texas, but with little or no success (Reagan & Bessin, 1989; Pfannenstiel & Browning, 1989). More encouraging results were reported by Spaull (1988, 1990) who obtained up to 56 % mortality of *Eldana saccharina* in Natal with a species of *Heterorhabditis*, designated isolate Hsp1. However, uneconomically large numbers of nematodes (130 × 10^9 infectives/ha) and large volumes of water (26 000 l/ha) were used to achieve this level of control. Lower concentrations and smaller volumes were tested subsequently but none gave more than moderate control (Spaull, 1990). Adding 10 % glycerol or 0.1 % carboxymethylcellulose to the spray mix was tested but neither chemical improved the performance of the *Heterorhabditis* (Spaull, 1990). However, certain spray additives tested by MacVean *et al.* (1982), and Kaya and Reardon (1982) increased the effectiveness of *Steinernema carpocapsae (Neoaplectana carpocapsae)* against foliar feeding insects. I tested one of these compounds, a methylcellulose polymer, Methocel J75 MS (Dow Chemical Co.), as a

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spray additive in an attempt to improve the performance of the *Heterorhabditis* species against *E. saccharina* in sugarcane. The results are presented in this paper.

Materials and methods

The *Heterorhabditis* isolate Hspl, which had been isolated from soil in Natal, South Africa, in 1985, was reared at 20 °C *in vivo* on larvae of *E. saccharina* from a laboratory culture. The infective stage juveniles were stored in 0.1 % formalin at 12 °C until required.

Six field tests were conducted to investigate the effects of adding Methocel J75 MS to the suspension of nematodes sprayed onto the cane. The pH of the Methocel was first adjusted to 8.5 by adding 1M NaOH. Single row plots of sugarcane, cultivar N13 or NCo376, comprising approximately equal numbers of stalks per field test and with up to 55 stalks per plot, were marked out in two or more adjacent rows of mature cane infested with *E. saccharina*. Suspensions of a known number of infectives, with or without 0.1 % of the surfactant Triton X100, and with or without 0.25 % or 0.5 % Methocel J75, were prepared and carried to the field in polythene bags. The nematodes were sprayed onto the lower two-thirds of the cane stalks, i.e. the region where most of the borings of *E. saccharina* occur. The borings were usually concealed by the old, dead, overlapping leaf sheaths that remain more or less firmly attached to the stalk. Except where the sheaths had fallen away from the stalks, access to the borings by the nematodes in the spray suspension was via the exposed surface of a leaf sheath down to the surface of the stalk within the confines of the older leaf sheath below.

In an attempt to ensure high relative humidity at the time of application the field tests were conducted after 17 h 00 except the third and sixth tests, which were conducted at mid-afternoon. In the third field test it was raining at the time of spraying, and in the sixth test light rain had fallen for most of the morning and the afternoon was overcast. The nematodes were applied at a rate of 85 000 to 100 000 infectives per stalk and in one test, also at an additional rate of 500 000 per stalk. Since there are approximately 130 000 stalks of cv. N13 and cv. NCo376/ha these concentrations are equivalent to about 11, 13 and 65 $\times$ 10^9 infectives/ha respectively. The volume of suspension applied ranged from 15 to 180 ml per stalk (1950 to 23 400 l/ha). Details of the treatments in each field test are given in Table 1.

Spraying was performed with a knapsack sprayer fitted with a battery driven pump and a Spraying Systems Co. T9520 nozzle that delivered 5 l per minute at 83 kPa. Treatments were randomised in blocks and replicated five times.

Ten to 13 days after spraying the cane, all the stalks in each of the plots were cut and split open and examined for larvae of *E. saccharina*. The larvae that were recovered were fourth, fifth and sixth instars, rarely third instars. No first or second instar larvae were found. All the recovered larvae were taken to the laboratory where, on the following day, dead individuals were examined for nematodes and/or the characteristic symptoms associated with their symbiotic bacterium.

To check the pathogenicity of the nematodes used in the field tests, a suspension containing 500 infectives/ml was carried to and from each field test. After overnight storage at 12 °C, 2 ml of the suspension were added to five *E. saccharina* larvae in a Petri dish lined with two filter papers. After 48 or 72 hours the number of larvae killed by the nematodes was recorded. Each pathogenicity check was replicated ten times.

In addition, observations were made on the retention and survival of infectives of *Heterorhabditis* Hsp1 when sprayed onto sugarcane. On six occasions a known volume and concentration of a suspension of nematodes were sprayed onto sugarcane as described above. After 10 to 30 min or 15 to 24 h, five stalks were cut and the leaf blades removed. Without delay the stalks and the leaf sheaths were washed and the number of live and dead infectives of *Heterorhabditis* in the washings were recorded. In six other tests similar methods were used to measure the retention and survival of the nematodes that had been sprayed onto sugarcane with or without 0.25 % or 0.5 % Methocel.

Results

The addition of 0.5 % Methocel to the nematode suspension generally improved the performance of the infectives of Hsp1 against larvae of *E. saccharina* (Table 1). Thus, in the second, third, fourth and sixth field tests, where the nematodes were sprayed onto the cane at a rate of 85-100 000 infectives in 26-60 ml suspension per stalk, mortality of *E. saccharina* was increased from a mean of 20 % when applied in water to 33 % in 0.5 % Methocel. The notable exception was in the fifth field test where the infectives were applied to the cane in only 15 ml/stalk. At this relatively low volume the Methocel treatment was ineffective, but so too were the Triton X100 and water treatments (Table 1). The lower concentration of Methocel used in the first field test did not improve the performance of the infectives.

In the first and fourth field tests there was some indication that the infectives were more effective when applied in larger volumes, but this is not supported by the results from the second and third tests (Table 1). Increasing the concentration of infectives from 100 000 to 500 000 per stalk almost doubled their effectiveness against *E. saccharina* (6th field test, Table 1). The combination of a high concentration of infectives and 0.5 % Methocel was the most effective of the treatments tested (Table 1).

The check on the pathogenicity of the infectives used in the field tests indicated that only in the third test was the infectivity of the nematodes lower than expected

Table 1. Efficacy of *Heterorhabditis* infectives sprayed onto sugarcane in water, or 0.1 % Triton X100 (= TX100) with or without 0.25 % or 0.5 % Methocel (each treatment replicated five times).

Field test No.; relative humidity and temperature on completion of test; mean number of stalks per plot; length of plots; sugarcane cultivar	Treatment per stalk	Mean number *E. saccharina* per plot	Mean percentage *E. saccharina* killed by nematodes ± SE
1; 80 %; 26 °C; 55 stalks; 3.5 m; N13	92 000 infectives in :		
	180 ml TX100	17	32.4 ± 4.4
	180 ml TX100 + 0.25 % Methocel	17	35.5 ± 6.3
	60 ml TX100	17	26.0 ± 9.9
	60 ml TX100 + 0.25 % Methocel	19	20.1 ± 8.1
2; 73 %; 27 °C; 54 stalks; 2.7 m; N13	93 000 infectives in :		
	180 ml TX100	16	26.6 ± 7.5
	60 ml TX100	18	24.5 ± 4.8
	60 ml TX100 + 0.5 % Methocel	17	32.9 ± 7.8
3; 100 %; 16 °C; 39 stalks; 2.6 m; N13	85 000 infectives in :		
	52 ml TX100	10	21.6 ± 6.6
	26 ml TX100	13	20.1 ± 2.3
	26 ml TX100 + 0.5 % Methocel	14	38.7 ± 5.3
4; 74 %; 19 °C; 33 stalks; 2.3 m; N13	100 000 infectives in :		
	30 ml TX100	14	10.2 ± 5.3
	30 ml TX100 + 0.5 % Methocel	11	19.5 ± 6.2
	15 ml TX100 + 0.5 % Methocel	13	9.7 ± 4.4
5; 71 %; 26 °C; 50 stalks; 2.6 m; NCo376	100 000 infectives in :		
	15 ml water	32	1.6 + 1.0
	15 ml TX100	33	0.9 ± 0.6
	15 ml TX100 + 0.5 % Methocel	30	0
6; 81 %; 19 °C; 29 stalks; 2.1 m; NCo376	100 000 infectives in :		
	30 ml TX100	5	25.6 ± 13.1
	30 ml TX100 + 0.5 % Methocel	5	39.2 ± 7.2
	500 000 infectives in :		
	30 ml TX100	7	49.1 ± 2.5
	30 ml TX100 + 0.5 % Methocel	9	74.9 ± 7.0

(Table 2). However, the field performance of the nematodes appears not to have been affected (Table 1).

In the six tests to determine the retention and survival of the *Heterorhabditis* isolate when applied to cane in water, only 12 % of the nematodes were recovered from the surface of the stalks or leaf sheaths within half an hour of spraying. This fell to 5 % within 24 hours. Three-quarters of the 12 % were alive but less than half of the 5 % were alive. In the other retention and survival tests it was found that the addition of 0.25 % or 0.5 % Methocel did not affect the total number of infectives retained on the stalks : compare 4.9 % retained with water with 4.8 % retained with Methocel (observations made after 18 h, mean of three tests). Similarly, Methocel had little effect on survival of *Heterorhabditis* sprayed onto sugarcane : compare 1.7 % survival of the nema-

Table 2. Pathogenicity of the *Heterorhabditis* infectives used in the field tests.

Field test No.	Percentage *E. saccharina* killed by nematodes (mean of 10 replicates with five *E. saccharina* larvae and 1000 infectives per replicate	
	48 h exposure	72 h exposure
1	92	—
2	90	—
3	13	87
4	66	96
5	100	—
6	75	94

todes applied in water with 2.3 % survival when applied in a 0.5 % solution of Methocel (observations made after 16 h, mean of six tests).

Discussion

Excluding the data from the fifth field test, the addition of 0.5 % Methocel increased the effectiveness of the *Heterorhabditis* isolate, on average, by more than 60 % (Table 1). These results confirm reports by MacVean *et al.* (1982) and Kaya and Reardon (1982) on the benefit of Methocel J75 as a spray additive. They found that the addition of Methocel to the nematode suspension retarded desiccation and thus prolonged survival of infectives of *Steinernema carpocapsae* that were being tested in the field to control foliage feeding nematodes. In laboratory tests MacVean *et al.* (1982) had found that the addition of 1 % Methocel J75 brought about a four-fold decrease in the rate of evaporation from an aqueous suspension in open Petri dishes and on potato leaves. Such a marked effect was not recorded in the present study. Methocel at 1 % and 0.5 % had no effect on the rate of water loss from Petri dishes over a 24 and 72 h period (unpubl. data). Also the average time taken for 0.02 ml drops of water to dry out increased from 1 h 31 min with 0.1 % Triton X100 on its own to 1 h 52 min with the addition of 0.5 % Methocel, an increase of only 21 min (mean of seven drops replicated seven times; unpubl. data). It seems unlikely that this would explain the notable increase in efficacy of the Hsp1 infectives. How the Methocel enhanced the performance of the *Heterorhabditis* in sugarcane is not known.

MacVean *et al.* (1982) additionally found that at 1 % concentration Methocel also increased the retention of the infectives on the foliage. They considered this to be a prime reason for the increased effectiveness of the nematodes. This was not the case in the present study where lower concentrations of Methocel were used. Indeed, for the control of *E. saccharina* in sugarcane, the spray medium must be sufficiently mobile on the surface of the cane to carry the nematodes to the entrance of the borings. Mobility is dependent not only on the viscosity of the spray medium but also on the volume applied. This may partly explain why, in previous field tests, the infectives of the *Heterorhabditis* were generally more effective when applied in larger volumes (Spaull, 1988, 1990).

The results of two earlier tests indicated that the addition of the surfactant, Triton X100, to the spray medium had no effect on the performance of the nematodes (Spaull, 1990). However it was included in the present field tests as it had been observed that the wetting of *E. saccharina* frass was considerably improved when the surfactant was present.

In the more than twenty field tests conducted with the *Heterorhabditis* isolate against *E. saccharina* since 1987, four factors were found to increase the efficacy of the nematodes : 1) applying the infectives when the relative humidity was high and when the time of application was followed by an extended period of high humidity; 2) the addition of 0.5 % Methocel; 3) applying the infectives in large volumes of water; 4) using a high concentration of infectives (Table 1; Spaull, 1988, 1990, 1991). Of these factors the concentration of infectives appears to be the most critical. With one exception, increasing the concentration of infectives two to ten times doubled or more than doubled the mortality of *E. saccharina* (Table 1, Spaull, 1988, 1991). However, this is not surprising in view of the observation that almost 90 % of the infectives never reached the stalks, and of those that did, most died within 24 hours.

The very poor recovery of infectives from the stalks is due, in part, to the observed interception, and consequent loss, of a notable proportion of the nematode suspension by the pendent blades of the dead leaves (Spaull, 1988). Surprisingly, removal of the leaf blades did not improve the performance of the nematodes (Spaull, 1990). Quite probably many nematodes were lost when the sprayed suspension passed through the cane row, even though it was directed, as near as possible, along the row rather than across it.

The results of these field tests to control *E. saccharina* with Hsp1 may explain why Reagan and Bessin (1989) failed to achieve any control of the sugarcane borer, *Diatraea saccharalis* with *Steinernema feltiae.* They applied the nematodes to sugarcane cultivar CP74-383 at a rate of about 24.7×10^6 infectives in 260 l water/ha. With approximately 94 000 stalks of CP74-383/ha (Ricaud & Arceneaux, 1989), this is equivalent to an application rate of about 260 infectives in 2.8 ml/stalk. This is a small fraction of the number of infectives and volume necessary to achieve even moderate control of *E. saccharina* with Hsp1 in sugarcane (Table 1).

In their field test to control *Eoreuma loftini* in sugarcane cv. NCo310, Pfannenstiel and Browning (1989) used larger numbers of *S. carpocapsae* and a greater volume of water, viz. approximately 57 000 infectives in 32 ml/stalk. This is assuming there were about 100 000 stalks of NCo310/ha (Thomas *et al.*, 1985). However, they achieved no more than 4 % mortality of the larvae of *E. loftini*, but the nematodes were applied during the middle of the morning in bright sunshine. Under similar conditions 50 000 Hsp1 infectives in 100 ml water/stalk caused 5 % mortality of *E. saccharina;* this increased to 9 % when the nematodes were applied in 200 ml/stalk (Spaull, 1988).

To conclude, data indicate that to achieve a high level of mortality of *E. saccharina* and presumably other sugarcane stalk borers by means of entomogenous nematodes, it is necessary to use many thousands of millions of infectives in dilute Methocel per hectare. In the United States the nematodes cost about 40 cents per million (Schroeder, 1991). Even if this were reduced to

10 cents per million the cost of 65 × 10^9 infectives would be far in excess of the value of the crop.

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