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An epizootic of *Heterorhabditis* spp. (Heterorhabditidae : Nematoda) in sugar cane scarabaeids (Coleoptera)

Raymond J. AKHURST *, Robin A. BEDDING *, Richard M. BULL ** and Donald R. J. SMITH ***

* *CSIRO Division of Entomology, G.P.O. Box 1700, Canberra, ACT 2601, Australia;*
** *Bureau of Sugar Experiment Stations, P.O. Box 651, Bundaberg, Qld 4670, Australia, and* *** *Biotech Australia Pty Ltd, P.O. Box 20, Roseville, NSW 2069, Australia.*

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Summary — Two *Heterorhabditis* species were found causing an epizootic among four species of scarabaeid grubs feeding on sugar cane. The epizootic extended over 5 ha and it was estimated that tens of thousands of grubs were infected at the time of sampling. *Heterorhabditis* were detected in 65 % of soil samples, with 3 % of samples containing both species. Infection rates among grubs collected from different parts of the fields varied between 26 % and 100 %.

Résumé — Heterorhabditis *spp. (Heterorhabditidae : Nematoda) épizootiques envers des scarabéides (Coleoptera) de la canne à sucre* — Deux espèces d'*Heterorhabditis* ont été trouvées qui causent une épizootie envers les larves de quatre espèces de scarabéides se nourrissant sur canne à sucre. Cette épizootie s'étend sur 5 ha et il est estimé qu'au moment de l'échantillonnage des dizaines de milliers de larves étaient infestées. *Heterorhabditis* a été détecté dans 65 % des échantillons dont 3 % contenaient l'une et l'autre espèces. Le taux d'infestation des larves d'insectes varie, dans les différentes parties du champ, de 26 % à 100 %.

Key-words : Epizootic, *Heterorhabditis*, Scarabaeid, sugar cane.

Heterorhabditis spp. are pathogens of a wide range of pest insects. The infective stage of these nematodes is a soil-dwelling, third instar juvenile that may survive months without feeding. Infective juveniles are attracted to insects (Bedding & Akhurst, 1975) and invade via natural orifices (Poinar, 1979) and through interskeletal membranes (Bedding & Molyneux, 1982). They penetrate to the haemocoel where they release cells of a symbiotic bacterium, *Xenorhabdus luminescens* (Thomas & Poinar, 1979) that have been carried within their intestines. These bacteria multiply, killing the host and generating conditions suitable for feeding and reproduction by the nematodes. After one or two cycles a new generation of infective juveniles carrying their symbiotic bacteria emerges from the cadaver to infect new hosts.

Use of a baiting method for detecting entomopathogenic nematodes in soil (Bedding & Akhurst, 1975) has shown that *Heterorhabditis* spp. are widely distributed throughout the world (Beavers *et al.*, 1988; Akhurst & Brooks, 1984; Akhurst & Bedding, 1986; Mráček & Jenser, 1988). However, there are few reports of natural infection by *Heterorhabditis* spp. and there has been only one report of an epizootic produced by a *Heterorhabditis* sp. (Sexton & Williams, 1981).

Although *Heterorhabditis* spp. are well distributed in sugar cane fields in Australia (Akhurst & Bedding, 1986) and scarabaeids are the major pests of sugar cane in Australia, there have been no reports of natural infection of cane scarabaeids by these nematodes. When one of us (RMB) detected infected scarabaeid grubs in a young first ratoon cane field near Bundaberg, the survey described in this paper was conducted to determine the extent and significance of the infection.

Materials and methods

The occurrence and distribution of nematodes and infected grubs was determined by taking 500 cm^3 samples to 0.2 m depth at approximately 15 m intervals throughout the three adjoining fields (5 ha) where infected grubs had first been detected. The soil was a coarse alluvial sand grading to fine silt, in which the pesticide lindane (BHC) applied in previous years was almost all degraded. Soil samples were collected in plastic food containers with snap-seal lids and transported to the laboratory where two final instar *Galleria mellonella* larvae were placed on top of the soil. The containers were inverted to keep the soil in intimate contact with the larvae and left at room temperature for one week. After this time the larvae were removed and retained on dry filter paper at room temperature until infective juvenile nematodes emerged.

Although the concentration of grubs was high for these species (about five per metre of row), it was too low for us to obtain an indication of the level of parasitism from the general survey. Therefore more intensive sampling was conducted in four areas around sites where infected grubs had been found during the general survey. In these areas a trench was dug to a depth of 1 m along the row of cane until approximately 40 grubs were collected (approx. 3 m^3). The grubs were transported to

the laboratory in peat, dissected and examined for nematodes.

Soil samples were taken from six other cane fields in the Bundaberg district (five to fifteen samples per field) and tested for the presence of entomopathogenic nematodes as described above.

The infectivity for third instar *Lepidiota negatoria* larvae of entomopathogenic nematodes isolated during this survey was tested by the method of Bedding *et al.* (1983).

Results

Hundreds of dead grubs of four scarabaeid species were seen during the sampling of the 5 ha of canefield in which the epizootic occurred. However, the extent of the area infected and the density of grubs present indicate that tens of thousands of grubs may have been infected. Very few insects other than scarabaeids were seen during the survey; none of these others were found to be infected.

Infection rates of grubs of 26 %, 68 %, 90 % and 100 % were recorded in the four samples of *ca* 40 grubs taken by completely sampling portions of rows. Some members of each of the four grub species present were infected : third instar larvae of *Lepidiota crinita, L. negatoria* and *Lepidiota picticollis* and pre-pupae and pupae of *Antitrogus consanguineus.*

Entomopathogenic nematodes were detected in 65 % of the 236 soil samples (Fig. 1). Two species of entomopathogenic nematode were recovered, both of the genus *Heterorhabditis.* Infective juveniles of species A (36 % of samples) averaged 0.7 mm in length (0.58-0.84 mm, n = 200) and carried an isolate of *X. luminescens* that was not bioluminescent (Akhurst & Boemare, 1986). Those of species B (32 %) averaged 0.59 mm in length (0.48-0.71 mm, n = 200) and carried a luminescent isolate of *X. luminescens.* Isozyme analysis (Akhurst, 1987) subsequently showed that species B was conspecific with other *Heterorhabditis* isolates obtained from tropical regions (northern Australia, Cuba, southern China) and that species A is also an undescribed species for which no other isolates are known.

Both species of nematode were detected in soil samples from a property adjoining that on which the epizootic was discovered. Species A was not detected in samples from six other properties in the district (6-30 km distant) and species B from only one; none of the grubs on any of these properties was found to be infected.

When the original field was randomly sampled five months later, only uninfected grubs were observed. Species B was recovered from 26 % of soil samples but species A from only 5 %.

The infectivity of both *Heterorhabditis* species for third instar *L. negatoria* in laboratory tests at 28 °C was poor, with less than 50 % mortality at dosages up to 10 000 nematodes/100 ml sand.

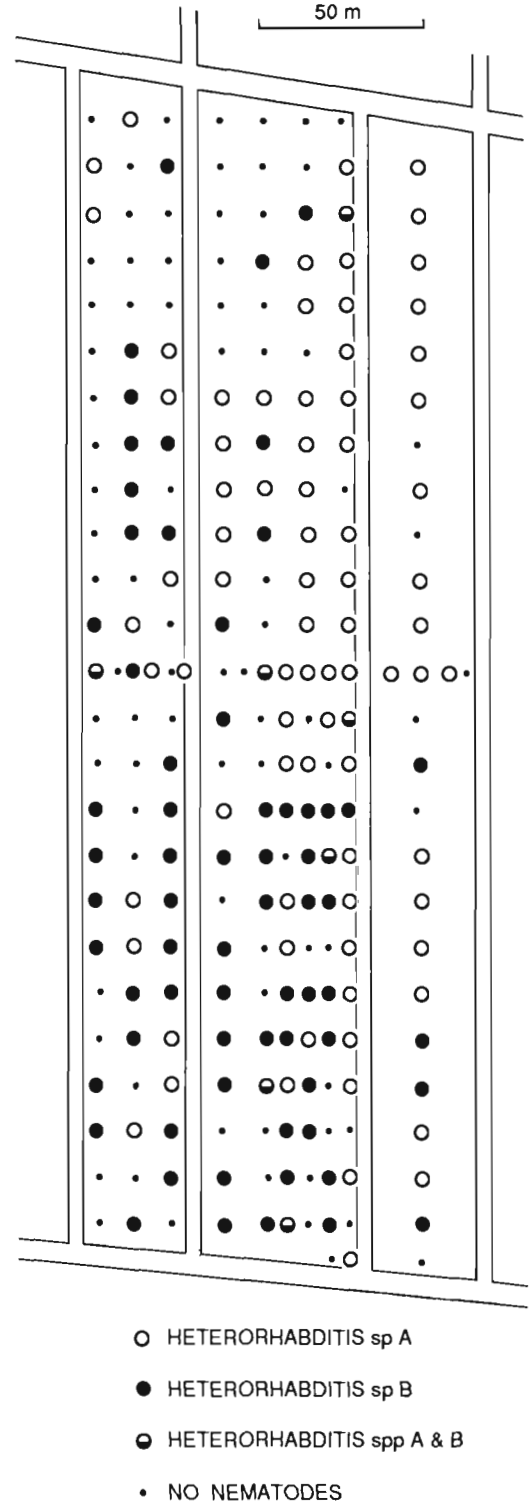

Fig. 1. Distribution of *Heterorhabditis* spp. in three sugar cane fields as determined by bioassay of soil samples.

Discussion

As in an epizootic in white-fringed weevil, *Graphognathus leucoloma* (Sexton & Williams, 1981), the epizootic reported here involved *Heterorhabditis* infecting insect species that were not very susceptible to infection by any entomopathogenic nematodes under laboratory conditions or in field trials, even with *Steinernema glaseri*, which has been found to be the most effective species (Bedding & Akhurst, unpubl.). It suggests that, under suitable conditions, *Heterorhabditis* spp. can be highly effective against insect species previously thought to be not amenable to control by nematodes. The involvement of two species of *Heterorhabditis*, neither of which was very infective in subsequent laboratory as says, suggests that conditions must have been particularly suitable in the three fields in which the epizootic occurred. Several factors may have contributed to the epizootic. The presence of four species of scarabaeid with different life cycles may have provided an abundant supply of susceptible hosts (e.g. early instars) over a longer period than normal. The soil type and moisture potential were highly suitable for the nematodes infectivity (Molyneux & Bedding, 1984) and flooding of the area some months previously may have aided their dispersal. *G. mellonella* larvae used to bait the soil samples were each infected by hundreds of nematodes, suggesting that the nematodes were present in high densities (Fan, 1989). The residual insecticide (BHC), though largely degraded, may have had a slight debilitating effect on the insects increasing their susceptibility to nematode infection. It was not possible to determine which of these factors, or combination of factors, was essential to the epizootic because there were no satisfactory control blocks. The neighbouring field was not a useful control because the sugar cane was in a different period of the rotation, grub density was lower and the field was elevated above the three in which the epizootic occurred.

The difference in ratio of recovery of the two *Heterorhabditis* spp. between the initial sampling and that of five months later may indicate the better survival of species B. Alternatively, it may reflect differences in the relative activity of the two species at the time of testing, as suggested by Fan's (1989) examination of *Steinernema feltiae (= S. bibionis)* populations.

Although the epizootic reported here demonstrates the potential of *Heterorhabditis* for controlling scarabaeid larvae, it gave no clear indications of the most effective means of using the nematodes in a control program. The conditions required to establish an epizootic of a particular pathogen can only be determined by examination of the common features of a number of such epizootics or by detailed experimental analysis of factors that might be contributory.

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