PROGRESS WITH THE USE OF NEMATODES AND BACTERIA FOR THE CONTROL OF GRASS GRUB

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SUMMARY

Artificially cultured parasitic nematodes (*Neoaplectana glaseri* and *Heterorhabditis bacteriophora*) (strain V16) and a bacterium (tentatively identified as *Hafnia alvei*) were applied to field plots infested with grass grub (*Costelytra zealandica*) larvae. After one month both nematode species were recovered from the soil and found in larval cadavers and grass grub population reductions in the nematode treated plots ranged from 0-92%. Two months after application of bacteria 18-27% of larvae in the treated plots displayed disease symptoms and a population reduction of 55% was recorded in one trial.

INTRODUCTION

There are many naturally occurring pathogens of grass grub larvae (*Costelytra zealandica*) that provide some level of biological control (e.g. Miln and Carpenter 1979). None of these have, in the past, been found to be capable of manipulation or multiplication to provide acceptable levels of control on demand.

Some of the entomophagous nematodes are, however, capable of being mass produced at low cost (Bedding 1981; Wouts 1981) and have also shown promise for biological control of grass grub (Hoy 1955, Jackson *et al* 1981). Additionally the feasibility of using artificially reared nematodes for insect control has been demonstrated (Bedding and Miller 1981 a, b).

More recently a bacterium (tentatively identified as *Hafnia alvei*) has been discovered and appears to be associated with high natural mortality of grass grubs in Canterbury (Trought *et al* 1982). The disease is widespread, easily cultured and transmissible under laboratory conditions to healthy larvae.

This paper briefly describes the progress being made towards grass grub control by inundative application of artificially cultured parasitic nematodes and bacteria to small field plots.

METHODS

The infective stages of the nematodes used in these experiments were reared from larvae of the greater wax moth (*Galleria mellonella*) in a manner similar to that described by Poinar (1979) and applied at a rate equivalent to 1000 million per hectare on all sites. The species under test were *Neoaplectana glaseri* and *Heterorhabditis bacteriophora* (strain V16) cultured from samples imported from Tasmania through Dr. R.A. Bedding (CSIRO).

The bacteria (*H. alvei*), originally isolated from diseased grub larvae from South Canterbury (Trought *et al* 1982) were applied as a peat based formulation containing approximately 10⁹ spores/gram at the equivalent of 75 kg formulation/ha.

The organisms were applied in aqueous suspension by watering-can evenly to cover plots 2 x 2 m in extent which were then irrigated to simulate 25 mm of rainfall. The trials were of randomised block design with three to five replicates. There were seven trials in which nematodes were applied; in two of these (1 and 7) bacteria were also applied to separate plots. The plots were sampled at intervals with a soil corer by taking six cores/plot, 6 cm in diameter to a depth of 10 cm. From these, grass grub larvae both live and dead were extracted, examined and counted. Dead grubs were examined for the

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presence of nematodes; soil samples were bioassayed using larvae of the greater wax moth for the presence or absence of nematodes (Bedding and Akhurst 1975). Bacterial infection was assessed visually in accordance with the symptoms described by Trought *et al* (1982).

Extensive sampling of the trial sites had shown no indication of either the presence of disease or nematodes in the grass grub populations in the trial sites prior to application of the test bio-control organisms. Irrigation of the plots continued at intervals throughout the dry season to maintain soil moisture levels of 18-25%.

RESULTS

Nematodes

The results of trials using nematodes are summarised in Table 1. From all sites but one nematodes were recovered from the soil by bioassay. During sampling some cadavers containing nematodes were found in four of the trials. Since grass grub larvae attacked by parasitic nematodes are killed and disintegrate rapidly, particularly under the high (20-30°C) soil temperatures prevalent during the experiment, it came as no surprise that only up to 5% of the grass grubs recorded were infected with nematodes. The cadaver appearance and the size of the extracted infective nematodes corresponded to those expected from the nematodes applied but no positive identification was made.

The reduction of the grass grub population in the nematode treated plots below the untreated population ranged from 0-92%. The greatest reduction occurred in Trial 1 where the grass grub population was significantly ($P < 0.01$) less than in the untreated plots. In trials 3-7 results were more variable; the reductions in population were not statistically significant due probably to the aggregated dispersal of grass grub, the small size of the plots and insufficient replication.

TABLE 1: Summary of the results of trials to evaluate the use of parasitic nematodes for the control of grass grub. Population/m² in untreated plots at time of sampling (1 month after nematode introduction) in parentheses.

Trial No.	Date of application	Nematodes recovered from soil	Cadavers found containing nematodes	% Population decline in nematode treated plots	
				N. glaseri	V16
1	18.12.81	+	+	●	92(690)
2	5.1.82	—	—	●	●
3	2.2.82	+	—	48	39 (320)
4	17.2.82	+	+	44	41 (630)
5	4.3.82	+	+	0	54 (300)
6	19.3.82	+	—	56	56 (211)
7	27.3.82	+	+	0	0 (297)

(+, positive result; —, negative result; ●, no measurement)

Bacteria

In trials 1 and 7 the incidence of larvae displaying disease symptoms was significantly ($P < 0.05$) higher in the bacterial treated plots than in the untreated plots (Table 2). A significant ($P < 0.05$) population reduction below untreated was recorded in the bacterial treatment in trial 1. At the time of sampling no population reduction was measured in trial 7 but 18% diseased larvae were recorded in the bacterial plots. Since diseased larvae can live for about 6 weeks (Trought *et al* 1982) sampling may have preceded a population decline in this case.

TABLE 2: Percentage of grass grub larvae showing disease symptoms 8 weeks after application of the bacteria. Population/m² in parentheses.

Trial	Untreated	Bacteria treated
1	3.0 (540)	27 (240)
7	3.9 (290)	18 (370)

DISCUSSION

The trials indicate that both nematodes and bacteria may, by inundative release of artificially bred cultures, infect and kill grass grub larvae under field conditions. Satisfactory levels of control of grass grubs with nematodes were achieved in some trials but the results were variable; work is continuing in order to determine whether the apparent failures were due to different levels of nematode vigour or changes in conditions at each time of application. A previous attempt to control grass grub using *N. glaseri* was unsuccessful (Hoy 1955). The work described here, however, indicates that some species or strains of nematodes can give satisfactory grass grub control. Commercial acceptability is enhanced by the low-cost techniques recently developed for their mass production (Bedding 1981; Wouts 1981).

In both tests using bacteria, disease was introduced into the population and in one test a significant population reduction was recorded. Microbial control of grass grub has been attempted previously in New Zealand by Kelsey (1966) and Fowler (1974) using "milky disease" (*Bacillus* sp) which had been used in successful programmes for the control of Japanese beetle in the USA (Hall 1963). These attempts were, however, only partially successful and development of milky disease as a microbial insecticide was further hindered by the difficulty of its artificial culture.

The availability of two biological agents, capable of easy and cheap artificial culture, and capable of killing grass grub larvae in the field indicate a new area for research into practical methods for the control of this pest.

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