Entomopathogenic nematodes as biocontrol agents in New Zealand agriculture: a case study

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Abstract

Entomopathogenic nematodes (EPNs) have been used experimentally to control insects in pastures and in this study we investigated the use of EPNs against clover root weevil. We tested the ability of two EPNs (*Steinernema carpocapsae* and *Heterorhabditis zealandica*) to control soil-dwelling stages of clover root weevil in a Waikato pasture over two spring seasons, with *Steinernema* being used at two rates in the second year. In the first year, *Steinernema* EPNs produced a significant reduction in weevil numbers, compared to untreated, 3 weeks after application in October but not 5 weeks after application in November. In the second year, the reduction was not significant after 3 weeks but, a further 2 weeks later, weevil numbers were slightly reduced in EPN-treated soil. No measurable effects were noted from application of *Heterorhabditis*. Lower soil temperatures likely limited the success of *Steinernema* in the second spring. Timing of EPN application appears important for controlling clover root weevil. The trial site used here had resident populations of both *Steinernema* and *Heterorhabditis.*

Keywords: *Sitona lepidus*, *Heterorhabditis bacteriophora*, larvae, pupae, *Galleria*, wax moth

Introduction

Insect-killing or entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* invade (usually larval) insects through their cuticle, whereupon they release symbiotic bacteria from their gut into the insect haemocoel which then kills the insect within 1–2 days (Adams & Nguyen 2002). The nematodes feed on the bacteria, multiply and eventually release large numbers of infective juveniles to attack more insect hosts. There are around 25 *Steinernema* and nine *Heterorhabditis* species currently described worldwide (Adams & Nguyen 2002). EPNs have been described from every continent except Antarctica (Hominick 2002). Most EPNs are sensitive to exposure to UV and desiccation as they are adapted to attack only soil-dwelling stages of insects.

The symbiotic relationship between EPNs and the bacteria they carry relies on the nematodes to vector the bacteria to host insects and the bacteria to kill the host and produce food for nematode development. Without the bacteria the nematodes cannot reproduce in the host as they have no food source, and without the nematodes the bacteria can infect insects to only a limited degree. The advantage for the bacteria is free host location and delivery, which can involve nematodes being attracted towards exudates of damaged plant roots, and therefore probable sites of insect activity (Rasmann *et al.* 2005).

The use of entomopathogenic nematodes as potential biocontrol agents for insects has been demonstrated many times in New Zealand (eg Jackson *et al.* 1985; Jackson & Wouts 1987; Kain *et al.* 1982), and the number of studies worldwide is very large with 125 publications on EPNs appearing annually from 1990 to 1998 alone (Hominick 2002).

Clover root weevil (*Sitona lepidus*) is a serious pest of white clover in the North Island of New Zealand (Gerard *et al.* 2007) which has the potential to spread throughout the rest of the country (Hardwick *et al.* 2004). Previous lab work with clover root weevil has shown that EPNs have the potential to be effective biological control agents of this pest (Bell *et al.* 2000). The current study moved this work to the field stage where spring was chosen as the best time to apply these entomopathogenic nematodes. There were two reasons for this: 1) the clover root weevil population at this time of year is large and consists almost exclusively of soil dwelling stages which are the targets of these nematodes; and 2) prevailing climatic conditions suit nematode survival and activity *viz.* high soil moisture and rising soil temperatures.

Methods

The EPNs used in these experiments were *Steinernema carpocapsae* isolated from soil beneath pasture in the Waikato and *Heterorhabditis zealandica* isolated from soil beneath kahikatea trees, also in the Waikato. Nematodes were reared in larvae of wax moth (*Galleria mellonella*) on moist filter paper in Parafilm-sealed petri dishes at 15°C. After *ca.* 2 weeks infected wax moth larvae were transferred to White traps (White 1927) at 20°C, in order to stimulate production of infective stage nematodes. Nematodes were collected from White traps and stored in aqueous suspension at 4ºC until required (*ca.* 1 week).

Initial numbers of clover root weevil soil stages were counted from twenty 10 cm diameter soil cores at 36, 0 and 0 days before Trials 1, 2 and 3 which were set up on 3 October 2001, 12 November 2001 and 1 October 2002 respectively on a farm at Springdale, Waikato (NZMS260

Table 1 Nematode treatments and rates applied in Trials 1–3.

Trial	Nematode treatment	Nematode rate/ ha
1	Control	0
(Oct 2001)	*Steinernema carpocapsae*	0.69×10^9
	Heterorhabditis zealandica	0.97×10^9
2	Control	0
(Nov 2001)	*Steinernema carpocapsae*	0.95×10^9
	Heterorhabditis zealandica	0.89×10^9
3	Control	0
(Oct 2002)	*Steinernema carpocapsae* – Low rate	0.49×10^9
	Steinernema carpocapsae – High rate	2.08×10^9

T13 368063). Forty randomised blocks, each containing one replicate of each treatment (see Table 1) were set up in soil cores. For Trial 1, 10 cm diameter cores were taken to *ca.* 10 cm depth without regard to the vegetation whereas in Trials 2 and 3 cores were taken over white clover using a soil corer. Cores were removed from the soil and the resulting hole lined with a PVC sleeve, the cores were then replaced in the sleeve. A visual estimate of the percentage of the surface of each core covered by white clover and by the total vegetation was recorded for both Trials 2 and 3. Trial 2 was set up on the opposite side of the same paddock as Trial 1, some 10 m separate, with Trial 3 being located in an adjacent paddock *ca*. 20 m separate.

For all trials the nematodes were applied in 1 ml of solution to a slot which was made across the centre of each core to *ca.* 3 cm depth. Solutions of nematodes were produced in tap water with the untreated control receiving 1 ml of tap water.

The low initial soil moisture level of Trial 1 meant that the cores were watered prior to nematode inoculation to assist in nematode establishment. Watering was not carried out in Trial 2 and soil moistures in Trial 3 were such that watering was unnecessary.

For Trial 1, the cores and their surrounding PVC sleeves were removed from the soil after 21 days (Week 3), for Trial 2 they were removed after 35 days (Week 5) and for Trial 3 they were removed 21 (Week 3, 20 cores) and 38 days (Week 5, 20 cores) after application. Sleeves and cores were placed individually in plastic bags and transported back to Ruakura where they were immediately placed in cool storage (*ca.* 4°C).

In Trial 1, clover root weevil larvae, pupae and adults were recovered from 35 soil cores per treatment by hand-crumbling cores and subsequent flotation in magnesium sulphate. In Trial 2, 10 replicates per treatment were assessed as for Trial 1 except that weevil eggs were also extracted from five of those replicates by wet sieving. In Trial 3, weevil life stages were assessed by hand-crumbling 20 cores per treatment for both the 3 and 5 week post-treatment samplings. In all trials any weevils recovered from soil which appeared to be EPN-infected were collected and subsequently dissected to confirm EPN infection. The genus of EPN infecting the weevils was determined by the colour of the insect: creamy white for *Steinernema* and red for *Heterorhabditis*.

To check for the persistence of applied EPNs at the trial site, 100 g of soil from each of five replicates of each treatment was collected at the end of Trial 1 and baited with five larvae of the greater wax moth (*Galleria mellonella*) in glass jars with a sealed lid at 20°C. After 1 week *Galleria* were removed from the soil and scored as alive, dead or killed by nematodes.

To compare the susceptibility of *Galleria* to EPNs with that of clover root weevil both insects were added to eight untreated soil samples collected from the trial site at the beginning of Trial 3. From each sample, a 100 g subsample was placed into a jar, insects added (five *Galleria* and 15 mixed stage weevil larvae) and kept in a 15°C controlled environment room for 11 days after which insects were recovered and assessed for nematode infection.

Data were analysed by ANOVA after $\log_{10}(n+1)$ transformation. The percentage of white clover and of vegetative cover in each core was used as co-variates in all analyses.

Results and Discussion

Although the temperature recordings in Table 2 are from Ruakura and not the trial site, they are indicative of the temperature differences between years. Visual assessment of clover cover in the cores appear to be higher in 2002 than 2001 but it should be remembered that in Trial 1 cores were taken randomly whereas in Trials 2 and 3 they were taken over clover so are biased towards greater clover levels.

There was a significant reduction in the weevil population in the *S. carpocapsae* treatment in Trial 1 but not in Trials 2 or 3 (Table 3). *H. zealandica* did not significantly reduce weevil numbers. The percent clover and vegetative cover co-variates both had a significant ($P<0.05$) effect on the difference between treatments in Trial 1 but not in Trials 2 or 3 which reflected the difference in selecting core positions randomly (Trial 1)

Table 2 Site characteristics for Trials 1 (3/10/01 to 24/10/01), 2 (12/11/01 to 17/12/01) and 3 (1/10/02 to 8/11/02). Figures in parentheses are the range.

Trial	Weeks post treatment	Soil moisture (0–5 cm depth)	Mean grass min.[1](°C)	Mean 10 cm soil[1](°C)	% clover
1	0	58.8	—	—	—
(2001)	3	74.4	7.3 (0.1–14.9)	15.2 (13.8–17.3)	9.9
2	0	59.1	—	—	—
(2001)	5	80.7	11.3 (2.7–18.1)	18.2 (16.2–20.0)	12.6
3	0	79.9	—	—	29.7
(2002)	3	65.9	1.7 (-5.4–9.7)	12.7 (10.8–14.4)	40.8
	5	77.7	5.1 (-2.0–9.5)	13.9 (12.4–15.4)	24.2

[1] Temperatures as recorded at Ruakura Research Centre, Hamilton.

Table 3 Mean number of live clover root weevil (CRW) post-egg individuals/m^2 and eggs/m^2 (figures in parentheses are log_{10} transformed data per core adjusted for percent clover and vegetative cover in cores). Note: Week 0 in Trial 1 is 6 weeks prior to trial initiation, all others are at trial initiation.

Trial	Week	Nematode treatment	Total CRW	SED[1]	CRW eggs	SED
1	0	Control	980 (0.85)	(0.072)	—	
	3	Control	593 (0.63)	(0.060)	—	
		H. zealandica	451 (0.54)		—	
		S. carpocapsae	342 (0.46)		—	
2	0	Control	248 (0.35)	(0.114)	—	
	5	Control	57 (0.15)	(0.108)	662 (0.79)	(0.128)
		H. zealandica	85 (0.10)		560 (0.67)	
		S. carpocapsae	57 (0.11)		433 (0.57)	
3	0	Control	828 (0.81)	(0.063)	—	
	3	Control	732 (0.77)	(0.081)	—	
		S. carpocapsae – Low	637 (0.68)		—	
		S. carpocapsae – High	598 (0.69)		—	
	5	Control	433 (0.55)	(0.081)	—	
		S. carpocapsae – Low	318 (0.47)		—	
		S. carpocapsae – High	293 (0.47)		—	

[1] Standard error of difference except for week 0 data which is standard error of mean.

or deliberately over clover (Trials 2 and 3). The considerably cooler temperatures experienced in 2002 compared to 2001 (Table 2), and the consequent lower nematode activity, may help explain the reduced control exerted on clover root weevil by the applied nematodes in that year (Table 3). There appeared to be too few weevils in the soil in Trial 2 to be able to detect any effect from applying EPNs (Table 3) with the reduction in clover root weevil numbers in the soil likely due to their development through to adults (which emerge from soil and feed above-ground).

The total weevil numbers significantly ($P<0.001$) declined across all treatments from weeks 3 (637/ m^2) to 5 (376/ m^2) in the Trial 3 and this was probably due to several factors: *viz.* adult weevil emergence (Hardwick *et al.* 2004), mortality from resident nematode infection, *Beauveria* fungal infection (Brownbridge *et al.* 2006) and other diseases. There was no significant effect of application rate of *S. carpocapsae* in Trial 3.

At Week 3 in Trial 1, EPN-infected clover root weevil cadavers were recovered only from nematode treated cores (15% of cores, maximum of one infected weevil per core). However, of the five replicates of cores baited with wax moth for each treatment at Week 3 in Trial 1, three replicates yielded nematode-killed wax moth larvae for the control treatment, one for the *H. zealandica* treatment and two for the *S. carpocapsae* treatment. The EPNs found to be killing wax moth in the control cores were later identified as *H. bacteriophora* (by male and female morphology), a different species to that applied. So it appeared there was a resident population of *Heterorhabditis* nematodes present at the site.

In Trial 3, EPN-killed weevils were recovered from Week 0 samples (10% of cores) and from untreated (5 and 25% for weeks 3 and 5 respectively), low rate (30 and 35%) and high rate treated cores (20 and 35%). Of the nematode-infected clover root weevil found in cores from the Week 5 sampling of Trial 3, some from the *Steinernema*-inoculated cores were found to be infected with either *Steinernema* or *Heterorhabitis* while some

Table 4 Percent reduction in clover root weevil (CRW) life stage numbers/m^2 and the total of all post-egg life stages, compared to untreated control. Note: positive values indicate an increase in number/m^2.

Trial	Week	Nematode treatment	Eggs	Larvae	Pupae	Adults	Total CRW
1	3	*H. zealandica*	—[1]	6.3	–54.8	–36.8	–15.4
		S. carpocapsae	—	–30.5	–61.9	–21.1	–37.8
2	5	*H. zealandica*	–15.4	50.0	nd[2]	—	50.0
		S. carpocapsae	–34.6	–25.0	nd	—	0.0
3	3	*S. carpocapsae* – Low	—	–16.7	25.0	0.0	–7.8
		S. carpocapsae – High	—	–16.7	16.7	–100.0	–11.2
	5	*S. carpocapsae* – Low	—	–22.7	–14.3	–20.0	–20.0
		S. carpocapsae – High	—	–11.4	–33.3	–80.0	–26.7

[1] = none in any cores or not assessed, [2] = not determined due to none in control cores

Table 5 Percent composition of the clover root weevil population in control cores.

Trial	Week (Date)	Eggs	Larvae	Pupae	Adults
1	3 (23 Oct 2001)	—	61.3	25.8	12.9
2	5 (12 Dec 2001)	89.7	10.3	0.0	0.0
3	3 (22 Oct 2002)	—	77.6	20.7	1.7
	5 (8 Nov 2002)	—	58.7	28.0	13.3

from untreated cores were found to be infected with *Steinernema*. The nematodes from untreated cores were subsequently identified as *S. carpocapsae* (D. Sturhan pers comm.), but it is considered unlikely that these were present as a result of cross-contamination from treated cores so are most likely resident nematodes.

In addition to clover root weevil larvae being infected by EPNs, larvae were also observed infected with fungi, including what appeared to be *Beauveria* fungi (25% of cores Week 0, Trial 3)

It appears that *Galleria* may be a less susceptible host than clover root weevil to the EPNs which infect the weevils because at the end of 11 days at 15°C all eight soil samples tested from the trial site (Week 0, Trial 3) produced infected weevil larvae but only three produced infected *Galleria*. This may be because clover root weevils stay in contact with soil for longer periods than do the mobile *Galleria* larvae so perhaps appear more susceptible as a result. Additionally, this could be a temperature effect with nematodes not being active enough to infect the larger, more mobile *Galleria* compared to the smaller, often stationary clover root weevil larvae.

There was a trend for *Steinernema* EPNs to reduce numbers of all life stages of clover root weevil, including teneral adults (Table 4). Infected cadavers of all stages were found from soil cores. The reduced egg numbers in Trial 2 was somewhat surprising as they are not likely to be attacked directly by EPNs so it is unclear why their number should be reduced.

The composition of the clover root weevil population reflected the effect of lower soil temperatures between years with pupal moulting to adults delayed *ca.* 2 weeks in 2002 (Trial 3) compared to 2001 (Trial 1) (Table 5). It may be that some aspect of clover root weevil population composition, such as percentage of the population that are pupae, could be used as a determinant of the ideal timing to apply EPNs to ensure good weevil control.

Conclusions

It appears that the trial site used in this study had resident populations of both *Steinernema* and *Heterorhabditis* nematodes. Despite this, the application of supplementary EPNs can give added control to clover root weevil, provided they are applied when both temperatures and weevil numbers are high enough to support population growth. Both the presence of resident EPNs and the influence of temperature on EPN effectiveness are issues that would need to be addressed before considering using EPNs in agricultural situations.

What effect the level of nematode infection seen in this study has on clover root weevil is of interest not only for late spring and summer populations but also over the longer term as the ability of nematodes to cycle through the weevils may improve the level of control over time. A study by Jackson & Wouts (1987) found an effect of applying EPNs was evident a year after application demonstrating that applied EPNs can persist in the pasture environment.

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