Antifeedant effects of diethyl toluamide and neem oil on native cave weta, cockroaches, and amphipods

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P.G. McGregor[1], P.G. Peterson[1], P.H. Berben[1], G.C. Arnold[1] and E.B. Spurr[2]

[1] Landcare Research, Private Bag 11052, Palmerston North 5321, New Zealand.
[2] Landcare Research, PO Box 69, Lincoln 8152 New Zealand.
Email: mcgregorp@LandcareResearch.co.nz

ABSTRACT

The antifeedant effects of both diethyl toluamide (DEET) and neem oil added at concentrations of 0.2% to cereal-based baits used for vertebrate pest control in New Zealand were investigated with three taxa of native invertebrates known to eat these baits. DEET reduced the numbers of captive cave weta and cockroaches feeding on treated non-toxic baits, but neem oil did not. Cinnamon oil, routinely added to bait used for possum control, also failed to reduce the numbers of captive cave weta and cockroaches feeding on treated non-toxic baits. Captive amphipods were not observed feeding on treated or untreated baits. Further tests are needed to confirm both the palatability and efficacy of toxic baits containing DEET to possums, rats, and mice, and the antifeedant effects of toxic baits containing DEET on invertebrates in the field. The environmental fate of DEET will also need to be investigated before it can be added routinely to baits used for control of possums and rodents in New Zealand.

Keywords: Invertebrates, insect repellents, antifeedants, baits, vertebrate pest control, New Zealand.

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1. Introduction

A range of invertebrate species have been observed eating baits containing toxicants, such as sodium monofluoroacetate (1080) and brodifacoum. These baits are used in New Zealand for the control of introduced vertebrate pests such as the brushtail possum (*Trichosurus vulpecula*), ship rat (*Rattus rattus*), Norway rat (*R. norvegicus*), kiore (*R. exulans*), and house mouse (*Mus musculus*). Invertebrates that eat baits include isopods (Isopoda), amphipods (Amphipoda), harvestmen (Opiliones), mites (Acarina), millipedes (Diplopoda), springtails (Collembola), cockroaches (Blattodea), earwigs (Dermaptera), weta (Orthoptera), beetles (Coleoptera), and ants (Hymenoptera) (Sherley et al. 1999; Spurr & Drew 1999; Lloyd & McQueen 2000). No deleterious impacts have been detected on invertebrate populations (Spurr 1994, 1996, 2000; Sherley et al. 1999; Spurr & Drew 1999; Lloyd & McQueen 2000), but concern has been expressed that invertebrates which eat baits may be transporting toxicants into the environment and, through the food chain, into insect-feeding birds such as the brown kiwi (*Apteryx australis*) and morepork (*Ninox novaeseelandiae*), and mammals such as the short-tailed bat (*Mystacina tuberculata*) (Innes & Barker 1999; Lloyd & McQueen 2000; Eason et al. 2002). If invertebrates could be prevented from eating baits then they would not be able to transport toxicants into the environment. An antifeedant that deters invertebrates but is palatable to possums and rodents could achieve this.

A review of the international literature (Spurr & McGregor 2002) identified six potential invertebrate antifeedants that could be added to baits used for vertebrate pest control: diethyl toluamide (DEET), dimethyl phthalate (DMP), citronella oil, eucalyptus oil, neem oil, and alpha-cypermethrin. Subsequent trials showed that 0.2% DEET and 0.2% neem oil were the most palatable of these compounds to possums, ship rats, and Norway rats (Spurr et al. 2002). The antifeedant effects of these two compounds on three taxa of native invertebrates known to eat baits were tested by Landcare Research, Lincoln and Palmerston North, for the Department of Conservation (DOC), between July 2001 and September 2002.

2. Objective

The objective of this study was to test the antifeedant effects of 0.2% DEET and 0.2% neem oil in cereal baits on cave weta, cockroaches, and amphipods.

3. Methods

DEET (99% pure) was obtained from Global Science & Technology, Christchurch, and neem oil (Neem 900 EC) from Suntec (NZ), Tokomaru. In April 2001, Animal Control Products (Wanganui) incorporated these putative invertebrate antifeedants into non-toxic, green-dyed, 6 g Wanganui No.7 cereal-based baits, at a concentration of 0.2%. Half the baits also contained 0.15% cinnamon oil. Cinnamon oil is routinely added to baits used in possum control operations, but not in rat control operations. Thus, there were six bait treatments:

S = untreated (i.e. no putative invertebrate antifeedants), as used for rat control; SC = 0.15% cinnamon oil, as used for possum control; D = 0.2% DEET; DC = 0.2% DEET plus 0.15% cinnamon oil; N = 0.2% neem oil; NC = 0.2% neem oil plus 0.15% cinnamon oil

Cave weta (Orthoptera: Rhaphidophoridae), native cockroaches (Blattodea: Blattidae), and amphipods (Amphipoda: Talitridae) were collected from the Palmerston North City Council's Turitea Water Works Reserve, Tararua Ranges, and placed in cages in the laboratory. The cages were constructed from plastic containers 20 cm wide × 30 cm long × 10 cm deep. Holes (6 cm in diameter) were drilled in the centre of the side walls of each container and at both ends of each lid, and 1-mm stainless steel mesh was glued over these holes. Air was expected to flow in through the side vents and up and out through the lid vents because it was being drawn from above. The airflow was strong enough to easily pin an A4 piece of paper to the vents in the roof of the laboratory. It was expected that this airflow would restrict general movement of the putative invertebrate antifeedant fumes throughout the cages.

Fresh leaf litter was added to each cage and any unwanted invertebrates were removed. The litter was misted with distilled water, and a small dish of water and cotton wool added to the centre of cave weta and cockroach cages. Additional food—dog biscuits, apple, and broadleaf (*Griselinia littoralis*) foliage—was added to cave weta cages, and dog biscuits to cockroach cages. Invertebrates were then added to the cages as follows: 5 cave weta × 10 cages, 5 cockroaches × 10 cages, and 10 amphipods × 10 cages. However, due to a shortage of cockroaches there were only 4 instead of 5 individuals in some cages. The difficulty of obtaining enough invertebrates caused the trial to be delayed until July 2001, about 10 weeks after bait manufacture.

Food was removed from the cages approximately 48 h before the first sampling occasion. The bait treatments were then applied in pairs (one treated bait and one untreated bait placed at opposite ends of each cage, on top of a thin layer of leaf litter) as follows:

D v. S; N v. S; DC v. SC; NC v. SC; S v. SC.

TABLE 1. EXPERIMENTAL DESIGN USED TO TEST ANTIFEEDANT EFFECTS (N = 30 CAGES PER SAMPLE).

SAMPLE NO. AND DATE†	1 5–6 JUL	2 8–9 JUL	3 11–12 JUL	4 14–15 JUL	5 17–18 JUL
Cage No.					
1	S v. SC	DC v. SC	N v. S	NC v. SC	D v. S
2	D v. S	N v. S	S v. SC	DC v. SC	NC v. SC
3	NC v. SC	S v. SC	D v. S	N v. S	DC v. SC
4	DC v. SC	D v. S	NC v. SC	S v. SC	N v. S
5	N v. S	NC v. SC	DC v. SC	D v. S	S v. SC
6	D v. S	NC v. SC	N v. S	DC v. SC	S v. SC
7	DC v. SC	N v. S	D v. S	S v. SC	NC v. SC
8	NC v. SC	DC v. SC	S v. C	N v. S	D v. S
9	S v. SC	D v. S	DC v. SC	NC v. SC	N v. S
10	N v. S	S v. SC	NC v. SC	D v. S	DC v. SC

† The first date is the set-up date and the second date is the measurement date for the 10 cages of each of the 3 invertebrate taxa. All dates refer to 2002.

Table 1 shows how these five treatment pairs were allocated to the ten cages of each invertebrate taxon in five time periods (samples) so that:

- Every treatment was used in every cage
- Every treatment was used twice in every time period
- Every treatment followed every other treatment twice

Due to an unfortunate miscommunication, the treatment pairs were not allocated randomly to the left or right sides of the cages. Instead, all treated baits were placed on the left, and all untreated baits on the right side of the cages. There is no evidence that this affected the results.

After the bait treatments were added to the cages, the invertebrates were left for at least 24 h (and not more than 30 h) before the following measurements were made (e.g. set-up at 1500 h one day meant start of measurements at 2000 h the next day).

3.1 NUMBER OF INVERTEBRATES ON BAITS

The number of invertebrates observed on baits (most, if not all, of the invertebrates observed were actively feeding) was counted, with the aid of a red light, three times at 1–2-h intervals on each sampling occasion, starting between 2000 h and 2230 h. Statistical analysis of the data was by Poisson regression. Comparisons between pairs of baits in the same cage should have had a different variance from comparisons between bait treatments, but as both sources of variability were under-dispersed (i.e. less variable than a Poisson distribution would predict, with a dispersion less than 1.0), a conservative approach to the dispersion for both was taken as 1.0. This was equivalent to using a chi-squared test on the deviances (rather than the *F*-test used below). The effects were so clear that this choice had no effect on the significance of the results.

The method used was to compare models of increasing complexity, noting the improvement in fit as each component of the treatment effect was added. Model 1 fitted the experimental structure, cage and sampling occasion (cage*occasion = cage + occasion + cage:occasion), which necessarily includes the average effect of the treated and untreated baits allocated to the cages. Model 2 added the difference between treated and untreated bait in each combination. Model 3 added DEET as a treatment effect. Model 4 added the remaining treatment effects (neem oil and/or cinnamon oil).

3.2 NUMBER OF INVERTEBRATES IN THE TREATED HALVES OF THE CAGES

At the end of each sample, the number of invertebrates located in each half of the cage was determined (left half = treated bait, right half = untreated bait). Cave weta were located and counted without removing the litter. Cockroaches and amphipods were located and counted by scooping the contents of one half of the cage into a large tub and sorting through this material until all active invertebrates were found. The contents of the other half of the cage were then checked in the same way. Statistical analysis was by logistic regression, with the number of invertebrates on the treated side of the cage taken as a proportion of the total number in the cage. To make the assumption 'invertebrates moved independently' unnecessary, the dispersion parameter was estimated, not assumed to be 1.0, and an *F*-test was used for the test of overall treatment effect. In the event, only amphipods showed evidence of over-dispersion (χ^2_{28} = 47.3, P = 0.013). The design enabled adjustment for any effects of cage, sampling occasion, and previous treatment, although their effects were small.

4. Results

4.1 NUMBER OF INVERTEBRATES ON BAITS

The mean number of cave weta feeding on baits was reduced by the addition of DEET (Tables 2 and 3). In Table 3, Model 2 shows that there was a significant difference between the numbers of cave weta feeding on treated and untreated baits. Model 3 shows that the difference between numbers of cave weta feeding on treated and untreated baits was the result of adding DEET to the baits. Model 4 shows that replacing DEET with neem oil, or adding cinnamon oil, did not have any effect on the numbers of cave weta feeding on treated baits compared with untreated baits.

The number of cockroaches feeding on baits was also reduced by the addition of DEET (Tables 4 and 5). The models in Table 5 show the same pattern of results as those for cave weta in Table 3.

Amphipods were not observed on baits (but may have been underneath some).

TABLE 2. MEAN (+ SED) NUMBER OF CAVE WETA OBSERVED ON TREATED AND UNTREATED BAITS (SED = STANDARD ERROR OF DIFFERENCE BETWEEN TREATMENT MEANS).

BAIT TREATMENT	DEET	DEET PLUS CINNAMON OIL	NEEM OIL	NEEM OIL PLUS CINNAMON OIL	CINNAMON OIL
Treated bait	0.2	0.0	0.4	0.6	0.8
Untreated bait	1.2	1.3	0.6	0.3	0.4
SED	0.3	0.3	0.3	0.3	0.3

TABLE 3. ANALYSIS OF DEVIANCE, FOR CAVE WETA, SHOWING RESIDUAL (RES.) AND TEST DEGREES OF FREEDOM (D.F) AND DEVIANCE (DEV.).

	MODEL AND TERM	RES. D.F	RES. DEV.	EFFECT TESTED	TEST D.F.	TEST DEV. (χ^2)	*P*
1	Cage*Occasion	50	64.45				
2	Cage*Occasion+Bait (treated v. untreated)	49	58.77	+Bait	1	5.68	0.017
3	Cage*Occasion+Bait*DEET	48	40.47	+Bait:DEET	1	18.30	< 0.001
4	Cage*Occasion+TreatmentEffects*Bait	45	35.72	Model 3 v. 4	3	4.75	0.191

TABLE 4. MEAN (+ SED) NUMBER OF COCKROACHES OBSERVED ON TREATED AND UNTREATED BAITS (SED = STANDARD ERROR OF DIFFERENCE BETWEEN TREATMENT MEANS).

BAIT TREATMENT	DEET	DEET PLUS CINNAMON OIL	NEEM OIL	NEEM OIL PLUS CINNAMON OIL	CINNAMON OIL
Treated bait	0.1	0.0	0.4	0.4	0.8
Untreated bait	1.2	1.7	0.6	0.4	0.4
SED	0.3	0.3	0.2	0.2	0.3

TABLE 5. ANALYSIS OF DEVIANCE, FOR COCKROACHES, SHOWING RESIDUAL (RES.) AND TEST DEGREES OF FREEDOM (D.F.) AND DEVIANCE (DEV.).

	MODEL AND TERM	RES. D.F.	RES. DEV.	EFFECT TESTED	TEST D.F.	TEST DEV. (χ^2)	*P*
1	Cage*Occasion	50	58.91				
2	Cage*Occasion+Bait (treated v. untreated)	49	47.26	+Bait	1	11.65	< 0.001
3	Cage*Occasion+Bait*DEET	48	25.95	+Bait:DEET	1	21.31	< 0.001
4	Cage*Occasion+TreatmentEffects*Bait	45	22.61	Model 3 v. 4	3	3.35	0.341

TABLE 6. PROPORTION OF INVERTEBRATES OBSERVED IN THE TREATED (LEFT) HALF OF THE CAGES.

BAIT TREATMENT	CAVE WETA		COCKROACHES		AMPHIPODS	
	PROPORTION†	LOG_E‡	PROPORTION†	LOG_E‡	PROPORTION†	LOG_E‡
DEET	0.54	+0.094	0.58	+0.323	0.45	–0.201
DEET + cinnamon	0.44	–0.389	0.51	+0.022	0.57	+0.282
Neem	0.51	+0.037	0.57	+0.278	0.44	–0.240
Neem + cinnamon	0.51	–0.088	0.49	+0.028	0.44	–0.251
Cinnamon	0.58	+0.377	0.65	+0.628	0.54	+0.198
SE range (28 d.f.)		0.36–0.39		0.32–0.34		0.24–0.26
$F_{(4,28)}$		0.61		0.60		1.12
P		0.66		0.67		0.37

† Proportion in treated half.

‡ Odds ratio.

4.2 NUMBER OF INVERTEBRATES IN THE TREATED HALVES OF THE CAGES

There were no significant differences in the numbers of cave weta, cockroaches, or amphipods found in the treated or untreated half of the cages for any of the bait treatments (Table 6).

5. Discussion

In our study, the addition of 0.2% DEET to Wanganui No.7 cereal-based baits had a clear antifeedant effect on captive cave weta and cockroaches. No data were obtained for amphipods because they were not observed feeding on the baits. In contrast, the addition of 0.2% neem oil did not show an antifeedant effect on captive cave weta and cockroaches. Higher concentrations of neem oil might reduce feeding on baits by invertebrates, but might also be unpalatable to possums and rodents. Baits containing 2% neem oil repelled captive ship rats and Norway rats in another study (Spurr et al. 2002). The addition of 0.15% cinnamon oil to baits also did not reduce the number of captive cave weta or cockroaches feeding on baits in our study. This is contrary to the results of an earlier field trial which found that the addition of 0.1% cinnamon oil to baits reduced the incidence of invertebrates feeding on the baits by more than 50% (Spurr & Drew 1999).

The results of our trial may have been influenced by the age of the baits. The trial was delayed for 10 weeks after bait manufacture because of the difficulty of collecting sufficient invertebrates. The concentration of cinnamon oil in baits is known to decline over time, to 40% of its original concentration after 8 weeks (Henderson & Frampton 1999). We do not know if the concentration of neem oil in baits also declines over time. If it does, this could explain why the baits containing neem oil showed no effect on our captive invertebrates. However,

our trial is still relevant because baits may be up to 12 weeks old when used in management operations. Twelve weeks is about the maximum storage life of baits (Henderson & Frampton 1999). The effect of bait age on antifeedant concentration and palatability to invertebrates needs further investigation.

The antifeedant effect of DEET was determined only 1 day after baits were put in the cages. We do not know how long this effect might persist. The effect was evident in baits that had been stored in bags for 10 weeks after manufacture. However, it is possible that the antifeedant effect of DEET may decline over time after baits are taken out of the bags. This needs to be determined, although even 1 day's antifeedant effect on invertebrates could be considered worthwhile.

In our study, DEET does not appear to have had a repellent effect (as distinct from an antifeedant effect) on the captive cave weta, cockroaches, or amphipods because its effect did not extend away from the bait. There was no significant difference in the numbers of these species found in the treated and untreated halves of the cages.

Further research is needed before we can recommend the routine addition of 0.2% DEET to baits used for possum and rodent control. First, the conflicting results of the two possum palatability trials need to be resolved (Spurr et al. 2002). Second, the palatability of DEET to kiore and house mice should be determined in case there are species-specific differences in palatability to rodents. Third, the palatability and efficacy of toxic baits containing DEET to both possums and rodents should be determined in case there is an interaction between the antifeedant and the toxicant. Fourth, the antifeedant effects of DEET on cave weta, cockroaches, amphipods, and other invertebrates should be confirmed in field trials. These trials should also investigate how long the antifeedant effect persists. Finally, the environmental fate of DEET will need to be investigated to ensure that there would be no adverse environmental impacts from its use in baits. If further research confirms the suitability of DEET as an invertebrate antifeedant, then the risk of dissemination of vertebrate toxicants into the environment through invertebrate feeding could be greatly reduced by routinely adding DEET to baits used for the control of possums and rodents in New Zealand.

6. Recommendations

- The palatability of 0.2% DEET to possums should be confirmed by further cage trials. It should also be determined for kiore and house mice in case there are species-specific differences in palatability to rodents.
- The efficacy of toxic baits containing 0.2% DEET on possums and rodents should be determined in cage trials.

- The antifeedant effects of 0.2% DEET on invertebrates should be confirmed in field trials. These trials should also investigate how long the antifeedant effect persists.
- The environmental fate of 0.2% DEET should be investigated to ensure that there would be no adverse environmental impacts from its addition to baits used for control of possums and rodents in New Zealand.

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