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Effects of cattle treatment with a cypermethrin/cymiazol spray on survival and reproduction of the dung beetle species *Euoniticellus intermedius* (Coleoptera: Scarabaeidae)

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Abstract

In a bioassay to determine non-target ecotoxicological effects of a pyrethroid spray (Ektoban®) on dung beetles, dung from both cypermethrin/cymiazol-treated and control cattle was collected one, two, three, five, seven, 14, 21 and 28 days after treatment and fed to a treatment and control group (respectively) of beetles of the species *Euoniticellus intermedius* (Reiche). This was done to assess whether a spray formulation of cypermethrin may affect dung beetles differently than previously tested pour-on formulations. Following three beetle generations for two weeks each, the experiment retrieved no significant differences in adult or larval survival, egg production, fecundity and fertility between the control and treatment group. These results demonstrated that the used spray formulation of cypermethrin is likely to be far less detrimental to dung beetles than previously tested pour-ons.

Keywords: antiparasiticide, cypermethrin, cymiazol, dung beetles, ecotoxicological effect, *Euoniticellus intermedius*, pyrethroid

Introduction

It is common practice in commercial livestock farming to control internal and external parasites using chemical compounds with insecticidal, acaricidal or anthelmintic properties, so-called endectocides. Most of these agents and/or their metabolites are to some extend voided in the faeces of the treated animals (Strong & Wall, 1990). Thus the residues may have deleterious non-target effects on beneficial organisms utilizing the dung, such as dung beetles (McKellar, 1997; see also review of Floate *et al.*, 2005). In many tropical and sub-tropical ecosystems, the dung-burying activity of dung beetles is a major force in breaking down dung and recycling the nutrients and organic matter into the soils. Therefore dung beetles are vital to the health of tropical and subtropical ecosystems (also agroecosystems; Stokstad, 2004) and disruptions in their populations may very likely lead to a loss of soil fertility and sustainability of pastoral ecosystems (Wardhaugh & Beckmann, 1996/97). Consequently, it is of high importance to determine unintended ecotoxicological effects of antiparasitic drugs in use. Many tests have been conducted on the toxicity of the widely used avermectins (e.g. Wardhaugh & Rodriguez-Menendez, 1988; McCracken & Foster, 1993; Ridsdill-Smith *et al.*, 1993; Sommer *et al.*, 1993; Strong *et al.*, 1993, 1996; Strong & Wall, 1994; Krüger & Scholtz, 1997, 1998a, 1998b; Floate, 1998; Dadour *et al.*, 1999; reviewed in Floate *et al.*, 2005). In contrast, only few studies (Bianchin *et al.*, 1998; Krüger *et al.*, 1998, 1999) have investigated the relative toxicity of pyrethroids against beneficial dung fauna, despite the fact that pyrethroids are used extensively for control of ectoparasites on farm livestock (Srivastava *et al.*, 1993; Franc & Cadiergues, 1994; Kok *et al.*, 1996). These compounds, derivatives of natural pyrethrins, are extremely active

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against insects and mites, but show low toxicity for birds and mammals (Wardhaugh & Beckmann, 1996/97). So far, no study has tested the effects of a cypermethrin spray formulation, which may have entirely different ecotoxicological effects on dung-dwelling non-target organisms than the tested pour-ons.

The product Ektoban® is a pyrethroid formulation on the South African market for chemotherapy against ticks, flies, lice and screw-worm on cattle. It comes as an emulsifiable concentrate containing $175\,\mathrm{g\,l^{-1}}$ cymiazol (amidine) and $25\,\mathrm{g\,l^{-1}}$ cypermethrin and is diluted 15 ml per 10 l water for plunge dipping or hand spraying.

Chapman (2002) emphasized the importance of choosing test taxa for ecotoxicological assays that are (or are at least similar to) resident taxa and have a high ecological relevance. *Euoniticellus intermedius* (Reiche) (Coleoptera: Scarabaeidae) is a widely distributed dung beetle species throughout the Afrotropical region (Ferreira, 1968–1969) and an integral part of South African dung beetle communities. The species belongs to the functional group IV (as described by Doube, 1990), the large slow-burying paracoprids. Beetles belonging to this group tunnel and build their nest in the soil beneath the dung pad. Furthermore, this beetle has a wide ecological tolerance (Blume, 1984) and has the capability to vary the nesting strategies with soil type and season (Halffter & Edmonds, 1982). This enables *E. intermedius* to survive under extreme edaphic and climatic conditions (Rougon & Rougon, 1982). The species has gained further importance by the fact that it was one of the dung beetle species to be successfully introduced into Australia and the USA for the biocontrol of dung-breeding flies and removal of accumulated cattle dung on the surface of pastures (Blume, 1984; Matthiesen *et al*., 1986). Its importance and ecological profile together with its ease of handling, rearing, and short generation times therefore make *E. intermedius* the ideal species of dung beetle in laboratory bioassays. The aim of this study was to assess any lethal and/or sublethal ecotoxicological non-target effects that cattle treatment with Ektoban® may have on *E. intermedius*.

Materials and methods

A group of five Friesian heifers were hand-sprayed once with cymiazol/cypermethrin (Ektoban®) strictly according to the manufacturer's instructions (dose rates: cymiazol 52.5 mg per 50 kg body weight, cypermethrin 7.5 mg per 50 kg body weight) and kept on a paddock at the experimental farm at the University of Pretoria. Simultaneously, another group of five heifers was kept untreated on another paddock 20 m away without any contact with the treated group. The cattle had not been treated with any antiparasitic agent for three months prior to this trial and were maintained on hay, grass, and water *ad libitum*.

Early in the morning one, two, three, five, seven, 14, 21, and 28 days after treatment, freshly deposited cattle dung was collected from both paddocks. For each collection day and cattle group the dung was thoroughly mixed and then frozen until needed. It was used in the trial within 12 weeks from the collecting date.

Laboratory colonies of the dung beetle species *E. intermedius* were established by collecting beetles from the field (Parys, Free State Province, South Africa, 26°54′S, 27°35′E) from a farm where no antiparasitic drugs had been used for more than three months prior to the collecting date. The experiments were started with laboratory reared F1 generation offspring from the field-collected beetles. The rearing of the beetles and consecutive breeding experiments were undertaken in an insectary at 27°C, 12 h photoperiod and 60% humidity.

The experimental design was as follows. For each dung collection day, ten pairs (one male and one female each) of unmated ten-day-old *E. intermedius* from the F1 generation were placed in 1 litre plastic buckets (13.5 cm in diameter, 12.3 cm height) three-quarters filled with moist, sandy soil and closed with a gauze lid. Five pairs were given 250 ml dung from cattle treated with cymiazol/cypermethrin and five pairs were supplied with 250 ml dung from untreated cattle. After four days the beetles were fed with another portion of 250 ml of the respective dung type. After seven days the contents of each bucket were sifted and the number of surviving adult beetles as well as the number of brood balls (each containing a single egg) were counted. The brood balls were transferred into a new bucket with moist sandy soil; the beetle pairs were placed into new buckets with fresh sand and dung and the procedure was repeated for another week. Only the beetles emerging from the brood balls of the second week were used for the second part of the breeding experiment, because brood ball production during the first week of the experiment could have been biased by the control dung fed to the F1 beetles during their maturation (Tyndale-Biscoe *et al*., 1981).

The second part of the experiment aimed to assess the effect of cymiazol/cypermethrin on the fecundity and fertility of *E. intermedius*. Ten pairs of unmated, ten-day-old beetles belonging to the F2 generation and reared on the respective dung type that their parents had been fed were paired up in buckets with fresh sandy soil. All ten pairs were fed with 250 ml control dung each. The above-described procedure was repeated. Fecundity was then measured via the number of brood balls formed (and thus the number of eggs laid), and fertility was indirectly measured via the number of adult F3 beetles emerging from these brood balls (Southwood, 1978).

The experiment was undertaken in two time-separated sets of five pairs per experimental category, with different field-collected F1 generation stocks and dung from two different groups of treated and untreated cattle.

The data on adult survival, number of brood balls, brood ball viability (larval survival and adult emergence), fecundity and fertility were analysed for pairwise comparisons per dung-sampling day with the non-parametric Mann-Whitney U test (Sokal & Rohlf, 1981) using STATISTIKA® (Version 7; Statsoft, Tulsa, Oklahoma) and (after log + 1-transformation and resultant normality of the data) with a two-way ANOVA approach testing for influences of the factors 'day' and 'treatment' using a significance threshold value of $P < 0.002$ in SIGMASTAT (Version 2.0; SPSS Inc.). Percentage corrected mortality was calculated using Abbott's formula (%mortality = [% alive control − % alive treatment/% alive control] × 100; Abbott, 1925).

Results

Adult survival

The data for adult survival were not normally distributed and included a lot of ties; hence the data was not suitable for analyses with with Mann-Whitney-U test nor

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Table 1. Mean number of adult F1 *Euoniticellus intermedius* (per pair) surviving seven and 14 days of exposure to dung from cattle treated with Ektoban® spray and to control dung.

Day	Week 1					Week 2				
	Ektoban®		Control		% mort	Ektoban®		Control		% mort
	n	m/25–75%s	*n*	m/25–75%s		*n*	m/25–75%s	*n*	m/25–75%s	
1	10	2/2–2	10	2/2–2	5.00	10	2/2–2	10	2/2–2	5.26
2	10	2/2–2	10	2/2–2	0.00	10	2/2–2	10	2/2–2	−5.26
3	10	2/2–2	10	2/2–2	0.00	10	2/2–2	10	2/1–2	−6.25
5	10	2/2–2	10	2/2–2	−11.11	10	2/2–2	10	2/2–2	−11.11
7	10	2/2–2	10	2/2–2	−11.11	10	2/2–2	10	2/2–2	−5.88
14	10	2/2–2	10	2/2–2	5.00	10	2/2–2	10	2/2–2	0.00
21	10	2/2–2	10	2/2–2	5.56	10	2/2–2	10	2/2–2	5.88
28	10	2/2–2	10	2/2–2	15.00	10	2/1–2	10	2/2–2	−6.25

n, number of F1 beetle pairs; m, median; 25–75%s, interquartile range. Percentage corrected mortality (% mort) was calculated following Abbott (1925).

Table 2. Mean number of F2 brood balls formed per pair F1 *Euoniticellus intermedius* with dung from cattle treated with Ektoban® spray and control dung.

Day	Week 1				Week 2			
	Ektoban®		Control		Ektoban®		Control	
	n	m/25–75%s	*n*	m/25–75%s	*n*	m/25–75%s	*n*	m/25–75%s
1	10	10.5/8–14	10	11/4–28	10	11.5/7–16	10	13/10–22
2	10	15/11–18	10	15/13–17	10	11.5/5–22	10	13.5/10–19
3	10	14/10–22	10	21/8–22	10	19.5/15–22	10	17/14–27
5	10	14/11–19	9	13/5–21	10	17.5/14–19	9	7/4–9
7	10	19/15–22	9	14/12–18	10	14.5/12–19	9	9/6–16
14	10	12/3–14	10	13.5/8–16	10	17/10–21	10	15.5/13–18
21	10	6/0–13	10	9/4–17	9	10/6–11	10	12/11–13
28	10	11/5–16	10	7.5/5–19	9	9/7–16	10	11/4–17

n, number of parental F1 beetle pairs; m, median; 25–75%s, interquartile range.

ANOVA. However, it was obvious from the data themselves (table 1) that the values in the treatment group did not deviate from the values in the control group. Furthermore, neither Mann-Whitney-U tests performed for each single dung-sampling day nor a global two-way ANOVA ($P = 0.661$ for week 1 and $P = 0.568$ for week 2) showed any statistically significant differences in the number of surviving adult F1 beetles between the groups exposed to dung from cymiazol/cypermethrin-treated cattle and the control groups (table 1). Throughout the experiment, the survival rates in treatment and control groups were similarly high. The percentage corrected mortalities were correspondingly low, in several cases even negative (table 1) indicating a higher survival rate in the treatment group than in the control. There was no detectable lethal effect of the cymiazol/cypermethrin spray on adult *E. intermedius*.

For all the following sections the data were analysed in two separate ways: seeing that the data were not normally distributed we performed non-parametrical Mann-Whitney-U test on pairwise comparisons between control and treated groups for each dung collection day and for both weeks separately. Furthermore, after log + 1-transformation the data were normally distributed and were then subjected to global ANOVA analyses.

F2 brood ball production

The number of F2 brood balls formed by the F1 generation one and two weeks after cattle treatment did not differ significantly between the treated and control groups (table 2, ANOVA $P = 0.529$ for week 1 and $P = 0.7$ for week 2). In some cases the number of brood balls produced was higher in the control group and in some cases the number was higher in the treatment group. There was no notable trend indicating any negative effects due to cymiazol/cypermethrin application. All single day comparisons between treatment and control group were statistically non-significant in both weeks (Mann-Whitney-U test).

Adult emergence (F2 generation)

For week 1 there was no significant difference in adult emergence of F2 *E. intermedius* reared on dung from treated cattle and control cattle on any of the post-treatment days (Mann-Whitney-U test, table 3). For week 2, 21 days after cattle treatment, the number of adult F2 beetles emerging was significantly lower in the treatment group than in the control group ($U = 10$, $P = 0.02$). This occurred very late in the course of the experiment and stands out as an unusual result against the background of otherwise non-significant

Table 3. Mean number of F2 *Euoniticellus intermedius* emerged from brood balls (per F1 parental pair) formed with dung from cattle treated with Ektoban® spray and control dung.

Day	Week 1				Week 2			
	Ektoban®		Control		Ektoban®		Control	
	n	m/25–75%s	*n*	m/25–75%s	*n*	m/25–75%s	*n*	m/25–75%s
1	10	5/4–9	10	8/2–15	9	6/3–8	10	6.5/4–14
2	10	11.5/5–18	10	12/12–14	10	6/1–12	9	9/5–11
3	10	8.5/1–15	10	14.5/8–17	9	14/6–18	10	13.5/11–17
5	10	7.5/4–13	9	9/5–12	10	5.5/4–7	8	4/1.5–7
7	10	10/7–16	9	8/8–11	10	8.5/6–11	8	6.5/3–13.5
14	10	4/0–9	10	6/6–12	9	11/9–17	9	12/10–14
21	10	4/0–8	10	4/0–9	8	7.5/3.5–10	8	12/8.5–14.5*
28	10	9.5/4–11	10	7.5/5–13	8	6/4.5–15.5	9	11/3–12

n, number of parental F1 beetle pairs; m, median; 25–75%s, interquartile range. *$P < 0.05$; Mann-Whitney-U test.

Table 4. Mean number of F3 brood balls formed per breeding pair F2 *Euoniticellus intermedius* reared on dung from cattle treated with Ektoban® spray and control dung.

Day	Week 1				Week 2			
	Ektoban®		Control		Ektoban®		Control	
	n	m/25–75%s	*n*	m/25–75%s	*n*	m/25–75%s	*n*	m/25–75%s
1	10	14/10–19	9	14/13–26	10	14.5/11–20	9	15/0–17
2	9	17/14–20	9	17/3–19	9	18/3–21	9	19/7–24
3	10	13.5/2–16	9	13/11–17	9	6/1–16	9	17/11–18
5	6	2.5/0–8	7	11/3–12	6	6.5/1–15	7	13/6–17
7	10	8/2–13	10	10/6–12	9	15/13–17	9	20/19–24*
14	10	11/7–20	10	14/4–19	10	23/17–24	10	17/12–24
21	10	7/0–12	10	11.5/3–17	10	13/5–16	10	21.5/20–24**
28	9	17/0–18	9	16/13–20	9	20/0–22	9	16/11–24

n, number of parental F2 beetle pairs; m, median; 25–75%s, interquartile range. *$P < 0.05$; **$P < 0.01$, Mann-Whitney-U test.

differences. Furthermore, the overall two-way ANOVAs for both week were non-significant ($P = 0.153$ for week 1 and $P = 0.177$ for week 2).

F3 brood ball production (fecundity)

For week 1 the numbers of F3 brood balls constructed by adult F2 *E. intermedius* did not significantly differ between the control and the treatment group (per sample-day Mann-Whitney-U tests; table 4). In week 2, seven and 21 days after cypermethrin/cymiazol treatment of cattle, the number of F3 brood balls was significantly higher for the control group than for the treatment group ($U = 15.5$, $P = 0.03$; and $U = 14.5$, $P < 0.01$). Furthermore, in 12 out of 16 cases the control group produced more brood balls than the group on cypermethrin/cymiazol-treated dung. This suggested that cypermethrin/cymiazol may have a slight and delayed negative effect on the fecundity of *E. intermedius*. However, the overall two-way ANOVAs for both weeks did not result in any significant differences due to the treatment ($P = 0.106$ for week 1 and $P = 0.058$ for week 2).

F3 broodball emergence (fertility)

Neither in week 1 nor in week 2 were there any significant differences in the number of emerged adult F3 beetles on any post-treatment day (Mann-Whitney-U tests; table 5). The mean numbers of emerged adults were almost always higher in the control group. However, the overall two-way ANOVAs for both weeks did not result in any significant differences due to the treatment ($P = 0.110$ for week 1 and $P = 0.148$ for week 2). Therefore, any possible negative effect of cypermethrin/cymiazol treatment of cattle on the fertility of *E. intermedius* would only be slight and non-significant.

As was the case for all previously mentioned two-way ANOVA tests for brood ball production and emergence, the difference in the mean values among the different levels of day (dung-sampling day: 1, 2, 3, 5, 7, 14, 21, and 28 days after cattle treatment) was greater than would be expected by chance after allowing for effects of differences in treatment (cypermethrin/cymiazol-sprayed or control cattle). However, in all cases the effect of different levels of day did not depend on what level of treatment was present; there was no statistically significant interaction between day and treatment.

Discussion

Under laboratory conditions, the cypermethrin/cymiazol spray formulation Ektoban® proved to be without notable ecotoxicological effect on adult survival of the dung beetle *E. intermedius* for over two weeks of exposure to experimental dung. This is in contrast to reports of lethal effects of cattle treatment with other pyrethroid formulations. For instance the deltamethrin pour-on Arrest® was lethal to adult dung beetles of the species *Onthophagus binodis* Thunberg for up to one week after treatment

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Table 5. Mean number F3 *Euoniticellus intermedius* emerged from brood balls per F2 breeding pair reared on dung from cattle treated with Ektoban® spray or control dung.

Day	Week 1				Week 2			
	Ektoban®		Control		Ektoban®		Control	
	n	m/25–75%s	*n*	m/25–75%s	*n*	m/25–75%s	*n*	m/25–75%s
1	10	7/4–12	9	7/1–11	10	7.5/5–10	9	4/0–9
2	9	5/3–17	9	10/2–14	9	3/0–12	9	7/2–11
3	10	3/0–9	9	11/8–11	9	2/0–6	9	11/8–11
5	6	2.5/0–4	7	4/1–9	6	6.5/0–8	7	4/1–11
7	10	4/0–9	10	3.5/0–5	9	9/4–12	9	12/7–14
14	10	7/5–10	10	11.5/3–13	10	13.5/9–18	10	14/8–18
21	10	3.5/0–8	10	5.5/2–14	10	8.5/5–10	10	11/9–16
28	9	10/0–11	9	12/10–14	9	13/0–22	9	12/8–19

n, number of parental F2 beetle pairs; m, median; 25–75%s, interquartile range.

(Wardhaugh *et al*., 1998). And the deltamethrin pour-on Arrest easy-Dose® caused mortality rates in adult *Euoniticellus fulvus* Goeze of 88–90% for up to four days after treatment (Wardhaugh & Beckmann, 1996/97). Vale *et al*. (2004) mentioned that the toxicity of different pyrethroids seems to vary, with deltamethrin being more potent than others. Additionally, the test species used (e.g. flies versus beetles) is another confounding factor for toxicity determination. All this may explain the discrepancies in observed mortality rates between the above cited studies and the data presented here.

Bianchin *et al*. (1992) observed mean mortalities among adult beetles of the species *Onthophagus gazella* Fabricius ranging from 51–61% for up to three weeks after treatment with various pyrethroid pour-on formulations. Sommer *et al*. (2001) reported increased larval and adult mortality of the dung fly *Neomyia cornicina* (Fabricius) (Diptera: Muscidae) due to alpha-cypermethrin treatment of calves. A study by Krüger *et al*. (1999) found that dung from cypermethrin-treated cattle caused mortality rates of 80–100% of adult *E. intermedius* for two to seven days after treatment. However, the applied dosage of cypermethrin (in the form of Blitzdip®) used by Krüger *et al*. (1999) was approximately 10× higher than in the present study (which does not necessarily correspond to a 10-fold increase in the dung concentration of the residue) and the formulations used in the above-mentioned studies were all pour-ons whereas in the presented study cypermethrin was administered as a spray. Both these factors, dosage and administration route, are well known to affect the intensity of any adverse effects (Bianchin *et al*., 1992); with active ingredients in pour-ons becoming systemic and therefore ending up in the faeces of the treated animals to a higher degree than when the same substances are applied as sprays. This could account for a much weaker or even missing ecotoxicological effect of a cypermethrin spray here in comparison to the strong effects of cypermethrin pour-ons in the literature.

The absence of lethal effects of the cypermethrin/cymiazol spray Ektoban® on *E. intermedius* documented here is in agreement with Krüger *et al*., (1998, 1999) who established that the correct use of flumethrin (also a pyrethroid) appeared to be non-lethal to adult dung beetles. However, Bianchin *et al*. (1997) reported lethal effects of flumethrin on the dung beetle *O. gazella*.

Furthermore, based on the data here, cattle treatment with the cypermethrin/cymiazol spray Ektoban® also did not have any notable sub-lethal effects on *E. intermedius* in terms of egg production, larval survival, fecundity (as measured by the production of F3 brood balls by F2 adults) and fertility (as measured by the emergence of F3 adult from the F3 brood balls).

In the relevant literature, even in cases where the applied pyrethroid had lethal effects on adult *E. intermedius*, there were no effects on fecundity or fertility (Krüger *et al*., 1999). Nonetheless, Wardhaugh *et al*. (1998) argued that juvenile stages of certain dung beetle species may be more sensitive to pyrethroid residues in dung and they reported a reduction in fecundity of *O. binodis* due to deltamethrin up to 28 days after treatment. However, this negative effect on fecundity was consistently present from day 1 after treatment and not sporadic and rare as in the case of the present study, where the only significant deviation in F2 adult emergence occurred 21 days after treatment in week 2 of exposure and the only two significant deviations in F3 brood ball production occurred 7 and 21 days after treatment in the second week. Furthermore, in the same study, Wardhaugh *et al*. (1998) noted that various species of dung beetles may well respond differently to antiparasitic drug residues.

In summary, adult and larval survival, fecundity and fertility of the dung beetle species *E. intermedius* appeared to be unaffected by exposure to faeces of cypermethrin/cymiazol-spray-treated cattle.

A survey by Spickett & Fivaz (1992) came to the conclusion that South African dairy and beef farmers in the Eastern Cape Province prefer pyrethroids for tick control, with over 62% of all used acaricides falling into this category. Furthermore, it showed that beef farmers preferred pour-on formulations over dips and sprays (Spickett & Fivaz, 1992). The results of this study in context with previous studies on cypermethrin suggested that the very same pyrethroid may be extremely detrimental to the beneficial dung beetle communities if used as a pour-on formulation, while it may be much more dung beetle friendly if used in a dip or spray application. It thus remains an important task to raise awareness among farmers that while a pyrethroid pour-on may be more convenient and faster in the application, a dip or spray application of the same compound may have the advantage of being environmentally safer towards beneficial dung degrading insects and therefore more supportive of sustainable farming. However, further research into this topic is necessary in order to corroborate this speculation.

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