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Short communication

Effects of cattle treatment with a fluazuron pour-on on survival and reproduction of the dung beetle species *Onthophagus gazella* (Fabricius)

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Abstract

While resistance against many other classes of acaricides has been described, products containing benzoylphenyl urea are currently still successfully used against the pesticide-resistant blue tick (*Boophilus decoloratus*) in South Africa. In order to assess any adverse impact of these tickicides on the important dung beetle (Coleoptera: Scarabaeidae) fauna, a bioassay was undertaken on the ecotoxicological effects of a fluazuron (benzoylphenyl urea) pour-on formulation (Acatak®) on the survival and reproduction of the African dung beetles species *Onthophagus gazella* (Fabricius). The experiment yielded no significant differences in adult or larval survival, egg production, fecundity and fertility between the control and treatment group following three beetle generations over. These results suggested that treatment of cattle with the fluazuron pour-on formulation Acatak® was not detrimental to the selected dung beetle species in any notable way.

Keywords: Acaricide; Benzoylphenyl urea; *Boophilus decoloratus*; Dung beetle; Ecotoxicological effect; Fluazuron; *Onthophagus gazella*

1. Introduction

In commercial livestock farming, internal and external parasites are controlled using so-called endectocides. Most of these agents are only partially metabolized; the active ingredients are to some extent excreted in the faeces of the treated animals (Strong and Wall, 1990) and may have deleterious non-target effects on beneficial organisms, such as dung beetles (McKellar, 1997). Dung beetles (Coleoptera: Scarabaeidae) are a major force in breaking down dung pads and recycling nutrients into the soils of tropical and sub-tropical ecosystems (Bornemissza, 1976; Davis, 1996). They are thus vital to the health of these ecosystems (including agroecosystems, Stokstad, 2004), and the determination of unintended ecotoxicological effects of antiparasiticides on dung beetles is of high importance (Wardhaugh and Ridsdill-Smith, 1998).

Little is known about the environmental impact of tickicides with the active ingredients of benzoylphenyl urea (also, fluazuron). These compounds are highly toxic against ticks (especially the blue tick, *Boophilus decoloratus*, Bull et al., 1996) and possess considerable action against fleas, lice and sheep blowflies by interfering with the chitin synthesis and thus interrupting the life cycle at many stages (Junk, 1987). Since there was no widespread resistance against benzoylphenyl urea chitin-synthesis inhibitors in pest populations (Wilson and Cain, 1997), they gained considerable popularity.

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The product Acatak® is a benzoylphenyl urea formulation on the South African market for chemotherapy against blue ticks. It comes as a ready-to-use pour-on containing 25 g/l fluazuron with a systemic mode of action and a peak concentration of the active ingredient in the faeces 2–4 weeks after application.

In tests conducted by Ciba-Geigy Australia Ltd., dung was spiked with discrete concentrations of Acatak® (0, 100, 500, and 10,000 ppb) and fed to laboratory kept dung beetles (Fisara, 1994). While *Euoniticellus intermedius* was not affected, there were notable reductions in brood production and viability in *Onthophagus taurus* and *Onthophagus gazella* (Fisara, 1994, 1996a). The same author found no change in brood production and emergence testing Acatak® on *E. intermedius* or *O. gazella* in a follow-up study (Fisara, 1995a,b). Cattle treatment with Acatak® at the rate of 2.5 mg/kg did not have any negative effects on the reproduction of *E. intermedius* (Fisara, 1996b).

So far, there has been no comprehensive laboratory based bioassay using dung voided from fluazuron pour-on-treated cattle documenting the lethal and sub-lethal effects on dung beetles through several generations.

O. gazella (Fabricius) is a widely distributed dung beetle species throughout the tropical and subtropical African region (Ferreira, 1968–1969) and an integral part of South African dung beetle communities. Its importance and ecological profile therefore make *O. gazella* the ideal species to use for laboratory assays on ecotoxicological effects of antiparasitic drugs on dung beetles. The aim of this study was to assess any lethal and/or sublethal ecotoxicological non-target effects that cattle treatment with Acatak® may have on *O. gazella*.

2. Materials and methods

A group of five Friesian heifers was treated once with a fluazuron pour-on (benzoylphenyl urea: 25 g/l, Acatak®) strictly according to the manufacturer's instructions (3 mg/kg or 6 ml/50 kg body mass) 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 to the treated group. The cattle had not been treated with any antiparasitic agent for 3 months prior to this trial and were maintained on hay, grass, and water *ad libitum*.

One, 2, 3, 5, 7, 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.

Laboratory colonies of *O. gazella* were established with beetles from a farm where no antiparasitic drugs had been used for more than 3 months prior to collecting date. The experiments were started with lab reared F1 generation offspring from the field-collected beetles and were undertaken in an insectary at 27 °C, 12 h photoperiod and 60% humidity.

For each dung collection day, 10 pairs (one male and one female each) of unmated 10-day-old *O. gazella* from the F1 generation were placed in 2 l plastic buckets (one pair per bucket) filled three-quarters with soil and closed with a gauze lid. Five pairs were given 250 ml dung from cattle treated with fluazuron, five pairs were supplied with 250 ml dung from untreated cattle. After 4 days the beetles were fed with another portion of 250 ml of the respective dung type. After 7 days the contents of each bucket were sifted and the number of surviving adult beetles was counted. The beetle pairs were placed into new buckets with fresh sand and dung and the procedure was repeated for another week. At the end of the second week, the number of F2 broodballs produced was counted (measure for fecundity of F1 generation) and the number of emerging F2 individuals from these broodballs was recorded (measure for fertility of F1 generation; after Southwood, 1978).

The second part of the experiment aimed to assess the effect of fluazuron on the fecundity and fertility of the F2 generation *O. gazella*. Ten pairs of unmated, 10-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 were fed with 250 ml control dung. The above-described procedure was repeated.

The entire experiment was repeated starting with the F1 generation obtained from new colonies of field-collected beetles and dung from new groups of treated and untreated cattle.

After square-root transformation the data were normally distributed and analyzed with a two-way ANOVA in SIGMASTAT (Version 2.0; SPSS Inc.). Percentage corrected mortality was calculated using Abbott's formula (Abbott, 1925).

3. Results and discussion

The data for adult survival were not normally distributed and included a lot of ties; they were therefore not analyzable with 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. The percentage corrected mortalities

Table 1
Mean number of adult F1 *Onthophagus gazella* (per pair) surviving seven to 14 days of exposure to dung from cattle treated with Acatak® pour-on and to control dung

Day	Week 1					Week 2				
	Acatak®		Control			Acatak®		Control		
	n	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	% Mortality	n	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	% Mortality
1	10	2.00 ± 0.00	10	1.90 ± 0.10	−5.26	10	1.90 ± 0.10	10	1.80 ± 0.13	−5.56
2	10	2.00 ± 0.00	10	1.70 ± 0.15	−17.65	10	1.80 ± 0.13	10	1.60 ± 0.16	−12.50
3	10	2.00 ± 0.00	10	1.90 ± 0.10	−5.26	10	1.80 ± 0.13	10	1.90 ± 0.10	5.26
5	10	2.00 ± 0.00	10	1.90 ± 0.10	−5.26	10	1.50 ± 0.22	10	1.70 ± 0.21	11.77
7	10	2.00 ± 0.00	10	1.90 ± 0.10	−5.26	10	1.80 ± 0.13	10	1.80 ± 0.13	0.00
14	10	2.00 ± 0.00	10	2.00 ± 0.00	0.00	10	2.00 ± 0.00	10	1.90 ± 0.10	−5.26
21	10	2.00 ± 0.00	10	2.00 ± 0.00	0.00	10	1.90 ± 0.10	10	2.00 ± 0.00	5.00
28	10	2.00 ± 0.00	10	1.90 ± 0.10	−5.26	10	1.90 ± 0.10	10	1.90 ± 0.10	0.00

Percentage corrected mortality was calculated following Abbott (1925); n, number of F1 beetle pairs.

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 notable lethal effect of the fluazuron pour-on on adult *O. gazella*.

The number of F2 brood balls formed by the F1 generation in the second week of exposure did not differ significantly between the treated and control groups (Table 2, ANOVA, $P = 0.195$).

The overall two-way ANOVAs for F2 adult emergence was non significant ($P = 0.508$). In some cases the mean number of F2 broodballs produced and of F2 beetles emerging was higher in the control group and in some cases the mean number was higher in the treatment group (Tables 2 and 3), but there was no evident trend indicating any negative effects on F1 fecundity or fertility due to fluazuron application.

The mean number of F3 broodballs and emerged F3 beetles was sometimes higher in the group reared on fluazuron-treated dung and sometimes in the control group (Tables 4 and 5). The overall two-way ANOVAs did not result in any significant differences in F2 fecundity nor fertility due to the treatment ($P = 0.866$ and 0.334).

The faecal concentration of fluazuron administered to cattle at a dosage of 3 mg/kg body weight peaks around 14–28 days after application. This bioassay has found no evidence for any detrimental affects of faecal fluazuron on three generations of the dung beetle species *O. gazella* from 1 to 28 days after drug application to cattle, covering the presumably most critical time period. This is in contrast to the reported negative effects of diflubenzuron and teflubenzuron on the dung beetle *Sphaeridium scaraboides* (as quoted in Strong and Wall, 1990) and on the grasshoppers *Aiolopus thalassinus* and *Eyprepocnemis plorans* (Schmidt et al., 1993), respectively. It may not be surprising that the tested chemical did not impact on adult survival of a dung degrading coleopteran, because its mode of action consists of interrupting the larval

Table 2
Mean number of F2 brood balls formed per parental F1 *O. gazella* pair in the second experimental week with dung from cattle treated with Acatak® pour-on and control dung (n, number of parental F1 beetle pairs)

Day	Acatak®		Control	
	N	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.
1	10	31.20 ± 8.18	10	22.90 ± 5.82
2	10	19.70 ± 5.82	10	19.50 ± 5.83
3	10	34.90 ± 6.10	10	17.40 ± 4.47
5	10	8.80 ± 4.03	10	13.30 ± 5.11
7	10	19.20 ± 5.05	10	20.40 ± 3.40
14	10	42.80 ± 4.59	10	42.70 ± 3.20
21	10	22.90 ± 5.36	10	10.30 ± 5.25
28	10	18.80 ± 5.54	10	19.50 ± 5.63

Table 3
Mean number of F2 *O. gazella* emerged from brood balls (per F1 parental pair) formed with dung from cattle treated with Acatak® pour-on and control dung (n, number of parental F1 beetle pairs)

Acatak®		Control	
n	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.
10	22.60 ± 6.56	8	20.50 ± 3.67
8	9.50 ± 2.17	8	11.13 ± 3.50
9	20.00 ± 2.53	9	11.22 ± 3.24
6	11.33 ± 5.02	6	4.00 ± 1.84
9	10.44 ± 2.52	10	8.40 ± 2.25
10	5.70 ± 2.20	10	8.50 ± 1.62
9	13.11 ± 3.36	5	11.20 ± 5.11
7	1.43 ± 0.57	7	6.14 ± 2.18

Table 4
Mean number of F3 brood balls formed by F2 *O. gazella* reared on dung from cattle treated with Acatak® pour-on or control dung (*n*, number of parental F2 beetle pairs)

Day	Week 2			
	Acatak®		Control	
	n	$\bar{x} \pm$ S.D.	*n*	$\bar{x} \pm$ S.D.
1	10	18.10 ± 3.48	10	5.60 ± 1.98
2	10	20.60 ± 6.02	10	12.00 ± 4.40
3	10	10.00 ± 3.69	10	7.50 ± 2.60
5	10	17.00 ± 5.02	9	19.88 ± 6.96
7	10	16.30 ± 3.60	10	12.70 ± 5.57
14	10	15.20 ± 5.78	10	20.50 ± 5.59
21	10	12.70 ± 3.82	10	27.40 ± 5.15
28	10	21.20 ± 7.03	10	28.50 ± 3.80

Table 5
Mean number of F3 *O. gazella* emerged from brood balls formed by F2 pairs reared on dung from cattle treated with Acatak® pour-on or control dung (*n*, number of parental F2 beetle pairs)

Acatak®		Control	
n	$\bar{x} \pm$ S.D.	*n*	$\bar{x} \pm$ S.D.
10	8.80 ± 1.74	7	2.71 ± 0.92
7	14.43 ± 4.25	7	6.00 ± 1.43
6	7.00 ± 2.21	6	6.33 ± 1.52
6	8.33 ± 1.54	5	10.00 ± 3.35
8	5.75 ± 1.52	5	7.00 ± 2.95
5	11.40 ± 2.29	8	4.63 ± 1.51
7	3.29 ± 1.11	8	10.88 ± 1.65
7	4.71 ± 1.13	10	6.90 ± 2.04

development of arthropods. However, following reports of disturbed growth and development of oocytes after diflubenzuron ingestion in another beetle species, *Tenebrio molitor* (Soltani_Mazouni, 1994) and potent toxicity of chlorfluazuron (another benzoylphenyl urea insecticide) on larvae of the red flour beetle, *Tribolium castaneum* (Gazit et al., 1989), fluazuron related reduced fecundity and/or fertility in *O. gazella* could have been expected. On the other hand, it is well established that the action of benzoylphenyl urea compounds is highly selective, and also various species of beetles have different tolerance thresholds to residues of antiparasitic drugs (Wardhaugh et al., 1998).

However, the data presented here are also different from the results generated by Fisara (1994, 1996b) on the same dung beetle species. These conflicting findings may be the result of different end-concentrations of the chemical in the dung, differing laboratory conditions (Forbes and Forbes, 1994) or genetic strains of *O. gazella* or a combination of those (compare Krüger and Scholtz, 1997).

The absence of negative effects of benzoylphenyl urea on *O. gazella* documented here is in agreement with the results of a long term field study by Kryger et al. (2005) who found no adverse changes in South African dung beetle communities after cattle treatment with the fluazuron pour-on Acatak®.

South African beef farmers in the Eastern Cape Province prefer pour-on formulations over dips and sprays (Spickett and Fivaz, 1992). The development of resistance in South African *B. decoloratus* to DDT, organophosphates, carbamates, synthetic pyrethroids and toxaphene (Maree and Casey, 1993) has lead to a situation where benzoylphenyl urea compounds are the only acaricides left where no severe resistance has occurred yet. The fluazuron-containing product Acatak® is the only parasiticide still effective against the blue tick in South Africa. This product combines this effectiveness with the ease of application of a pour-on (and therefore popularity) and with an environmental friendliness towards beneficial dung beetles. As long as farmers use it according to the manufacturers' specifications (avoiding overdosing and only on affected livestock) its widespread use would therefore not raise concerns. However, it remains important to stress that the product should not be used more often than necessary, in order to slow the advent of tick resistance (Maree and Casey, 1993) as well as in order to conserve beneficial coprophagus beetles. Furthermore, it is crucial to propagate the continued existence of susceptible ticks in refugia by avoiding the ‘‘drench-and-move’’ practice and by leaving some animals untreated (see Van Wyk, 2001).

The relative impact on the dung beetle fauna may become more pronounced with an accumulation of the compound in the ecosystem. For sustainable farming, promoted by efficient dung removal and consequently healthy pastures, the frequency and timing of any insecticide application should therefore be sensitive to the dynamics in the dung beetle populations.

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