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Topical treatment of calves with synthetic pyrethroids: effects on the non-target dung fly *Neomyia cornicina* (Diptera: Muscidae)

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Abstract

Dung from calves treated with synthetic pyrethroids negatively influenced, in varying degrees, survival, reproduction and size of the common dung fly *Neomyia cornicina* (Fabricius). This was documented in assays where the coprophagous larvae and adults of *N. cornicina* were exposed to dung collected from calves dosed with topical preparations of deltamethrin, flumethrin, cyfluthrin, and α-cypermethrin. Larval mortality was significantly increased in dung collected up to at least seven days after treatment with deltamethrin, α-cypermethrin and cyfluthrin. Alpha-cypermethrin caused significant mortality of adults allowed to feed on moist dung. Nulliparous flies fed for six days on dung collected three days after treatment of calves with α-cypermethrin or deltamethrin showed little or no ovarian development. A tendency for a comparable effect with flumethrin was also observed. A connection between ovarian development and inhibition of feeding was indicated by the observation of significantly lowered excretion rates in flies exposed to residues of deltamethrin, α-cypermethrin and flumethrin. Larvae that survived exposure to dung from calves dosed with deltamethrin, α-cypermethrin, or cyfluthrin gave rise to smaller flies. The effect on adult fly size decreased when larvae were exposed to dung collected at longer times after treatment of the calves. Adult fly size was significantly reduced in dung collected up to 14 days (α-cypermethrin) or up to 28 days after treatment (deltamethrin and cyfluthrin). Fluctuating asymmetry of a wing vein character did not reflect the anticipated levels of exposure. The study strongly indicated that the use of synthetic pyrethroids affected the insect dung fauna and that such use may reduce dung decomposition.

Introduction

The activity of more than 200 insect species associated with cattle dung (Skidmore, 1991) plays a vital role in the decomposition of dung organic matter in temperate pastures (e.g. Holter, 1977, 1979). By comparison, only a few of these insects are veterinary pests, the most important being *Hydrotea* spp., *Musca autumnalis* De Geer, and *Haematobia irritans* (Linnaeus) (Diptera: Muscidae). In order to control these flies, insecticides may be administered routinely to the cattle either by impregnated eartags or as pour-ons. Based on earlier reports of susceptibility of dung insects to veterinary medicines (e.g. review by Strong & Wall, 1990), the use of insecticides naturally raises the question of whether possible residues in dung may affect the insects and their function.

Studies of side-effects of synthetic pyrethroids against dung insects have focused on afrotropical dung beetles

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(Bianchin *et al*., 1992, 1997, 1998; Krüger *et al*., 1998; Wardhaugh *et al*., 1998) and only a single study reports on larvicidal effects against a dung breeding fly. Wardhaugh *et al*. (1998) observed increased mortality when larvae of the Australian bush fly *Musca vetustissima* Walker (Diptera: Muscidae) developed in dung collected one to two weeks after cattle received a topical dose of deltamethrin. To study the possible lethal and sublethal effects against a common dung fly from the temperate region, *Neomyia cornicina* (Meigen) (Diptera: Muscidae) was chosen for study. Adults of this fly species are attracted to dung from grazing cattle, place clusters of eggs in cavities they make near the surface in the fresh dung pat, and feed on the dung liquid (Skidmore, 1985). The fly larvae also feed on dung constituents and both adults and larvae may therefore be exposed to residues of synthetic pyrethroids. Consequently, adult and larval bioassays were used to investigate the influence of dung from calves that receive topical doses of deltamethrin, flumethrin, cyfluthrin and α-cypermethrin.

Materials and methods

Two types of assay were conducted as follows: (i) larval bioassays in which insects surviving to the adult stage were counted, scored for head width and wing vein asymmetry; and (ii) adult bioassays, based on measurements of mortality, faecal excretion, and ovarian development.

Dung from calves dosed with deltamethrin or flumethrin and control dung from untreated calves were used in the first set of larval assays. New calves were then treated with deltamethrin and flumethrin and dung collected again for the first set of adult bioassays. A second set of adult and larval assays used similar procedures to compare effects of dung from calves dosed with α-cypermethrin and cyfluthrin with dung from a group of untreated calves.

Treatment of calves, collection and preparation of dung

Two or three male calves were allocated into each of three groups; two groups of treated calves and one untreated control group. Calves weighed 143–344 kg and were allocated so as to ensure that total body weight per group was approximately equal. The animals had not been treated with any veterinary medicine for at least three months prior to the administration of synthetic pyrethroids. Doses recommended by the manufacturer of 10 ml per animal of deltamethrin (Coopersect®, 10 mg ml^{-1}), cyfluthrin (Bayofly®, 10 mg m^{-1}) or α-cypermethrin (Flusa®, 15 mg ml^{-1}) were applied along the back-line or between the shoulders (deltamethrin) of the calves. Flumethrin (Bayticol®, 10 mg ml^{-1}) was given as a pour-on along the back-line at a recommended dose of 20 ml per 100 kg. The calves were fed a mixture of one-third oat, one-third pelleted green fodder and one-third mixture of ground barley, soybean cake and wheat (and other minor components). In all cases feeding was supplemented with hay. Individual calves, or calves belonging to different groups, were placed in separate enclosures to avoid physical contact and possible cross contamination with the synthetic pyrethroids.

Dung was collected *per rectum* or from the floor and care was taken that no urine or straw bedding was mixed into the dung. Larval assays used dung collected at days 1, 3, 7, 14 and 28 after treatment. Dung from untreated cattle was collected on the same days. Dung from each collection was thoroughly mixed and deep-frozen (−29°C) for a minimum of one week. Dry matter was then determined in two subsamples for each combination of drug treatment and day after treatment. Dry matter was adjusted to 12% by addition of water and portions of 80 g were transferred to plastic cups and frozen until use in the larval assays.

Dung collected three days after treatment together with corresponding control dung were used for the adult bioassays. All dung was stored at 3–5°C for three to five days until dry matter was determined and corrected to 13% by the procedure described above. Dung was then placed in 90 mm Petri dishes and frozen at −29°C until use.

Larval bioassay

Three replicates were used for each combination of drug treatment and day after treatment. Plastic cups with 80 g of dung were seeded with 25 first stage larvae of *N. cornicina* by gently transferring them to the dung surface with a small brush. Sawdust was added to limit desiccation and the cups were placed in a filter paper cylinder, height 85 mm, with 90 mm Petri dishes serving as lid and base. The cups were placed at 27°C, 65% rh. Emerging flies were counted and kept for later measurements of head width and wing vein asymmetry.

Neomyia cornicina kept in a culture maintained at the Danish Pest Infestation Laboratory was used for larval and adult bioassays (rearing procedures may be obtained from the Danish Pest Infestation Laboratory).

Analyses of head width and fluctuating asymmetry

Head widths of flies from larval bioassays were measured with an ocular micrometer under a stereomicroscope and measurement of each male was scaled to approximate female size by multiplying by the mean female size and dividing by the mean male size.

The fly wings were mounted in Euparal and the linear length from the junction of the postical crossvein and postical vein to the junction of the postical crossvein and discal vein (see e.g. Fonseca, 1968) was measured in a digital image system. Departures from a normal distribution (mean of zero) of left (L) minus right (R) lengths were tested by Kolmogorov-Smirnov D-statistic (Sokal & Rohlf, 1981) for each drug treatment and day after treatment. $\Sigma(L\text{-}R)^2/N$ was used as an index for fluctuating asymmetry.

Adult bioassay

Groups of eight male and seven female *N. cornicina*, that had been allowed access to sugar and water, were transferred to each of twelve 48 × 29 × 29 cm cages. The flies were three to seven days old and the females were nulliparous. Cages were supplied with water and a sugar cube and kept at 24–27°C, and approximately 65% rh. After 24 h, a Petri dish with freshly thawed dung was placed in each cage. Every 24 h, Petri dishes were replaced, and dead flies were recorded and removed. When the first lot of Petri dishes was replaced, a 16 × 17.5 cm piece of filter paper was placed under each dish to facilitate counting of faecal spots. The filter papers were then changed every 24 h together with the Petri dishes. The dung was searched for eggs immediately and the presence of egg-clusters was noted and counted. After seven days, all remaining flies were dissected and scored for ovarian development, which was classified into three groups (comparable to groups 0–I, II–III, IV–V in Vogt *et al.*, 1974). Ovaries of females that had laid eggs during the experiment were classified as group III. The rate of faecal excretion of the flies in each cage was measured by calculation of the number of faecal spots per fly per day (number of flies was the mean number of live flies at two consecutive changes of filter papers).

Results

Larval bioassay

There was a marked difference in larval mortality induced by the synthetic pyrethroids (table 1). When larvae were exposed to dung from calves treated with flumethrin no significant negative effects were recorded. By contrast, no larvae survived through to the adult stage in dung from calves treated one or three days earlier with deltamethrin, α-cypermethrin, or cyfluthrin. In the same groups, mortality was significantly increased in dung collected seven days after treatment (Chi-square test, $P < 0.001$). No significant reduction in larval mortality was observed in any treatment when dung was collected 14 or 28 days after dosing. The mean control mortality varied between 56.0 and 73.3%, which is in accordance with control mortality found in a previous study of temperate dung breeding flies including *N. cornicina* (Sommer *et al.*, 1992).

Analyses of head width and fluctuating asymmetry

Significantly reduced head widths were seen in flies emerging from dung collected up to 28 days after dosing with deltamethrin or cyfluthrin (fig. 1a,b). With α-cypermethrin a reduction in head width was evident up to 14 days after dosing (t-tests, $P < 0.001$) (fig. 1b). For these three drug treatments, size generally increased with the number of days after treatment, that is, size increased as the expected concentration of drug residues in the dung decreased. Flies developing in treated cattle dung from flumethrin were smaller, or of equal size to those in the corresponding controls, but without any apparent order relative to the time after treatment (fig. 1a).

Mean wing vein length based on 16 repeated measurements was 20.1 μm with a variance of 0.026. As this variance value was approximately 2% of the average asymmetry value, it was assumed that any errors in measurement would not contribute significantly to the estimates of asymmetry.

The fluctuating asymmetry was independent of size (absolute differences between left and right wing vein regressed on average length was non-significant ($P > 0.88$)). $\Sigma(L\text{-}R)^2/N$ was therefore used as an index for fluctuating asymmetry, because this index is most likely to detect differences between groups (Palmer & Strobeck, 1986).

The asymmetry distribution did not prove significantly different from a normal distribution (Kolmogorov-Schmirnov D-statistic, $P > 0.05$) in 21 of the 24 groups tested (table 2). In the remaining three groups, P-values were less than 0.05, with one P value = 0.047. The measures, left minus right, were therefore assumed to be normally distributed. In the two control series, the fluctuating asymmetry index at different days 'after treatment' (table 2) varied by a factor 2.5 or 2.8 respectively. This variation in control groups was larger than for any other groups, and fluctuating asymmetry in control and treated groups could therefore not be

Table 1. Total number of *Neomyia cornicina* completing their lifecycle in three replicates each seeded with 25 first stage larvae. Dung was collected at 1, 3, 7, 14, or 28 days after treatment of calves with synthetic pyrethroids or collected from untreated control calves on the same days.

	Days after treatment				
	1	3	7	14	28
Control	28	21	23	24	20
Flumethrin	29	33	21	35	22
Deltamethrin	0	0	6	24	29
Control	26	27	31	33	33
α-cypermethrin	0	0	8	29	37
Cyfluthrin	0	0	8	30	37

Table 2. Fluctuating asymmetry of a wing vein character of *Neomyia cornicina* calculated as $\Sigma(\text{Left-Right})^2/N$ for all drug treatments at different days after topical treatment of calves with synthetic pyrethroids (units of μm^2).

	Days after treatments				
	1	3	7	14	28
Control	0.40 (27)	1.00 (19)	0.47 (23)	0.59 (24)	0.50 (20)
Flumethrin	0.42 (24)	0.50 (32)	0.44 (20)	0.60 (34)	0.56 (21)
Deltamethrin	–	–	0.52 (6)	0.44 (23)	0.30 (28)
Control	0.86 (25)	0.30 (26)	0.79 (29)	0.32 (32)	0.56 (33)
α-cypermethrin	–	–	0.27 (7)	0.30 (28)	0.46 (35)
Cyfluthrin	–	–	0.45 (8)	0.43 (30)	0.63 (35)

N is given in parenthesis.

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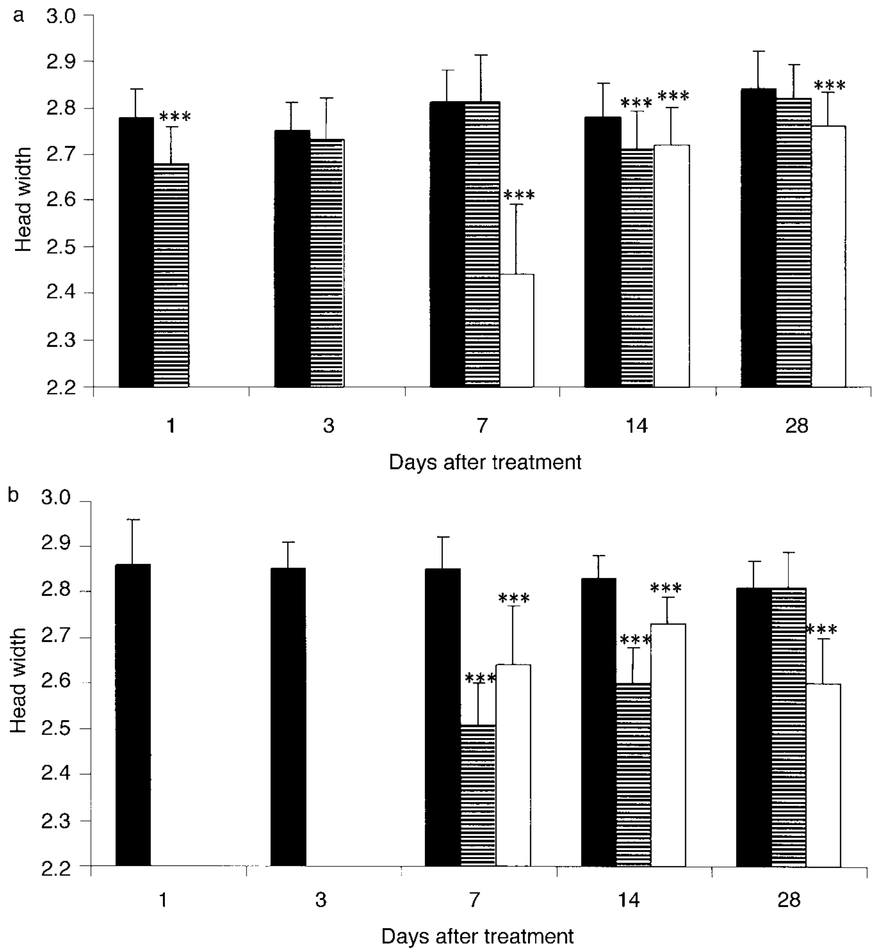

Fig. 1. Head width (in mm) of adult *Neomyia cornicina* completing their life cycle in dung collected at different days after treatment (± s.d.) with (a) flumethrin (▤) and deltamethrin (□) and (b) α-cypermethrin (▤) and cyfluthrin (□). Controls represented by ■ in both a and b. Male head width was scaled to female size (see text). ***: $P < 0.001$.

compared in a sensible way. Only in the deltamethrin group did the fluctuating asymmetry index decrease with increasing number of days after treatment (cf. decreasing concentration of residues with time after treatment, as mentioned above) but the fluctuating asymmetry measures for this group were lower than the controls for days 14 and 28 after treatment. No further analysis was performed on the data.

Adult bioassay

When adult *N. cornicina* were allowed to feed on dung collected three days after treatment with α-cypermethrin, a significant increase in mortality was observed (Yates Chi-square test, $P < 0.025$) (table 3). Mortality was not significantly affected in any of the other groups, although control mortality was high when dung with flumethrin and deltamethrin residues was tested. Paralysis of hind and middle legs or elevation of the hind part of the body was observed in flies exposed to dung from α-cypermethrin treated calves.

Ovarian development was significantly influenced by exposure to dung containing α-cypermethrin and deltamethrin residues (Chi-square test, both $P < 0.001$). The observed effect of flumethrin was not significant (Chi-square test, $0.2 < P < 0.1$) as was the effect of cyfluthrin. Some females from the control groups and the cyfluthrin group had fully matured eggs after four, five, and six days of feeding as evidenced by eight egg-clusters found in Petri dishes with control dung and three egg-clusters observed in dung with cyfluthrin residues. All eggs were viable and hatched except for one control egg-cluster.

The excretion rate of flies, based on counts of faecal spots per day over six days was significantly affected by each of the four treatments (ANOVA, each treatment versus control, $P < 0.001$). Flies offered dung from calves dosed with deltamethrin, flumethrin and α-cypermethrin produced fewer faecal spots than flies feeding on control dung, whereas flies given dung from calves dosed with cyfluthrin produced significantly more spots. The number of faecal spots per fly per day did not differ between control groups. In both control groups, in the flumethrin and in α-cypermethrin groups, numbers of faecal spots were significantly influenced by the number of feeding days (regression of faecal spots on number of days, $P \leqslant 0.001$). The excretion rate decreased with duration of feeding in the

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Table 3. Accumulated number of live and dead adult *Neomyia cornicina* of both sexes, and number of surviving females with ovarian stages I, II or III. The adult flies were allowed access for six days to dung from untreated calves or dung collected three days after treatment with synthetic pyrethroids.

	Nos of		Ovarian stage of female *N. cornicina*		
	live flies	dead flies	I	II	III
Control	47	13	5	3	12
Flumethrin	42	19	7	1	7
Deltamethrin	49	10	15	0	0
Control	59	0	1	3	23
α-cypermethrin	53	7	22	1	2
cyfluthrin	60	0	1	6	20

two controls and the slopes were not significantly different ($P > 0.05$). In the flumethrin group the decrease in excretion rates was less pronounced but the slope was not significantly different from the slope of the pooled controls ($P < 0.05$). By contrast, the excretion rate increased in the α-cypermethrin group and the slope was significantly different from the slope of pooled controls ($P < 0.01$). Details are given in table 4.

Discussion

The present study shows that if flies oviposit in dung voided up to at least seven days after treatment with deltamethrin, cyfluthrin or α-cypermethrin, significantly fewer adult flies will emerge from the dung (table 1). This is in agreement with the effects of deltamethrin reported by Wardhaugh et al. (1998) who found significant larval mortality of *Musca vetustissima* up to seven or 14 days after applications of two different pour-on formulations.

Larvae that survive exposure to faecal residues of deltamethrin, α-cypermethrin, or cyfluthrin develop into adults of smaller size (fig. 1a,b). This is of interest because there is a positive correlation between fecundity and size in several species of *Muscidae* (e.g. Schmidt & Blume, 1973; Ball *et al.*, 1985). Hence, reduction in size implies a reduction in the reproductive potential of *N. cornicina*. It is noteworthy that this sublethal effect was detected for a considerable length of time, i.e. up to a minimum of 14 or 28 days (the maximum number of days investigated) after treatment for α-cypermethrin, and cyfluthrin/deltamethrin, respectively.

In addition to a lowered reproductive output as indicated by a reduced size, there were also significant direct effects of drug treatments on ovarian development (table 3), which could further reduce the number of eggs produced. This effect was confined to adults fed on dung from deltamethrin and α-cypermethrin treated calves, but with a tendency for a comparable effect in the flumethrin treatment. The observation of significantly fewer faecal spots per fly per day in the deltamethrin, α-cypermethrin and flumethrin groups suggests a connection between reduced feeding rates and ovarian development. When offered dung from α-cypermethrin treated cattle the flies' feeding rate was clearly suppressed and in contrast to the other groups, increased for each day of feeding (slope of regression positive, table 4). However, the toxic effects of α-cypermethrin residues are unlikely to be reversed by more prolonged feeding as the increase in feeding rate was weak and likely to cause an increase in mortality. Whether impaired ovarian development was caused by an anti-feeding effect or a repellent effect was not investigated for any of these compounds.

The degree of sublethal stress on feeding fly larvae is expected to decrease as the synthetic pyrethroid is eliminated from the calves. This was clearly reflected in the size of the adult flies up to 14 or 28 days after treatment (fig. 1a,b). Size may thus hold potential as a sensitive bioindicator of pyrethroid residues (parent drug and/or metabolites) in the dung. Wardhaugh *et al.* (1998) used gas chromatography to detect deltamethrin residues directly. Due to a high level of detection this was only successful in three (of 10) samples collected at one, three and seven days after dosing of cattle with *c.* 75 mg deltamethrin per 100 kg (*c.* 40 mg deltamethrin per 100 kg was used in this study). The other bioindicator used here, the degree of wing vein asymmetry, did not conform to expectations; fluctuating asymmetry index values were both highest and most variable in controls, and only in the deltamethrin group did the level of fluctuating asymmetry decrease with number of days after treatment

Table 4. Faecal excretion measured as mean number of faecal spots per fly per day and linear regression of number of faecal spots per fly on the number of days (1–6) dung was offered to *Neomyia cornicina*. Dung was collected from untreated calves or from calves treated three days earlier with synthetic pyrethroids.

	Mean number of faecal spots per fly per day	Slope of linear regression	R-square
Control	34.3	−5.9	0.59
Flumethrin	8.3	−1.8	0.41
Deltamethrin	2.8	n.s.	–
Control	32.9	−4.0	0.40
α-cypermethrin	8.7	1.9	0.69
Cyfluthrin	44.9	n.s.	–

(table 2). Clarke & Ridsdill-Smith (1990) and Strong & James (1993) have shown increased levels of fluctuating asymmetry in *M. vetustissima* and *Scathophaga stercoraria* (Linnaeus) (Diptera: Scathophagidae) of a character identical to the wing vein length measured in the *N. cornicina*. The fluctuating asymmetry was highly sensitive in the sense that a significant increase was observed in flies developed in dung from cattle treated up to 35 days earlier with ivermectin or developed in dung with as low as 0.0005 ppm of ivermectin. Significantly increased fluctuating asymmetry of the same wing vein character could not be detected in a study by Wardhaugh *et al*. (1993) where *M. vetustissima* were developed in dung from sheep dosed with ivermectin or levamisole/oxfendazole. These studies, together with our results, show that fluctuating asymmetry, at least of the wing vein character measured in this study, has limited use as a general stress indicator across insecticides and fly species.

Bianchin *et al*. (1992, 1997, 1998) and Wardhaugh *et al*. (1998) report on the effects of synthetic pyrethroids on beetles belonging to the guild of small tunnelling dung beetles (Halffter & Edmonds, 1982; Doube, 1990). Adults of *Onthophagus gazella* (Fabricius) (Coleoptera: Scarabaeidae) suffered significantly increased mortality when fed on dung voided up to eight days after pour-on treatment with deltamethrin, cyalothrin, alphamethrin, flumethrin or cypermethrin (spray), with an increased mortality still evident up to approximately 18 days after treatment (Bianchin *et al*., 1992, 1997, 1998). Likewise, both *Euoniticellus fulvus* (Goeze) and *Onthophagus binodis* Thunberg (Coleoptera: Scarabaeidae) were killed by deltamethrin residues up to one week after treatment of cattle with deltamethrin (Wardhaugh *et al*., 1998). These authors also recorded reduced brood production and increased duration of juvenile development. Krüger *et al*. (1998) have investigated the dung beetle colonization and burial of naturally deposited pats from cattle treated with a topical formulation of flumethrin. Flumethrin dosing apparently did not affect the burial of dung (pats were visually inspected after 24 h and after three days) or the number of beetles in the dung remaining above ground. Contrary to Bianchin *et al*. (1992, 1998) and in line with Krüger *et al*. (1998) no toxic effects of residues from flumethrin treatments were recorded in our study with the exception of a reduced rate of faecal excretion and an indication of a reduced ovarian development. Also, the results confirm the toxic properties of deltamethrin including the effects on fecundity reported by Wardhaugh *et al*. (1998).

The lethal and sublethal effects of veterinary medicines on non-target dung fauna have been a cause of concern for more than ten years. This concern is motivated by the potential consequences for the process of dung decomposition and for the populations of dung insects. As dung organic matter is mineralized by the decomposer system, which includes a highly diverse insect fauna, it is feared that decomposition rates may be lowered if fewer insects are present in the dung. Indeed, the observed reduced rates of dung decomposition under temperate conditions have been attributed to larvicidal effects (e.g. Madsen *et al*., 1990; Sommer *et al*., 1992) and possibly to a repellent effect (Holter *et al*., 1993; Floate, 1998). By contrast, no immediate negative effect has been observed under (sub)tropical conditions, where dung burial is carried out by adult dung beetles (Wardhaugh & Mahon, 1991; Sommer & Nielsen, 1992; Sommer *et al*., 1993, Krüger *et al*., 1998). Instead, longer-term effects may result from exposure of susceptible populations (see below). Hence dung decomposition can be negatively influenced by an immediate larvicidal effect, by a repellent effect and by a longer-term population effect.

Lethal and sublethal effects such as those reported here may have the potential to suppress insect population sizes and, in particular, populations of dung insects with vulnerable life strategies. For example, the obligate dung breeding fly *Mesembrina meridiana* (Linnaeus) (Diptera: Muscidae) lays a total of only five or six eggs in her lifetime (Séguy, 1923; Skidmore, 1985). Also many subtropical and tropical coprophagous Scarabaeinae have low reproductive performances, with the extreme represented by the female *Kheper nigroaeneus* (Tribe) that lays, at most, one or two eggs per year (Edwards, 1988). That veterinary medicines may reduce populations of dung beetles that produce even 140 eggs in a lifetime has been predicted from a model based on available life history characteristics of tunnelling dung beetles (Wardhaugh *et al*., 1998). A 75% reduction in dung beetle activity (an index of cumulative total of beetles when deltamethrin was used versus cumulative total of beetles when no treatment was applied) was predicted when cattle receive a single dose of deltamethrin at the time of year when the majority of tunnelling dung beetles are non-parous. More serious is the prediction of local extinction when cattle are dosed repeatedly. The study by Wardhaugh *et al*. (1998) has evidently underlined that knowledge on the basic ecology of dung insects is warranted, if we are to predict, to any reasonable degree, the influence of perturbations such as those mediated by veterinary medicines.

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