Evaluation of the toxicity and sublethal effects of lambda-cyhalothrin against horse flies (Diptera: Tabanidae) via bioassays and exposure to treated hosts

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Abstract

Mortality of *Tabanus fuscicostatus* Hine females, engorged to repletion on bullocks treated with lambda-cyhalothrin impregnated ear tags, or placed on treated bullocks for periods of 15, 30, 45 or 60 s but not allowed to feed, was equal to or greater than 96%, while from 0% to 8% mortality was observed for controls. Average feeding time was significantly lower in *T. fuscicostatus* fed on treated bullocks (33%) than in flies fed on control bullocks, but average engorged weight was not significantly different between treatments. Mortality of *T. americanus* Forster females, placed on the backs of treated bullocks but not allowed to feed for periods of 15, 30, 45 or 60 s, was 16%, 44%, 76% and 100%, respectively. Results of bioassays in which flies were exposed to treated filter paper (LC_{50}) or were topically treated (LD_{50}) are presented per fly and per weight for *T. fuscicostatus* and *T. lineola* complex.

Introduction

Tabanids are considered to be major pests of ungulates world-wide. Livestock production losses from tabanid attack are attributed to annoyance and blood losses (Drummond, 1987) and disease transmission (Foil, 1989; Foil & Issel, 1991). In spite of their acknowledged importance, there are few control strategies for horse flies and deer flies (Anderson 1985). Several insecticide formulations have been shown to kill tabanids (Bay et al., 1976; Harris & Oehler, 1976, Presley & Wright, 1986; Foil et al., 1990; Leprince et al., 1991) but few have practical application under field conditions. Lambda-cyhalothrin has been shown to be more toxic than resmethrin, deltamethrin or permethrin against mosquitoes (Sulaiman et al., 1991; Weathersbee et al., 1991), but effects of lambdacyhalothrin on horse flies attacking treated livestock have not been investigated. The purpose of this study was to establish baseline information on the toxicity of lambda-cyhalothrin to horse flies using topical and contact bioassays, and to evaluate the effects of contact with bullocks treated with lambda-cyhalothrin ear tags on the survival, engorgement weight and feeding time of horse flies.

Materials and methods

Horse flies were collected at the Thistlethwaite Wildlife Management Area (WMA) located in central Louisiana (St Landry Parish, 30°39'N, 92°00'W). Females of Tabanus fuscicostatus Hine, T. lineola complex (T. lineola Fabricius and T. subsimilis Bellardi) and T. americanus Forster were collected in June 1991 from canopy traps (Hribar et al., 1991) synergized with carbon dioxide. As voucher specimens for unfed weight, 101 unfed females of T. fuscicostatus and 131 females of T. lineola complex were collected on 20 June, immobilized at -20°C, and weighed. In the T. lineola complex, 51 flies were T. subsimilis and the remaining flies were T. lineola. Flies for assays were removed from the traps every 2 h, transported to the field laboratory on the periphery of the WMA and transferred into 35 ml transparent plastic cups. The flies were used in assays or transferred into cardboard containers (Zyzak et al., 1989) for subsequent assays. The holding containers had two sugar cubes glued to the

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side; water and sucrose were available ad libitum and flies were maintained at $23.5 \pm 1^{\circ}$ C.

Animal assays

Six Jersey bullocks (ca. 200 kg) were used. Three bullocks were tagged three weeks prior to the experiment with two ear tags (Saber™, 10-% lambda-cyhalothrin, Coopers Animal Health, Kansas City, KS 66103) per animal on 28 May 1991, and the remaining three bullocks received insecticide-free identification tags. Treated and control bullocks were kept in noncontiguous pastures at the St Gabriel Research Station (St Gabriel, LA), and then transported in separate compartments of an animal trailer to the WMA where they were maintained in two separate portable pens. Flies used in the animal studies, which were conducted on 20 and 21 June 1991, were processed the day they were collected. In the first experiment, T. fuscicostatus females were placed individually on bullocks for 1 min but not allowed to feed. Flies that attempted to feed were dislodged by lateral movement of the inverted plastic cup. Groups of 25 flies were each exposed to the backs, front legs and back legs of 1 control and 3 treated bullocks. The front and back leg areas corresponded to areas above each leg in the region 3 described by Mullens & Gerhardt (1979). In the second experiment, T. fuscicostatus and T. americanus females were placed individually under cups on the backs of treated bullocks but not allowed to feed for periods of 15, 30, 45 or 60 s, and for 60 s on the back of a control bullock; groups of 25 flies were accumulated. In the third experiment, T. fuscicostatus females were allowed to engorge individually, until feeding ceased, on the backs of three treated and one control bullocks; groups of 25 flies were exposed on each bullock. All flies were transferred from cups into holding containers which were 2.2 1 for T. fuscicostatus and 4.4 1 for T. americanus. Mortality, which was equivalent to inability to translocate, was recorded 24 h after treatments in all assays.

Feeding time and engorgement weight

Two groups of 100 individuals of *T. fuscicostatus* were allowed to engorge until feeding ceased on the back of one treated and one control bullock. Feeding time of each fly was recorded. Engorged flies were sealed in the plastic cup with a paper top and the container was immediately deposited on dry ice to stop metabolic activities and then stored at -20°C. Specimens were thawed at room temperature for an hour, then weighed to the nearest 0.1 mg using a Mettler AE balance. After weighing, each fly was dissected and the weights of flies without blood in the midgut were not included in the analysis.

In a second trial, T. fuscicostatus were collected on 10 July 1991 and two groups of 19 flies were exposed individually for 1 min to filter papers treated with acetone or $20 \,\mu g/cm^2$ lambda-cyhalothrin in acetone, a dose that was known to induce 100% mortality within 24 h. Flies were then placed individually under plastic cups on the shaved back of an untreated bullock. Feeding time and engorged weight were recorded for each fly.

Body size

Wing length was added to the model to control for the potential confounding effect of body size in the different treatments related to feeding time and engorgement weight. Total wing length of engorged specimens was measured from the base of the costa to the tip of the wing after the wing was deposited between two slides (Leprince & Bigras-Poulin, 1988). Several specimens had damaged wing tips which prevented direct evaluation of wing length. Total wing length in mm was correlated with the distance from the base of the costa to the anterior cross vein (ACV), and from the base of the costa to the bifurcation of veins R4-R5 (R5) in 26 T. fuscicostatus. When total wing length could not be measured from either the left or the right wing, ACV or R5 was measured and transformed into total wing length values for data analysis.

Topical assay (LD₅₀)

Two-fold dilutions of lambda-cyhalothrin from 0.0005 to 5.0 µg/ml were prepared in acetone. Each individual was treated on the dorsum of the thorax with 1.0 µl of insecticide solution; controls were treated with 1.0 µl of acetone. After treatment, flies were transferred into holding containers and mortality was recorded after 24 h. The topical assay for the *T. lineola* complex was conducted on the day that the flies were collected (20 June 1991) at the WMA; there were 25 flies per dose which ranged from 0.0005 to $2.5 \,\mu\text{g/ml}$. The assay for unfed T. fuscicostatus was conducted at Louisiana State University (LSU) on 29 June; 7 d after they were collected, there were 25 flies per dose which ranged from 0.033 to 5.0 µg/ml. The assay for fed *T. fuscicostatus* was conducted at LSU on 27 June, 5 d after they were collected; there were 30 flies per dose which ranged from 0.033 to 5.0 µg/ml. The flies were fed to repletion on the shaved backs of ponies prior to treatment.

Contact assay (LC_{EO})

Solutions of lambda-cyhalothrin were made in acetone and applied in 1 ml volume to 9 cm filter papers (Whatman 1); doses in 2-fold dilutions ranged from 1.25 to $15 \,\mu g/cm^2$ (Sheppard & Hinkle, 1987). Filter papers were placed in the top and the bottom of petri dishes and flies were introduced for 1 min in groups of 2 or 3. Ten flies per dose for *T. lineola* complex (on 20 June) and 20 flies per dose for *T. fuscicostatus* (on 21 June) were processed on the day they were collected at the WMA. Mortality was recorded after 24 h.

Statistical analyses

Total wing length (mm) was regressed on ACV and R5 using the SAS regression procedure (SAS Institute Inc., 1988). Comparisons of the feeding time (s), engorged weight (mg) and body size (total wing length) of *T. fuscicostatus* fed to repletion on control and treated bullocks were made using the SAS t-test procedure (SAS Institute Inc., 1988). Weight of engorged *T. fuscicostatus* was regressed on body size (SIZE), feeding time (TIME) and treatment (TRT; 0 = control bullock, 1 = treated

bullock) using SAS general linear model procedure (SAS Institute Inc., 1988). Mortality and insecticide concentrations of topical and contact assays were analysed by probit-analysis (MicroProbit 3.0, T.C. Sparks & A. Sparks) after Finney (1971).

Results

Animal assays

Mortality of T. fuscicostatus females placed for 60 s without feeding on the control bullock was 3% (n = 100); 8% (n = 25) for the front leg, 2% (n = 50) for the back and 0% (n = 25) for the back leg. There was 100% mortality (n = 225) for T. fuscicostatus females placed for 1 min without feeding on the three body regions of the three treated bullocks. Mortality within groups of 25 females of *T. fuscicostatus* placed on the backs of treated bullocks for 15, 30, 45 and 60 s was 96%, 96%, 100% and 100%, respectively. No mortality was recorded for the 25 T. americanus females exposed 60 s to control bullocks. Mortality within groups of 25 females of T. americanus placed on the backs of treated bullocks without feeding for 15, 30, 45 and for 60 s was 16%, 44%, 76% and 100%, respectively. Mortality of T. fuscicostatus feeding on the back of a control bullock was 8% (n = 25) versus 100% (n = 75) for the flies feeding on the backs of the three treated bullocks.

Feeding time and engorgement weight

Linear regressions of total wing length (WING) in mm on ACV and R5 of T. fuscicostatus were both significant (P < 0.01): WING = 1.90ACV + 1.18, $R^2 = 0.91$; WING = 1.25R5 + 0.61, $R^2 = 0.97$) and either value could adequately be used to estimate total wing length. Totals of 85 and 93 individuals of T. fuscicostatus were considered engorged on control and treated bullocks, respectively. Wing length of T. fuscicostatus females fed on control $(9.02 \pm 0.51 \text{ mm} \pm \text{SD})$ and treated bullocks $(8.96 \pm 0.49 \text{ mm})$ did not vary significantly (t-test, df = 176, P > 0.05). The engorged weight of T. fuscicostatus females fed on control (82.4 ± 18.2 mg) and treated bullocks $(86.3 \pm 18.8 \text{ mg})$ did not vary significantly (ttest, df = 176, P > 0.05). The average feeding time of flies fed on treated bullocks (104.8 \pm 49.8 s) was significantly smaller (33%) than flies fed on controls (156.1 \pm 93.3 s) (ttest, df = 176, P < 0.01). A linear regression analysis of engorged weight of T. fuscicostatus indicated that SIZE

contributed significantly to the variation of engorged weight (P < 0.001) while variables TRT and TIME, which were added to control for treatment and feeding time effect, did not relate to engorged weight (P > 0.05; table 1).

A total of 38 individuals of T. fuscicostatus, equally divided between treatments, engorged successfully on an untreated bullock after being exposed for 1 min to filter papers impregnated with acetone and lambdacyhalothrin. The size of T. fuscicostatus females exposed to acetone treated filter papers (8.82 ± 0.51 SD mm) did not vary significantly from the size of flies exposed lambda-cyhalothrin treated filter $(8.82 \pm 0.54 \text{ mm})$ (t-test, df = 36, P > 0.05). The engorged weight of T. fuscicostatus females exposed to acetone and treated filter papers prior to engorgement on bullocks was not significantly different $(80.9 \pm 19.6 \text{ mg})$ versus 82.1 \pm 14.9 mg, respectively; t-test, df = 36, P > 0.05). The average feeding time of control flies (97.4 \pm 52.1 s) was 21% greater than treated flies (79.9 \pm 44.2 s) but this difference was not statistically different due to a small sample size and large variance (t-test, df = 36, P > 0.05).

Topical and tarsal assays

The LD_{50} for topical assays and LC_{50} for filter paper assays of lambda-cyhalothrin to T. fuscicostatus and T. lineola complex are presented in table 2. Mortality in all controls averaged 6%. The LD_{50} s for unfed and engorged T. fuscicostatus were 0.243 ng/fly and 0.197 ng/fly, respectively; when LD_{50} results were adjusted for weight, the differences increased (6.38 ng/g for unfed and 2.40 ng/g for engorged flies). The LD_{50} for unfed T. lineola complex was less than that calculated for T. fuscicostatus (table 2), but the LC_{50} s for the two species were similar (3.495 µg/cm² versus 3.169 µg/cm², respectively).

Discussion

When bullocks were fitted with two ear tags containing 10% lambda-cyhalothrin three weeks prior to assay, there was enough insecticide at three body areas to kill all tabanids (*T. fuscicostatus*) that stayed on the host for at least 1 min (fed or unfed). Furthermore, mortality of flies (*T. fuscicostatus*) remaining in contact with hosts for 15 s was 96%. The feeding time for flies (*T. fuscicostatus*) was reduced by 33% by treatment but the engorged weight was not affected. The difference in feeding time was not related to differences in host's haircoat between treatments since the reduction in feeding time was also

Table 1. Linear regression of engorged weight on body size, treatment (control and lambda-cyhalothrin ear tagged bullocks) and feeding time for 178 *Tabanus fuscicostatus* females^a

Variable	Parameter estimate	Standard error	t	P value	
Intercept	-0.1343	0.0194	-6.91	< 0.001	
SIZE	0.0244	0.0021	11.71	< 0.001	
TRT	0.0041	0.0021	1.90	0.059	
TIME	-0.00002363	0.00001412	-1.67	0.0960	

The residual sum of squares for the regression was 0.0288 (3 df) and the error sum of squares was 0.0321 (174 df), the model was highly significant (P < 0.001).

observed for flies exposed to lambda-cyhalothrin and then fed on shaved bullocks. Differences in body size between treatments also did not influence these results (table 1). This is the first report of a commercially available ear tag having such activity against tabanids. We previously reported reduced feeding time and engorged weight of tabanids feeding on cattle sprayed with 0.05% fenvalerate (Foil *et al.*, 1990). Up to 79% mortality was reported for tabanids feeding on cattle sprayed with 0.02% fenvalerate but uncorrected mortality for fenvalerate tag treatments was only 15% (Leprince *et al.*, 1991). There have been no previous studies on feeding time and engorged weight for tabanids feeding on livestock treated with ear tags.

Mortality of T. fuscicostatus that remained on the treated bullocks for 45 s was 100% and the average engorgement time for the flies feeding on tagged bullocks was 104 ± 50 s. All T. fuscicostatus females that were fed to repletion on treated animals died. Since we saw no evidence that tag treatments prevented feeding by tabanids, we speculate that the majority of tabanids equivalent in size to T. fuscicostatus would be killed after feeding on animals treated in a similar way.

The largest horse fly in North America is T. americanus (Pechuman et al., 1983). The average unfed weight of T. americanus, 522 ± 93.2 mg (Zyzak et al., 1989), is more than 10 times greater than T. fuscicostatus (table 2). The average feeding time of T. americanus females on untreated cattle is 153 ± 122 s (n = 10; L.D. Foil & D.J. Leprince, unpublished data). If there were a reduction in the engorgement time of T. americanus feeding on treated bullocks, similar to that recorded for T. fuscicostatus (33%), the average engorgement time would still be 1.7 times (103 s) greater than the one minute exposure that induced 100% mortality for T. americanus. Blood ingestion does not decrease susceptibility of T. fuscicostatus females to topical application of fenvalerate (Zyzak et al., 1989) or lambda-cyhalothrin (table 2) when LD₅₀s are expressed in µg/g of engorged weight. Therefore, under the present conditions, the survival of tabanids feeding on animals treated three weeks previously with two lambda-cyhalothrin ear tags appears unlikely.

The fact that the LD₅₀s for T. lineola complex and T. fuscicostatus were different and the LC₅₀s were the same was probably influenced by the pretreatment handling (table 2). The LC₅₀s were derived from flies processed immediately after capture, as was the LD₅₀ for the T. lineola complex. The flies used for the topical assays for T. fuscicostatus were held for 5-7 days in conditions where they had sucrose available ad libitum. In tabanids, carbohydrates are directed to the diverticulum (Stoffolano, 1983).

It has been observed that the diverticulum of tabanids held under laboratory conditions can be greatly distended when compared to field collected flies (D.J. Leprince, unpublished observation). In the evaluation of the toxicity of fenvalerate to tabanids, Zyzak et al. (1989) assayed flies on the same day that they were collected without providing them with sucrose prior to the treatment. The T. lineola complex was treated similarly in this study and comparison of the LD₅₀s for fenvalerate and lambdacyhalothrin between studies indicate that lambdacyhalothrin is 100-fold more toxic than fenvalerate.

Lambda-cyhalothrin ear tags are the first ear tags found to be effective in killing tabanids that attack cattle. The duration of this activity post-tagging still needs to be ascertained. As the concentration of insecticide on animals decreases over the season, a proportion of the horse fly populations may be subjected to a sublethal dose of insecticide. Recent studies have shown that an estimated 75% of the tabanids exposed to a sublethal knockdown dose of fenvalerate sprayed on cattle survived and attempted to seek a subsequent host (Foil *et al.*, 1991).

The impact of mortality of tabanids attacking cattle on the seasonal and yearly burden of flies is unknown. Studies on the parity rates of tabanids trapped in areas where treated and untreated cattle have been held, indicate that there is a portion of tabanid populations that return to the site of a successful bloodmeal for a subsequent bloodmeal (Foil et al., 1989). However, those studies were conducted on one species of Tabanidae (T. fuscicostatus) in areas contiguous to larval habitats. There are no studies that can be used to estimate the impact that the use of ear tags that effectively kill tabanids subsequent to feeding on treated hosts could have, or might have already had, on local tabanid populations. Therefore, the application of this study could be more in the introduction of a tool (the lambda-cyhalothrin tag) to study tabanid population associations with domestic livestock. For example, incidence and parity rate of tabanid populations attacking tagged and control herds could be compared. The results of this study may also be applicable to control strategies for other large dipterous pests of livestock, such as tsetse flies.

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Table 2. Topical LD₅₀, tarsal LC₅₀ and their fiducial limits (95% FL) of lambda-cyhalothrin on a per fly and per weight (WT) in mg basis.

Species	Treatment	Status	No. flies	LD_{50} or LC_{50}	No. flies	WT±SD	LD_{s_0} or LC_{s_0}	Slope (SE)
T. fuscicostatus	Topical	Unfed	300	0.243 (0.209 - 0.293)a	101	38.1 ± 8.2	6 38 (5.47 - 7.69) ^b	3 83 (0.66)
T. fuscicostatus	Topical	Engorged	200	0.197 (0.153 - 0.251) ^a	85	82.4 ± 18.1	2 40 (1.86 - 3.04) ^b	2.81 (0.68)
T. lineola complex	Topical	Unfed	250	0.132 (0.103 - 0.162) ^a	131	59.9 ± 16 1	2.20 (1.72 - 2.72)b	3.53 (0.63)
T. fuscicostatus	Tarsal	Unfed	160	3,169 (2,597 - 3,926) ^c	101	38.1 ± 8.2	83 1 (68.1 - 103.0)d	3.54 (0.87)
T. lineola complex	Tarsal	Unfed	250	3,495 (2,021 - 5,339)°	131	59.9 ± 16.1	58 3 (33.7 - 89.1) ^d	3.53 (0.63)

 $^{^{}a}LD_{50}$ ng/fly, $^{b}LD_{50}$ ng/g, $^{c}LC_{50}$ ng/cm², $^{d}LC_{50}$ ng/cm²*g.

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