Survival and Dispersal of Horse Flies (Diptera: Tabanidae) Feeding on Cattle Sprayed with a Sublethal Dose of Fenvalerate

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ABSTRACT A mark–recapture study was undertaken to determine the fate of tabanids feeding on cattle sprayed with a sublethal dose of fenvalerate. A total of 15,465 tabanids belonging to at least 17 species and five genera were marked individually; flies were divided equally between control and fenvalerate-sprayed bullocks. Data were summarized for the four most abundant species: *Tabanus fuscicostatus* Hine, *T. lineola* F., *T. pallidescens* Philip, and *T. wilsoni* Pechuman. The recapture rate of tabanids was 6.6% for the control and 3.4% for the fenvalerate treatment. When the proportion of flies predicted to die or be unaffected after feeding on the sprayed bullocks was considered, an estimated 75% of the flies exposed to a sublethal knockdown dose survived and attempted to seek a subsequent host. Dispersal of tabanids was not affected by treatment. The recapture date after the first day of marking was significantly delayed for tabanids that fed on fenvalerate-sprayed bullocks.

KEY WORDS Insecta, *Tabanus* spp., knockdown, pyrethroids

HORSE FLIES ARE MAJOR PESTS of ungulates worldwide. Livestock production losses from tabanid attack are attributed to annoyance, blood losses (Drummond 1987), and disease transmission (Krinsky 1976, Foil 1989). In spite of their importance, there are few control strategies for horse flies and deer flies (Anderson 1985). The use of residual insecticides to protect livestock from tabanid attack is limited, although several insecticide formulations have been shown to kill tabanids (Wilson 1968, Bay et al. 1976, Harris 1976, Harris & Oehler 1976, Presley & Wright 1986). Sublethal effects of a pyrethroid, fenvalerate, including reduction in bloodmeal size and feeding time of tabanids by 30%, also have been reported (Foil et al. 1990).

Tabanids collected from cattle that are sprayed with fenvalerate either die within a specified time, are unaffected, or are incapable of coordinated movement during a period after collection but survive under appropriate laboratory conditions (i.e., are exposed to a knockdown dose). Because the concentration of pyrethroids to which flies are exposed varies with time and type of treatment (Taylor et al. 1985, Yeung et al. 1988), the survival of flies exposed to sublethal doses in nature should be considered.

Our study was designed to determine if a knockdown dose in nature would result in death. The objective of our experiment was to assess survival, dispersal, and subsequent host-seeking activity of horse flies feeding on cattle sprayed with a sublethal dose (for some tabanids) of fenvalerate under field conditions.

Materials and Methods

Determination of a Knockdown Dose. Four untreated Jersey bullocks were held in pastures at the St. Gabriel Research Station, St. Gabriel, La., and were transported to the Thistlethwaite Wildlife Management Area (WMA) in central Louisiana (St. Landry Parish, 30°39'N, 92°00'W). The bullocks were individually sprayed with 0.001, 0.002, 0.0025, and 0.005% (AI) of a fenvalerate water dispersible liquid mixture (Fermenta Animal Health, Kansas City, Mo.) at a volume of 4–6 liters of the mixture applied at 690–1,380 kPa (100–200 psi). Bullocks were placed individually in portable stanchions at the WMA, and 25 engorged (ceased feeding without subsequent probing) tabanids (a mixture of the four most abundant species: *Tabanus fuscicostatus* Hine, *T. lineola* F., *T. pallidescens* Philip, and *T. wilsoni* Pechuman) were collected at their natural feeding site from each of the treated bullocks. A 30-ml transparent plastic cup was placed over the fly and a paper lid was inserted between the fly and the hair of the animal. Flies were then transferred in a 2.2-liter cardboard holding container (Zyzak et al. 1989) containing sugar cubes glued to the side, and water was offered ad libitum. Holding containers were maintained at a temperature of 23.5 ± 1°C for 24 h in a laboratory at the WMA. Symptomology of the flies was recorded at 2 h, and mortality was recorded at 24 h after treatment. All

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flies that could not walk after probing at 24 h were recorded as dead.

Predation Experiment. Horse flies were allowed to engorge on two controls and two bullocks sprayed with 0.002% fenvalerate. The control and the treated bullocks were placed in portable stanchions <50 m apart at the WMA, and fly collection was simultaneous at both sites. Tabanids were collected in plastic cups, as described above, and held in standard plastic food trays suspended above frozen ice-packs inside ice chests for periods of 15–30 min. Each fly then was placed individually under the catch container of a Pherocon boll weevil trap (Hercon Laboratories Corp., South Plainfield, N.J.). The bottom of this "trap" is a screen cone (9 cm tall) with a base 11 cm in diameter. The cone protrudes ≈4 cm into the top portion of the trap, which is a plastic cylinder (5.5 cm tall) with a diameter of 5 cm. A total of 100 traps were placed along three transects parallel to the forest margin: 50 m into the forest (20 stations), forest margin (15 stations), and 30 m into an open grassy area (15 stations). Stations along each transect were separated by 5 m and comprised two traps, one for a control fly and one for a fly collected on a sprayed bullock. The ground was cleared and flattened to prevent escape of the flies from apertures between the ground and the side of the traps. However, there was sufficient space for small predators to enter the traps through the screen cone and under the bottom edges of the traps. Flies were considered alive if they or their remnants were in the top of the trap at 24 h and dead if they did not move to the top part of the trap. No live flies were found in the bottom portion at 24 h. The experiment was replicated three times on 13, 14, and 22 June 1987.

Mark–Recapture Experiment. Jersey bullocks (two control and two sprayed) were placed daily in portable stanchions in the WMA from 6 to 13 June 1988 between 1030 and 2030 hours (CST). The distance between the marking sites for the control and the treated bullocks was 120 m. The two treated bullocks were sprayed with a fenvalerate solution (0.002%) every morning and washed with ≈100 liters of water at the end of each day. Horse flies were allowed to land on the host and were marked with latex paints when engorgement was imminent. A small dot of latex paint was applied to the thorax of a tabanid with a paint brush, and flies were allowed to disperse upon repletion. Different colors were used to allow identification of flies marked on control and treated bullocks during the first and second half of the marking period. Marking site, treatment (color), date, time, species, and location of the feeding site were recorded for each marked fly. Each day, 25 engorged flies were collected from control and treated bullocks to evaluate knockdown and mortality as described above.

A total of 22 canopy traps (Catts 1970) baited with carbon dioxide (dry ice) was placed in a circular pattern along roads and a pipeline at 0–3.1 km from the marking sites; collections were made from 6 June to 4 July. After the marking period (June 14), one canopy trap was set at each marking station. Traps were located at distances of 0 km (two traps after the marking period), 0.8 km (four traps), 1.6 km (seven traps), 2.0 km (four traps), and 3.1 km (five traps) from the marking sites.

Statistical Analyses. Chi-square tests were used to test for independence between two classification variables in the predation experiment and two and four variables in the dispersal experiment. Each recaptured fly was assigned a recapture date according to the first day of the 4-d marking period (6–9 June or 10–13 June). The value 1 was given to the first marking day of each period. Because dry-ice baiting of and daily collection from canopy traps occurred once daily in the morning, the catch was considered that of the preceeding day. *T* tests and analyses of variance were used to compare the average recapture period of tabanids feeding on control and sprayed bullocks (SAS Institute 1988).

Results

Knockdown Dose. The percentages of knockdown at 2 h and mortality at 24 h for the different concentrations of fenvalerate were 50 and 0% at 0.001%, 84 and 28% at 0.002%, 100 and 60% at 0.0025%, and 100 and 88% at 0.005%, respectively. Control flies were not collected during this preliminary study, but <1% mortality and no knockdown was recorded for control flies collected from 6 to 13 June 1990. The 0.002% fenvalerate concentration was selected as the standard dose for the remainder of this study because it gave the highest proportion of knockdown with minimal mortality.

Predation Experiment. The survival rate was 96% in the 150 flies collected on control bullocks and 37% in the 150 flies collected on fenvalerate-sprayed bullocks. No statistical differences were detected in survival among transects for flies fed on control bullocks ($\chi^2 = 0.03$, df = 2, $P > 0.05$) or fenvalerate-sprayed bullocks ($\chi^2 = 0.51$, df = 2, $P > 0.05$). Based upon the 84% knockdown and 28% mortality for flies collected from bullocks sprayed with 0.002% fenvalerate, 31 of the flies from treated bullocks that survived should have undergone knockdown.

Mark–Recapture Experiment. Seventeen tabanid species belonging to four genera and some unidentified *Chrysops* spp. were marked on control and sprayed bullocks (Table 1). The numbers of engorging flies marked was 7,779 on controls and 7,686 on sprayed bullocks. The most abundant species marked on the bullocks in descending order were *T. wilsoni*, *T. pallidescens*, *T. lineola*, and *T. fuscicostatus* (Table 1).

The proportion of knockdown and unaffected flies after 2 h and mortality at 24 h was evaluated daily for pools of 25 flies of the four most abundant tabanid species (Table 2). The LD_{50} for fenvalerate for these four species was not significantly different

Table 1. Species composition of tabanids marked on control and fenvalerate-sprayed bullocks and recaptured on bullocks or in canopy traps, and unmarked flies collected in canopy traps

Species	Marked			Recaptured			Canopy trap
	% Control	% Treatment	% Total	% Control	% Treatment	% Total	% Traps
T. fuscicostatus	7.2	4.9	6.1	14.2	13.0	13.8	45.1
T. lineola lineola	11.5	11.0	11.3	13.4	21.8	16.2	31.4
T. pallidescens	35.6	35.6	35.6	31.5	18.0	26.9	13.0
T. wilsoni	43.3	47.1	45.2	35.9	42.5	38.1	6.4
Other tabanid species[a]	2.4	1.4	1.9	5.0	4.6	0.6	4.2
n	7,779	7,686	15,465	515	261	776	275,522

[a] *Chlorotabanus crepuscularis* (Bequaert), *Diachlorus ferrugatus* (F.), *T. americanus* Forster, *T. atratus atratus* F., *T. equalis* Hine, *T. calens*, L., *T. limbatinevris* Macquart, *T. molestus* Say, *T. proximus* Walker, *T. stygius* Say, *T. turbidus* Wiedemann, *T. trimaculatus* Palisot de Beauvois, *Whitneyomyia beatifica atricorpus* Philip, and some unidentified *Chrysops* spp.

(Zyzak et al. 1989); therefore, survival data of flies of these four species also were combined for comparisons between flies marked on control and treated bullocks.

A total of 7,595 engorged tabanids belonging to the four most abundant species were marked on control bullocks. When corrected for a 4% mortality in the rearing container for the flies collected on the last marking day from control bullocks, 7,568 flies were considered marked. A total of 7,550 engorged tabanids were marked on sprayed bullocks. Based on the daily symptomology of collected flies, 1,420 flies should have been unaffected by the treatment, 3,251 should have received a knockdown dose and 2,879 should have died (Table 2).

A total of 275,522 flies distributed in seven genera and at least 21 species were collected from canopy traps. *Hybomitra lasiophthalma* (Macquart), *Leucotabanus annulatus* (Say), *T. nigripes* Wiedemann, and *T. venustus* Osten Sacken were collected only from canopy traps. The four most abundant species of tabanids collected from canopy traps by descending order were *T. fuscicostatus*, *T. lineola*, *T. pallidescens*, and *T. wilsoni* (Table 1).

A total of 776 flies were recaptured: 6.6% (n = 515) of the flies marked on control and 3.4% (n = 261) of the flies marked on treated bullocks (Table 1). Tabanids were recaptured between day 3 and day 26 of the experiment. The average time until recapture for tabanids feeding on control bullocks was 7.06 ± 3.04 ($\bar{x} \pm$ SD) d, which was significantly lower than 7.53 ± 3.10 d for tabanids feeding on sprayed bullocks ($t = -1.99$, df = 736, $P < 0.05$). Based on the recapture rate for flies marked on control cattle, 92 of the 1,420 flies that should have been unaffected after feeding on sprayed bullocks should have been recaptured. A total of 249 flies of the four predominant species were marked on sprayed bullocks and recaptured. Therefore, 157 of the recaptured flies should have undergone knockdown, which extrapolated to a rate of 75% recovery from knockdown.

The average dispersal of flies was 0.57 ± 0.80 km from the marking sites; 80% of the recaptured flies were collected within 0.8 km from the release point. Dispersal of flies marked on control and sprayed bullocks and captured from 0–3.1 km from the marking sites did not vary significantly within *T. fuscicostatus* ($\chi^2 = 1.30$, df = 3, $P > 0.05$), *T. lineola* ($\chi^2 = 2.81$, df = 3, $P > 0.05$), *T. pallidescens* ($\chi^2 = 2.08$, df = 2, $P > 0.05$), or *T. wilsoni* ($\chi^2 = 1.34$, df = 3, $P > 0.05$). Therefore, the total number of recaptured flies was used to compare dispersal of the four species; dispersal patterns were different ($\chi^2 = 89.90$, df = 9, $P < 0.01$) (Table 3). Sixty-four percent of 588 flies of the four species that were recaptured on days 1–8 of the experiment were recaptured on the bullocks at the marking sites. Conversely, of 101 flies recaptured on days 9–12 and 150 flies recaptured on days 9–27, only 38 and 30%, respectively, were recaptured in canopy traps placed at the marking sites.

Discussion

Survival rate in the predation experiment was similar in the open, the forest margin, and under the forest canopy, suggesting that recovery was not habitat-related. Survival of horse flies predicted to receive a knockdown dose of fenvalerate was estimated to be 37% in the predation experiment,

Table 2. Number of flies belonging to the four most abundant *Tabanus* species marked on fenvalerate-treated bullocks, percentage of unaffected flies (2 h), mortality (24 h), and number of predicted survivors based on 25 engorged flies collected from bullocks

Day	No. marked	% Unaffected	% Mortality	Knockdown survivors predicted
June 6	984	16	28	551
June 7	979	24	28	470
June 8	982	20	20	589
June 9	975	16	24	585
June 10	995	28	44	279
June 11	989	4	72	237
June 12	991	36	28	357
June 13[a]	655	0	72	183
Total	7,550	19	38	3,251

[a] Corrected for 4% mortality in control.

which was half the survival predicted by the mark–recapture experiment. The greater mortality rate in the predation experiment may have been related to habitat modification (setting traps and flies on bare soil) or the duration and the artificial nature of the captivity. Furthermore, flies receiving a knockdown exposure (but not confined) may have maintained a certain level of wing or leg movement that could prevent or delay the attack of small predators such as ants. The results of this preliminary predation experiment indicated that some percentage of flies receiving a knockdown exposure did survive and justified the much more labor intensive mark–recapture experiment.

Horse flies feeding on sprayed bullocks dispersed similarly to those fed on controls, indicating that the flight capacity of surviving flies was not influenced by knockdown. The majority of the horse flies exposed to a knockdown dose of fenvalerate survived, but there was a delay in the average time of recapture by half a day.

The primary objective of this paper was to describe the survival and dispersal of flies that fed on cattle sprayed with a "knockdown dose" of a pyrethroid. Differences in the dispersal (recapture after assumed oviposition) (Table 3) of different tabanids could be attributed to many factors beyond the scope of this experiment, including the location of larval habitats and weather conditions. We plan to conduct more detailed analyses on several aspects of the data (e.g., population estimates). The percentage of different tabanid species obtained from the catches from bullocks and canopy traps cannot be directly compared because of temporal and spatial differences in trapping efforts, but the differences do warrant further investigation (Table 1). If tabanid sampling differences (other than those of magnitude) between animals and traps exist, the interpretation of tabanid population studies using a mixture of traps and animals should and will have to weigh "trap" differences.

The fact that 64% of the flies recaptured on days 1–8 were caught on the bullocks adds to the concept that the dry ice-baited canopy traps and cattle differ in attraction of host-seeking flies. Only comparisons of survival and dispersal differences between treated and control flies were made in this study, and both treatments were equally sampled by the two sampling tools. However, future studies will be required to determine whether the magnitude and diversity differences are due to differences in sampling radius, trap efficacy, or differences in the physiological status of captured flies.

The recovery of flies after a knockdown exposure to a pyrethroid was unexpected and contradicted the dogma associated with pyrethroid use. Crop insects that receive a knockdown dose of pyrethroids may have subsequent pyrethroid exposure before escaping the treated area because they refeed or fall upon treated substrates. Conversely, horse flies are exposed to insecticide only during probing and feeding on their treated host. The first

Table 3. Dispersal of the four most abundant species of horse flies marked on control and fenvalerate-sprayed steers

Tabanus spp.	Distance, km				Total
	0	0.8	1.6	>1.6[a]	
T. fuscicostatus	34	43	16	14	107
T. lineola	51	36	16	23	126
T. pallidescens	132	52	20	5	209
T. wilsoni	205	40	37	14	296
Total	422	171	89	56	738

[a] 2.0 and 3.1 km combined.

30 s on the host are determinant, as feeding times greater than 30 s do not influence mortality (Foil et al. 1990). Because recovery of horse flies occurs in an untreated environment, mortality may be lower than that for crop insects. Adult hematophagous Diptera (e.g., horn flies) that use the vertebrate host as an exclusive source of energy may develop knockdown resistance as a mechanism of survival. However, selection pressure for knockdown resistance in Diptera that use vertebrate blood primarily for oogenesis would appear to be absent if oviposition does occur after exposure to a knockdown dose of insecticide.

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