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IN VITRO ASSAY FOR PERMETHRIN PERSISTENCE AND INTERFERENCE WITH BLOODFEEDING OF *CULICOIDES* (DIPTERA: CERATOPOGONIDAE) ON ANIMALS

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ABSTRACT. Permethrin (65% AI) was applied to the dorsum of 3 goats (3–4 ml/animal). Hair clippings were taken before treatment and 4–6, 18–20, 39–41 and 67–69 days after treatment. *Culicoides variipennis* fed upward through a thin layer of hair and through a membrane on an artificial feeder containing cattle blood. Midge knockdown was usually complete after the 45 min exposure period, but many bloodfed first. Belly hair residues did not statistically reduce engorgement beyond 4–6 days. Head hair residues reduced engorgement by up to 98% at 18–20 days, but did not significantly reduce engorgement at 67–69 days. Back hair residues significantly reduced engorgement for the entire period (99–100% through day 39–41 and 66% by day 67–69). Partial engorgement was higher in the treatments.

INTRODUCTION

Efficacy testing of insecticidal or repellent compounds on bloodfeeding Diptera commonly is done with human volunteers, who can note the number of bites (Harbach et al. 1990). The information can then be extrapolated to estimate whether treatments might reduce disease agent transmission. This method obviously is not possible in assessing treatment efficacy on animals. Typically the residual effects of insecticides applied to animals are assessed by direct observation of feeding insects in the field (Foil et al. 1990). This may risk bias due to the proximity of a human observer, but, more significantly, observation generally is not possible for many of the smaller crepuscular or nocturnal species. An alternative method is to expose insects directly to animals or treated substrates under controlled conditions. Holbrook (1986), for example, took hair clippings from ear-tagged (permethrin-treated) and untreated cattle, all held under field conditions, and showed that the treated hair was toxic to *Culicoides variipennis* (Coquillett) adults for at least 70 days after treatment.

The present studies were initiated after it was found that free-ranging desert bighorn sheep in southern California were subject to high lamb mortality influenced by hemorrhagic disease viruses, presumably transmitted by *Culicoides* spp. (Mullens and Dada 1992). Persistent pesticides might be used to treat field-captured animals and protect against *Culicoides* attack (Holbrook 1986).

MATERIALS AND METHODS

Domestic goats, which have a hair coat similar to that of bighorn sheep, were used as a model. Hair was clipped from the upper side of one goat. Preliminary laboratory experiments used 36–72-h-old *Culicoides variipennis* reared in the laboratory (Jones et al. 1969). Approximately 200–300 adult midges (mixed sexes; approximately half females) from the same cohort were lightly anesthetized with CO_2 and added to each of a number of clean 23 ml (0.75 oz) plastic cups. A piece of sheer nylon stocking (15 denier) was stretched tightly across the top of each cup (surface area 19.6 cm^2) and affixed with a small rubber band. Approximately 50–70 mg of hair from the goat then was arranged loosely and evenly on the top of the nylon. Blood feeders (Lillie Glassblowers, Inc., 3431 Lake Drive, Smyrna, GA 30082) were used to warm cattle blood (EDTA added to prevent clotting) to 37°C above a stretched parafilm membrane (Hunt and McKinnon 1990). Each cup then was raised immediately to contact the membrane. The midges would move upward to the feeding surface and probe successfully through the nylon mesh, hair and membrane to obtain a blood meal. A period of 45 min was allowed for bloodfeeding. A pretreatment batch of hair similarly was clipped from the upper side of each goat and used as control hair throughout the tests.

Three castrated male goats (47.7–70.5 kg body weight) used in the test were housed in individual, contiguous, chain-link fence enclosures. They were outside during the day under shade cloth and were inside at night. When outside, they received approximately 1–3 hours of direct sun/day. The tests were done in the fall, with mean maximum daytime temperatures generally 25–35°C. A permethrin formulation containing 65% AI, packaged in 1-ml plastic ampules, was selected for the tests and applied at either 3 or 4 ml/animal (depending on body weight). This high concentration formulation, used commercially for ectoparasite control on dogs, was intended to enhance persistence in the field. The amount re-

quired per animal could easily be carried in a researcher's pocket and applied quickly to sheep captured for study in remote bighorn sheep habitats, thus minimizing stress to the animal. On day 0 the permethrin was applied to one goat along the dorsal midline. After 2 days with no apparent ill effects, the other 2 goats were treated similarly.

On day 6 (4–6 days post-treatment) hair clippings were taken from near the ventral midline, the head around the base of the ears, and the dorsal midline (in that order, cleansing the clippers thoroughly with soap and hot water between animals). This was done because some *Culicoides* spp. have preferred body regions for feeding (e.g., Schmidtmann et al. 1980, Braverman 1988) and we wanted to determine how the permethrin might become distributed around the body. Care was taken not to remove hair from exactly the same spot on different dates. Hair samples were placed in separate clean plastic petri dishes identified by animal and body region.

In the laboratory, three glass feeders were arranged in series. Adult *C. variipennis* were placed in clean, labelled cups as above, and a set of controls (untreated hair from each goat) was run to establish a baseline feeding rate for that cohort of midges. After the 45-min feeding period, the cups were removed, and new membranes and blood were used in the feeders. Treated hair from the same body region of each animal quickly was applied to the top of the nylon, and the cups were brought into contact with the membranes. After the feeding period, the procedure was repeated with hair from another body region.

After each feeding period we observed the midges to determine the number knocked down (midges on the bottom of the container incapable of directed movement) or alive (crawling, usually on the sides or top of the container). The containers then were frozen for 1–24 hours and the midges sexed and counted under a dissecting microscope. Midges with visible blood in the gut were counted as engorged. If the blood occupied ≥3 abdominal segments, the midge was considered fully engorged; less was scored as partial engorgement. Hair was clipped and the procedures were repeated on days 18–20, 39–41 and 67–69. The percentage engorgement was transformed to arcsin $\sqrt{\text{proportion}}$ for analysis of variance (ANOVA) and *t*-tests to compare different animals, test dates and body regions.

RESULTS AND DISCUSSION

A two-way ANOVA first was done to test for differences among dates and animals for untreated (control) hair. In no case were more than 6 midges knocked down in any control cup throughout the test. There were significant differences among days ($F = 9.21$; $df = 3,6$; $P < 0.01$), with days 18–20 having a significantly lower engorgement rate using Tukey's test to separate means. There was no difference among animals ($F = 0.147$; $df = 2,6$; $P > 0.05$), so control data were pooled for each date to obtain an estimate of control engorgement. This was used to calculate the reduction in engorgement for midges feeding through treated hair from each body region for that test date (Table 1).

Table 1. Engorgement of *Culicoides variipennis* allowed to feed on cattle blood (parafilm membrane, artificial blood feeder) through hair from different body areas of permethrin-treated goats. Treatment applied to dorsal midline.

Days after treatment	Body area	% engorgement (SD)[1]	% reduction in engorgement[2]
4–6	Control[3]	82.2 (1.3)a	—
	Belly	40.2 (1.1)b	51.1
	Head	5.2 (7.8)c	93.7
	Back	0.2 (0.7)c	99.8
18–20	Control	41.3 (1.0)a	—
	Belly	15.4 (3.4)ab	62.7
	Head	1.0 (3.1)bc	97.6
	Back	0.1 (0.3)c	99.8
39–41	Control	70.6 (0.9)a	—
	Belly	55.8 (1.3)a	21.0
	Head	29.4 (0.8)b	58.4
	Back	0 (0)c	100.0
67–69	Control	65.7 (0.1)a	—
	Belly	79.6 (0.7)a	0.0
	Head	45.9 (1.6)ab	30.1
	Back	22.6 (7.1)b	65.6

[1] Mean (standard deviation) of arcsin $\sqrt{\text{proportion}}$-transformed data, backtransformed for table. Means within a column for a given feeding day followed by the same letter are not significantly different ($P > 0.05$) using Tukey's test.

[2] [(% engorgement in control − % engorgement in treatment)/% engorgement in control] × 100.

[3] Pretreatment control hair from upper side of animal.

One-way ANOVAs were conducted on the data for each test date, using Tukey's test to separate means (Table 1). Partial data for one goat (treated head and back hair) were omitted from the day 67–69 analysis due to complete lack of feeding by those midges, possibly due to inadequate membrane stretching. We did not have enough adult midges from the same cohort to repeat the feeding attempts on those hair samples for that date. Residue on back hair consistently resulted in complete knockdown of the midges by the end of the 45-min feeding period. It also significantly

reduced engorgement (≥99.8% reduction relative to controls through days 39–41), though reduction had declined to 65.6% by days 67–69. Residues on head hair also knocked down all midges by the end of the 45-min feeding period and resulted in intermediate reductions in feeding. Reduction was very high early in the test, but declined to 30.1% (not significantly different from controls) by days 67–69. Hair from the belly was least active; a few midges still were active after the 45-min feeding period on days 4–6, and approximately 10% still were active after the exposure period by days 67–69. Residues on belly hair significantly reduced feeding only on days 4–6 post-treatment, though the reduction in percentage terms actually was slightly higher on days 18–20. By days 67–69, engorgement through belly hair slightly exceeded engorgement through control hair, even though most midges were knocked down by the end of the feeding period.

The frequency of partial feeding (% of those fed that were partially engorged) was significantly higher for flies feeding through treated hair (chi-square ≥ 5.40, 1 df, $P < 0.05$) for all test dates except day 39–41 (chi-square = 0.882, 1 df, $P > 0.05$). Partial engorgement was highest on days 4–6 (22.5% for treated hair and 5.9% for control hair) and days 18–20 (27.8% for treated hair and 2.9% for control hair). Partial engorgement was only 2.0% through treated hair on days 39–41 and 4.9% on days 67–69 (versus 0.9% and 1.3% for the respective controls).

Foil et al. (1990) observed and captured adult tabanids visiting control and fenvalerate-treated (0.5% AI) cattle in the field (day of treatment) and documented significantly reduced feeding times and a reduction in engorged weights for some species attacking treated cattle. The increased frequency of partial engorgement reported here agrees with the tabanid findings. Nasci et al. (1990) did not conduct direct animal observations, but showed that the frequency of bloodfeeding in field-collected, resting *Psorophora columbiae* (Dyar and Knab) and *Anopheles quadrimaculatus* Say was significantly reduced in treated versus control areas following permethrin treatment (0.05%) of cattle herds in those areas; bloodfeeding success was somewhat higher 10 days versus 3 days after treatment. Nasci and co-workers did not see a reduction in engorgement by *An. crucians* Wied. or *Culex salinarius* Coq., and also did not observe a significant reduction in parity in *Ps. columbiae*. The present study shows that dorsally applied permethrin moved around the body, was less concentrated ventrally, and also declined in activity over time. Maximum activity appeared to be 18–20 days after treatment.

Schmidt et al. (1985) used a somewhat similar assay to quantify permethrin resistance in *Haematobia irritans* (Linn.) in the laboratory, placing treated cotton cloth between flies and a feeding surface (cotton soaked with beef blood). The present assay, however, is superior to other contact toxicity assays in allowing an assessment of both feeding success and contact toxicity. It is much simpler than similar tests done directly on animals, particularly for very small insects. It has the added, somewhat more realistic advantage of the flies falling away, or being repelled from, a treated surface (rather than being continuously confined and in contact with it, as in a petri dish or vial), and thus could be used in assessing short-term knockdown effects.

This test does require a species that readily will attempt to feed on a blood source in the laboratory. The *C. variipennis* in the present tests are very aggressive feeders, probably presenting a good approximation of insects in the final stages of host orientation and attack. Finally, while the present assay is somewhat realistic, it does not simulate whole-body contact likely made when insects crawl through treated hair. Nevertheless, the assay did show quite clearly relative insecticide distribution and persistence on an animal's body over time and indicated that *C. variipennis* will feed readily and successfully prior to intoxication. While a treated animal thus becomes toxic to attacking flies (and may contribute to a decline in the local vector population), it is not itself necessarily protected against pathogen exposure. This assay could be useful for other vector species that either transmit pathogens of veterinary importance or serve as hosts for zoonotic pathogens.

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