IN VITRO TESTING OF CHEMICALS FOR REPELLENCY AGAINST *CULICOIDES BREVITARSIS* (KIEFFER) (DIPTERA: CERATOPOGONIDAE)

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Summary

Culicoides brevitarsis is a biting midge and the main vector of the bluetongue and Akabane viruses, which impede the export of livestock from Australia. Sixteen chemical products were tested for repellency against *C. brevitarsis* with an *in vitro* technique using sprayed netting over light-traps. Flyaway®, Pyrethroid-T, deltamethrin and fenvalerate significantly reduced numbers caught in the traps. These products are proposed for testing on animals or for acceptance as protectants on livestock to be moved to ports for export. The test procedure was unsuitable for products with oil or paraffin bases as the midges were caught on nets.

Keywords: *Culicoides brevitarsis,* repellency, light traps, livestock, export protocols

INTRODUCTION

Exports of Australian livestock to many countries are dependent on animals being certified free of infection with bluetongue (BLU) and Akabane viruses. In 1997-98, large areas of Australia, previously considered by these trading nations as being BLU infected were accepted as free of BLU based on the epidemiology of the virus and the occurrence of its principal vector *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae). Similar certification was not possible for *C. brevitarsis* and BLU endemic areas across northern Australia and down the coastal plains of Queensland and central/northern NSW. Many movements of livestock from free areas to nearest ports for export would ideally cross these infected areas or include the cost of transport to BLU free ports in distant southern Australia. Protocols are being developed to propose safe movements of livestock (principally cattle) to nearest ports based on seasonal or daily activity of the vectors and virus. In addition, it is proposed that non-persistent chemicals be used to protect animals while in transit across a potential vector zone.

Animal protectants can act on arthropods through their toxic or repellent effects. Repellency has an advantage over toxicity as it should prevent vectors from feeding and possibly transmitting virus during the time that is takes for conventional insecticides to act. However, some typically toxic compounds could also have a repellent effect or act quickly enough to prevent feeding. Testing for repellency has mainly concentrated on mosquitoes and human hosts (Schreck 1977) although several tests have involved activity against *Culicoides* spp. (Schreck and Kline 1983; Trigg and Hill 1996) and other Ceratopogonids (Perich *et al.* 1995). A few studies have considered *Culicoides* and their animal hosts (Blume *et al.* 1971; Braverman and Chizov-Ginzburg 1997; Kitaoka *et al.* 1965) and the use of animals as attractants is common to many test procedures. Unfortunately, results on repellency are not always consistent within genera and some variability can exist between *Culicoides* species (Schreck *et al.* 1979). Information on chemical repellency against *C. brevitarsis* is sparse and largely anecdotal. The response by *C. brevitarsis* to potential repellents needs to be evaluated if the strategy for short-term protection of livestock during their movement to ports is to be effective.

In this study, we used an *in vitro* technique to test a range of compounds and formulations for repellency against *C. brevitarsis.* Our aim was to propose products suitable for testing on livestock or acceptable for use because of current registration.

MATERIALS AND METHODS

All tests were carried out at the CB Alexander Agricultural College at Tocal (32°38'S, 151°35'E) in the Hunter Valley, NSW. Most products were registered for use on livestock (Table 1); cyfluthrin was tested because of its potential as an area protectant (eg. feedlots and animal quarters) and a plant derative (PD1) was included because its active ingredient had shown potential overseas [AG1000 - Braverman and Chizov-Ginzburg (1997)]. The test method was modified from that described by Braverman and Chizov-Ginzburg (1997) and used standardised light-traps (Dyce *et al.* 1971 and

modified by HA Standfast, pers. comm.) to catch insects. Sections of polyester bed netting (25 holes/ cm^2) were sewn as open ended bags 34 cm long x 72 cm in circumference. Bags were sprayed with each product at its recommended rate (Table 1) until run-off to fully cover and saturate the nets. The products were applied once only, prior to the nets being attached in the hour before sunset of the first day. The nets were allowed to air dry for 0.5 h and were then stretched over the entry section of the traps and attached by drawstrings. Each experiment was carried out over two nights.

Traps were hung 2 m above the ground at equal distances around the perimeter of a complex of holding yards containing about 30 cattle spread equally through the yards to act as attractants. Treatments were arranged in a randomised complete block with partial neighbour balance. This design allowed a nearest neighbour analysis when there was evidence of treatment effects on adjacent traps. Treatments were restricted to 16 to 20 light-traps. In 1999, two replicates of eight chemical treatments and 4 untreated control traps (water only); three replicates of five treatments and the control plus one of Flyaway®; and, three replicates of four treatments and 4 control traps were used in experiments 1, 2 and 3 respectively. Four replicates of four treatments and a control (experiment 4) and five replicates of three treatments and a control (experiment 5) were used in 2000 (Table 1). Catches were made into 250 mL plastic bottles containing 70% alcohol. Bottles were changed every 2 h for the first nights of experiments 1 and 2 to record any short-term changes to the time that products might exhibit repellency. Temperatures (°C) were taken with wet and dry bulb thermometers and these used to calculate relative humidity each time bottles were changed. Wind speed was measured with an ANEMO hand-held wind-speed indicator. Other weather data were obtained from the College weather station.

Catches of *C. brevitarsis* were counted under a binocular microscope in the laboratory. Sexes were differentiated (1999 and 2000) and females further subdivided into nulliparous, bloodfed/gravid and post parous stages in the 1999 experiments only. Insects were observed attached to the netting of two treatments in experiment 1 and although not counted, some were identified as *C. brevitarsis*. These two treatments were repeated in experiments 2 and 5 and the netting removed after the two nights and stored in plastic bags. Insects were washed from the nets with 70% alcohol and the numbers of *C. brevitarsis* counted.

Total count data were analysed for the first and second nights separately using Generalized Linear Mixed Modelling (Schall 1991) assuming a gamma distribution with a log link function to relate total counts to treatment effects. The log-transformed means of treatments were compared to the control using Dunnett's procedure (Dunnett 1955). Chi-squared tests were performed to examine the relationships between treatments and the parous stage of females present each night. Two hourly counts over the first nights (≈ 12 h) of experiments 1 and 2 were graphed and changes in repellency assessed visually. Male numbers were too low to be analysed separately in every experiment.

RESULTS

Experiment 1

The experiment was conducted 9-11 March 1999 when sunset was around 1930 h. Temperatures fell from 22°C immediately after sunset to a low of 14°C at 2400 h, wind speeds were <1 km/h and humidity reached 100% at 2300 h on the first night. Weather conditions were similar on the second night although more adults were caught on this night. Total numbers of *C. brevitarsis* caught with flumethrin, permethrin and two formulations of Musca-ban® were significantly lower than the control on the first night (Table 1). Of these, permethrin was no longer different on the second night. No associations were established between the parous stages of females and the treatments and there was no obvious change in the response of *C. brevitarsis* to treatments from 2 h to 12 h after sunset.

Experiment 2

The experiment was conducted 24-26 March 1999 when the sun set near 1900 h. Temperatures fell from 19°C immediately after sunset to a low of 12.5°C at 0600 h, wind speeds were between 0-1 km/h and the air was saturated by 0400 h on the first night. Weather conditions were similar on the second night although numbers were again higher. Total numbers caught in the traps with Flyaway® and flumethrin were significantly lower than the control on both nights (Table 1).

Significant numbers of *C. brevitarsis* were attached to the nets of the flumethrin and Musca-ban® treatments. None was attached to the nets of other treatments. No associations were established between parous stages and treatments and *C. brevitarsis* did not change its response to treatments from 2h to 12h.

Table 1. Effect of chemical treatments on numbers of *Culicoides brevitarsis* in traps and caught on treated netting over the traps over two nights in March-April 1999 (Expts 1-3) and March-April 2000 (Expts 4 and 5) [# significantly different from their respective controls using Dunnett's test (P < 0.05)]

Compound	Reps	Trade name®	Rate	Predicted numbers		
				Night 1	Night 2	Nets (Nights 1 + 2)
Experiment 1						
Control	4			112.0	243.1	
Cypermethrin+	2	Cypafly	40 mL/10 L	118.5	207.1	
Diazinon+	2	Di-Jet	0.5 L/10 L	109.0	203.0	
Mixture	2	Supershield+	Undiluted	73.0	160.9	
Deltamethrin+	2	Coopafly (Pour-on)	Undiluted	59.0	146.5	
Permethrin+	2	Permoxin (Pour-on)	50 mL/4 L	18.0#	59.7	
Flumethrin+	2	Bayticol	Undiluted	16.5#	41.0#	
Mixture	2	Musca-ban 1*+	Undiluted	18.0#	30.6#	
Mixture	2	Musca-ban 2**	Undiluted	3.0#	20.3#	
Experiment 2						
Control	3			115.8	225.8	0
Permethrin+	3	Permoxin	50 mL/4 L	84.8	144.3	0
PD1	3		40 mL/1 L	66.2	142.1	0
Lambda-cyhalothrin+	3	Outlaw (Pour-on)	Undiluted	41.1	105.9	0
Mixture	3	Musca-ban 2**	Undiluted	31.5	101.7	52.6#
Flumethrin+	3	Bayticol (Pour-on)	Undiluted	12.9#	46.8#	107.2#
Mixture	1	Flyaway+	Undiluted	9.6#	1.4#	0
Experiment 3						
Control	4			20.3	32.8	
Permethrin+	3	Rinse & Spray Conc.	20 mL/800 mL	18.9	22.7	
Cyfluthrin	3	Tugon	0.1 kg/25 L	15.5	19.0	
Pyrethroid-T++	3		10 mL/1 L	9.4	11.8	
Mixture	3	Flyaway+	Undiluted	1.2#	2.2#	
Experiment 4						
Control	4			1234.2	866.5	
Fenvalerate+	4	Sumifly	50 mL/10 L	736.4	414.5#	
Deltamethrin+	4	Coopafly (Pour on)	Undiluted	518.6#	352.7#	
Pyrethroid-T++	4		10 mL/ 1 L	371.6#	408.7#	
Mixture	4	Flyaway+	Undiluted	273.7#	116.3#	
Experiment 5						
Control	5			61.0	262.6	0
Flumethrin+	5	Bayticol (Spray)	1 L/ 1000 L	44.6	154.4	0
Flumethrin+	5	Bayticol (Pour-on)	Undiluted	2.0#	5.0#	69.0#
Mixture	5	Musca-ban 1*+	Undiluted	0.2#	0.8#	87.8#

+Registered for use on livestock, ++(Braverman and Chizov-Ginzburg 1997, *with pyrethrin, **with permethrin

Experiment 3

The experiment was conducted 12-14 April 1999 when the sun set around 1734 h. Temperatures (daily min-max) ranged between 10°C and 26.1°C and wind speeds were negligible over the two days. *C. brevitarsis* numbers were in decline in response to lower seasonal temperatures. Counts in the traps with Flyaway® were significantly lower on both nights (Table 1). Numbers in Pyrethroid-T treatments were different at $P < 0.1$. No *C. brevitarsis* were attached to any nets. No associations were established between parous stages and treatments.

Experiment 4

The experiment was conducted 28-30 March 2000 when the sun set near 1755 h. Temperatures (daily min-max) ranged between 15.5°C and 30.2°C over the two days and there was little wind. Only midge counts in the fenvalerate treatment were not different from those in the control on the first night and counts for all treatments were less than those for the control on the second night (Table 1).

Experiment 5

The experiment was conducted 10-13 April 2000 when the sun set near 1737 h. Temperatures (daily min-max) ranged between 12.5°C and 24.1°C over the two days and there was negligible wind. Possible repellency by flumethrin (Bayticol Pour-on) and Musca-ban® was again confounded by numbers of *C. brevitarsis* caught on nets. Counts in flumethrin treatments without the oil base (Bayticol Spray) did not differ from the control (Table 1).

DISCUSSION

Some level of activity was evident in all chemical treatments as numbers were consistently less than, but not always significantly different from, the controls in each experiment. Flyaway® was the only product that exhibited a consistently high level of repellency against *C. brevitarsis*. Repellency was also indicated for Pyrethroid-T, deltamethrin and fenvalerate. Significant differences between deltamethrin (not detected in experiment 1), Pyrethroid-T ($P < 0.1$ in experiment 3) and the control were obtained by greater replication and less variance between trap catches in experiment 4. Permethrin appeared promising initially but this was not confirmed over time or by repeat experiments. Flumethrin and Musca-ban® were initially identified as potential repellents but this conclusion was confounded by the large numbers of *C. brevitarsis* caught on the nets. Flumethrin was subsequently shown to have little repellency when used as the EC formulation. Musca-ban® is marketed as both an insecticide and a repellent. It is a mixture of compounds and component effects could not be assessed separately.

Repellency can be tactile as well as a response to vapours (Schreck 1977). This mode of action is recognised to exist in some pyrethroids. In our experiments, repellency was masked by the "stickiness" of some products' components (eg. Citronella oil, paraffins and synergists in Musca-ban®) or base chemicals (eg. an oil base is used for flumethrin in the pour-on formulation) that trapped *C. brevitarsis* allowing them to be killed before possibly being repelled. Therefore, if contact is involved, the testing procedure is unsuitable for these types of product. The net could not be regarded as an accurate method of quantifying activity and the results are open to a range of interpretations. However, oils that prevent *Culicoides* spp. from biting by trapping them on the skin have been used effectively in the absence of suitable repellents (Schreck and Kline 1981).

In each of our experiments, similar responses to the chemical treatments were recorded over two nights although total *C. brevitarsis* numbers often changed dramatically. This is the approximate time required for the protection of livestock during their movement to ports. There was no obvious indication that the repellency of any product varied over the first 12 h in the field. Many products, which include natural substances and di-ethyl toluamide (DEET, the most commonly used personal repellent), have been shown to have short-term repellency against *Culicoides* spp. (Braverman and Chizov-Genzburg 1997) sometimes down to a few hours. Differences through the latter half of the first 12 h in our experiments were difficult to analyse because numbers peaked in the first 2 h after sunset and then declined quickly. This is common in the study area and is a response to declining night temperatures (Bishop *et al.* 1995).

This investigation identified Flyaway®, Pyrethroid-T, deltamethrin and fenvalerate as products capable of actively repelling *C. brevitarsis*. Although not always exhibiting significant repellency, no product could be discounted because insecticidal properties on animals may be all that is required to prevent infections with BLU. Some products may even exhibit greater or less repellency if formulated differently. Before progressing to testing any proposed products on animals it was noted that there has frequently been a failure to establish correlations

(Schreck 1979). Such relationships can be affected by variability in the host [eg. differences in skin characteristics (texture, wetness, colour, hairiness and absorption)], the vector (eg. species, sex, stage and age - differences that were not evident here) and the environment (time of day and temperature). However, three of the products identified as a repellent are already registered for use on livestock. It is possible that these could be registered quickly for the purpose of protecting animals during their transport to ports for export. For example, deltamethrin and fenvalerate are currently registered for cattle. Flyaway® is registered for horses and as an area protectant (animal quarters). As these products are known insecticides or have insecticidal components, repellency in addition to toxicity would be an positive enhancement for their proposed use. Deltamethrin, permethrin and fenvalerate are being tested on cattle in the Northern Territory (L. Melville, DPIF, Northern Territory, personal communication) and deltamethrin and cypermethrin in Queensland (W. Doherty, QDPI, personal communication). Use of any proposed products will ultimately depend on their availability, the development of suitable application methodology, residual activity, ease at which the products can be registered for the proposed purpose and cost.

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