Short communication

The repellent effect of organic fatty acids on *Culicoides* midges as determined with suction light traps in South Africa

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Abstract

The efficacy of a 15% (w/w) mixture of octanoic, nonanoic and decanoic acids in light mineral oil to repel *Culicoides* biting midges (Diptera; Ceratopogonidae) was determined in three replicates of a 4 x 4 Latin square design under South African field conditions. The fatty acids were applied to ±0.07 m^2 polyester meshes with a mesh size 2-3 mm fitted to 220 V 8 W Onderstepoort downdraught light traps. To reduce the relatively strong attraction of the light trap, the black light tubes in the Onderstepoort trap were replaced with 8 W 23 cm white light tubes. The traps were operatingf overnight next to cattle. Two traps treated with the mixture of fatty acids collected 1.7 times fewer midges than two untreated traps. Although this mixture of fatty acids had shown a repellent effect against a number of blood-feeding insects this is the first indication that it also has a significant repellent effect against *Culicoides* species and especially *Culicoides* (*Avaritia*) *imicola* Kieffer when applied to polyester mesh.

Key words: African horse sickness, Bluetongue, control, *Culicoides imicola*

1. Introduction

Culicoides biting flies (Diptera; Ceratopogonidae) can transmit a wide range of viruses, bacteria, protozoa, nematodes and helminths to humans and animals (see Meiswinkel et al., 2004 for review). At least three orbiviruses (*Reoviridae*) namely African horse sickness (AHSV), bluetongue (BTV) and epizootic haemorrhagic disease (EHDV), transmitted by these midges, cause diseases of such international significance that they have been classified as notifiable to the Office International des Epizooties (OIE).

In addition to the use of vaccines (if available) the use of insect repellents and/or insecticides will unavoidably have to form part of integrated control programmes against these diseases (Carpenter et al., 2008). Although it is generally accepted that animals that are stabled at night are safe against pathogens transmitted by these midges (Meiswinkel et al., 2004), it has been shown that *Culicoides* midges do enter stables, and that animals are protected only if the stables are adequately closed (Barnard, 1997; Meiswinkel et al., 2000; 2008; Baylis et al., 2010). In one study a 14-fold reduction in the numbers of *Culicoides* midges collected inside a stable was achieved by closing the stables with gauze (Meiswinkel et al., 2000). The application of insect repellents and/or insecticides to the gauze may increase the efficacy (Meiswinkel et al., 2000).

Several insecticides, e.g. permethrin (Stevens et al., 1988; Mullens et al., 2000), fenvalerate pyrethroid (Holbrook, 1986), cypermethrin (Papadopoulus, 2010) and alpha-cypermethrin (Papadopoulos et al., 2009), have shown significant negative effects on colony-reared *Culicoides* (*Monoculicoides*) *sonorensis* Wirth and Jones and *Culicoides* (*Monoculicoides*) *nubeculosis* Meigen. Susceptibility to deltamethrin was found to be higher in colony reared *C. nubeculosus*, than in field populations of *Culicoides* (*Avaritia*) *obsoletus* Meigen or *Culicoides* (*Avaritia*) *imicola* Kieffer (Venail et al., 2011). In bioassay determinations of the efficacy of permethrins against *C. sonorensis* it was shown that midges were able to feed, and thus potentially transmit pathogens, before being incapacitated (Mullens et al., 2000). Increasing concerns of the impact of chemicals on the environment resulted in a decline in the number of agents available for livestock pest management. Reliance on only a few active ingredients may create problems with insecticide resistance.

In addition to the use of insecticides, the application of repellents has been recommended to decrease *Culicoides* attack rate (White and Evans, 2002). In South Africa 15% N,N-diethyl-3-methylbenzamide (DEET) was shown to have a significant repellent effect against *Culicoides* species and especially *C. imicola* the most abundant vector of BTV and AHSV virus in South Africa (Page et al., 2009).

Fatty acids are common in nature and are normal constituents of vertebrate skin. Some fatty acids have been shown to repel mosquitoes attempting to bite or oviposit (Skinner et al., 1970; Hwang et al., 1982). It was shown that a mixture of octanoic acid (C8), nonanoic acid (C9) and decanoic acid (Cl0), fatty acids which occur naturally in a variety of plants and also on the surface of human skin, were highly repellent to houseflies (*Musca domestica*) and horn flies (*Haematobia irritans*) (Mullens et al., 2009). Furthermore, it has been reported that this mixture of fatty acids will provide relief from stable flies (*Stomoxys calcitrans*), face flies (*Musca autumnalis*), cattle lice (*Bovicola bovis, Solenopotes capillatus* and *Linognathus vituli*), and ticks (*Ixodes pacificus, Amblyomma americanum*) (Reifenrath, 2010).

The objective of the present study was to determine the potential repellent efficacy of a mixture of fatty acids, known as C8910 (Afrivet (Pty) Ltd.), against *Culicoides* species when applied to polyester mesh and tested with a downdraught suction light trap.

2. Materials and Methods

2.1 *Fatty acids tested*

C8910 is a 15% (w/w) mixture of octanoic ($CH_3(CH_2)_6COOH$), nonanoic ($CH_3(CH_2)_7COOH$), and decanoic ($CH_3(CH_2)_8COOH$) acids in light mineral oil. While octanoic and decanoic acids come from coconut or palm kernel oil, nonanoic acid is derived from oleic acid present in animal tallow.

2.2 *Test procedure*

To determine the potential repellency of C8910 on the numbers of *Culicoides* midges collected as well as species composition, the results obtained with two Onderstepoort 220V 8W downdraught white light traps fitted with commercially available polyester mesh treated with C8910 were compared to that of two traps fitted with untreated mesh. The Onderstepoort trap is described in detail by Goffredo and Meiswinkel, 2004 and Venter et al., 2009.

Each light trap was fitted with white polyester mesh (surface area ±0.07 m^2; mesh size 2-3 mm), surrounding the light source. Under normal trapping conditions the purpose of the mesh is to exclude moths and bigger insects from the collections (Goffredo and Meiswinkel, 2004). According to the manufacturer's instructions, 12 ml of C8910 was sprayed evenly onto the nets until dripping wet. To prevent *Culicoides* midges from sticking to the oily nature of the product, treated nets were air-dried at room temperature in a closed room for three days. After three days, the nets were stored individually in sealed Ziploc (Johnson and Son, South Africa) plastic bags until attached to the traps immediately before midge collection each night. Each mesh (treated and untreated) was used only during one trapping occasion.

Light traps were suspended 1.8 m above ground-level next to open-sided barns housing between 20-40 cattle each at the ARC-Onderstepoort Veterinary Institute (25°39' S, 28°11' E; 1 219 m above sea level). Traps were hung as close to the cattle as practically possible. To ensure that treatment means were independent of any effects due to site or occasion, trap treatments at the four collection sites were alternated in three replicates of a 4 x 4 randomized Latin square design (Perry et al., 1980). To minimise interference between light traps, the collecting sites were located at least 15 m apart. Light trap operating procedure was conducted as described by Venter *et al*., 2009.

Black or UV light is known to be more effective than white light for the attraction and collection of *Culicoides* midges (Wieser-Schimpf et al., 1990) including *C. imicola* (Venter and Hermanides, 2006), the most abundant vector of BTV and AHSV in South Africa. The strong attraction of the black light source renders the Onderstepoort light trap less useful for the evaluation of repellents against *Culicoides* midges (Venter et al., 2009). To reduce the relatively strong attraction of the light trap, the black light tubes in the Onderstepoort trap were replaced with 8 W 23 cm white light tubes.

Trapping was conducted on 12 nights in autumn from 15 April to 6 May 2010. Climatic variables (outside temperature and relative humidity) were recorded using a Tinytag Explorer (Gemini Data Loggers, UK, Ltd.).

After retrieval in the morning, the captured insects were transferred to 80% ethanol. Large collections were sub-sampled following the method of Van Ark and Meiswinkel (1992). *Culicoides* midges were sorted to species level by examination of wing patterns. For age-grading, the females of all species were classified according to the abdominal pigmentation method of Dyce (1969) into unpigmented (nulliparous), pigmented (parous), gravid females (with eggs visible in the abdomen) and freshly blood-fed females. The numbers of males and other insects collected were also recorded.

Analysis of variance (ANOVA) was used to differentiate between the trap treatment effects at the 5% level. Treatment means were separated using Fisher's protected t-test least significant difference (LSD) at the 5% significance level (Snedecor and Cochran, 1980). Chi-square (X^2) tests were applied to differentiate between proportions of insect counts at the different trap heights. Data were analyzed using the statistical program GenStat® (Payne et al., 2010).

3. Results

A total number of 81 721 *Culicoides* midges were collected in 48 collections among 12 nights between 15 April and 6 May 2010 (Table 1). The total number of midges collected in the treated traps (30 076) was significantly fewer ($P = 0.020$) than the number collected in the untreated traps (51 645) (Table 1). Of the 21 different species collected, 18 and 19 species were caught in the untreated and treated traps respectively.

In both trapping regimes, the most abundant *Culicoides* species collected was *C. imicola*. Of the 21 different *Culicoides* species collected it was also the only species to be present in each of the 48 collections made. The total number of specimens of *C. imicola* collected with the traps treated with C8910 (29 460) was significantly lower ($P = 0.021$) than the total number collected in the untreated traps (50 830) (Table 1). The percentage representation of this species, however, did not differ significantly ($P = 0.448$) between the treated (98.0%) and untreated traps (98.4%).

Culicoides (*Hoffmania*) *zuluensis* de Meillon was the second most abundant species to be collected in both the untreated and the treated traps. It was present in all 24 collections made with the treated traps and in 21 of the 24 collections made with the untreated traps. The number of specimens collected in the treated traps (204) was not significantly lower ($P = 0.951$) than the number collected in the control traps (271) (Table 1).

No statistically significant differences could be found in the portion of unpigmented (nulliparous), pigmented (parous), gravid and freshly blood-fed of *C. imicola* females and males between the treated and untreated traps (Table 1). Similarly no significant difference ($P = 0.385$) was found in the total numbers of non-blood feeding insects collected (Table 1).

The outside temperature during the trapping period (15 April to 6 May 2010) ranged between 8.2ºC and 28.5ºC (average 17.0ºC). The relative humidity varied between 23.2% and 100% (average 78.5%).

4. Discussion

These results indicate that the mixture of fatty acids used had a significant repellent effect against *Culicoides* species and especially *C. imicola*, when applied to polyester mesh and tested with suction light traps. Based on its confirmed vector status, host preference and relative abundance around livestock, this Afro-Asiatic *Culicoides* species *C. imicola,* is considered the principle vector of BTV and AHSV in South Africa (Nevill *et al.*, 1992; Meiswinkel *et al*., 2004) and BTV in southern Europe (Mellor *et al.*, 1985; Boorman, 1986; Mellor, 1992; Mellor *et al.*, 2000; 2009).

Transovarial transmission of BTV is not known to occur in the genus *Culicoides* (Nelson and Scrivani, 1972; Nunamaker et al., 1990) and therefore the number of parous individuals is of importance in evaluating the vector potential of a specific *Culicoides* population. In the present study no statistically significant differences could be found in the number of nulliparous and parous *C. imicola* females collected with the treated and untreated traps (Table 1). This indicates that C8910 is equally effective at repelling both these stages with equal efficiency.

A drawback of light traps is that they appear not to attract male and/or blood-fed and gravid females (Venter et al., 2009). It was therefore not possible to determine the repellency of C8910 in these groupings. These groups will be of less importance in the transmission of pathogens as they do not seek blood meals on a host.

In a previous study done during the peak of the summer season in South Africa, it was found that light traps fitted with mesh treated with a 15% DEET suspension, considered as the gold standard for insect repellency (Fradin, 1998), collected 2.5 times fewer *Culicoides* midges compared to control traps (Page et al., 2009). In the present study, done in autumn, traps treated with C8910 collected 1.7 times fewer midges than the untreated traps. Because these two studies were not done during the same season and the possibility that ambient temperature and other climatic variables may influence the dispersal and efficiency of the repellent the results of these two studies are not very comparable.

The relatively strong attractant effect of the Onderstepoort light trap for *Culicoides* species coupled to the strong downdraught of the fan may counteract a repellent effect of the compound tested (Venter et al., 2009). Studies using stabled cattle or horses as the attractant should give more credible results Despite the limitations of the light trap testing technique, a significant repellent effect of C8910, when applied three days previously to polyester mesh, was confirmed against *Culicoides* species. The duration of its efficacy, beyond three days has not been determined. It must also be considered that on animals, the far more complex nature of natural mixtures and concentrations of various fatty acids may interact with other chemicals to increase or decrease attractiveness (Bosch et al., 2000).

Although the success of live attenuated vaccines as the primary control measure in endemic situations is evident in South Africa, control measures that will reduce the *Culicoides* midge attack rate and limit the dissemination of virus amongst animals during high risk periods, are important. It will also be beneficial to reduce biting rates during outbreak situations where the use of live attenuated vaccines may not be appropriate (Mellor and Hamblin, 2004). Under these conditions, the application of proven *Culicoides* repellents to animals and their stable environment, along with stabling and meshing of stables, will be of primary importance.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1

Summary of *Culicoides* midges collected in three replicates of a 4 x 4 Latin square design trial at the ARC-OVI. Collection were made from 15 April to 6 May 2010 to determine the effect of a mixture of fatty acids on *Culicoides* numbers, species composition, age grading results and physiological status of the population collected with white light downdraught traps (* *P* values ≤ 0.05 indicate a statistical significant difference)

Light trap treatment	Untreated	Treated	Statistical significance (*P* value)
No. of collections made	24	24	
No of species collected	18	19	
Total *Culicoides* collected	51 645	30 076	0.020*
Average collection size	2 152	1 253	
Range in collection size	59 – 7 315	91 - 6 012	
Total non blood-feeding insects collected	6 607	7 264	0.385
C. imicola			
Total collected	50 830	29 460	0.021*
Average collected	2117.9	1227.5	0.448
Range in collection size	57 - 7 204	77 - 5 908	
% of total *Culicoides* collected	98.4%	98.0%	
C. zuluensis			
Total collected	271	204	0.951
Average collected	11.3	8.5	0.596
Range in collection size	0 – 56	1- 46	
% of total *Culicoides* collected	0.5%	0.7%	
Age grading results in *C. imicola*:			
Nulliparous	1 485	850	0.614
Parous	553	336	0.453
Freshly blood fed	16	4	0.186
Gravid	2	3	0.502
Males	62	35	0.983