Assessment of the repellent effect of citronella and lemon eucalyptus oil against South African *Culicoides* species

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The use of insect repellents to reduce the attack rate of *Culicoides* species (Diptera: Ceratopogonidae) should form part of an integrated control programme to combat African horse sickness and other diseases transmitted by these blood-feeding midges. In the present study the repellent effects of a commercially available mosquito repellent, a combination of citronella and lemon eucalyptus oils, on *Culicoides* midges was determined. The number of midges collected with two 220 V Onderstepoort traps fitted with 8 W 23 cm white light tubes and baited with peel-stick patches, each containing 40 mg of active ingredient, was compared with that of two unbaited traps. Two trials were conducted and in each trial the four traps were rotated in two replicates of a 4 x 4 randomised Latin square design. Although more midges were collected in the baited traps, the mean number in the baited and unbaited traps was not significantly different. This mosquito repellent did not influence either the species composition or the physiological groups of *Culicoides imicola* Kieffer. The higher mean numbers in the baited traps, although not statistically significant, may indicate that this mosquito repellent might even attract *Culicoides* midges under certain conditions.

Introduction

Small (< 3 mm) blood-feeding flies of the genus *Culicoides* (Diptera: Ceratopogonidae) are associated worldwide with the transmission of several pathogens to a variety of hosts (Borkent 2005; Meiswinkel, Venter & Nevill 2004). Of the more than 66 viruses (Borkent 2005) isolated from *Culicoides* midges, African horse sickness virus (AHSV) is probably the most devastating to South Africa owing to its effect on the horse industry. Despite the widespread use of live-attenuated African horse sickness (AHS) vaccines, more than 1500 horses in South Africa have succumbed to AHS since 2005 (African Horse Sickness Trust 2012). This relatively high death rate can partly be ascribed to the large numbers of vectors, *Culicoides* species, present in the endemic AHS areas of South Africa. *Culicoides imicola* Kieffer (*C. imicola*), the most abundant livestock-associated *Culicoides* species, is considered to be a proven vector of AHSV (Meiswinkel *et al.* 2004; Mellor, Boorman & Baylis 2000). Under favourable conditions, more than a million *C. imicola* females can be collected overnight in a single light trap near livestock (Meiswinkel *et al.* 2004). Although these traps intercept only a relatively small percentage of the active blood-seeking females (Meiswinkel *et al.* 2004) and do not always reflect the true biting rate on livestock (Carpenter *et al.* 2008; Gerry *et al.* 2009; Scheffer *et al.* 2012; Viennet *et al.* 2011), these numbers give an indication of the potentially high attack rate that horses and other livestock can be exposed to in endemic areas. This perceived high attack rate emphasises the need for supportive control measures, for example the use of insect repellents and/or insecticides, in addition to vaccination, as part of an integrated control programme. Repellents and insecticides can be applied directly to livestock and/or to their immediate surroundings, for example gauze coverings of stable windows (Meiswinkel, Baylis & Labuschagne 2000).

Since the 1960s increasing concerns about the environmental impact of insecticides and an increased resistance in pest species have resulted in a decline in the number of agents available for pest management. An additional shortcoming of insecticides is that infected midges may be able to feed and potentially transmit pathogens before being incapacitated (Mullens *et al.* 2000).

In addition to the use of insecticides, repellents can be used to reduce the *Culicoides* attack rate and potential transmission of pathogens (White & Evans 2002). The evaluation of repellents against *Culicoides* midges is hampered by their small size (3 mm to 4 mm) and mainly nocturnal activity. To date in South Africa only two compounds have been shown to repel *Culicoides* midges from a light trap when applied to the polyester mesh surrounding the trap. These products are a 15% N,N-diethyl-3-methylbenzamide (DEET) (Page *et al.* 2009) and a mixture of octanoic acid

(C8), nonanoic acid (C9) and decanoic acid (Cl0) (Venter *et al.* 2011), fatty acids that occur naturally in a variety of plants and on the surface of human skin.

The apparent repellent effect of citronella on mosquitoes and other pest insects was already observed in 1901 (Granett 1940) and numerous reports on the efficacy of citronella or citronella-derived components as repellents against a variety of arthropods have been published since (Fradin & Day 2002; Kongkaew *et al*. 2011; Osmani, Anees & Naidu 1972; Revay *et al.* 2013; Tawatsin *et al.* 2001, 2006). Depending on the mode of application, additives and test procedures, the efficacy varies between species (Kongkaew *et al*. 2011; Osmani Anees & Naidu 1972). It has been found that some plant-derived repellents, including citronella, can give up to 9 h protection against mosquitoes and blackflies (Tawatsin *et al.* 2006). The principle constituent of citronella, Citronellol (3,7-dimethyl-6-octen-8-ol), is a volatile unsaturated aldehyde (terpene) with a characteristically strong, sweet smell (Botha & McCrindle 2000).

Because certain plant-derived mosquito repellents are regarded as safe for use on vertebrates, studies in Israel have begun to focus on their use to protect livestock against *C. imicola* (Braverman & Chizov-Ginzburg 1998; Braverman, Chizov-Ginzburg & Mullens 1999; Braverman, Wegis & Mullens 2000). Braverman *et al*. (1999) found that a plant-derived mosquito repellent based on the oil of *Eucalyptus maculata* can attract large numbers of *C. imicola*. However, other studies have shown that it will repel the Scottish species, *Culicoides impunctatus* Goetghebeur, from humans in the field (Trigg 1996) and the North American species, *Culicoides variipennis* Coquillett, from humans in the laboratory (Trigg & Hill 1996). In Spain, it was shown that citronella-based products will repel biting flies (*Simulium* and *Culicoides* species) from fumigated bird nests for up to three days (Martínez-de la Puente *et al.* 2009). These findings highlight the fact that the genus *Culicoides* is biologically highly diverse and that extrapolation of data from one species to another is not recommended (Tabachnick 1992).

Plant-derived repellents, if effective, may provide a cheap, environmentally safe and practical alternative to protect horses and other livestock against midges. In the present study, light traps were used to determine the potential repellent efficacy of a commercially available mosquito repellent against *Culicoides* species – a combination of citronella and lemon eucalyptus oils. This data may help to identify compounds that can be further evaluated for their use on livestock and will enable researchers to provide scientific advice to veterinarians and stock owners on the effectiveness of these products.

Material and methods

To determine the repellent effects of a combination of citronella and lemon eucalyptus oil, commercially available (Lifecare Medical Instruments Co., Ltd., Taiwan) peel-stick patches (diameter: 40 mm x 1 mm x 1.0 g) were used. Each patch, containing 40 mg active ingredient, is individually sealed in an aluminium package and can, according to the manufacturer, give up to 12 h protection against mosquitoes when stuck to clothing. As in previous studies conducted on repellents in South Africa (Venter *et al*. 2011), the number of midges collected with two 220 V down-draught Onderstepoort light traps baited with the repellent was compared with that of two unbaited control traps. A new patch was stuck on to the top of a light trap each night immediately before trapping was started. To reduce the relatively strong attraction of the light trap, the black light tubes were replaced with 8 W, 23 cm white light tubes (Venter & Hermanides 2006). Moths and larger insects were excluded by polyester netting (hole size 2 mm), which was placed around the entrance portals of the traps.

Two trials were conducted. In the first, trapping was carried out for eight nights in autumn, between 11 May 2009 and 03 June 2009. The second was conducted for eight nights in the height of summer between 27 January 2010 and 20 February 2010. Light traps were suspended 1.8 m above ground-level under the eaves of open-sided barns housing between 20 and 30 cattle; each were at the Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI) (25°39'S 28°11'E; 1219 m above sea level). At all four sites, the traps were hung as close to the cattle as practicably possible. To minimise interference between traps, sites were located at least 15 m apart (Venter *et al.* 2012). To eliminate any effects due to site or occasion, trap treatments at the four collection sites were alternated in three replicates of a 4 × 4 randomised Latin square design (Perry, Wall & Greenway 1980). Light trap operating procedure was conducted as previously described (Venter *et al.* 2009).

After retrieval in the morning, insects were transferred to 80% ethanol. Large collections were sub-sampled (Van Ark & Meiswinkel 1992) and *Culicoides* midges were counted, sexed and sorted to species level. Females were classified according to their abdominal pigmentation (Dyce 1969), these were: unpigmented (nulliparous); pigmented (parous); gravid (with eggs visible in the abdomen); or freshly blood-fed. The numbers of males and other insects were also recorded.

Data analyses

Analysis of variance (ANOVA) was used to differentiate between 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 (VSN International 2012). Evenness in species distribution and diversity for each treatment site was calculated with the Shannon Wiener index (Al Young Studios 2012). Species abundance in the different treatments was compared using linear regression GraphPadInStat Version 3 (GraphPad, USA).

Ethical considerations

Materials used in the experiment posed no health risk to researchers and no animals were harmed. The study was done as part of a project on National Assets at the ARC-OVI (project OV 7/03/P002–Insect Collection).

Results

In the first trial (11 May 2009 to 03 June 2009), 144 323 *Culicoides* midges were collected in 32 collections made over eight nights. Taking into account the substantial day-to-day variation in the numbers per trap per night, the higher mean in the 16 collections made with the two repellent traps (4660.6) was not significantly greater ($p = 0.559$) than the mean of that of the two unbaited control traps (4359.8) (Table 1).

Nine different *Culicoides* species were collected in the control and ten in the baited traps. The most abundant species, representing 95.0% in the control and 95.5% in the baited traps, was *C. imicola* (Table 1). The higher mean number of *C. imicola* in the baited traps (4449.3) was not significantly ($p = 0.662$) different from that (4359.8) in the control traps (Table 1). The second most abundant species in both trapping regimens was *Culicoides enderleini* Cornet and Brunhes (*C. enderleini*). This species represented 3.8% of the midges in the control traps and 4.0% of the midges collected in the baited traps (Table 1). The higher mean number in the baited traps (188.7) was not significantly different ($p = 0.534$) from that of the control traps (165.8) (Table 1).

A breakdown of the physiological stages of *C. imicola* showed that 99.2% (control traps) and 99.1% (baited traps) were females actively seeking a blood meal. The mean numbers of unpigmented (nulliparous) females collected in the baited (3309.5) and control (3029.8) traps were not significantly different ($p = 0.563$). Similarly the mean numbers of pigmented (parous) females in the baited (1098.4) and control traps (1079.7) were not significantly different ($p = 0.932$). Freshly blood-fed females (0.1%), gravid females (0.5%) and males (0.2%) represented 0.8% of the total number of *C. imicola* collected in the control traps, whilst in the baited traps blood-fed females (0.1%), gravid females (0.6%) and males (0.3%) represented 1.0% of the total number (Table 1). There was no significant difference ($p = 0.889$) in the numbers of other insects collected (Table 1).

In the second trial in summer (27 January 2010 to 20 February 2010), 123 941 *Culicoides* midges were collected in 32 collections. Of these, 59.5% were in the two baited traps. As in the previous trial, the higher mean collected with the baited traps (4605.8) was not significantly different ($p = 0.481$) from that in the control traps (3139.6) (Table 1).

Whilst 19 different *Culicoides* species were collected in the control traps, 22 were present in the baited traps (Table 1). *Culicoides imicola*, representing 97.9% in both regimens, was the dominant species with no significant difference ($p = 0.500$) in the mean numbers in baited traps (4509.2) or control traps (3073.3) (Table 1). Also, for *C. enderleini* (the second most dominant species) the higher mean in the baited traps (30.4) was not significantly ($p = 0.114$) different from that of the control traps (10.4) (Table 1).

Active blood-seeking *C. imicola* females represented 90.7% and 92.0% of the midges in the control traps and baited

TABLE 1: Summary of the *Culicoides* midges collected with white light suction traps in two trials at the Agricultural Research Council-Onderstepoort Veterinary Institute to determine the effect of a combination of citronella and lemon eucalyptus oil on *Culicoides* numbers, species composition and physiological status of the population.

Species	Light trap treatment	Collection date									
		May 2009 – June 2009					January 2010 – February 2010				
		Untreated		Treated		Statistical significance (*p*-value)	Untreated		Treated		Statistical significance (*p*-value)
		n	%	*n*	%		*n*	%	*n*	%	
Culicoides imicola	Total collected	66 293	95.0	71 189	95.5	0.662	49 172	97.9	72 147	97.9	0.500
	Mean number collected	4359.8	-	4449.3	-	-	3073.3	-	4509.2	-	-
	Range in collection size	169–10 115	-	217–11 946	-	-	489–7896	-	373–21 001	-	-
	Physiological status:	-	-	-	-	-	-	-	-	-	-
	• Nulliparous	48 477	73.1	52 952	74.4	0.563	27 683	56.3	43 080	59.7	0.179
	• Parous	17 275	26.1	17 575	24.7	0.932	16 895	34.4	23 323	32.3	0.130
	• Freshly blood fed	99	0.1	36	0.1	-	469	1.0	540	0.7	-
	• Gravid	326	0.5	437	0.6	-	297	0.6	217	0.3	-
	• Males	116	0.2	189	0.3	-	3828	7.8	4988	6.9	-
Culicoides enderleini	Total collected	2653	3.8	3019	4.0	0.534	166	0.3	486	0.7	0.114
	Mean number collected	165.8	-	188.7	-	-	10.4	-	30.4	-	-
	Range in collection size	24–463	-	17–589	-	-	0–56	-	0–252	-	-
Number of collections made		**16**	**-**	**16**	**-**	**-**	**16**	**-**	**16**	**-**	**-**
Number of species collected		**9**	**-**	**10**	**-**	**-**	**19**	**-**	**22**	**-**	**-**
Total *Culicoides* collected		**69 753**	**-**	**74 570**	**-**	**0.559**	**50 249**	**-**	**73 692**	**-**	**0.481**
Total Mean *Culicoides* collected		**4359.8**	**-**	**4660.6**	**-**	**-**	**3139.6**	**-**	**4605.8**	**-**	**-**
Range in collection size		**211–10 602**	**-**	**201–11 397**	**-**	**-**	**498–7994**	**-**	**435–21 294**	**-**	**-**
Shannon-Weiner index		**0.24**	**-**	**0.22**	**-**	**-**	**0.15**	**-**	**0.15**	**-**	**-**
Species evenness		**0.11**	**-**	**0.10**	**-**	**-**	**0.05**	**-**	**0.05**	**-**	**-**
Total other insects collected		**1084**	**-**	**1025**	**-**	**0.889**	**25 504**	**-**	**27 228**	**-**	**0.495**

p-values < 0.05 indicate a statistical significant difference in treatments.

traps respectively. There were no significant differences in either the mean number of nulliparous (p = 0.179) or parous (p = 0.130) *C. imicola* collected (Table 1). Freshly blood-fed females (1.0%), gravid females (0.6%) and males (7.8%) represented 9.4% of the totals in the control, whilst in the baited traps, blood-fed females (0.7%), gravid females (0.3%) and males (6.9%) represented 7.9% of the total numbers (Table 1). As in the first trial, there was no significant difference (p = 0.495) in the numbers of other insects collected (Table 1).

In both trials a strong linear correlation (R^2 = 100%) was found in the proportion of different species collected with the control trap and repellent trap. Species diversity and evenness as reflected by the Shannon-Weiner index, which describes the evenness in distribution of species abundances taking sample size into account, was nearly identical between treatments (Table 1).

Discussion

In the present evaluation using light traps, a combination of citronella and lemon eucalyptus oil did not appear to have any repellent effect on *Culicoides* midges. Females of *C. imicola* looking for a blood meal were the dominant grouping in both trapping regimens and no significant differences were found in the different physiological stages. Higher numbers as well as a larger number of species were collected with the baited traps. However, owing to the substantial day-to-day variation in the numbers these differences were not statistically significant. These results are in agreement with those of Page *et al*. (2009), who found that higher mean numbers of *Culicoides* midges, including *C. imicola,* were collected with light traps baited with 0.6% citronella oil.

Owing to the hole size (2 mm) of the netting used on the Onderstepoort trap, mosquitoes and insects larger than *Culicoides* midges were excluded from the trap, so the repellent effect of this combination of oils on the numbers of these insects could not be evaluated. The higher mean number of insects other than *Culicoides* midges collected in the baited traps was not significantly different from those of the controls.

In evaluating repellents, the influence of the ambient temperature, wind speed and other factors that affect the dispersal capacity of the repellent must be taken into consideration. During May to June the mean numbers collected with the baited traps was 1.1 times higher than those collected in the control traps. During the warmer period of January to February the baited traps collected on average 1.5 more midges than the control traps.

A shortcoming of the present evaluation is that the relatively strong attraction of the light trap for *Culicoides* species coupled with the strong downdraught of the fan could have counteracted any repellent effect of the compound tested. Attraction of insects to a light source is somewhat artificial and it may not be comparable to that of a warm-blooded host animal. It should be borne in mind that on mammals the far more complex nature of natural mixtures and concentrations of various organic components found on the skin might interact with other chemicals to increase or decrease attractiveness of a product (Bosch, Geier & Boeckh 2000). Despite these shortcomings, light traps were used efficiently to determine the repellent effect of DEET (Page *et al.* 2009) and a mixture of organic fatty acids (Venter *et al.* 2011) for *Culicoides* midges.

The apparent repellence of citronella for various species of mosquitoes and other pest insects has been documented since 1901 (Granett 1940). Even with some encouraging results having been published (Martínez-de la Puente *et al.* 2009; Tawatsin *et al.* 2006), citronella is generally rated as less effective than repellents with synthetic active ingredients (Kongkaew *et al.* 2011). Despite this, it has long been used in a number of commercial preparations (Curtis *et al.* 1987). In a review by Fradin and Day (2002) it is stated that none of thousands of plant extracts were able to repel mosquitoes for more than 1 h and 30 min and that most, regardless of their active ingredients and formulations, gave very short-lived protection, ranging from 3 min to 20 min.

In endemic situations like South Africa, vaccination will always remain the key control strategy for diseases caused by viruses transmitted by *Culicoides* midges. However, effective repellents can help in decreasing *Culicoides* attack rates (White & Evans 2002). In order to develop and evaluate repellents successfully an understanding of the molecular basis of insect olfaction will be essential (Bohbot & Dickens 2010; Logan & Birkett 2007). Repellents can either be applied directly on to the animals and/or to their immediate surroundings (Meiswinkel *et al*. 2000). To date, only two repellents, DEET (Page *et al*. 2009) and a combination of organic fatty acids (Venter *et al*. 2011), have been found to effectively repel *Culicoides* midges as determined with light traps under field conditions in South Africa. In an effort to control viral diseases transmitted by *Culicoides* midges it will be worthwhile to test these products for efficiency and safety on livestock. The evaluation of a great number of potential repellents on various livestock species will, however, be labour intensive, expensive and in certain instances, unethical. Light trap results can be used to screen products before their evaluation on livestock.

Conclusion

In the present study no evidence could be found to support the use of a combination of citronella and lemon eucalyptus oil as a repellent for *Culicoides* midges. The higher mean numbers, although not significant, collected with the baited traps may indicate that citronella, under certain conditions, might even attract *Culicoides* midges. An apparent attraction effect of citronella for *C. imicola* was also found in previous studies (Braverman *et al*. 1999; Page *et al*. 2009).

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

G.J.V. (Agricultural Research Council-Onderstepoort Veterinary Institute) was responsible for the project design. K.L. (Agricultural Research Council-Onderstepoort Veterinary Institute) did all the *Culicoides* species analyses and age grading of the collections. S.N.B.B. (Agricultural Research Council-Onderstepoort Veterinary Institute) was responsible for the collection of the *Culicoides* midges and the rotation of the light traps. L.M. (Agricultural Research Council-Biometry Unit) was responsible for most of the statistical analyses. G.J.V. compiled the data and the draft of the manuscript.

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