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Short communication

The effect of 1-octen-3-ol and 4-methylphenol on *Culicoides* midge numbers collected with suction light traps in South Africa

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ABSTRACT

Despite some shortcomings, suction light traps are the primary monitoring tool for the collection of *Culicoides* species (Diptera: Ceratopogonidae). Factors that may increase the efficiency of these traps need to be investigated. In the present study the numbers of *Culicoides* midges collected with two Onderstepoort black light traps baited with a mixture of 1-octen-3-ol and 4-methylphenol, as a potential olfactory cue, were compared to those of two unbaited traps. Comparisons were done in two and three replicates of a 4×4 randomized Latin square design in the presence and absence of cattle. The addition of 1-octen-3-ol and 4-methylphenol, released at 9.1 and 15.5 mg/h, respectively, did not influence species richness, numbers collected, sex ratios or age-grading results. Comparisons of *Culicoides* numbers and especially the abundance of *Culicoides imicola* Kieffer in collections done in the presence and absence of cattle confirm previous findings that show that host animals will be the primary attraction for *Culicoides* midges and that light traps mostly sample midges already in the near vicinity of the host.

1. Introduction

Several species of *Culicoides* (Diptera: Ceratopogonidae) can be involved in the biological transmission of the viruses that cause bluetongue (BT) and African horse sickness (AHS) in the same geographical area (Mellor and Pitzolis, 1979; Caracappa et al., 2003; Paweska et al., 2003; Torina et al., 2004; Savini et al., 2005; Carpenter et al., 2009). The success of an integrated control programme and disease risk analysis will depend on the ability to detect all potential vectors in an area. The involvement of a variety of *Culicoides* species, each with a unique biology, greatly increases the complexity of such a programme.

The most practical and extensively used tool for the monitoring of *Culicoides* species is various models of suction light traps (Venter et al., 2009). Light traps intercept a relatively small portion (<0.0001%) of the active blood-seeking females (Meiswinkel et al., 2004). These numbers can be influenced by the design of the trap (Venter et al., 2009) as well as a variety of physical and environmental factors. Light traps are poorly attractive to males and/or blood-fed and gravid females. Diurnal species that may play a role in the transmission of pathogens will not be collected. Some studies have shown that the number of *Culicoides* collected with light traps is not necessarily comparable to species diversity and host bite rate (Carpenter et al., 2008; Gerry et al., 2009).

Because of their impact on veterinary health, there is considerable interest in understanding how *Culicoides* midges use olfactory cues in host location. Olfactory cues, as an additional attractant to light, may increase the efficiency of these traps. Various chemicals such as carbon dioxide, 1-octen-3-ol, phenols, ketones and acetone, found in the breath of large herbivores, have been shown to have some degree of attraction for blood-sucking insects such as tsetse flies (Vale and Hall, 1985) and mosquitoes

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(McIver and McElligot, 1989; Takken and Kline, 1989; Kline et al., 1990, 1991). Studies in North America and Australia have shown that 1-octen-3-ol on its own or in combination with carbon dioxide and/or phenol can attract *Culicoides* midges to suction traps in the absence of a light source (Kline et al., 1994; Ritchie et al., 1994; Braverman et al., 2000; Cilek and Kline, 2002). Electroantennogram studies on *Culicoides impunctatus* Goetghebuer in Scotland demonstrated responses from antenna stimulation with 1-octen-3-ol (Blackwell et al., 1996, 1997) and Bhasin et al. (2001) showed that 1-octen-3-ol released at 0.06 mg/h will attract this species under field conditions. In most instances a combination of two or more of these chemicals will have a synergistic effect on the number of *Culicoides* midges collected (Ritchie et al., 1994; Cilek and Kline, 2002). It was, however, also shown that 1-octen-3-ol can repel certain North American *Culicoides* species (Kline et al., 1994). The role that olfactory cues may play in the collection of African and European *Culicoides* species, especially that of the proven vector of bluetongue virus, *Culicoides imicola* Kieffer, is not known.

The aim of the present study was to determine if the addition of olfactory cues to the Onderstepoort light trap might increase the range and numbers of *Culicoides* species collected and if this could have an effect on the age-grading and sex ratio results of the population.

2. Materials and methods

To determine the differential attraction of a 1-octen-3-ol/4-methylphenol mixture on the numbers of *Culicoides* midges collected and species composition, the results obtained with 220 V down-draught black light traps baited with the mixture were compared to those of unbaited traps. The 1-octen-3-ol/4-methylphenol mixture was dispensed from heat-sealed sachets (7 cm × 9 cm) made from low-density polyethylene tube (wall thickness 150 microns) placed directly on top of the trap. The release rate of the 1-octen-3-ol was 9.1 and that of the 4-methylphenol was 15.5 mg/h. This release rate was similar to that used by Kappmeier and Nevill (1999) for the collection of tsetse flies in South Africa who determined the release rate by weighing the sachets 24-hourly under field conditions. The average concentration of 1-octen-3-ol in natural ox odour is about 0.05 mg/h and the dose used in the present study equals that of about 182 head of cattle.

Two trials were conducted at the ARC-Onderstepoort Veterinary Institute (25°39′S: 28°11′E; 1219 m above sea level). In the first trial, conducted at the end of summer, from 27 April to 5 May 2009, four light traps, two baited with the mixture and two unbaited, were operated for eight nights. Traps were suspended 1.8 m above ground level next to open-sided barns housing between 20 and 40 cattle each. In the second trial, to avoid the effect of the presence of the cattle, trapping was repeated 500 m away from any livestock on 12 nights in spring, from 11 to 25 September 2009, using two baited and two unbaited traps. In both trials, to prevent interference between traps, trap sites were located at least 15 m apart. Trapping was conducted from dusk to dawn as described by Venter et al. (2009). Based on abdominal pigmentation (Dyce, 1969) the females of all species were age-graded into unpigmented (nulliparous), pigmented (parous), gravid or freshly bloodfed. Males and all other insects captured were also counted. To ensure that treatment means were independent of any effects due to site or occasion, trap treatments at the four sites were alternated in either two (trial 1) or three (trial 2) replicates of a 4 × 4 randomized Latin square design (Perry et al., 1980).

Analysis of variance (ANOVA) was used to differentiate between the trap treatment effects. The data was normally distributed and testing done at the 5% level. Treatment means were separated using Fisher's protected *t*-test least significant difference (LSD) at the 5% level of significance (Snedecor and Cochran, 1980). Data was analyzed using the statistical program GenStat® (Payne et al., 2007).

3. Results

During the first trial, conducted near cattle, 98,722 *Culicoides* midges were present in 16 collections made with unbaited light traps (Table 1). During the same period 100,165 *Culicoides* midges were present in 16 collections made with light traps baited with the 1-octen-3-ol/4-methylphenol mixture. The total number of midges collected by baited and unbaited traps was not statistically different (Table 1). *Culicoides* midges belonging to 13 species were collected with the unbaited traps and 10 species in the traps baited with the 1-octen-3-ol/4-methylphenol mixture (Table 1). *C. imicola* was the dominant species in both the unbaited (94.1%) and the baited traps (94.8%) and no statistical difference was found in the total numbers collected (Table 1). *Culicoides enderleini* Cornet & Brunhes was the second most abundant species in both the unbaited (4.9%) and the baited traps (4.3%) (Table 1). All other *Culicoides* species collected made up <1% of the total catch number.

In the second trial, conducted away from livestock, 11,477 *Culicoides* midges were collected in 24 unbaited traps and 9608 *Culicoides* in 24 traps baited with the 1-octen-3-ol/4-methylphenol mixture (Table 1). This difference was not statistically significant (Table 1). *Culicoides* midges belonging to 19 species were collected with the unbaited and 22 species in the baited traps. *Culicoides leucostictus* Kieffer, considered a bird feeder, was the dominant species in both the unbaited (44.3%) and the baited traps (43.0%) (Table 1). *C. imicola*, representing 36.8% in the unbaited and 37.2% in the baited traps, was the second most abundant species. The third most abundant species, *Culicoides bedfordi* Ingram & Macfie, also considered a bird feeder, represented 10.2% of the *Culicoides* collected in the unbaited and 11.2% in the baited traps (Table 1). None of these differences were statistically significant (Table 1). All other species collected made up <5% of the number of *Culicoides* midges collected.

Except for the number of *C. enderleini* males collected in the first trial, no other statistically significant differences could be found in the number of unpigmented, pigmented, gravid and blood-fed females and males between the baited and unbaited traps (Table 1). No statistically significant differences were found in the total numbers of all other insects collected in either trial 1 ($P = 0.294$) or trial 2 ($P = 0.955$).