Duration of repellency of N,N-diethyl-3-methylbenzamide, citronella oil and cypermethrin against *Culicoides* species when applied to polyester mesh

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Abstract

The repellent efficacy of 15% N,N-diethyl-3-methylbenzamide (DEET), 0.6% citronella oil, and 0.3% α-cyano-cypermethrin against Culicoides species was compared in three 5 × 5 Latin squares (15 replicates) under South African field conditions. DEET, citronella oil or α-cyano-cypermethrin were applied to polyester meshes that were fitted to down-draught suction 220 V UV light traps which were operated overnight. No significant repellent effect against Culicoides was found for the citronella oil or the α-cyano-cypermethrin. DEET had a significant repellent effect against Culicoides species and C. imicola for all catches made from after sunset to before sunrise.

1. Introduction

Due to the viruses they transmit *Culicoides* biting midges (Diptera: Ceratopogonidae) are of economic and veterinary significance worldwide (Mellor et al., 2000 and Meiswinkel et al., 2004). Of principal importance to equids in sub-Saharan Africa are *Culicoides* (*Avaritia*) *imicola* Kieffer and *Culicoides* (*Avaritia*) *bolitinos* Meiswinkel which have been implicated in the transmission of African horse sickness (AHS) virus (Du Toit, 1944, Meiswinkel, 1998, Venter et al., 2000 and Meiswinkel and Paweska, 2003). Both *C. imicola* and *C. bolitinos* can also become infected with and permit replication of equine encephalosis (EE) virus (Paweska and Venter, 2004), which is associated with similar clinical signs, but markedly lower

mortality than AHS in horses (Paweska et al., 1999 and Howell et al., 2004). Various species of *Culicoides* have been associated with equine insect hypersensitivity, also referred to as summer seasonal recurrent dermatitis (Braverman, 1988 and Greiner, 1995), the most common skin allergy affecting horses (Pascoe and Knottenbelt, 1999).

The recent spread of bluetongue (BT) into northern Europe has highlighted the risk of introduction and rapid spread of vector-borne diseases outside their traditional boundaries (Purse et al., 2005, Thiry et al., 2006 and Nolan et al., 2007). *Culicoides imicola* and members of the *Culicoides obsoletus* and *Culicoides pulicaris* complexes have been implicated in the outbreaks of BT in Europe (Nolan et al., 2007), whilst both *C. imicola* and *C. bolitinos* have been implicated as vectors of the BT virus in South Africa (Du Toit, 1944 and Venter et al., 1998).

In endemic areas, vaccination is the primary AHS control measure (Coetzer and Guthrie, 2004). A polyvalent, live attenuated AHS vaccine is commercially available for compulsory vaccination of horses in South Africa. Until 1990 the attenuated live-virus vaccine comprised two quadrivalent vaccines, one containing serotypes −1, −3, −4 and −5 and the other serotypes −2, −6, −7 and −8. Due to safety problems, the vaccine strain of AHSV-5 was discontinued in 1990 (Van Dijk, 1998). As part of an integrated control program other recommended measures to prevent diseases associated with *Culicoides* in equids include stabling at night, meshing of stables, and application of insect repellents both to the animal and its stable environment (Perris, 1995 and Meiswinkel et al., 2004). Whilst stabling is recommended for control it has been shown that *Culicoides* do enter stables, and horses are protected from *Culicoides* bites only if the stables are adequately closed (Barnard, 1997 and Meiswinkel et al., 2000). Closing stables by meshing with synthetic gauze resulted in a 14-fold reduction in the numbers of *Culicoides* entering (Meiswinkel et al., 2000).

The effects of repellents against *Culicoides* and the duration of their activity on humans have been documented, with most studies reporting the repellency of various compounds against that of N,N-diethyl-3-methylbenzamide (DEET) (Braverman et al., 2000). Assessment of efficacy of repellents applied to horses against *Culicoides* species and especially *C. imicola* is hampered by their relatively small size and their nocturnal activity which make direct observation difficult. A novel method for preliminary field screening of potential repellents to protect animals against *Culicoides* has been reported (Braverman and Chizov-Ginzburg, 1997). Studies using this method, which utilizes light traps and repellent-impregnated polyester mesh, have provided an indication of the duration of repellency of the various

compounds tested (Braverman and Chizov-Ginzburg, 1998, Braverman et al., 1999 and Braverman et al., 2000).

Citronella oil and cypermethrin are included in various topical ectoparasiticides registered for use on horses in South Africa, whilst DEET is registered for human use. No published data are available on the efficacy and duration of repellency of insect repellents against *Culicoides* in sub-Saharan Africa (Meiswinkel et al., 2004). The aim of this study was to determine and compare repellent efficacy of 15% DEET, 0.6% citronella oil, and 0.3% α-cyano-cypermethrin against *Culicoides* species when applied to polyester mesh under South African conditions.

2. Materials and methods

Five 220 V down-draught suction light traps equipped with 8 W, 23 cm UV-light tubes (Venter and Meiswinkel, 1994) were used to collect *Culicoides* midges in a randomised, blinded, field experiment. The study was conducted at the height of the *Culicoides* season, during February 2004, at the Faculty of Veterinary Science, Onderstepoort (25°38′51.42″ S, 28°10′45.96″ E, 1238 m).

Each light trap was fitted with white polyester mesh (area $0.07 \ m^2$; mesh size 3–4 mm), covering the entrance portal to the trap. Meshes were weighed, immersed in the test preparations for 30 min, air dried for 30 min, reweighed, and kept individually wrapped in tin foil inside sealed Ziploc® (S.C. Johnson and Son, South Africa) plastic bags prior to being attached to the light traps with elastic bands. A new mesh was prepared each afternoon for each of the light traps, i.e. each mesh was used only once, for one test article application. Meshes were treated with 15% DEET (MGK, USA), 0.6% citronella oil (Nicola-J Flavours and Fragrances, South Africa), 0.3% α-cyano-cypermethrin (Polytrin 200EC, Villa Crop Protection (Pty) Ltd., South Africa), or ethanol solvent, along with an untreated control mesh. The required dilutions of the test articles were prepared by titration with 70% ethanol. Meshes were removed from the light traps 14 h after application, re-wrapped in tin foil, and kept in sealed plastic bags before being reweighed. The amount of active ingredient absorbed per m^2 of net was calculated according to the method of Schreck and Self (1985).

Light traps were suspended in a row with the entrance portals at a height of 1.8 m, and a distance of 5.6 m between traps. The locations of the light traps in five outside camps were randomised for 15 nights in three 5×5 Latin square designs (Snedecor and Cochran, 1980). A horse in each camp served as an attractor for *Culicoides* midges. Light traps were operated from before sunset to after sunrise. Catches were made into 500 ml plastic beakers containing

200 ml 0.5% Savlon® (Johnson and Johnson, South Africa) and water solution that were replaced hourly between 19:00 and 06:00 h. Collections were decanted and stored in 70% ethanol prior to the *Culicoides* being counted and analysed to species level. Climatic variables (outside temperature, relative humidity, rainfall and wind speed) were recorded hourly using a Weather Monitor II® and Weatherlink® data logger (Davis, USA).

2.1. Statistical analyses

Midge numbers were cube root transformed which produced near-normality, both overall and within treatment groups, before data analysis. Statistical analyses were done using Stata version 8.2 (StataCorp, College Station, TX, USA) and NCSS 2004 (NCSS, Kaysville, UT, USA). Mean numbers of *Culicoides* species and *C. imicola* were compared between treatment groups whilst controlling for the effects of camp, time and day, using analysis of variance (ANOVA). Separate ANOVA's were also done at each time point whilst controlling for camp and day. Where *F*-ratios were significant, all pairwise comparisons were done between treatment groups using Tukey's HSD test. In order to estimate the effect of temperature, wind speed, humidity and rainfall on counts, multiple regression (Huber's method) was used. $P < 0.05$ was considered significant.

3. Results

A total of 107,204 *Culicoides* midges were collected in hourly light-trap collections made over 15 nights from 5 light traps operated simultaneously. Of 34 *Culicoides* species caught *C. imicola* was the most abundant and comprised 79.1% of midges collected, followed by *C. bolitinos* which comprised 5.3%.

The mean number of *Culicoides* midges (Fig. 1) as well as the mean number of *C. imicola* (Fig. 2) collected hourly with DEET was significantly ($P < 0.05$) lower than for all other treatments at all times except the first (19:00 h) and the last (06:00 h) sampling points. During the period of collection sunset fell between 18:40 and 18:58 h, and sunrise between 05:44 and 06:00 h. Light-trap catches of the total number of *Culicoides* and *C. imicola* increased with increasing temperature ($P < 0.001$), and decreased with increasing wind speed and rainfall ($P < 0.001$).
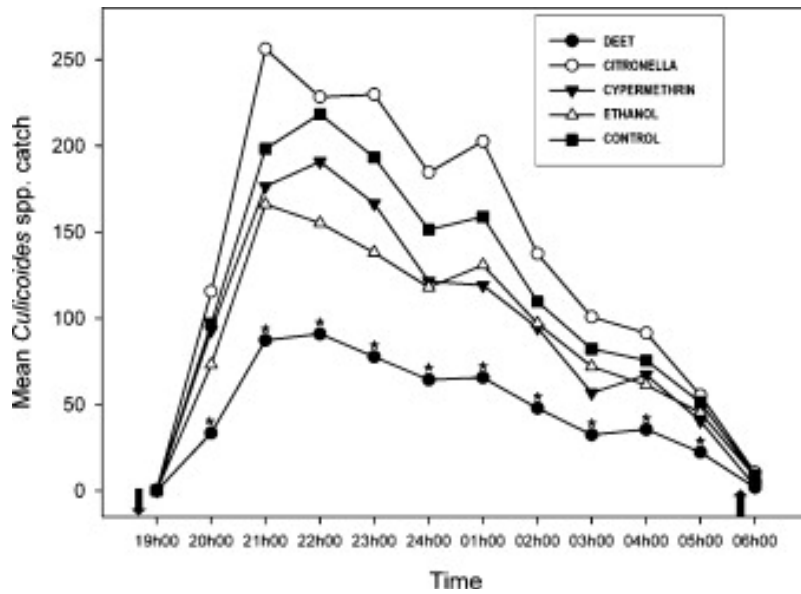
Fig. 1. *Mean number of Culicoides midges collected hourly with light traps fitted with polyester mesh treated with DEET, citronella, cypermethrin, ethanol and a control: ↓ = sunset and ↑ = sunrise. *P < 0.05.*
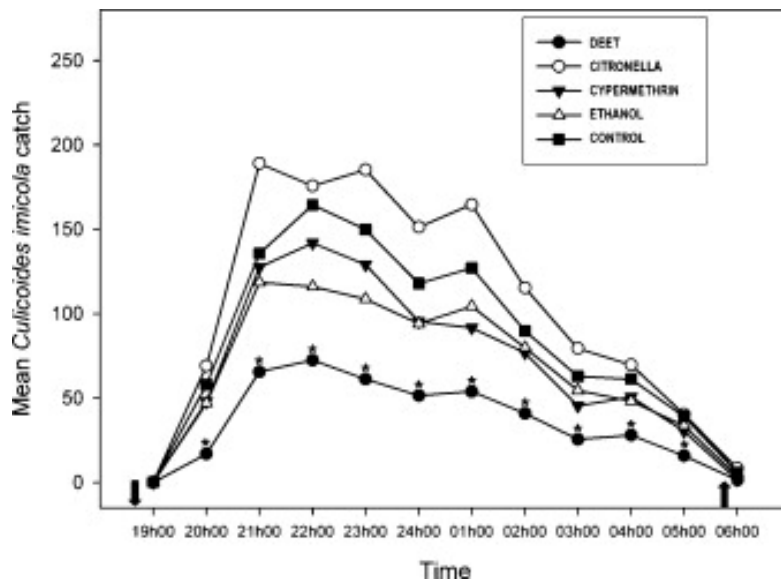

Fig. 2. *Mean number of C. imicola collected hourly with light traps fitted with polyester mesh treated with DEET, citronella, cypermethrin, ethanol and a control: ↓ = sunset and ↑ = sunrise. *P < 0.05.*

The amounts of active ingredient absorbed by the meshes before application to the light-traps were 11.0, 0.04 and 0.02 g/m^2 for the DEET, citronella oil and α-cyano-cypermethrin, respectively. The residual percentage of active ingredient remaining on the meshes 14 h after

application to the light traps were 69.1, 10.4 and 40.6% for the DEET, citronella oil, and α-cyano-cypermethrin, respectively.

4. Discussion

DEET had a significant repellent effect against *Culicoides* species, including *C. imicola*, for all catches made from after sunset to before sunrise, when applied to polyester mesh as tested with a down-draught suction light trap. Previous studies in Israel using light-traps and repellent-impregnated polyester mesh have documented durations of repellency of 2 h (Braverman et al., 1999) and 4 h for 15% DEET (Braverman and Chizov-Ginzburg, 1998). The amount of DEET absorbed per m^2 polyester mesh used in the present study was greater than previously reported (Braverman and Chizov-Ginzburg, 1998 and Braverman et al., 1999), and may have prolonged the duration of repellency. The meshes treated with DEET retained a high residual percentage of DEET after overnight application in comparison to the citronella oil and cypermethrin treated meshes. Depletion of active ingredient was most likely due to volatility and evaporation. Residual efficacy of the used meshes was not investigated further, however.

DEET is the principal active ingredient in human insect repellent formulations (Barnard, 2000), and remains the gold standard of currently available insect repellents (Fradin, 1998). The effect of six, topically applied, DEET aerosol formulations to horses has been investigated by Palmer (1969), who noted no adverse effect after single application of 3.75–75% concentrations. However, after 60 daily applications, excess of oil in the hair coat considered due to hypersteatosis was seen when concentrations of 15% or greater were applied, and cracking of the skin and ulceration was noted in two horses exposed to 50 and 75% concentrations (Palmer, 1969). Similar exfoliative lesions were reported in a horse after repeated exposure to 50 and 75% DEET concentrations (Blume et al., 1971). The concentration of DEET in commercial products registered for human use in South Africa is below the threshold at which adverse effects have been reported in horses. DEET applied to mesh screens would present no direct risk of exposure to high concentrations for horses in the stable area.

Other compounds previously evaluated via repellent-impregnated mesh have included a Meliaceae-derived plant extract named Ag 1000; Oregano; Herbipet, a mixture of plant extracts comprising oils of sage, rosemary and oregano; Tri-Tec14, containing cypermethrin and pyrethrins; Pyrethroid-T, a type II pyrethroid containing the α-cyano, 3-phenoxybenzyl moiety; Stomoxin, containing permethrin; Mosi-guard with *Eucalyptus* extract containing p-

methane-3,8-diol plus isopulegol and citronellol; Lice Free, containing plant extracts (Braverman and Chizov-Ginzburg, 1998, Braverman et al., 1999 and Braverman et al., 2000). Of these compounds the type II pyrethroid was superior to DEET in one study and demonstrated duration of repellency of 9 h (Braverman and Chizov-Ginzburg, 1998). A 4% permethrin pour-on provided an 86% positive response in clinical signs due to *Culicoides* hypersensitivity in horses (Stevens et al., 1988). No significant repellency was demonstrated for the cypermethrin synthetic pyrethroid used in this study. This may be due to the formulation of pyrethroid used, low percentage of cypermethrin applied to the mesh or other factors which were not investigated in the study.

No significant repellency was demonstrated for the citronella oil in the present study. A citronella fragrance was noted whilst the citronella oil was drying on the mesh. Any repellent effect due to the fragrance may have thus been reduced prior to mesh application to the light trap. Most of the plant derived essential oils tend to give short protection, usually less than 2 h (Fradin, 1998). Oil of neem has recently been evaluated against *Culicoides* (*Culicoides*) *impunctatus* Goetghebeur, with significant 'distance' repellency demonstrated for a 1% concentration and significant reduction in blood feeding on membranes treated with neem (Blackwell et al., 2004).

The relatively strong attractant effect of light traps for *Culicoides* species, including *C. imicola* (Venter and Hermanides, 2006), may counteract a repellent effect of the compound, and is a potential disadvantage of using light traps with repellent-impregnated mesh for screening of compounds for repellency. Despite this limitation a significant repellent effect of DEET when applied to polyester mesh was confirmed against *Culicoides* species and *C. imicola*.

Whilst the success of live attenuated AHS vaccines as the primary AHS control measure in endemic situations is evident, secondary control measures to reduce the *Culicoides* biting rate and thereby limit dissemination of virus amongst horses during high risk periods are important. Application of proven *Culicoides* repellents, such as DEET, to horses and their stable environment for this purpose is justified. Under epidemic situations, where the use of live attenuated AHS vaccines may not be appropriate and no commercial inactivated or sub-unit vaccines are available (Mellor and Hamblin, 2004), protection of horses by reducing biting rates is vital. Under such conditions, application of proven *Culicoides* repellents to horses and their stable environment, along with stabling and meshing of stables, is of primary importance.

Acknowledgements

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