Spatial repellency and vapour toxicity of transfluthrin against the biting midges *Culicoides nubeculosus* and *C. sonorensis* (Ceratopogonidae)

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**Abstract**

Biting midges (Diptera; Ceratopogonidae; *Culicoides* spp.) are biological vectors of disease agents, and they cause nuisance and insect bite hypersensitivity. Currently, there are no effective means to control biting midges as screening is impractical and the application of insecticides or repellents is of limited efficacy. Spatial repellents have the advantage over contact repellents that they can create a vector-free environment. Studies have shown the efficacy of spatial repellents to protect humans against mosquitoes, also outdoors, but no data are available for biting midges. We tested the spatial repellency and toxicity (knockdown effect) of the pyrethroid transfluthrin against the laboratory-reared biting midges *Culicoides nubeculosus* (Meigen) and *Culicoides sonorensis* (Wirth and Jones) and the mosquito *Aedes aegypti* (Linnaeus) in a high-throughput tube setup. Observations were made 15, 30 and 60 min. after application of the repellent. In addition to transfluthrin, the non-volatile pyrethroid permethrin and DEET, the gold standard of repellents, were included. Spatial repellency by transfluthrin was observed against both biting midge species and *Ae. aegypti*, already at the first observation after 15 min. and at much lower concentrations than DEET. Permethrin was spatially repellent only to *C. sonorensis* at the highest concentration tested (10 μg/cm²). Knockdown of biting midges and mosquitoes by transfluthrin, both by vapour or contact toxicity, was observed even at low concentrations. DEET had little to no effect on the knockdown of the insects, neither by direct contact nor vapour toxicity, while permethrin caused a high proportion of knockdown when direct contact was possible. In case these results can be confirmed in field experiments, spatial repellents could become a novel tool in integrated control programmes to reduce biting by *Culicoides* spp.

**Introduction**

Biting midges (Diptera; Ceratopogonidae; *Culicoides* spp.; ‘no-sees-ums’) are of veterinary importance, mainly as biological vectors of disease agents, such as e.g. bluetongue virus (sheep, cattle) and African horse sickness virus, but also as causative agents of nuisance (also for humans) and insect bite hypersensitivity, mainly in equids. Currently, there are no effective methods to control biting midges (Harrup et al., 2016). The most effective measures to protect humans from mosquito bites are to create a physical barrier with bed nets or screen houses (Lengeler, 2004). However, screening against biting midges is impractical because their small size (1–3 mm) requires the use of very fine-meshed nets that reduce air flow and might cause discomfort among the animals (discussed in Lincoln et al., 2015). The application of insecticides (Harrup et al., 2016; Vennal et al., 2011) or contact repellents (Blackwell et al., 2004; Venter et al., 2011; Robin et al., 2015; Gonzalez et al., 2014; Carpenter et al., 2005), even in a combination (Lincoln et al., 2015), to animals had limited and/or short-lived efficacy (Carpenter et al., 2008). Therefore, there is a need for effective and alternative control options.

Spatial repellents have the advantage over contact repellents that they do not have to be applied to the skin or clothes because they can diffuse easily through an area and protect from a distance (Norris and Coats, 2017). Spatial repellency is defined by the WHO as a range of insect behaviours induced by airborne chemicals that result in a reduction in human-vector contact and therefore personal protection (WHO, 2013). The behaviours can include movement away from a chemical stimulus, interference with host detection (attraction inhibition) and feeding response (WHO, 2013). Spatial repellents need to be very volatile for easy diffusion. Most spatial repellents are volatile pyrethroids (Bibbs and Kaufman, 2017) although some plant essential oils have been identified (Norris and Coats, 2017). In recent years, several studies have shown the potential of volatile pyrethroids to reduce house entry and biting, even outdoors, of mosquitoes (Culicidae). For example, eave ribbons treated with transfluthrin up to 83% protection against malaria mosquitoes (Mwanga et al., 2019). Outdoors, strips
impregnated with transfluthrin reduced biting exposure of humans to several species of mosquitoes at up to five meters distance (Ogoma et al., 2017). Another volatile pyrethroid often used as a spatial repellent is metofluthrin which has shown to be effective against Aedes aegypti mosquitoes when tested indoors (Darbro et al., 2017).

Studies on spatial repellents have focused on their use against mosquitoes. A few botanical compounds and fatty acids have been tested for their spatial repellency against biting midges, with varying results (Gonzalez et al., 2014; Venter et al., 2014). Pyrethroid-based spatial repellents have been tested in numerous studies against mosquitoes, most often with transfluthrin as the main pyrethroid repellent (Norris and Coats, 2017). To the best of our knowledge, there are no reports on the efficacy of pyrethroid spatial repellents on biting midges.

Here, we tested the spatial repellency as well as vapour and contact toxicity (knockdown) of repellents in a previously described high-throughput screening setup (Jiang et al., 2019). Next to the volatile pyrethroid transfluthrin, the non-volatile pyrethroid permethrin was included which is commonly used on bednets (Lengeler, 2004), as well as DEET as the gold standard of topical repellents with limited spatial repellency due to low volatility (Norris and Coats, 2017). Laboratory-reared Culicoides nubeculosus and C. sonorensis were exposed to the repellents. Aedes aegypti mosquitoes, also from a laboratory colony, were included as a reference and positive control (Jiang et al., 2019).

**Materials & methods**

**Insects**

_Aedes aegypti_ (Institut Pasteur New Caledonia; in colony for > 15 years) were reared as previously described (Verhulst et al., 2020). In brief, adult mosquitoes were kept in cubic cages (BugDorm, Taiwan) of 30 × 30 × 30 cm in a climate chamber (Kälte 3000, Switzerland) at 27°C, 85% RH, 16:8 h light-dark cycle including dusk/dawn phases of 1 h. The mosquitoes had continuous access to a 5% glucose solution through saturated dental wicks (IVF Hartmann AG, Switzerland). Three times a week, anticoagulated (EDTA) cow blood from a local slaughterhouse was offered through a Parafilm membrane at 37°C using the Hemotek feeding system (Hemotek Ltd., UK). Eggs were laid on seed germination paper (Enchor Paper, USA) in an oviposition cup half filled with deionised water. After drying the eggs for one week at room temperature, they were stored at 10°C until use. Eggs were hatched in 1 l dH2O and larvae fed with pulverised Tetramin (Qualipet, Switzerland) fish food.

_Culicoides nubeculosus_ (The Pirbright Institute, UK) were reared basically as described previously (Boorman, 1974) at 24 ± 0.5°C, 85 ± 5% relative humidity, long-day conditions (LD 17:7 h, 2 h dawn and dusk) in a climate chamber (Kälte 3000). Adults were kept in cardboard cylindrical cages (Whatkins and Doncaster, UK) with access to a 10% sucrose solution provided through saturated cotton on top of the cages. Once a week, the midges were fed with cow blood added to the concave bottom of plastic beakers, covered with a Parafilm membrane, at approximately 37°C (pre-warmed water in beaker). Eggs were laid on moist filter papers (2.5 cm diameter, Whatman, Germany). After hatching in pans with 3 l dH2O, the larvae were offered pulverised Tetramin fish food.

_Culicoides sonorensis_ (1955, PIR-s-3) pupae were kindly provided by The Pirbright Institute (UK). Pupae were hatched in the same cardboard containers as _C. nubeculosus_ and were kept under the same conditions until use.

**Repellents**

DEET (98.5%), permethrin (>95%), transfluthrin (99.6%) and the solvent acetone were purchased from Sigma-Aldrich (Switzerland). The concentration ranges tested were based on the results obtained with _Ae. aegypti_ by Jiang et al. (2019). DEET was tested at 1, 10, 50 and 100 μg/cm², permethrin at 0.1, 0.5, 1, 10 μg/cm² and transfluthrin at 0.01, 0.1, 0.5 and 1 μg/cm². Fifty μl of the corresponding stock solutions was applied to a filter paper (2.5 cm diameter, Whatman) which was dried in a fume hood for 15 min. Disks were stored separately in aluminium foil at 4°C (max. 1 h) until use.

**Assays**

The high-throughput spatial repellency assay was adopted from Jiang et al. (2019). The experiments were performed in a small laboratory room with heating, humidification and TL light. The conditions were 25.1 ± 0.29°C (standard error of the mean, SEM), 75.8 ± 0.90% relative humidity (RH) for the experiments with _Ae. aegypti_ and 22.1 ± 0.71°C and 63.1 ± 0.93% RH for the biting midges. Biting midges were sorted on a chill table (BioQuip, USA), and 15 females transferred with forceps to a glass tube (length 12.5 cm, 2.5 cm diameter, TriKinetcs, USA) that was covered with netting on both sides (Fig. 1). Female mosquitoes (15 per tube) were selected directly from their cage and transferred with a mouth aspirator to the glass tubes. After recovery of the insects for at least 15 min. and their spread over the tube, filter papers with the repellent or the solvent were placed in conical caps cut from 50 ml polypropylene tubes and attached to either side of the tube. Because of the netting, the insects could not get in contact with the filter papers.

In each experiment, eight tubes with the insects were placed on a white Styrofoam board with wooden sticks to hold the tubes in position at a distance of 10 cm. A black line indicated the middle of each tube, and the positions of the insects were recorded after 15, 30 and 60 min. Each treatment was tested six times for each of the concentrations described above (2.2). Treatments were randomised over the tubes, including control tests with filter paper with the solvent only on both sides of the tube.

Repellency was calculated by the proportion of insects on the side containing the repellent, whereby a value of 0 indicates 100% repellency, a value of 0.25 50% repellency and 0.5 (50:50 distribution) indicates no effect (Jiang et al., 2019). Knockdown is the partial paralysis upon contact with an insecticide which usually precedes death but can also last only few minutes, with the insects recovering (Wickham et al., 1974). Knockdown was recorded after 60 min. by dividing the number of insects that were lying on the bottom of the tube divided by the total number of insects in the tube. To determine the difference in knockdown with and without contact (vapour versus contact toxicity), the experiments were repeated with _Ae. aegypti_ and _C. sonorensis_ without the netting. Knockdown was recorded after 60 min. as described before.

After use, the tubes were washed in a laboratory washing machine and baked in an oven at 180°C for at least 12 h. The netting was discarded, and the conical caps washed and re-used only when they had been used for the controls.

**Statistics**

A Generalised Linear Model (GLM, binomial model, linked in logit) was used to investigate repellency, expressed as the fraction of insects residing on the side of the tube with the repellent divided by the total number of insects in the tube (binomial total). Occasionally, less than four mosquitoes were alive, and these observations were removed from the analysis. A similar GLM was used to test the effect of the repellents on the knockdown of the insects in the tube (number of insects lying on the bottom of the tube divided by the total number of insects).

The effects of concentration, compound and position of the tube, side of the treatment as well as their interactions were fitted in the GLM, and non-significant factors were removed. Models were compared by the corrected Akaike’s information criterion (AICC). No effects of the position of the tube and side of the treatment were found. Differences between concentrations were tested using pairwise comparisons with Least Square Differences (LSD) correction, and _P < 0.05_ was considered.
statistically significant. All statistical analyses were performed using SPSS, version 26 (IBM, USA).

Data availability

Data is provided in excel files as supplementary material.

Results

Repellency

Both transfluthrin and DEET were spatially repellent to *Ae. aegypti* and both Culicoides species in an approximate dose-dependant manner (Fig. 2). Permethrin did not repel *Ae. aegypti* and *C. nubeculosus*, and it repelled *C. sonorensis* but only at the highest concentration used (Fig. 2). Observations at 30 and 60 min. were similar to the observations at 15 min. but with stronger effect of the repellents. Because 60 min. equals the length of the knockdown experiments, detailed analyses of results are done for the observations after 60 min.

Except for the lowest concentration tested, the distribution of *Ae. aegypti* over the test tubes was different from a 50:50 distribution with transfluthrin and DEET (95% confidence interval (CI), GLM, Fig. 2). When permethrin (Fig. 2) or the tubes with only the controls were tested (Supplementary Fig. A1), no differences were found, demonstrating that both transfluthrin and DEET are spatial repellents for *Ae. aegypti* but permethrin is not. Transfluthrin repelled *Ae. aegypti* at much lower concentrations than DEET, whereby the two higher concentrations of transfluthrin tested (0.1 and 1 μg/cm²) were significantly more repellent than the two lower concentrations (0.1 and 1 μg/cm², GLM, Wald $X^2_{1.65} = 86.0, P \leq 0.00496$). DEET was most repellent at a concentration of 50 μg/cm², and at this concentration only 11.7 ± 2.1% (repellency 76.6%) of the mosquitoes were present on the repellent side of the tube, although this was not significantly different from the highest concentration of 100 μg/cm² DEET (GLM, Wald $X^2_{1.65} = 86.0, P = 0.304$, Fig. 2).

Effects of the repellents on the two midge species *C. nubeculosus* and *C. sonorensis* were similar or even stronger than those observed with *Ae. aegypti* (Fig. 2). Both Culicoides species were repelled by transfluthrin and DEET but not permethrin, except for the highest concentration of permethrin which was repellent to *C. sonorensis* (CI 0.14–0.46, GLM, Wald $X^2_{1.65} = 82.1$ for *C. sonorensis* and $X^2_{1.65} = 94.8$ for *C. nubeculosus*, $P < 0.05$, Fig. 2). The repellency of DEET and transfluthrin increased by dose), although for *C. sonorensis* the repellency did not increase anymore at the highest dose (GLM, Wald $X^2_{1.65} = 82.1, P > 0.05$, Fig. 2). The highest repellency was found when transfluthrin was tested against *C. nubeculosus* at a concentration of 1 μg/cm². At this concentration, only 6.3 ± 6.3% of the Culicoides were found at the repellent side after one hour (Fig. 2) (repellency of 87.4%).

Knockdown

Each of the three repellents tested had a different effect on the knockdown of *Ae. aegypti* and *C. sonorensis* (Fig. 3, controls see Supp. Fig. A2) determined after 60 min. DEET did not affect the knockdown of *Ae. aegypti* and *C. sonorensis* neither with nor without contact, except for one concentration (50 μg/cm², direct contact, *Ae. aegypti*). Permethrin did not affect the knockdown of *Ae. aegypti* when no contact was possible. However, when contact was possible 75.5 ± 1.8% of the mosquitoes were lying at the bottom of the tube at the highest concentration (10 μg/cm²) but only 11.0 ± 4.6% at the second highest concentration (1 μg/cm²), which was significantly different from the two lower concentrations tested (GLM, Wald $X^2_{1.65} = 185.6, P \leq 0.006$, Fig. 3). The effects of permethrin on *C. sonorensis* were similar to *Ae. aegypti*, although there was already a significant knockdown effect of permethrin at lower concentrations (0.5 μg/cm²) when direct contact was possible (GLM, Wald $X^2_{1.65} = 55.6, P \leq 0.006$, Fig. 3). There was a knockdown effect of transfluthrin on both insect species with both direct but also vapour contact, though the effect was more pronounced when direct contact was possible (Fig. 3). Vapour toxicity was virtually zero for *Ae. aegypti* at the lowest concentration tested (0.01 μg/cm²), but considerable (knockdown > 80%) at the highest concentration (1 μg/cm²). In *C. sonorensis*, a knockdown effect was observed already at the lowest concentration and reached around 60% at the three higher ones.

Discussion

The spatial repellency of transfluthrin, DEET and permethrin to colony populations of *Ae. aegypti* and two species of biting midges was tested in a high-throughput setup. Our main finding was that these compounds exerted similar repellency towards both *C. nubeculosus* and *C. sonorensis* as towards *Ae. aegypti*. Transfluthrin repelled *Ae. aegypti* at low concentrations, in line with another study with the same setup and the same mosquito species (Jiang et al., 2019). Transfluthrin vapour could both repel and knockdown biting midges in efficiencies that were comparable or, at higher concentrations with regard to repellency, more pronounced than found for *Ae. aegypti*. Because results can differ between colony and field populations of the same species, field populations will need to be tested.

In contrast to DEET (see below), virtually nothing is known on the mechanisms behind the behavioural effects of synthetic pyrethroid spatial repellents, like transfluthrin. Just the involvement of antennal perception has been demonstrated (referred in Norris and Coats, 2017). The toxic effects of pyrethroids are well understood. They affect the sodium ion channels in the nervous system of the insect and cause paralysis leading to knockdown and death (Zhu et al., 2020). The pyrethroid permethrin has a low volatility; spatial repellency was only found for the highest concentration tested against *C. sonorensis* (Fig. 2). This is consistent with studies on mosquitoes in which no spatial repellent effect of pyrethroids with a low volatility was shown (Spitzen et al., 2014;
Cooperband and Allan, 2009). Permethrin and other pyrethroids are widely used against mosquitoes, for example on bednets or spraying the walls of houses (Lengeler, 2004; Fluess et al., 2010). Pyrethroids are not widely used against biting midges. In a study by Melville et al. (2001), the topical application of permethrin on cattle reduced the number of biting midges obtaining a blood meal. However, in another similar study, no significant decrease in the number of engorged Culicoides was found (Mullens et al., 2000). A major shortcoming of topical insecticides is the difficulty to achieve a complete coverage of the whole body surface of the animal (Harrup et al., 2016; Mullens et al., 2000). Spatial repellents, applied in the environment or on the animals, would provide a more complete protection. Although resistance against transfluthrin has been reported in mosquitoes (Wagman et al., 2015) we have found no reports of resistance against any pyrethroids in Culicoides. In a comprehensive study conducted in 2015 on the susceptibility of field-collected and laboratory-reared Culicoides to a range of insecticides, no evidence for insecticide resistance was found (Venail et al., 2015). Repelling an insect with transfluthrin instead of killing it with an insecticide could result in a delayed or diminished development of insecticide resistance by minimising the intensity of selection pressure from contact-mediated toxicity mechanisms (Achee et al., 2012). A disadvantage of a spatial repellent could be that insects may be able to feed, and thus potentially transmit pathogens, before being immobilised by an insecticide (Mullens et al., 2000).

Fig. 2. Spatial repellency of transfluthrin, DEET and permethrin against *Aedes aegypti*, *Culicoides nubeculosus* and *C. sonorensis*. Given are the proportions of insects residing in the tubes on the side containing the repellent, at different concentrations of the repellents and at different time points after start of the experiments (red circles: 15 min., brown triangles: 30 min., blue squares: 60 min.). Symbols are the mean ± SEM based on six replicates with 15 insects. * indicates a difference from a 50:50 distribution based on the 95% Wald confidence interval (GLM estimates). For each repellent-insect combination the means not sharing the same letter differ significantly at *P* < 0.05 (GLM, followed by LSD, df = 63). NS = No significant differences found. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
DEET is the best known and most commonly used repellent against mosquitoes, but also repels ticks, leeches and bedbugs (Wang et al., 2013; Ogawa et al., 2016; Tawatsin et al., 2006). In our study, DEET repelled *Ae. aegypti* and the two species of biting midges, but at much higher concentrations (x100) than transfluthrin. This is consistent with previous studies that indicated that DEET repels mosquitoes from close distance and upon contact, although with two different mechanisms involved. From a distance, DEET interferes with the olfactory system in the antennae and maxillary palps required to detect a host (DeGennaro et al., 2013). Upon contact, DEET is detected by sensors on the legs (Dennis et al., 2019).

Previous work has shown that DEET repels biting midges when applied to high-density polyester mesh wrapped around down-draught suction UV light traps (Page et al., 2009; Braverman et al., 1999) or the skin of humans (Magnon et al., 1991; Trigg, 1996). In contrast, DEET in combination with permethrin when used on horses had no significant effect in reducing *Culicoides* (Lincoln et al., 2015). In a laboratory study by Gonzalez et al. (2014), DEET applied on filter paper was tested for its repellency against field-collected *Culicoides obsoletus* (Meigen) in a y-tube olfactometer. Interestingly, the lowest concentration of DEET tested in their setup (1 μg or 1.27 μg/cm²) was still repellent against *C. obsoletus*, while the lowest concentration of DEET tested in our setup (4.9 μg/cm²) was not repellent.
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