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Adaptation of the repellency response to DEET in *Rhodnius prolixus*

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ABSTRACT

For many years it has been accepted that DEET interferes with the detection of odours from the host instead of having a repellent effect. However, recent work showed that DEET acts as an odorant molecule and elicits a behavioural response in the absence of other stimuli. Therefore, DEET must promote some phenomenon connected with the stimuli–sensory system interaction, such as a sensory adaptation, where the sensory system regulates its sensitivity to different stimuli intensities during continuous or repetitive exposure. In this work, we studied different aspects of the insect–DEET interaction through behavioural observations. Previous exposure of fifth instar *Rhodnius prolixus* nymphs to DEET decreased the behavioural response to this repellent. We observed a decrease in repellence after different times of continuous stimulation with DEET in a time-dependent manner. The response to DEET was recovered 10 min after exposure, when insects were continuously stimulated during 5 or 10 min; maximum repellence was recovered 20 min after exposure when insects were stimulated for 20 min. DEET produced a repellent effect when nymphs were exposed only to its vapours. These results suggest that exposure to DEET produces adaptation in *R. prolixus* nymphs, and that the behavioural response elicited by DEET occurs via olfaction when no other stimuli are present.

1. Introduction

An insect repellent has been defined as a chemical that in insects produces oriented movements away from its source (Dethier et al., 1960). It is currently accepted that these compounds act as repellents in the vapour phase (Barton Browne, 1977). *N*,*N*-diethyl-3-methylbenzamide (DEET) is an insect repellent used worldwide. The repellent properties of DEET were discovered in 1946. Ten years later, it arrived on the market and become a successful product due to its effectiveness, persistence and low human toxicity (Frances, 2007). The effect of DEET has been proved in several insect species, including the haematophagous bugs *Triatoma infestans* (Alzogaray et al., 2000; Sfara et al., 2006) and *Rhodnius prolixus* (Sfara et al., 2008).

Although DEET has been commercially available since 1956, little is known about its mode of action. It has been widely accepted that it interferes with the detection of odours emanated from the host rather than having a repellent effect in the absence of other odour molecule stimuli (Ditzen et al., 2008; Dogan et al., 1999; McIver, 1981). However, electrophysiological and biochemical evidence shows that DEET acts as an odorant molecule (Alzogaray et al., 2000; Davis and Rebert, 1972; Syed and Leal, 2008).

As an odour molecule, DEET should promote some phenomenon associated with the stimulus–receptor interaction, such as sensory adaptation, a property of all sensory cells (Dolzer et al., 2003). In this phenomenon, the sensory system regulates its sensitivity to different stimulus intensities (Zufall and Leinders-Zufall, 2000), preventing saturation of the cellular transduction machinery and allowing the retention of high sensitivity during continuous or repetitive exposure to stimuli. Three types of sensory adaptations that differ in the time the effect lasts, the time of recovery and pharmacological properties have been identified in the olfactory receptor neurons of vertebrates and insects (Zufall and Leinders-Zufall, 2000). The duration and intensity of the stimulus determine the type of adaptation elicited. Adaptation is associated with a shift in the stimulus–response curve to higher concentrations (Borroni and Atema, 1988).

R. prolixus is a haematophagous bug distributed in the north of South America and some countries of Central America. As other haematophagous insects, it has conspicuous host-seeking behaviours associated with the detection of chemical cues, mainly olfactory, emanated by the host (Lehane, 1991). Repellents also elicit an easily measurable behavioural response in these insects (Sfara et al., 2008, 2009). Hence, we studied different aspects of insect–DEET interaction via behavioural observations. In this work we described a phenomenon of adaptation in *R. prolixus* nymphs exposed to DEET and the repellent effect of this substance in the absence of other stimuli.

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2. Materials and methods

2.1. Chemicals

N,*N*-diethyl-3-methylbenzamide (DEET) was from Aldrich (Milwaukee, WI, USA). Acetone was from Merck (Darmstadt, Germany).

2.2. Biological material

Experiments were performed using fifth instar *R. prolixus* nymphs from a colony reared in our laboratory. Insects were starved for 7–30 days after moulting and kept in an environmental chamber at 28 °C under a 12:12 L:D photoperiod. All experiments were performed at the beginning of the scotophase.

2.3. Evaluation of repellency

The device used to quantify the repellent effect of DEET is showed in Fig. 1. A circular piece of Whatman No. 1 filter paper (Whatman International Ltd., Miadstone, UK) (diameter: 11 cm) was cut into halves (Zone I and Zone II). Zone I was treated with 0.35 ml of acetone alone, and Zone II was treated with 0.35 ml of DEET dissolved in acetone (100 mg/ml). After acetone evaporation, both filter paper halves were fitted together and located on the test arena floor. A glass ring (high: 4.5 cm; diameter: 9 cm) was used to prevent the insect from leaving the filter paper. A fifth instar nymph was gently released in the centre of the filter paper and recorded with a closed-circuit digital camera (Sony, Tokyo, Japan) connected to a colour monitor (Sony, Tokyo, Japan). The image of the nymph was monitored visually and the time spent in Zone II was recorded during 300 s using a chronometer.

Results were expressed as Repellency Coefficients [RC = (Total Experimental Time − Time in Zone II)/Total Experimental Time]. RC values vary between 0 (maximum attraction) and 1 (maximum repellency). RC = 0.5 indicates that the insect spent the same time in both zones (random distribution of the insects). As controls, one insect was located in an arena where both halves were treated with acetone alone. Ten independent replicates were performed for each bioassay.

2.4. Adaptation to DEET

A circular piece of Whatman No. 1 filter paper (Whatman International Ltd., Miadstone, UK) (diameter: 9 cm) was treated with 0.5 ml of a DEET solution in acetone (200 mg/ml). After solvent evaporation, the filter paper was placed on the bottom of a circular plastic container (high: 4 cm; diameter: 9 cm) with a lid. The container was closed for 5 min to stabilize the system. A fifth instar nymph was placed into the container through a little opening, and was allowed to walk on the DEET-treated filter paper during the exposure time. Then, the repellent effect of DEET was evaluated for each individual at 0, 10, 20 and 30 min after exposure. Insects were kept in an open plastic container with a clean filter paper on its floor (DEET free atmosphere) for 5 min between measurements. Four exposure times were evaluated (1, 5, 10 or 20 min), and ten nymphs were used per exposure time. As controls, a nymph was placed on a filter paper treated with 0.5 ml of acetone alone. Ten independent replicates were performed for each bioassay.

2.5. Repellency: smell or contact?

The device showed in Fig 2A was used to evaluate the repellency produced by DEET vapours. A circular filter paper (diameter: 11 cm) was divided in half. One half was treated with 0.35 ml of acetone alone and the other half with 0.35 ml of a DEET solution in acetone (100 or 500 mg/ml). Both halves were fitted together and placed in the bottom of a plastic lid. The lid was covered with a piece of gauze, and a circular plastic container (diameter: 9 cm) was placed on the lid, keeping the gauze in place 1 mm above the treated filter paper. This prevented the nymph from coming into direct contact with the source of DEET. A fifth instar nymph was gently placed into the container through a little opening and repellency was evaluated as described above.

In another similar experiment both halves were treated with DEET (100 mg/ml); one half was placed in the bottom of the lid, while the other half was placed on the gauze (Fig 2B). This way, the nymph was exposed to DEET vapours and was able to contact the repellent while walking on the filter paper but was only exposed to DEET vapours when walking on the gauze. Repellency was evaluated as described above.

2.6. Shift of the stimulus–response curve

In order to study the shift of the stimulus–response curve of DEET after exposure to the repellent, the following experiments were performed. Repellency of three concentrations of DEET (100, 200 and 340 mg/ml) was determined in two groups of insects: one group was previously exposed to a surface treated with

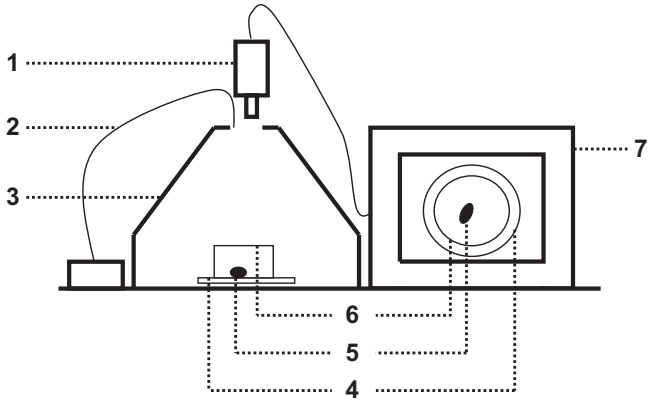

Fig. 1. Experimental arena used to determine the spatial distribution of the insects. (1) video camera; (2) optic fibre of light source; (3) dark chamber; (4) filter paper; (5) insect; (6) glass ring; (7) monitor.

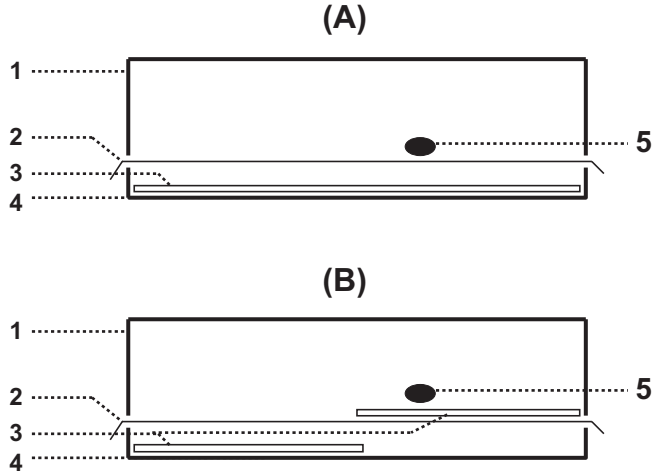

Fig. 2. Experimental device used to determine repellency caused by DEET via only vapours (A) and both contact or vapours (B). (1) plastic container; (2) gauze; (3) filter paper; (4) lid; (5) insect.