

Insecticide Resistance and Resistance Management

Deltamethrin-resistant German Cockroaches Are Less Sensitive to the Insect Repellents DEET and IR3535 than Non-resistant Individuals

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Abstract

The German cockroach, *Blattella germanica* (L.) (Blattodea: Blattellidae), is a serious worldwide pest with a considerable economical and sanitary impact. It is mainly controlled by the application of synthetic insecticides, but repeated use of these substances has promoted the appearance of resistance in cockroach populations throughout the world. The aim of this study was to compare the behavior of deltamethrin-susceptible (CIPEIN colony) and deltamethrin-resistant (JUBA and VGBA colonies) first instar nymphs exposed to the repellents *N,N*-diethyl-3-methylbenzamide (DEET) and ethyl 3-[acetyl(butyl)amino]propanoate (IR3535). Firstly, the behavior of the nymphs was assessed in an experimental arena in the absence of repellents. The parameters *Distance Traveled*, *Velocity*, *Mobility Time*, and *Time Spent* (in each half of the arena) were quantified using an image analyser, and showed that the behavior elicited by the three colonies was similar. After this, the behavior of the nymphs was quantified in an arena, half of which had been treated with repellent. The repellency of DEET increased as a linear function of log concentration for the three colonies. DEET elicited repellency as from a concentration of 97.49 µg/cm² for the CIPEIN and JUBA colonies and 194.98 µg/cm² for the VGBA colony. The repellency of IR3535 was weaker and started at a concentration of 389.96 µg/cm² for the CIPEIN colony, 779.92 µg/cm² for JUBA, and 1559.84 µg/cm² for VGBA. Finally, nymphs were exposed to 3:1, 1:1, and 1:3 DEET:IR3535 mixtures, and a synergistic effect was observed only in the CIPEIN colony.

Key words: *Blattella germanica*, insecticide resistance, insect repellent, synergism

The German cockroach, *Blattella germanica* (L.) (Blattodea: Blattellidae), is one of the most common pests in human houses and buildings (WHO 1997). It is distributed worldwide and is associated with any human dwelling that provides a habitat with adequate temperature, humidity, and food (Cochran 2003). It is a health-hazard concern because it acts as a mechanical vector for different pathogenic microbes (Mpuchane et al. 2006). In addition, particles from their feces and exuviae are allergenic to some people and cause asthma in others (Gao 2012). It also causes economic losses as it is an omnivore and feeds on a great variety of food sources and other human goods (Rust et al. 1995). In Argentina, these insects are found over a wide geographical range, from the province of Jujuy in the north to the province of Chubut in the south (Crespo and Valverde 2008).

A great variety of strategies are used to control *B. germanica*, including the application of liquid, gel, solid, and foam formulations containing an equally diverse array of active ingredients such as imidacloprid, fipronil, sulfluramid, hydramethylnon, boric acid,

cypermethrin, and deltamethrin, among others (Bennett et al. 2012, CPIA 2015). Repeated use of these products promoted the development of insecticide resistance in *B. germanica* populations.

Resistance is a genetically based characteristic that allows insects to tolerate concentrations of insecticides that are lethal to other individuals of the same species (Tabashnik et al. 2014). The proliferation of resistant individuals makes it more difficult to control the insects and subsequently damage caused by the pest increases. Sometimes, resistance leads to incorrect practices like increasing the frequency or quantity of insecticide treatments. With resistance reports for 42 active ingredients in different parts of the world, *B. germanica* is seventh in the 'Top 20' chart of the most insecticide-resistant insect species (Whalon et al. 2008). In Argentina, the first scientific report of insecticide resistance in *B. germanica* was published at the beginning of this century (Tairiol et al. 2001). More recently, resistance to deltamethrin was detected in 40 German cockroach samples collected in the city of Buenos Aires and other cities in the provinces of Buenos Aires and Córdoba (Alzogaray et al., unpublished data).

Carrying the resistance genotype entails an energetic cost that can decrease the fitness of these insects compared with their susceptible conspecifics. This characteristic has been reported in species represented by Diptera, Hemiptera, Lepidoptera, and Coleoptera (Kliot and Ghanim 2012). A decrease in fitness associated to resistance is evidenced in the reduction of physiological parameters such as survival rates, development, copulation, and fertility (Feng et al. 2009, Puinean et al. 2010).

The resistant to insecticides genotype might also entail alterations in the behavioral response to odorants. Insecticide-resistant *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) aphids showed a reduced behavioral response to alarm pheromones emitted by their conspecifics compared with susceptible insects (Foster et al. 2007). Likewise, the behavioral and electroantennographical response of insecticide-resistant codling moths, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), was higher when exposed to the attractant kairomone from ripe pears than the response of susceptible individuals (Saufhanor et al. 2007). Also, an alteration in the behavioral response to the repellent effect of the pyrethroid transfluthrin was observed in *Aedes aegypti* (L.) (Diptera: Culicidae) mosquitoes resistant to this insecticide (Wagman et al. 2015).

A repellent is a substance that 'makes insects perform orientated movements that carry them away from the source producing it' (White and Moore 2015). *N,N*-Diethyl-3-methylbenzamide (DEET) is the most widely used insect repellent in the world (Frances 2007). Its success is due to its high efficacy (practically unparalleled), broad spectrum of insecticidal activity, and low toxicity to mammals (EPA 2000). The repellent effect of DEET and many other substances, both synthetic and natural, has been studied thoroughly in mosquitoes that transmit human diseases (Goodyer et al. 2010), to a lesser degree in hematophagous holometabolous insects, and hardly at all in non-hematophagous hemimetabolous (Dolan and Panella 2011).

Taking the above into consideration, the objective of this study was to assess the behavior of susceptible and deltamethrin-resistant first instar nymphs of *B. germanica* in an experimental arena, in the absence and presence of the synthetic repellents DEET and ethyl 3-[acetyl(butyl)amino]propanoate (IR3535).

Materials and Methods

Biological Material

For the studies, we used first instar nymphs of *B. germanica*, 1–5 d old, from stable colonies kept at the Centro de Investigaciones de Plagas e Insecticidas (UNIDEF-CITEDEF-CONICET-CIPEIN). One susceptible colony (CIPEIN) and two deltamethrin-resistant colonies (JUBA and VGBA) were used. The CIPEIN colony has been reared under laboratory conditions in the absence of insecticides for over 20 yr. The JUBA colony was established in March 2013 from a sample collected in the city of Junin (Buenos Aires, Argentina), whereas the VGBA colony was established in June 2012 from a sample collected in the locality of Villa Gesell (Buenos Aires, Argentina). The colonies are kept in a chamber under a temperature of $25 \pm 1^\circ\text{C}$, 60–90% relative humidity, and a 12:12 (L:D) h photoperiod. Powdered beer yeast (Virgen, Buenos Aires), rabbit food pellets, and water were provided ad libitum.

Chemicals

DEET (97%) was purchased from Sigma-Aldrich (Buenos Aires, Argentina) and acetone (technical grade) from Merck (Darmstadt, Germany). IR3535 (99.6%) was kindly provided by Merck

Argentina. Deltamethrin (98.4%) was donated by Chemotecnica S.A. (Buenos Aires, Argentina).

Toxicity Bioassays

Deltamethrin toxicity was quantified by exposing insects to insecticide films on circles of filter paper (7 cm in diameter). Each circle was treated with 0.3 ml of a deltamethrin solution in acetone. Based on results from preliminary assays, nymphs from the CIPEIN colony were exposed to the following concentrations of deltamethrin: 0.49, 0.97, 1.95, 3.90, and 7.80 $\mu\text{g}/\text{cm}^2$. Nymphs from the JUBA and VGBA colonies were exposed to a single limit concentration of 1950 μg deltamethrin/ cm^2 , which in the preliminary tests produced very low mortality. This concentration was obtained by treating the filter paper with 0.3 ml of a solution of 250 mg deltamethrin/ml, which is half the solubility limit of this substance in acetone (WHO 1990). All assays included a control treatment using a piece of filter paper treated with 0.3 ml of acetone alone.

Once the papers were treated, the solvent was left to evaporate for 1 hr. A glass ring was placed on each filter paper (2.5 cm high \times 5 cm diameter) with the inside wall covered in vaseline to prevent the nymphs from escaping during the assays. Ten *B. germanica* nymphs were placed on each piece of filter paper. After 1 hr, the exposure was ended and the nymphs were transferred to a plastic container (10 cm high \times 8 cm diameter) with water and food ad libitum. Mortality was assayed 24 hr later. Four independent replicates were made for the CIPEIN and JUBA colonies and three replicates were performed for the VGBA colony. The Median Lethal Concentration (LC50) was calculated for the CIPEIN colony.

Usually, the resistance to insecticides is quantified by calculating the *Resistance Ratio* (= LC50 of the resistant population / LC50 of the reference susceptible population) (Tabashnik et al. 2014). Here, the LC50 values could not be calculated for the JUBA and VGBA colonies because a concentration near the limit of solubility of deltamethrin in acetone produced very low mortality in these nymphs. For this reason, we calculate the *Resistance Ratio* in the way it is calculated in cases like this one (Sfara et al. 2006):

$$\text{Resistance Ratio} = \frac{\text{maximum concentration applied to the resistant colony}}{\text{LC50 for the susceptible colony}}$$

Behavioral Bioassays

In an Untreated Arena

The experimental arena consisted of a circle of filter paper surrounded by a glass ring as described earlier. A line was drawn softly on the paper circle with a pencil dividing it into halves (zones A and B). The paper circle was placed on the floor of a wooden cabinet lined with melamine (1 \times 0.4 \times 0.4 m). A circular tube of cold light (22W, Luxa, Shanghai, China) was placed in the centre of the roof providing an uniform source of light. A web camera with a resolution of 640 \times 480 pixels (Logitech, Switzerland) was placed 20 cm above the experimental arena. The glass ring was set on the paper circle and a nymph was placed inside the circle. The door of the cabinet was closed and the behavior of the nymph was recorded during 10 min. The position of zones A and B was rotated 90° between each replicate.

To see whether the behavior of nymphs varied throughout the day, six independent replicates were carried out at each of the following intervals of time: 10:00–10:45, 13:00–13:45, and 16:00–16:45.

All assays took place under the same conditions of temperature and humidity as used to breed the colonies. The recordings were saved on the hard disc of a personal computer and analysed with Ethovision XT 10.0 software (Noldus 2013). This program calculates the following parameters: *Distance Traveled* (cm), *Velocity* (cm/s), *Mobility Time* (s), and *Time Spent* (in each half of the arena) (s). A *Distribution Coefficient* (DC) was calculated as follows:

$$DC = (Tt - TA) / Tt$$

where *Tt* is the experimental time and *TA* is the time spent by a nymph in the repellent-treated zone (modified from Alzogaray 2016). The value of *DC* ranges from 0 to 1, with values close to 1 indicating repellence, and values close to 0 attraction. Values around 0.5 indicate that the insect spent approximately the same amount of time in both zones.

In an Arena Treated With Repellent

The same procedure was used as described earlier, but this time each circle of paper filter was cut into halves (zones A and B). Zone A was impregnated with 0.15 ml of an acetone solution containing a repellent and zone B was impregnated with acetone alone. Based on previous preliminary results, the following concentrations were used for DEET: 24.37, 48.7, 97.49, 194.98, and 389.96 $\mu\text{g}/\text{cm}^2$, and for IR3535: 194.98, 389.96, 779.92, 1559.84, and 3119.68 $\mu\text{g}/\text{cm}^2$. Papers with both halves treated with acetone alone were used for the controls. The solvent was left to evaporate for 5 min and then both halves were attached together with adhesive tape. The filter paper was placed on the floor of the cabinet with the side with adhesive tape facing down and a glass ring was placed on top. Then, a nymph was placed in the centre of the ring and its activity was recorded during 10 min. Six independent replicates were carried out for each treatment in each colony.

In another experimental series, mixtures of both repellents were used. The repellent effect of three mixtures of DEET and IR3535 was compared with the effect of these substances alone in order to assess whether some sort of interaction occurred between them (synergy or antagonism). To compare, we chose a concentration of DEET that had produced an intermediate repellent effect in the CIPEIN colony in the previous experiments (97.49 $\mu\text{g}/\text{cm}^2$, which corresponds to a *DC* = 0.75). The same concentration was used for IR3535 which, as evidenced in the previous tests, produced no repellency. We assessed the mixtures DEET:IR3535 3:1, 1:1, and 1:3. The mass of repellent was maintained constant in the three mixtures (97.49 $\mu\text{g}/\text{cm}^2$). Thus, if the mixture produced *DC* values significantly higher than when using DEET alone, it would be evidence of a synergistic interaction. On the contrary, *DC* values significantly lower would suggest the presence of an antagonistic interaction.

Statistical Analysis

The LC50 of deltamethrin for the CIPEIN colony and its 95% Confidence Interval (95% CI) were calculated using the Polo Plus 2.0 program (LeOra Software 2002).

The results of the behavioral tests in an untreated experimental arena were analysed by two-way ANOVA (colony and time). The results of the repellency test were analysed by i) lineal regression and ii) one-way ANOVA followed by Tukey test.

Results

Both the JUBA and VGBA colonies showed a high *Resistance Ratio* when exposed to deltamethrin (Table 1). The LC50 for the CIPEIN colony (susceptible to insecticides) was 2.88 $\mu\text{g}/\text{cm}^2$. Exposure to 1950 $\mu\text{g}/\text{cm}^2$ deltamethrin produced an average of 5.0 and 11.67% mortality in the JUBA and VGBA colonies, respectively. As the concentration of deltamethrin applied was close to its solubility limit in acetone, it was impossible to use higher concentrations. For this reason, the values of LC50 could not be calculated for these colonies. Therefore, we can only report that the values of *Resistance Ratio* were higher than 676.61, indicating an extreme resistance to deltamethrin.

In the next experimental series, the behavior of the nymphs was quantified in an arena comprising a glass ring and a circle of filter paper (Table 2). The nymphs from the three colonies showed a similar behavior. The mean *Distance Traveled* during the experimental time ranged between 325.14 and 515.15 cm; the mean *Velocity*, between 0.64 and 0.99 cm/s; the mean *Mobility Time*, between 487.32 and 517.65 s, and the mean values of *DC*, between 0.45 and 0.55. No significant differences were observed for the 'colony' factor, the 'time' factor, nor the interaction between them (For *CD*, colony: $F = 0.963$, $df = 2, 2, 4, 45$, $P = 0.389$; time: $F = 1.214$, $df = 2, 2, 4, 45$, $P = 0.307$; colony \times time: $F = 0.204$, $df = 2, 2, 4, 45$, $P = 0.935$).

Figure 1 shows representative walking tracks that demonstrate how the behavior of the cockroaches varied when half the experimental arena was treated with DEET. In the absence of DEET, the cockroaches roamed all over the arena and spent most of the time close to the side of the glass ring that prevented them from escaping (Fig. 1A). In the presence of DEET, the cockroaches avoided the treated side of the arena, and as the concentration of the repellent increased they spent more time in the untreated half (Fig. 1B and D).

The values of *DC* in these experiments varied as a linear function of log repellent concentration for the three colonies (Fig. 2; Table 3). According to the multiple linear regression analysis, the values of the three colonies fit the linear model ($P < 0.0001$). The slope and y-intercept of the JUBA and VGBA colonies were not significantly different to that of the CIPEIN colony (for the y-intercepts: CIPEIN vs JUBA, $P = 0.79$; CIPEIN vs VGBA = 0.88; for the slopes CIPEIN vs JUBA, $P = 0.24$; CIPEIN vs VGBA, $P = 0.19$). The minimum concentrations of DEET that produced a significantly different behavior were 97.49 $\mu\text{g}/\text{cm}^2$ for the CIPEIN and JUBA colonies and 194.98 $\mu\text{g}/\text{cm}^2$ for the VGBA colony ($P < 0.05$) (Table 4). The values of *DC* for the maximum concentration of DEET applied (389.96 $\mu\text{g}/\text{cm}^2$)

Table 1. Toxicity of deltamethrin on three colonies of *B. germanica*

Colony	<i>n</i>	Slope (SE)	LC50 ($\mu\text{g}/\text{cm}^2$)	95% CI	<i>Resistance Ratio</i> ^a
CIPEIN ^b	239	1.700 (0.33)	2.882	(0.941–6.924)	–
JUBA ^c	85	–	>1950 ^d	–	>676.61
VGBA ^c	66	–	>1950 ^e	–	>676.61

^aMaximum concentration applied to resistant colony / LC50 for susceptible laboratory colony.

^bSusceptible laboratory colony.

^cRecently field collected colonies.

^dExposure to 1950 $\mu\text{g}/\text{cm}^2$ produced $5.0 \pm 2.89\%$ of mortality in this colony.

^eExposure to 1950 $\mu\text{g}/\text{cm}^2$ produced $11.67 \pm 7.26\%$ of mortality in this colony.

Table 2. Behavior of susceptible and deltamethrin-resistant first instar nymphs of *B. germanica* in an untreated experimental arena

Colony	Distance Traveled (cm) (SE)		
	10:00 a.m.	13:00 p.m.	16:00 p.m.
CIPEIN	325.14 (24.09)	419.26 (53.94)	378.89 (78.20)
JUBA	515.15 (130.99)	496.75 (54.11)	473.47 (48.89)
VGBA	493.32 (115.95)	396.22 (35.88)	381.58 (56.21)
Colony	Velocity (cm/s) (SE)		
	10:00 a.m.	13:00 p.m.	16:00 p.m.
CIPEIN	0.64 (0.04)	0.82 (0.09)	0.76 (0.15)
JUBA	0.98 (0.20)	0.99 (0.08)	0.91 (0.08)
VGBA	0.96 (0.17)	0.77 (0.06)	0.76 (0.10)
Colony	Moving Time (s) (SE)		
	10:00 a.m.	13:00 p.m.	16:00 p.m.
CIPEIN	504.04 (7.96)	502.86 (30.07)	493.31 (16.10)
JUBA	500.70 (38.98)	495.80 (20.38)	517.65 (13.28)
VGBA	487.32 (34.07)	508.74 (13.80)	496.14 (14.67)
Colony	DC ^a (SE)		
	10:00 a.m.	13:00 p.m.	16:00 p.m.
CIPEIN ^b	0.55 (0.03)	0.52 (0.02)	0.52 (0.03)
JUBA ^c	0.51 (0.04)	0.45 (0.05)	0.51 (0.04)
VGBA ^c	0.51 (0.03)	0.47 (0.04)	0.49 (0.05)

^aDistribution Coefficient = $(Tt - TA / Tt)$; where Tt is the experimental time, and TA is the Spent Time (by one nymph in zone A). In this experimental series, zones A and B were untreated.

^bSusceptible laboratory colony.

^cDeltamethrin-resistant colonies.

were 0.94, 0.93, and 0.92 for the CIPEIN, JUBA, and VGBA colonies, respectively.

The behavioral response produced by exposure to IR3535 was weaker compared with the effect of DEET (Fig. 3 and Table 5). Only the DC for the JUBA colony varied as a linear function of log repellent concentration ($DC = 0.29 \log C + 0.12$; $R^2 = 0.88$). IR3535 produced a significant effect as from a concentration of 389.96 $\mu\text{g}/\text{cm}^2$ in the CIPEIN colony, 779.92 $\mu\text{g}/\text{cm}^2$ in JUBA and 1559.84 $\mu\text{g}/\text{cm}^2$ in VGBA ($P < 0.05$). The maximum concentration of IR3535 applied produced DC values of 0.69 for CIPEIN and JUBA colonies, and 0.65 for VGBA.

The last experimental series consisted in exposing the nymphs to different mixtures of DEET:IR3535 (3:1, 1:1, and 1:3), while keeping a constant total mass (Table 6). According to the results shown in Table 5, the mass of IR3535 was not enough to produce repellency in all the

mixtures. The three mixtures elicited a synergistic effect on the CIPEIN colony as their corresponding values of DC were significantly higher than the DC corresponding to DEET alone (DEET vs mixture 3:1, $P < 0.001$; DEET vs mixture 1:1, $P < 0.001$; and DEET vs mixture 1:3, $P = 0.011$). In the JUBA and VGBA colonies, the values of DC for the mixtures with the highest concentrations of DEET (3:1) were slightly higher than the values of this repellent alone, but these differences were not significant (for JUBA: DEET vs mixture 3:1, $P = 0.69$; DEET vs mixture 1:1, $P = 0.36$; and DEET vs mixture 1:3, $P = 0.02$; for VGBA: DEET vs mixture 3:1, $P = 0.05$; DEET vs mixture 1:1, $P = 0.07$; and DEET vs mixture 1:3, $P = 0.92$).

Discussion

In this study, the repellent effect of IR3535 on *B. germanica* was assessed for the first time and compared with the repellency produced by DEET. It is also the first time the effect of IR3535 was evaluated on the behavioral response of insecticide-resistant insects. Compared with the susceptible individuals, the deltamethrin-resistant nymphs of *B. germanica* showed a reduced behavioral response to both repellents when applied separately. A synergistic effect was observed when susceptible nymphs were exposed to DEET:IR3535 mixtures.

To begin with, the behavior of deltamethrin-susceptible and resistant German cockroaches was compared in an untreated experimental arena. Individuals from the three colonies behaved similarly. All nymphs exhibited the behavior previously described for this species when it is in a confined space (Jeanson et al. 2003). The distinctive trait of this behavior is that in the absence of specific stimuli, the nymphs spend most of their time walking near the edge of the arena. Moreover, the values of DC were very close to 0.5, evidencing that the nymphs spent almost the same amount of time on each side of the experimental arena. No variations in behavior were observed within the time lapse from 10:00 to 16:00. Therefore, we were able to carry out the bioassays using repellents within a flexible working time. Once we had determined that the deltamethrin-susceptible and resistant cockroaches behaved similarly in an untreated arena, we went on to study their behavior in similar arenas treated with insect repellents.

The comparative repellent effects of DEET and IR3535 vary among species. For example, IR3535 is a good repellent against ticks, while in most laboratory and field assays DEET was relatively ineffective on these arachnids (Frances 2007, Puccetti 2007). However, both compounds elicited a very similar response in nymphs of the blood-sucking bugs *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) and *Rhodnius prolixus* (Stål) (Hemiptera: Reduviidae) (Alzogaray 2016, Reynoso et al. 2017). In the first instar nymphs of *B. germanica* used in this study, the repellency of IR3535 was much weaker than DEET in the three colonies studied.

There are very few studies on the behavior of insecticide-resistant insects exposed to repellents. Hereditary insensitivity against the repellent effect of the pyrethroid transfluthrin has been reported in *Ae. aegypti* mosquitoes and is associated to a decreased toxicological response to the same insecticide (in this case it is important to note that the substance used as a repellent is the same as the one the mosquitoes developed resistance against, and, therefore, it is possible to think that the same mechanism responsible for the resistance could be modifying the behavioral response to the repellent effect) (Wagman et al. 2015). In another study, pyrethroid-resistant nymphs of *T. infestans* showed the same behavior as susceptible nymphs when exposed to DEET (Sfara et al. 2006). In the present study, the behavioral response of *B. germanica* nymphs exposed to DEET and

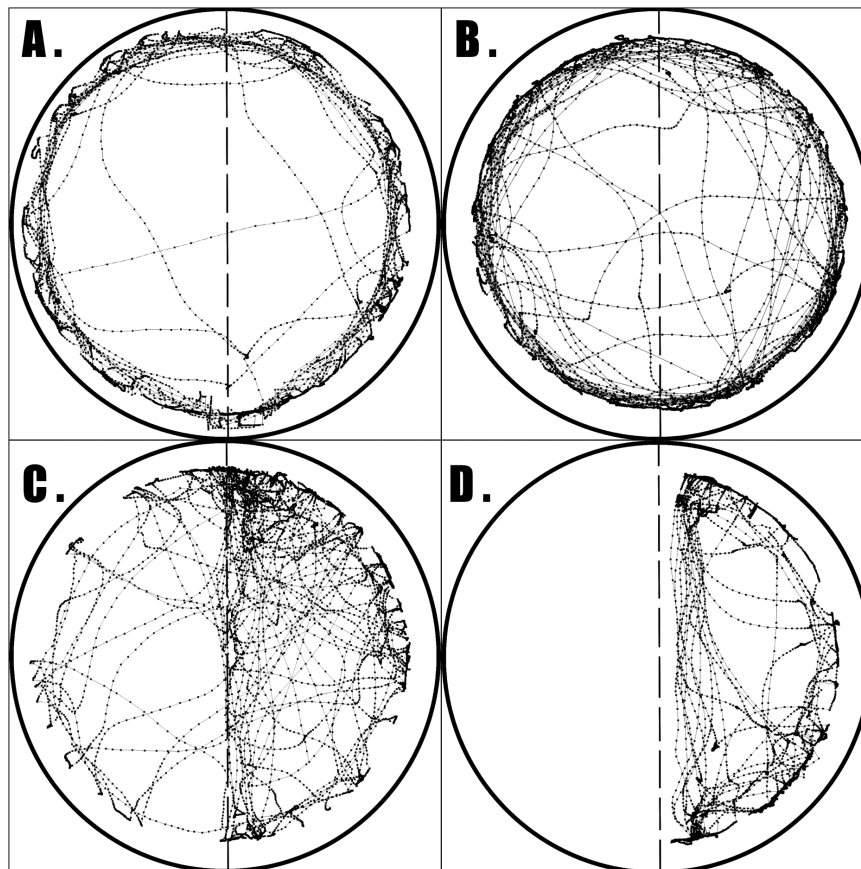


Fig. 1. Representative walking maps showing how the nymphs behavior varied when exposed to different concentrations of DEET. (A) Both halves of the arena were treated with acetone alone. (B–D) The right half of the arena was treated with acetone alone; the left half, with 24.31, 97.25, and 389 $\mu\text{g}/\text{cm}^2$ of DEET, respectively.

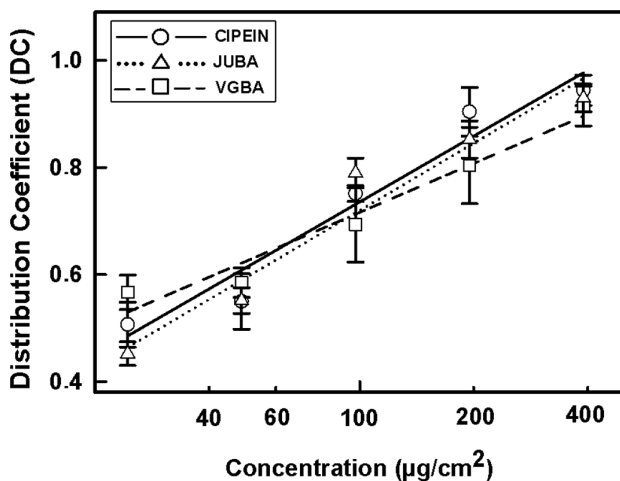


Fig. 2. Variation of DC values as a function of log of DEET concentration for susceptible and deltamethrin-resistant first instar nymphs of *B. germanica*. DC : Distribution Coefficient = $(Tt - TA / Tt)$; where Tt is the experimental time and TA is the Spent Time by one nymph in zone A. Each symbol is the mean of six independent replicates. Vertical lines are standard errors.

IR3535 was lower in the deltamethrin-resistant colonies. DEET produced repellency as from 97.49 in the CIPEIN and JUBA colonies, and from 194.98 $\mu\text{g}/\text{cm}^2$ in the VGBA colony. The differences were more noticeable for IR3535 that only produced a repellent effect at

Table 3. Lineal regression equations for Distribution Coefficient (DC) as a function of the log of DEET concentration for susceptible and deltamethrin-resistant first instar nymphs of *B. germanica*

Colony	Regression ^a	R^2
CIPEIN ^b	$DC = -0.07 \log C + 0.40$	0.95
JUBA ^c	$DC = -0.12 \log C + 0.42$	0.95
VGBA ^c	$DC = 0.10 \log C + 0.31$	0.97

^aIn the equations, $DC = (Tt - TA / Tt)$, where Tt is the experimental time and TA is the Spent Time (by one nymph in zone A); C : DEET concentration. In this experimental serie, zone A was treated with a solution of repellent in acetone, and zone B was treated with acetone alone.

^bSusceptible laboratory colony.

^cDeltamethrin-resistant colonies.

a concentration of 389.96 $\mu\text{g}/\text{cm}^2$ in the CIPEIN colony, 779.92 $\mu\text{g}/\text{cm}^2$ in the JUBA colony and 1559.84 $\mu\text{g}/\text{cm}^2$ in the VGBA colony.

The mechanisms conferring resistance to deltamethrin in the JUBA and VGBA colonies used in this study are still unknown. The main mechanisms responsible for pyrethroid-resistance are enhanced metabolic activity and voltage-gated sodium channel insensitivity (Khambay and Jewess 2005). Theoretically, any of these mechanisms could also modify the behavioral response to repellents. For instance, there is evidence that DEET is metabolized by microsomal mixed-function oxidases in *R. prolixus* (Alzogaray 2016). On the other hand, modifications to the voltage-gated sodium channels

Table 4. Mean *Distribution Coefficient (DC)* values for susceptible and deltamethrin-resistant first instar nymphs of *B. germanica* exposed to different concentrations of DEET

DEET ($\mu\text{g}/\text{cm}^2$)	<i>DC</i> ^a (SE)		
	CIPEIN ^b	JUBA ^c	VGBA ^c
0	0.50a (0.02)	0.50a (0.02)	0.51a (0.03)
24.37	0.51a (0.04)	0.45a (0.02)	0.56a (0.02)
48.7	0.55a (0.05)	0.55a (0.02)	0.59a (0.03)
97.49	0.75b (0.01)	0.79b (0.03)	0.69ab (0.07)
194.98	0.90bc (0.05)	0.85bc (0.03)	0.80bc (0.07)
389.96	0.94c (0.03)	0.93c (0.03)	0.92c (0.04)

In each column, numbers followed by the same letter are not significantly different ($P > 0.05$).

^a*Distribution Coefficient* = $(Tt - TA / Tt)$, where Tt is the experimental time and TA is the *Spent Time* (by one nymph in zone A). In this experimental serie, zone A was treated with a solution of repellent in acetone, and zone B was treated with acetone alone.

^bSusceptible laboratory colony.

^cDeltamethrin-resistant colonies.

Table 5. Mean *Distribution Coefficient (DC)* values for susceptible and deltamethrin-resistant first instar nymphs of *B. germanica* exposed to different concentrations of IR3535

IR3535 ($\mu\text{g}/\text{cm}^2$)	<i>DC</i> ^a (SE)		
	CIPEIN ^b	JUBA ^c	VGBA ^c
0	0.51a (0.03)	0.50a (0.01)	0.52a (0.02)
194.98	0.53ab (0.02)	0.57ab (0.02)	0.59ab (0.04)
389.96	0.66bc (0.02)	0.56ab (0.03)	0.61ab (0.02)
779.92	0.64bc (0.03)	0.62bc (0.01)	0.67ab (0.03)
1559.84	0.69c (0.04)	0.68cd (0.01)	0.69b (0.05)
3119.68	0.69c (0.03)	0.69d (0.02)	0.65ab (0.05)

In each column, numbers followed by the same letter are not significantly different ($P > 0.05$).

^a*Distribution Coefficient* = $(Tt - TA / Tt)$, where Tt is the experimental time and TA is the *Spent Time* (by one nymph in zone A). In this experimental serie, zone A was treated with a solution of repellent in acetone, and zone B was treated with acetone alone.

^bSusceptible laboratory colony.

^cDeltamethrin-resistant colonies.

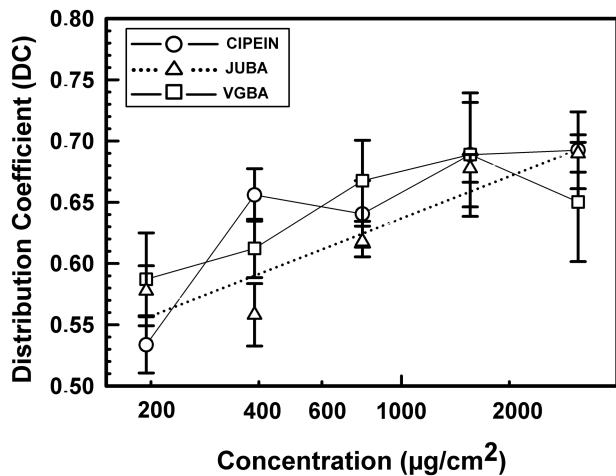


Fig. 3. Variation of *DC* values in susceptible and deltamethrin-resistant first instar nymphs of *B. germanica* exposed to IR3535. *DC*: *Distribution Coefficient* = $(Tt - TA / Tt)$; where Tt is the experimental time and TA is the *Spent Time* by one nymph in zone A. Each symbol is the mean of six independent replicates. Vertical lines are Standard Errors.

could alter the olfactory pathway from the receptor to the central nervous system.

The last experiment presented here consisted in evaluating the effects of different DEET:IR3535 mixtures. Studies with combinations of repellents are very scarce. The repellency produced by essential oils or mixtures of essential oils has been studied (Hieu et al. 2010, Kim et al. 2012), but these models are not appropriate for studying interactions. Essential oils comprise many components and the end result can be the combination of different types of interactions (synergy, potentiation, antagonism).

Table 6. Mean *Distribution Coefficient (DC)* values for susceptible and deltamethrin-resistant first instar nymphs of *B. germanica* exposed to different mixtures of DEET:IR3535

DEET: IR3535 ^a	<i>DC</i> ^b (SE)		
	CIPEIN ^c	JUBA ^d	VGBA ^d
0:0	0.54a (0.02)	0.54a (0.03)	0.50a (0.01)
1:0	0.70b (0.02)	0.77bc (0.03)	0.67bc (0.04)
0:1	0.61ab (0.03)	0.57a (0.03)	0.63ab (0.03)
3:1	0.94c (0.02)	0.84b (0.03)	0.79c (0.03)
1:1	0.88c (0.04)	0.67ac (0.04)	0.78c (0.05)
1:3	0.83c (0.03)	0.60a (0.05)	0.68bc (0.03)

In each column, numbers followed by the same letter are not significantly different ($P > 0.05$).

^aIn all cases, the concentration of repellent was 97.49 $\mu\text{g}/\text{cm}^2$.

^b*Distribution Coefficient* = $(Tt - TA / Tt)$, where Tt is the experimental time and TA is the *Spent Time* (by one nymph in zone A). In this experimental serie, zone A was treated with a solution of repellent in acetone, and zone B was treated with acetone alone.

^cSusceptible laboratory colony.

^dDeltamethrin-resistant colonies.

In the present study, three mixtures of DEET:IR3535 showed a synergistic interaction in *B. germanica* nymphs from CIPEIN colony. Although the three mixtures contained a concentration of IR3535 that was not enough to produce repellency, all of them produced a

behavioral response that was higher than the response produced by the same mass of DEET alone.

It is difficult to speculate why the repellent effect of IR3535 is weaker compared with DEET in *B. germanica* nymphs. The same can be said for the synergistic effect observed in the CIPEIN colony. This difficulty is based on how little we know about the way repellents act. Studies carried out in the last years indicate that the perception of these substances is a highly complex phenomenon.

Synthetic repellents activate olfactory receptors, elicit responses from olfactory sensory neurons, and modify insect behavior (Bohbot and Dickens 2012). ‘Generic repellent receptors’ to different repellents have been discovered in *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) (Syed et al. 2011). One of these receptors responded to DEET, IR3535, and picaridin. However, another receptor in this fly is only sensitive to picaridin. Other studies suggest that DEET might be acting on different molecular targets in different species (Leal 2014, Xu et al. 2014). As the molecular basis of repellent perception in insects is revealed, we will be able to understand the causes of the variations in behavioral response between species and the nature of the interactions that occur when insects are exposed to different combinations of repellents.

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