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Lethal and sublethal effects of eucalyptol on *Triatoma infestans* and *Rhodnius prolixus*, vectors of Chagas disease

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Abstract

Eucalyptol is the common name for a cyclic ether monoterpene found in essential oils from *Eucalyptus* species and other plants. Several reports showed its insecticidal activity. In this work, visible symptoms of intoxication, effect on locomotor activity, knock-down, and repellence produced by eucalyptol were evaluated on nymphs of *Triatoma infestans* Klug and *Rhodnius prolixus* Ståhl (both Hemiptera: Reduviidae). Both insects are among the main vectors of Chagas disease in Latin America. Visible symptoms of intoxication were similar to those observed for neurotoxic insecticides. A video tracking technique was used to evaluate locomotor activity and repellence by exposing the nymphs to impregnated papers. Hyperactivity (a non-directional increase in locomotor activity) is a symptom of intoxication that is used to detect triatomines in rural houses, because it causes the insects to leave their refuges. Eucalyptol produced hyperactivity only in *T. infestans* at a concentration 1 000× higher than the positive control, deltamethrin [(*S*)-cyano(3-phenoxyphenyl)methyl (1*R*,3*R*)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate]. It also produced repellence on both species at a concentration 10× higher than the positive control, DEET (*N*,*N*-diethyl-3-methylbenzamide). Knock-down effect was evaluated by exposing the nymphs to impregnated papers in closed containers (contact and fumigation simultaneously). Values of knock-down time for 50% of exposed nymphs (KT_{50}) were calculated for various concentrations of eucalyptol. The onset of knock-down occurred more rapidly as the concentration increased. In the best cases, eucalyptol was 12–15× less toxic than the positive control dichlorvos (2,2-dichlorovinyl dimethyl phosphate). After these results, eucalyptol seems discouraged as a hyperactivant agent for monitoring insects in rural houses. Nevertheless, its knock-down and repellence effect on vectors of Chagas disease deserve further investigation.

Introduction

During the second half of the 20th century, the indiscriminate overuse of synthetic insecticides (organochlorines, organophosphorus, and carbamates) produced environmental contamination, wildlife destruction, as well as undesirable effects on human health (Alavanja et al., 2004; Rattner, 2009). Recognition of these problems came by the hand of the ban or restriction of many compounds. At the same time, insect pest populations started to become resistant to insecticides (Parakrama Karunaratne, 1998). Because of this, it is essential to have alternative products to replace those who are no longer efficient.

Nowadays there is a constant search for new insecticides. In this context, plants have proven to be an important source of molecules showing insecticidal activity in laboratory bioassays (Isman, 2006; Dayan et al., 2009). In the last years, many works have reported the insecticidal properties of essential oils and their monoterpene components (Shaaya & Rafaeli, 2007).

Eucalyptol is the common name of 1,3,3-trimethyl-2-oxabycyclo[2.2.2]octane, a cyclic ether monoterpene

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also known as 1,8-cineole. At environmental temperature, it is a colorless liquid with camphor smell (Hawley, 1993). Eucalyptol is commonly found as the main component of *Eucalyptus* spp. essential oils (Coppen, 1995). It is also produced by plants of the genera *Achillea*, *Alpinia*, *Blumea*, *Cinnamomun*, *Eugenia*, *Laurus*, *Lavandula*, *Melaleuca*, *Mentha*, *Rosmarinus*, and *Salvia* (Lee et al., 2004). Eucalyptus essential oil was categorized as 'Generally Regarded as Safe', classified as 'non-toxic' and approved for use on human skin by the United States Food and Drug Authority (USEPA, 1993; Rehman et al., 2014). However, some cases of human poisoning upon ingestion of this oil have been reported (Spoerke et al., 1989; Webb & Pitt, 1993; Barnes, 1996; Darben et al., 1998).

The bioactivity of eucalyptol has been documented extensively. It has shown broad antibacterial and antifungal activity (Vilela et al., 2009; Mulyaningsih et al., 2010; Stojkovic et al., 2011; Shukla et al., 2012), antioxidant effect (Tepe et al., 2007; Wang et al., 2008), and anti-inflammatory and vasorelaxant action in rats (Santos et al., 2004; Pinto et al., 2009). Eucalyptol also has a broad insecticidal activity reported against stored grain beetles (Papachristos & Stamopoulos, 2002; Lee et al., 2004), human lice (Yang et al., 2004; Toloza et al., 2010), German cockroaches (Alzogaray et al., 2011), house flies and blow flies (Sukontason et al., 2004; Tarelli et al., 2009; Kumar et al., 2012), sandflies (Maciel et al., 2010) and mosquitoes (Batish et al., 2008; Lucía et al., 2009), among others species.

In addition to mortality, pesticides produce sublethal effects on insects. These are defined as 'effects (either physiological or behavioral) on individuals that survive exposure to a pesticide (the pesticide dose/concentration can be sublethal or lethal)' (Desneux et al., 2007). Repellence, metamorphosis interference, and reduced fecundity and egg hatchability are sublethal effects that have been reported for eucalyptol in insects (Stamopoulos et al., 2007; Alzogaray et al., 2011; Liška et al., 2011).

The blood-sucking bugs *Triatoma infestans* Klug and *Rhodnius prolixus* Ståhl (both Hemiptera: Reduviidae) are among the most important vectors of *Trypanosoma cruzi* (Chagas), the protozoon that causes Chagas disease (Stevens et al., 2011). This is the most severe parasitic disease of the American continent (Organización Panamericana de la Salud, 2006). In Argentina, there are 2 300 000 infected people (7.2% of total population) and 7 300 000 people are exposed in endemic areas, turning it into the main endemic in this country (Gimenez & Mitelman, 2010). During 2009 and 2010, several indexes related with entomological surveillance and vector control have improved, but the goals considered acceptable have not yet been reached (Ministerio de Salud de la Nación, 2013).

Essential oils containing eucalyptol have shown antiparasitic action against *T. cruzi* (Raposo Borges et al., 2012), but evaluations on Chagas vectors are scarce. On *T. infestans*, the effects of eucalyptol had not been evaluated before this study. On *R. prolixus*, eucalyptol has only been reported to show good performance as a fumigant on first instars (Sfara et al., 2009). The aim of this study was to evaluate the visible intoxication symptoms, the effect on locomotor activity, the knock-down, and the repellence produced by eucalyptol on first instars of *T. infestans* and *R. prolixus*.

Materials and methods

Biological material

First instars of *T. infestans* and *R. prolixus*, 1–5 days old and starved since eclosion, were used in all experiments. The *T. infestans* nymphs emerged from eggs provided by the Centro de Referencia de Vectores (Santa María de Punilla, Córdoba, Argentina), whereas the *R. prolixus* nymphs came from a stable colony reared at the Centro de Investigaciones de Plagas e Insecticidas (CIPEIN, Villa Martelli, Buenos Aires, Argentina).

Resistance to the pyrethroid deltamethrin was reported for *T. infestans* from several places in Argentina (Vassena et al., 2000; Picollo et al., 2005). For this reason, prior to performing the bioassays, the toxicity of deltamethrin was evaluated on the insects applying a diagnostic dose (which kills 100% of individuals from a susceptible colony) (ffrench-Constant & Roush, 1990). Every time we received a sample of *T. infestans*, we applied the diagnostic dose on a group of 1- to 3-day-old first instars. In all cases, mortality was 100%, suggesting lack of resistance.

Chemicals

All the chemicals used in this work were technical grade. Eucalyptol (99%) and *N*,*N*-diethyl-m-toluamide (DEET, 97%) were bought from Sigma Aldrich (Buenos Aires, Argentina); DEET was used as positive control in repellence bioassays. Deltamethrin (98%) and dichlorvos (96.5%) were used as positive controls in bioassays of locomotor activity and knock-down, respectively. Both of them were a gift from Chemotecnica (Spegazzini, Argentina). Analytical grade acetone (Merck, Darmstadt, Germany) was used as solvent.

Recording equipment

A video camera black and white closed-circuit (VC, 1910; Sanyo Electrical, Tokyo, Japan) and an image analyzer (Videomex V, Columbus, OH, USA) were used to evaluate repellence and locomotor activity. The video camera captures the image of the insects placed on a circular piece of treated filter paper. The image analyzer converts the analogue signal input from the video camera into digital data with a resolution of 256 × 192 pixels and an acquisition and processing speed of 30 frames s^{-1}. On the screen, the video signal colors are inverted: white objects appear black and vice versa. Therefore, the presence of insects on the filter paper is determined by visual contrast between the subjects (white) and the paper background (black), and is scored as the number of enlightened pixels. To quantify nymph movement, Videomex-V uses the Multiple Zone Motion Monitor software that compares consecutive frames captured by the camera and records the number of pixels that changed from 'on' to 'off' and vice versa. The sum of pixels that changes during experimental time is called 'motion' (M). The software also calculates the average number of pixels 'on' during experimental time. This parameter is called 'area' (A), and represents the average area occupied by the insects on the video image.

The illumination during testing was provided by a cold-light lamp (22 W; Luxa, Shanghai, China) placed at the zenith of the arena. Temperature was maintained at 26 ± 2 °C. Each set of data was imported and processed in a personal computer.

Description of visible intoxication symptoms

A disc of filter paper (4 cm diameter) was impregnated with 0.09 ml of a solution of eucalyptol in acetone. After acetone evaporated, the filter paper was placed at the bottom of a plastic container (4 cm diameter, 4 cm high). Ten first instars were placed on the filter paper and the container was closed with a plastic lid. As a control, 10 nymphs were placed on a filter paper treated with acetone alone. The normal triatomine locomotor behavior was confirmed experimentally with the control groups. Their visible changes (modifications or complete loss of normal movement of legs during walking) and the appearance of other visible unexpected behavior (grooming and/or feeding movements in the absence of triggering stimuli) were considered intoxication symptoms and recorded. Symptoms were divided into two phases according to their severity: first phase (minor to moderate) and second phase (severe). The observations were done throughout the knock-down bioassays (7 h maximum). Five concentrations of eucalyptol were studied: 39, 390, 780, 1 950, and 3 900 μg cm^{-2}. Six independent replicates were performed.

Quantification of locomotor activity

Discs of filter papers (70 mm diameter, 101 FAST; Hangzhou Xinxing Paper Industry, Fuyang, China) were impregnated by pipette (ensuring total and even distribution of the solution) with 0.3 ml of solution of eucalyptol or deltamethrin (positive control) in acetone. Through preliminary bioassays, the lowest effective concentration observed (i.e., the lowest concentration which increases nymph locomotor activity) was determined for both eucalyptol and deltamethrin. After this, bioassays were performed using serial dilutions (10×) including the lowest effective concentration observed. This approach allowed us to identify threshold concentrations and whether the effect on nymph locomotor activity varies with concentration.

The following concentrations were used: 39, 390, and 3 900 μg cm^{-2} for eucalyptol, and 0.0039, 0.039, 0.39, and 3.9 μg cm^{-2} for deltamethrin. Each bioassay had a negative control (acetone alone). After the solvent evaporated, the filter paper disc was placed on a horizontal surface and a glass ring (2.5 cm high, 5.5 cm diameter) was placed on top. Finally, four-first instars were gently placed in the center of the arena enclosed by the ring. In preliminary bioassays, after adjusting the control of the digitized video image to remove noise and get optimal tracking of the subject image, a poor repeatability was observed when using only one nymph. This is because first instars of triatomines are very small (3 mm long, 1.2 mm wide). For this reason, small changes in nymph position caused important variation in the area occupied by the insects (pixels 'on' in the video image). However, when four nymphs were placed on the experimental arena repeatability was good. No obvious aggregation occurred in the spatial distribution of the four nymphs on the arena.

To determine the nymph's locomotor activity we calculated the quotient M/A (Alzogaray et al., 1997). The results were expressed in units of pixels per area.

Evaluation of knock-down

During the bioassays for recording the symptoms of intoxication (see above), the number of knocked-down insects was recorded every 10 min. Insects were considered knocked down when they were unable to stand. Each trial was interrupted when 90% of the insects were knocked down or after 7 h of exposure. Dichlorvos, an organophosphate fumigant insecticide, was used as positive control (same concentrations as eucalyptol). Six replicates (10 nymphs each) were made for each trial. The results were used to calculate values of knock-down time for 50% of exposed nymphs (KT_{50}).

To assess knock-down recovery, nymphs of both species were exposed to the highest concentration of eucalyptol that showed important toxicity in the above described bioassays (3 900 μg cm^{-2}), for two durations: KT_{50} and KT_{90}. Nymphs were then moved to clean containers with untreated filter papers and checked for knock-down 24 and 48 h later. Two replicates (10 nymphs each) were made for each trial. If a knock-down recovery phenomenon exists, the number of knocked-down nymphs at 48 h would be lower than at 24 h (because some of the knocked-down nymphs at 24 h will be asymptomatic at 48 h).

Evaluation of repellence

The experimental design was similar to that used to measure locomotor activity but with the following modifications: (1) each filter paper was divided into two equal parts, one half being treated with 0.15 ml of a solution of the monoterpene in acetone, the other half with 0.15 ml of the solvent alone; and (2) the image analyzer was programmed to record the motion parameters on each zone separately. All trials lasted 15 min, consisting of 30 recording sessions of 30 s each. The design was completely randomized, with six independent replicates for each trial and three concentrations of eucalyptol (39, 390, and 3 900 μg cm^{-2}). Each bioassay had a negative control (acetone alone). DEET, used as positive control, was applied at 3.9, 39, and 390 μg cm^{-2}. We expressed repellence by a distribution coefficient (Sfara et al., 2011): DC = (AT-At)/AT, where AT is the total area occupied by nymphs on the arena, and At is the area occupied by nymphs on the treated zone, both during the experimental time. This coefficient varies between 0 and 1; 0 corresponds to the case where the substance produces maximum attraction, and 1 to the case where it produces maximum repellence. The 0.5 value corresponds to an equal distribution of the insects between treated and untreated zones (random distribution).

Statistical analysis

All analyses were done using the InfoStat 2014/e statistical package (Di Rienzo et al., 2014). Data from locomotor activity and repellence bioassays were analyzed (independently per each species dataset) using one-way ANOVA. If ANOVA indicated significant treatment effects, Fisher's least significant difference (LSD) test for post-hoc comparisons was used to explore differences between treatment pairs. If ANOVA assumptions were not met, data were analyzed using Kruskal–Wallis ANOVA on-ranks followed by Dunn's post-hoc comparisons. Percentage of abdomen distension was analyzed by t-test. KT_{50} values were calculated with their respective 95% confidence interval (CI) using the statistical software for correlated data developed by Throne et al. (1995). Differences between KT_{50} values were considered significant if the 95% CIs did not overlap.

Results

Visible intoxication symptoms

Nymphs exposed to eucalyptol showed leg paralysis, abnormal movements of proboscis and antennae, inability to return to their normal position after accidentally getting into dorsal decubitus position, and other symptoms resembling toxic effects on the nervous system (Table 1). Neither species-specific nor concentration-specific symptoms were identified. Not all symptoms were necessarily observed in the same individual and the order of appearance was sometimes different.

Exposure to two concentrations of eucalyptol also produced abdominal distension in nymphs of both species (Table 2). Control groups did not show it in any case. Both concentrations of eucalyptol produced the effect on *T. infestans* and *R. prolixus* nymphs (t-test, *T. infestans*: t = 4.365, d.f. = 5, P = 0.007; *R. prolixus*: t = 9.851, d.f. = 5, P<0.001), but only the effect of the higher one was significantly different from the control.

Table 1 Visible symptoms of intoxication with eucalyptol observed in first instars of *Triatoma infestans* and *Rhodnius prolixus*

First phase (minor to moderate)	Second phase (severe)
• Abnormal rest position: ventral contact of full body with support surface (at normal rest position, the body remains suspended over the support surface) • Reverse or lateral walk • Flexion of antennal flagella 90° outwards • Proboscis extension (in absence of phagostimulants) • Paralysis of hind legs • Antennas aligned forward	• Reverse walk with elevated abdomen or normal walk with leaning abdomen • Slow vertical oscillation of body over antero-posterior axis • 'Praying mantis' position: forelegs flexed beneath elevated thorax • Unable to return to normal position after accidentally getting in dorsal decubitus position • Total immobility

Effect on locomotor activity

Eucalyptol induced an increase in locomotor activity in both species (Figure 1), but this was significantly different from its control only for *T. infestans* exposed to 3 900 µg cm^{-2} (Dunn's test: H = 19.88, d.f. = 5, P = 0.001). Deltamethrin (positive control) hyperactivated both *T. infestans* (at 0.39 µg cm^{-2}) and *R. prolixus* (at 0.039 µg cm^{-2}) (Fisher's test: *T. infestans*: $F_{4,29} = 3.04$, P = 0.036; *R. prolixus*: $F_{4,29} = 3.47$, P = 0.022).

Knock-down

The values of KT_{50} for eucalyptol and dichlorvos (positive control) applied as films on filter papers are shown in Figure 2. KT_{50} value for the lowest concentration of eucalyptol on *R. prolixus* was not calculated because <50% of knocked-down nymphs were observed after 7 h of exposure. Dichlorvos was at least 12-15× more toxic than the monoterpene for both species. No recovery was observed in knocked-down nymphs exposed to 3 900 µg cm^{-2} of eucalyptol (the number of knocked-down nymphs at 48 h was never lower than at 24 h).

Table 2 Mean (± SE) percentage abdomen distension observed in first instars of *Triatoma infestans* and *Rhodnius prolixus* exposed to eucalyptol

Dose (µg cm^{-2})	*T. infestans*	*R. prolixus*
0 (control)	0	0
390	10 ± 5.2	18.3 ± 7.5
3900	15 ± 4.3*	23.3 ± 4.2*

*Significantly different from control (t-test: P<0.05).

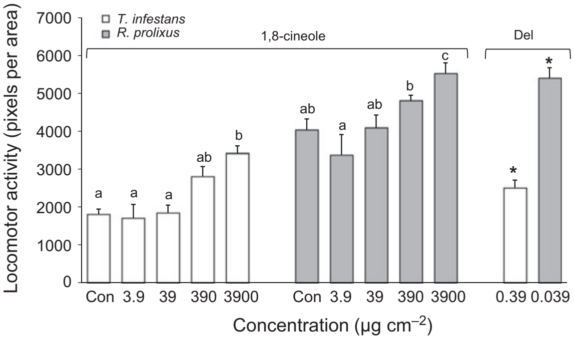

Figure 1 Mean (+ SE) locomotor activity of *Triatoma infestans* and *Rhodnius prolixus* nymphs exposed to filter paper treated with different concentrations of eucalyptol. Each bar represents the mean of six independent replicates. Con: control, Del: deltamethrin (only the lowest effective concentration is shown). Means within a group marked with the same letter are not significantly different (Dunn's test: P<0.05 for *T. infestans* and Fisher's LSD: P<0.05 for *R. prolixus*). The asterisks for deltamethrin indicate significant difference from corresponding controls (Fisher's LSD: P<0.05).

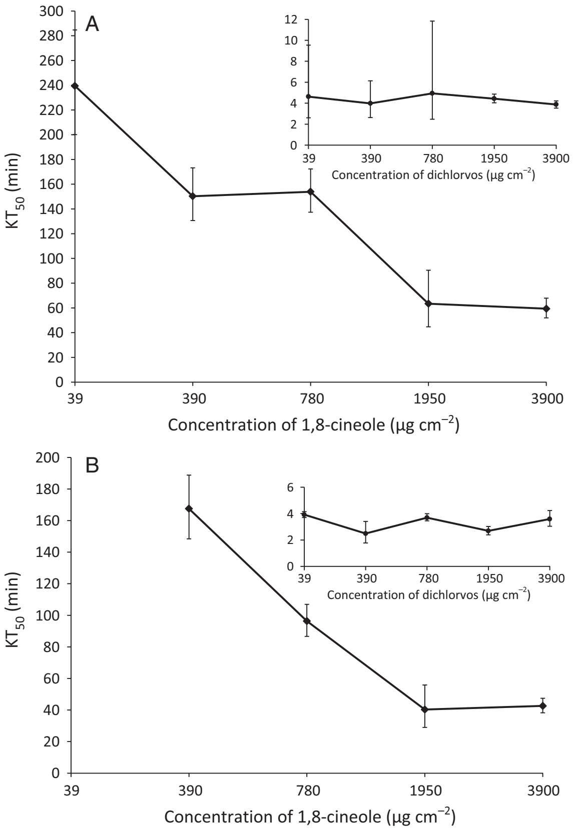

Figure 2 Mean KT_{50} values (+ 95% confidence interval) for eucalyptol on first instar (A) *Triatoma infestans* and (B) *Rhodnius prolixus*. Each symbol represents the mean of six independent replicates. Insets show KT_{50} values for dichlorvos (positive control). All KT_{50} values for eucalyptol are significantly different from positive control (95% CIs do not overlap).

Repellence

The distribution coefficients for both *T. infestans* and *R. prolixus* nymphs exposed to eucalyptol indicated significant repellence at 3 900 µg cm^{-2} (Figure 3). DEET (positive control) at 390 µg cm^{-2} produced a repellent effect significantly higher than the negative control, for both species (Fisher's test: *T. infestans*: $F_{3,23} = 18.3$; *R. prolixus*: $F_{3,23} = 17.35$, both P<0.001).

Discussion

In this study, we describe the visible intoxication symptoms and evaluate the changes in locomotor activity, knock-down, and repellence produced by the monoterpene eucalyptol on first instars of *T. infestans* and

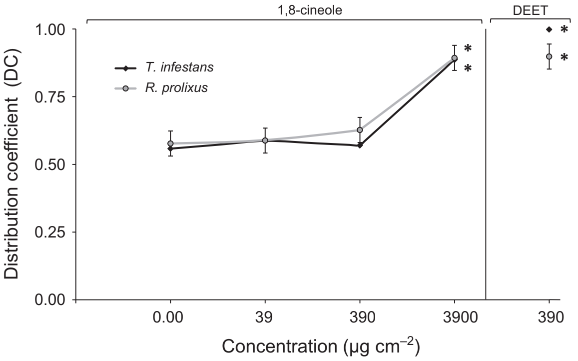

Figure 3 Distribution coefficient values for eucalyptol on nymphs of *Triatoma infestans* and *Rhodnius prolixus*. Each symbol represents the mean of six independent replicates. 0.00: control, DEET: *N*,*N*-diethyl-m-toluamide (only the lowest effective concentration is shown). Symbols marked with asterisks are significantly different from their corresponding controls (Fisher's LSD: P<0.05).

R. prolixus, vectors of Chagas disease. The mode of action of monoterpenes is still relatively unknown and may not be represented by a single mechanism (Grodnitzky & Coats, 2002). Our findings suggest that eucalyptol could affect the insect's nervous system, as intoxicated nymphs of *T. infestans* and *R. prolixus* displayed hyperactivity, incoordination, paralysis, and other symptoms resembling the response elicited by neurotoxic insecticides as organophosphates (Wood et al., 1982) and pyrethroids (Alzogaray et al., 1997). It is also noticeable that eucalyptol caused abdominal distension in both species studied here. *Triatoma infestans* and *R. prolixus* are hematophagous insects able to ingest several times their own weight in blood. This is possible because their cuticle can stretch rapidly to accommodate large meals. When *R. prolixus* takes a blood meal, the abdomen surface is quadrupled and the cuticle becomes thinner (Orchard et al., 1988). This phenomenon, called plasticization, is initiated by blood-sucking activity and is under control of the central nervous system. Abdomen distension in absence of any stimulants was also reported in instars of *T. infestans* intoxicated with the pyrethroid *cis*-permethrin (Alzogaray, 1996). However, no abdominal distension was observed in similar experiments with alcohol monoterpenes (Moretti et al., 2013). How and why these starved insects intoxicated with eucalyptol or *cis*-permethrin undergo cuticle plasticization remains to be investigated.

Locomotor hyperactivation (a non-directional increase in locomotor activity) is the first intoxication symptom observed in insects exposed to pyrethroids (Gammon, 1978; Miller & Adams, 1982). This symptom is exploited to detect vectors of Chagas disease in rural houses, because hyperactivated insects end up leaving their refuges (flushing-out phenomenon), making it easier to estimate their presence and abundance (Pinchin et al., 1980). Tetramethrin is a flushing-out agent used for Chagas disease vectors. This pyrethroid exerts a very slow lethal effect though a good flushing-out activity for large nymphs and adults of triatominae (Wood et al., 1993; Gürtler et al., 1999). In Argentina, specialized staff from the Servicio Nacional de Chagas sprays aerosolized tetramethrin on the walls and roofs of rural houses (Gürtler et al., 1995). Triatomines that leave their refuges are collected (1 man-hour per house). With one triatomine detected, a house is considered positive (infested).

At present, alternative flushing-out agents need to be identified because resistance to pyrethroids, and therefore to hyperactivation (Sfara et al., 2006), has been detected in *T. infestans* populations from Argentina and Bolivia (Vassena et al., 2000; Picollo et al., 2005). Taking this into account, we had firstly planned the use of tetramethrin as a positive control. Unexpectedly, preliminary bioassays indicated that this pyrethroid does not hyperactivate first instars of neither *T. infestans* nor *R. prolixus* when applied as films on filter papers. So, we used deltamethrin as positive control instead. Eucalyptol showed a low hyperactivation effect on first instars of *T. infestans* and only applied at a concentration 1 000× higher than deltamethrin. Moreover, it failed to hyperactivate nymphs of *R. prolixus*. When interpreting these results, one must keep in mind that deltamethrin is among the most powerful insecticides used for controlling pest insects. Furthermore, eucalyptol demonstrated very poor toxicity as contact triatomicide (in another experiment, it was about 5 000 000× less toxic than deltamethrin when topically applied on *T. infestans* and *R. prolixus* (AN Moretti, EA Zerba & RA Alzogaray, unpubl.).

Phosphine, ethyl bromide, and the organophosphate dichlorvos are fumigant insecticides restricted or banned in several countries due to its high toxicity in humans. During the search for alternative compounds, monoterpene vapors proved less toxic than the above mentioned fumigants (Rice & Coats, 1994a,b; Rajendran & Sriranjini, 2008; Lucía et al., 2009). In terms of lethal effects, eucalyptol appeared more potent than other monoterpenes against stored grain pests (Lee et al., 2004) and triatomines (Sfara et al., 2009), and a synergistic or additive action between eucalyptol and CO_2 was reported in a sealed fumigation system (Lee et al., 2004). In the German cockroach, the variation in the KT_{50} values for essential oils from various species of *Eucalyptus* was partially explained by the concentration of eucalyptol (Alzogaray et al., 2011). When tested alone, eucalyptol was about 3× less toxic than dichlorvos to first instars of this cockroach. In the present work, the KT_{50} values for different concentrations of eucalyptol were 10–60× higher than those for dichlorvos (positive control). KT_{50} values herein presented are far more promising in comparison with the ones obtained for other monoterpenes (Moretti et al., 2013).

Repellents are chemicals that cause insects to move directly away from its source (Dethier et al., 1960). More than 60 years after its discovery, *N*,*N*-diethyl-m-toluamide (DEET) remains the most widely used insect repellent (Frances, 2007). However, it occasionally affects human skin (inflammation, itchiness, and irritation) and nervous system (convulsions, especially in children) (Osimitz et al., 2010), which could be due to the ability of inhibiting acetylcholinesterase activity in insects and mammals (Corbel et al., 2009). Given these undesirable effects, there is a constant search for new repellents and plants have proven to be a good place to look for. Several monoterpenes produced repellence on both haematophagous and non-haematophagous insects (Nerio et al., 2010; Moretti et al., 2013). Eucalyptol has already shown promising results against various mosquito species (Batish et al., 2008). We found that eucalyptol was 10× less effective than DEET on *T. infestans* and *R. prolixus*. This opens the gate to further investigation in synergistic mixtures.

The poor effect of eucalyptol on nymph locomotor activity, reported here, discourages us to consider this monoterpene as a potential diagnostic tool of triatomine infestation in rural houses. Its repellent effect deserves further investigation. It may be worth to look for synergistic interactions with monoterpenes of higher repellency. The knock-down effect showed the more promising results, pointing to eucalyptol as a potentially good triatomicide.

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