Behavioral and Toxicological Responses of *Rhodnius prolixus* and *Triatoma infestans* (Hemiptera: Reduviidae) to 10 Monoterpene Alcohols

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ABSTRACT The effect on locomotor activity, the repellency, and the knock-down produced by 10 monoterpene alcohols were evaluated on first-instar nymphs of *Rhodnius prolixus* and *Triatoma infestans*, vectors of Chagas disease. A video tracking technique was used to evaluate locomotor activity and repellency by exposure to papers impregnated with monoterpenes. Eugenol on *R. prolixus* and (S)-*cis*-verbenol on *T. infestans* did not modify the locomotor activity. The remaining monoterpenes produced hyperactivity on both species, although the concentration required was at least a 1,000 times higher than that of deltamethrin (positive control). Carvacrol, eugenol, and geraniol resulted as repellent as *N*,*N*-diethyl-m-toluamide (positive control) for both species. A similar result was observed for almost every monoterpene on *T. infestans*. Knock-down effect was evaluated by exposing the nymphs in closed recipients. The order of increasing toxicity on *R. prolixus* was (KT_{50} values in min): geraniol (213.7) < α-terpineol (164.5) < linalool (124.2) < carvacrol (111.6) < eugenol (89.8) < thymol (78.9), and on *T. infestans*: α-terpineol (289.8) < eugenol (221.3) < carvacrol (164.2) < linalool (154.9) < thymol (96.7). All monoterpenes were less toxic than the positive control, dichlorvos (3.6 min for *R. prolixus* and 3.9 min for *T. infestans*). After 7 h of exposure, (-)-carveol, citronellol, and menthol (on both species) and geraniol (on *T. infestans*) produced <50% of knock-down. After these results, it is worthwhile to explore more deeply the potential of these compounds as tools for controlling Chagas disease vectors.

KEY WORDS *Rhodnius prolixus*, *Triatoma infestans*, locomotor activity, repellency, knock-down

The use of conventional synthetic insecticides (i.e., organophosphorus, carbamates, and pyrethroids) is questioned because of the many associated environmental and health risks (Isman 2006). Therefore, a significant commercial opportunity and a renewed interest in natural low-impact alternatives have been created (Isman 2000, 2010). Although the use of plant derivatives against pests has been known for millennia, just four groups of botanical insecticides are in commercial use today: pyrethrum, neem, rotenone, and essential oils (Isman 2006). The latter, traditionally used in the Mediterranean region and Southeast of Asia, are composed of highly complex mixtures of monoterpenes, phenols, and sesquiterpenes. They are widely indicated as both safe to nontarget organisms and environmentally friendly (Isman 2000). Essential oils are obtained especially from foliage of Myrtaceae and Lamiaceae families and examples include eucalyptol, the major constituent of oils from rosemary and eucalyptus; eugenol from clove oil; thymol from garden thyme; and menthol from various species of mint (Isman 2006, 2010). Within the components of essential oils, monoterpenes have a broad spectrum of insecticidal activity on stored grain Coleoptera (Park et al. 2003, Sahaf et al. 2008), Diptera (Tarelli et al. 2009, Santos et al. 2011), American and German cockroaches (Appel et al. 2001, Jang et al. 2005, Alzogaray et al. 2011), carpenter ants (Jang et al. 2005), human lice (Toloza et al. 2008), and triatomines (Fournet et al. 1996, Laurent et al. 1997, Abramson et al. 2007, Sfara et al. 2009).

In addition to lethal effects, there are several sublethal effects of monoterpenes on insects extensively documented: feeding deterrence (Hummelbrunner and Isman 2001, Petrakis et al. 2005), reduction in survival and oviposition rate (Yang et al. 2010), and reduction in hatchability, pupation, and adult emergence (Zahran and Abdelgaleil 2011). Repellency has been a hot topic of research for decades. The wide efficiency afforded by the synthetic insect repellent *N*,*N*-diethyl-m-toluamide (DEET) has not yet been overcome by any natural compound. However, concerns with the safety of DEET and other synthetic repellents, especially to children, have resulted in the identification of several plant oils or their components as natural alternatives, despite the limited duration of

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their effects (Isman 2006, 2010; Nerio et al 2010; Sritabutra et al. 2011).

Chagas is a Latin American disease caused by the protozoon *Trypanosoma cruzi* (Chagas), which is vectorized by triatomines. It is the most severe parasitic disease of the American continent (Organización Panamericana de la Salud [OPS] 2006). In Argentina, it is estimated that 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 and Mitelman 2010). *Rhodnius prolixus* (Stål), a classic model of insect physiology, is the most important vector in Central America, Colombia, and Venezuela, whereas *Triatoma infestans* (Klug) is its main vector in Argentina.

In addition, and taking into account the onset in Argentina of populations of *T. infestans* resistant to pyrethroids (Vassena et al. 2000, Picollo et al. 2005), the search for natural substances that could replace these insecticides in the Chagas vectors' control constitutes a primary goal. Cinnamon oil affected molting and, therefore, survival on *R. prolixus* (Abramson et al. 2007); eucalyptol showed good performance as a fumigant; and geraniol and menthyl acetate presented an important repellent effect (Sfara et al. 2009). Several terpenes of essential oils extracted from Bolivian plants showed ovicidal and larvicidal effects on *T. infestans* (Fournet et al. 1996, Laurent et al. 1997).

Botanical monoterpenes present a wide variety of chemical structures. In general, they can be grouped into alcohols, aldehydes, ketones, acids, esters, hydrocarbons, and others (Jang et al. 2005). As a first step to explore the potential of the monoterpenes as new tools for controlling the vectors of Chagas disease, the aim of the current study was to evaluate the effect on locomotor activity, and the repellency and the knockdown produced by 10 monoterpene alcohols on *R. prolixus* and *T. infestans.*

Materials and Methods

Biological Material. First-instar nymphs of *R. prolixus* and *T. infestans*, 1–5-d-old starved since the eclosion, were used in all the experiments. The former emerged from a stable colony reared at the Centro de Investigaciones de Plagas e Insecticidas (CIPEIN, Villa Martelli, Buenos Aires, Argentina), the latter from eggs provided by the Servicio Nacional de Chagas de Argentina (Santa María de Punilla, Córdoba, Argentina).

Chemicals. Monoterpenes (carvacrol, (-)-carveol, citronellol, eugenol, geraniol, linalool, menthol, α-terpineol, thymol, and (S)-*cis*-verbenol) and DEET were bought from Sigma Aldrich (Buenos Aires, Argentina). Deltamethrin and dichlorvos were a donation from Chemotecnica S.A. (Spegazzini, Argentina). Acetone was bought from Merck (Darmstadt, Germany).

Recording Equipment. A video camera black and white closed-circuit (VC 1910; Sanyo Electrical Co., Tokyo, Japan) and an image analyzer (Videomex V, Columbus, OH) were used to evaluate locomotor activity and repellency. The video camera captures the image of the insects placed on a circular piece of filter paper. The image analyzer converts the analog signal input from the video camera into digital data with a resolution of 256 by 192 pixels and an acquisition and processing speed of 30 frames/s. 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's 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 recording started immediately after placing the insects onto the experimental arena. The illumination during testing was provided by a cold light lamp (22 watts) Luxa (Shanghai, China) placed at the zenith of the arena. Temperature was maintained at 26 ± 2°C. Each set of data were imported and processed in a personal computer.

Evaluation of Locomotor Activity. Circles of filter papers (7 cm in diameter) were impregnated with 0.5 ml of solution of monoterpene in acetone. After the solvent was evaporated, groups of four nymphs were allowed to walk on the experimental arena within which they were contained because of the presence of an opaque glass ring. First instars of triatomines are very small (3 mm in length, 1–2 mm in width, approximately). 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 (because of very small changes in nymph's position, which caused variations in the area occupied by the insect on the video image). However, a good repeatability was obtained when four nymphs were placed on the experimental arena. Distribution of the four nymphs on the arena was consistently random (no aggregation was observed).

At the beginning of each trial, the four nymphs were gently placed in the geometric center of the experimental arena. All trials lasted 30 min, consisting of 60 recording sessions of 30 s each. The design was completely randomized. Four concentrations were tested for each substance. After screenings, the concentrations chosen were 3.9, 39, 390, and 3,900 $\mu g/cm^2$. Each bioassay had a negative control (acetone alone). Deltamethrin (3.9×10^{-3}, 3.9×10^{-2}, 0.39, and 3.9 $\mu g/cm^2$) was used as positive control. Each experiment was independently replicated six times (replicates were performed on different days, using different nymphs from a replicate to the next).

Because of the changes in nymph's position mentioned previously, the number of pixels on varies during the experimental time. To standardize the data for the size of the nymphs, we calculated the quotient $M/A = Locomotor\ Activity$ (Alzogaray et al. 1997). The results were expressed in units of pixels/area.

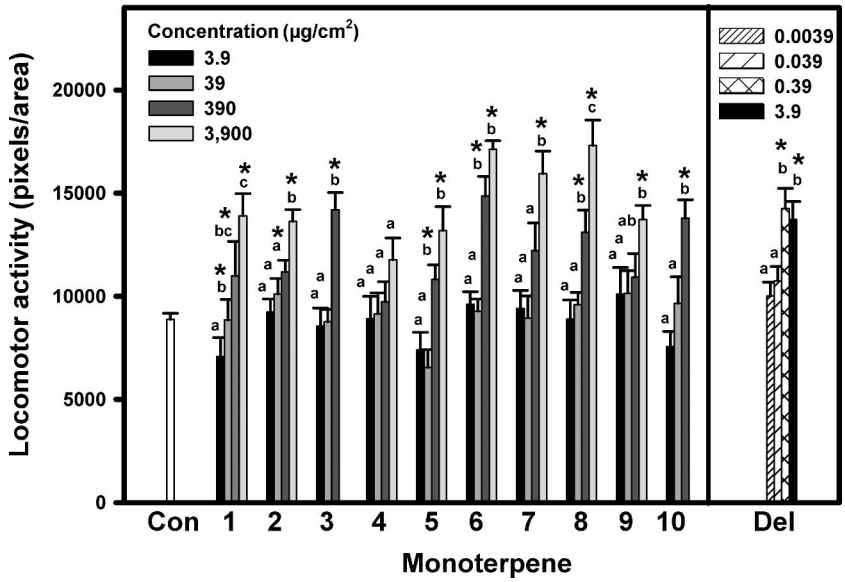

Fig. 1. Locomotor activity of *R. prolixus* nymphs exposed to filter papers treated with different concentrations of monoterpenes. Con: control, Del: deltamethrin (positive control), 1: carvacrol, 2: (-)-carveol, 3: citronellol, 4: eugenol, 5: geraniol, 6: linalool, 7: menthol, 8: α-terpineol, 9: thymol, 10: (S)-*cis*-verbenol. Each bar represents the mean of six independent replicates. Vertical lines are SE. Inside each group, bars marked with the same letter are not significantly different (Fisher test; $P > 0.05$). Asterisks indicate significant difference from control (Fisher test; $P < 0.05$).

Evaluation of Repellency. The experimental design was similar to that used to measure locomotor activity, 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 substance to be evaluated in acetone, and 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 for each substance (39, 390, and 3,900 $\mu g/cm^2$). Each bioassay had a negative control (acetone alone). DEET, used as positive control, was applied at 3.9, 39, and 390 $\mu g/cm^2$.

We expressed the data by using a *Distribution Coefficient:*

$$DC = (AT\text{-}At)/AT$$

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 nontreated zones (random distribution).

Evaluation of Knock-Down. Groups of 10 nymphs were exposed to monoterpenes inside plastic containers with lids (4 cm in diameter by 4 cm in height). The base of each container was covered with a filter paper circle. Filter papers were impregnated with 0.09 ml of solution of monoterpene in acetone (3,900 $\mu g/cm^2$) or with acetone alone (control), 5–10 min before starting each experiment, so that the solvent evaporates.

The number of knocked-down insects was recorded every 10 min. Insects were considered knocked-down when they lay on the paper, unable to walk.

Replicates were interrupted when 90% of the insects were knocked-down or after 7 h of exposure. Dichlorvos was used as positive control (3,900 $\mu g/cm^2$). Six independent replicates of each experiment were performed.

Statistical Analysis. Data from locomotor activity and repellency bioassays were analyzed by using 1-way analysis of variance (after passing normality and homocedacia tests). In cases where $P < 0.05$, Fisher post hoc comparisons (LSD method) were used to detect significant differences between pairs of treatments. The series of data that did not pass homocedacia tests were analyzed by using the nonparametric Kruskal–Wallis analysis of variance by ranks. In cases where $P < 0.05$, Tukey's test was used to detect significant differences between pairs of treatments.

Knock-down time 50% (KT_{50}) values were calculated with their respective 95% confidence limits by using the statistical software for correlated data developed by Throne et al. (1995). Differences between values were considered significant ($P < 0.05$) if the respective 95% confidence limits did not overlap.

Results

Locomotor Activity. The locomotor activity of *R. prolixus* and *T. infestans* nymphs exposed to monoterpenes is showed in Figs. 1 and 2, respectively. In all

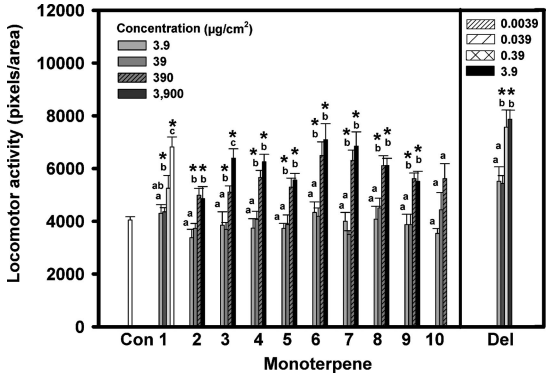

Fig. 2. Locomotor activity of *T. infestans* nymphs exposed to filter papers treated with different concentrations of monoterpenes. Con: control, Del: deltamethrin (positive control, only the highest concentration tested is shown), 1: carvacrol, 2: (-)-carveol, 3: citronellol, 4: eugenol, 5: geraniol, 6: linalool, 7: menthol, 8: α-terpineol, 9: thymol, 10: (S)-*cis*-verbenol). Each bar represents the mean of six independent replicates. Vertical lines are SE. Inside each group, bars marked with the same letter are not significantly different (Fisher test; $P > 0.05$). Asterisks indicate significant difference from control (Fisher test, $P < 0.05$).

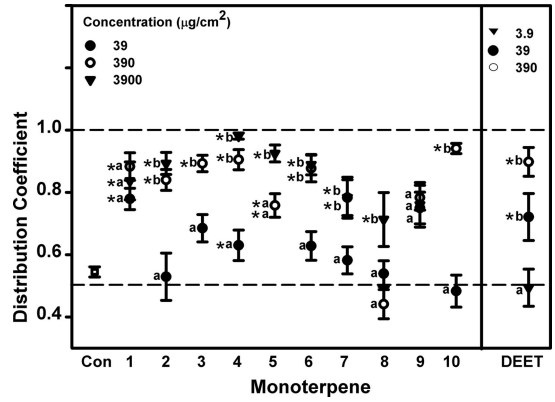

Fig. 3. Values of DC for monoterpenes on nymphs of *R. prolixus*. Con: control, DEET: *N, N*-diethyl-m-toluamide (positive control), 1: carvacrol, 2: (-)-carveol, 3: citronellol, 4: eugenol, 5: geraniol, 6: linalool, 7: menthol, 8: α-terpineol, 9: thymol, 10: (S)-*cis*-verbenol. Each symbol represents the mean of six independent replicates. Vertical lines are SE. Inside each group, symbols marked with the same letter are not significantly different (Fisher test; $P > 0.05$). Dashed lines indicate 0.5 and 1 DC values. Asterisks indicate significant difference from control (Fisher test; $P < 0.05$).

cases where hyperactivity was observed, it varied in a concentration-dependent way. Most of the 10 monoterpenes evaluated produced hyperactivity from 390 $\mu g/cm^2$ on both species. On *R. prolixus*, carvacrol produced hyperactivity from 39 $\mu g/cm^2$; menthol, thymol, and (S)-*cis*-verbenol only at 3,900 $\mu g/cm^2$. Eugenol did not modify the nymphs' locomotor activity. Citronellol was not tested for the highest concentration on *R. prolixus* because nymphs started to show symptoms of intoxication. On *T. infestans*, (S)-*cis*-verbenol failed to modify nymphs' locomotor activity. (S)-*Cis*-verbenol was not tested for the highest concentration in any species, because it exceeded its limit of solubility in acetone. Results of analyses of variance are shown in Table 1.

Repellency. The values of DC for *R. prolixus* and *T. infestans* nymphs exposed to monoterpenes are shown in Figs. 3 and 4, respectively. On *R. prolixus*, carvacrol, eugenol, and geraniol produced a repellent effect from 39 $\mu g/cm^2$; (-)-carveol, citronellol, linalool, menthol, and (S)-*cis*-verbenol produced repellency from 390 $\mu g/cm^2$; α-terpineol from 3,900 $\mu g/cm^2$; and thymol did not produce repellency. Citronellol was not tested for the highest concentration on *R. prolixus* because nymphs started to show symptoms of intoxication. On *T. infestans*, all monoterpenes produced a strong repellent effect. On both species, DEET pro-

Table 1. Results of ANOVA for locomotor activity bioassays on *R. prolixus* and *T. infestans*

Compound	*R. prolixus*			*T. infestans*		
	F	df	*P*	*F*	df	*P*
Carvacrol	4.809	4, 25	0.005	12.273	4, 25	<0.001
(-)-Carveol	9.447	4, 25	<0.001	5.389	4, 25	0.003
Citronellol	24.31	4, 25	<0.001	11.268	4, 25	<0.001
Eugenol	1.864	4, 25	0.148	13.454	4, 25	<0.001
Geraniol	12.27	4, 25	<0.001	9.66	4, 25	<0.001
Linalool	20.545	4, 25	<0.001	10.43	4, 25	<0.001
Menthol	5.89	4, 25	0.002	15.022	4, 25	<0.001
α-Terpineol	14.852	4, 25	<0.001	6.65	4, 25	<0.001
Thymol	3.821	4, 25	0.015	7.351	4, 25	<0.001
(S)-*Cis*-verbenol	7.396	3, 20	0.002	1.818	3, 20	0.176
Deltamethrin	8.938	4, 24	<0.001	8.478	4, 25	<0.001

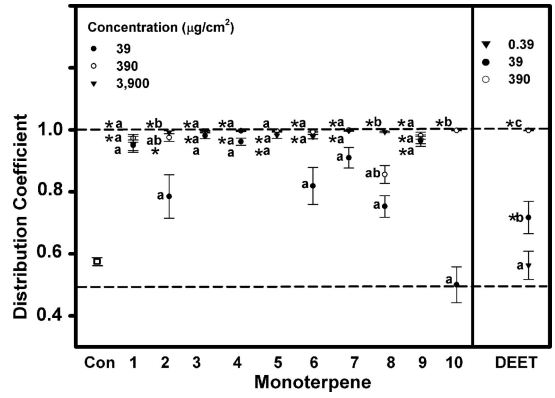

Fig. 4. Values of DC for monoterpenes on nymphs of *T. infestans*. Con: control, DEET: *N,N*-diethyl-m-toluamide (positive control), 1: carvacrol, 2: (-)-carveol, 3: citronellol, 4: eugenol, 5: geraniol, 6: linalool, 7: menthol, 8: α-terpineol, 9: thymol, 10: (S)-*cis*-verbenol. Each symbol represents the mean of six independent replicates. Vertical lines are SE. Inside each group, symbols marked with the same letter are not significantly different (Fisher test; $P > 0.05$). Dashed lines indicate 0.5 and 1 DC values. Inside each group, bars marked with the same letter are not significantly different (Tukey's test for ANOVA by ranks; $P > 0.05$). Asterisks indicate significant difference from control (Fisher test; $P < 0.05$).

Table 2. Results of ANOVA for repellency bioassays on *R. prolixus* and *T. infestans*

Compound	*R. prolixus*			*T. infestans*		
	F	df	*P*	H	df	*P*
Carvacrol	8.475	3, 20	<0.001	13.664	3	0.003
(-)-Carveol	24.716	3, 20	<0.001	17.973	3	<0.001
Citronellol	11.662	3, 20	<0.001	14.392	3	0.002
Eugenol	50.232	3, 20	<0.001	19.036	3	<0.001
Geraniol	28.717	3, 20	<0.001	13.365	3	0.004
Linalool	12.14	3, 20	<0.001	18.007	3	<0.001
Menthol	4.548	3, 20	0.014	18.496	3	<0.001
α-Terpineol	5.389	3, 20	0.007	19.207	3	<0.001
Thymol	1.486	3, 20	0.249	13.72	3	0.003
(S)-*Cis*-verbenol	40.238	2, 15	<0.001	11.392	2	0.003
	H	df	*P*	H	df	*P*
DEET	5.967	3	0.113	15.874	3	0.001

duced repellency from 39 $\mu g/cm^2$. In this way, carvacrol, eugenol, and geraniol showed a repellent effect similar to DEET on *R. prolixus*, whereas almost all the monoterpenes gave results similar to DEET on *T. infestans*. (S)-*Cis*-verbenol was not tested for the highest concentration in any species, because it exceeded its limit of solubility in acetone. Results of analyses of variance are shown in Table 2.

Knock-Down. The values of KT_{50} for monoterpene alcohols applied as films on filter papers on *R. prolixus* and *T. infestans* are shown in Table 3. On *R. prolixus*, the monoterpenes presented the following order of increasing toxicity (KT_{50} values in min): geraniol (213.7) < α-terpineol (164.5) < linalool (124.2) < carvacrol (111.6) < eugenol (89.8) < thymol (78.9). On *T. infestans*, the results were α-terpineol (298.9) < eugenol (221.3) < carvacrol (164.2) < linalool (154.9) < thymol (96.7). After 7 h of exposure, (-)-carveol, citronellol, and menthol (on both species) and geraniol (on *T. infestans*) produced <50% of knock-down. Dichlorvos (positive control) was more toxic than any monoterpene for both species, showing a KT_{50} of 3.6 min for *R. prolixus* and 3.9 min for *T. infestans*. (S)-*Cis*-verbenol was not tested in any species, because the concentration to apply exceeded its limit of solubility in acetone.

Discussion

In this work, we evaluated the effect on locomotor activity, and the repellency and knock-down produced by 10 monoterpene alcohols on the vectors of Chagas disease *R. prolixus* and *T. infestans* (results are summarized in Table 4).

An increase in locomotor activity (hyperactivity) is the first symptom of intoxication produced by pyrethroids in insects (Miller and Adams 1982, Alzogaray et al. 1997, Alzogaray and Zerba 2001). Hyperactivity is of practical use for entomological pests because it causes the flushing-out phenomenon: hyperactivated insects leave their refuges, making it easier to estimate their presence and abundance by controllers (Pinchin et al. 1980, Gualtieri et al. 1985). This method has been applied to detect vectors of Chagas disease for decades (Wood et al. 1993). Hyperactivity was also observed in

Table 3. KT_{50} and KT_{95} values for 10 monoterpenes with an alcohol group on first-instar nymphs of *R. prolixus* and *T. infestans*

Compound	*R. prolixus*					*T. infestans*				
	N^a	Slope (SE)	KT_{50} min (95% CL)	KT_{95} min (95% CL)	χ^2	N^a	Slope (SE)	KT_{50} min (95% CL)	KT_{95} min (95% CL)	χ^2
Thymol	60	6.2 (0.64)	78.9a (67.3–92.7)	145a (118.5–204)	16.2	60	3.9 (0.40)	96.7a (78.2–120.4)	255.4a (191.6–401.4)	26.6
Eugenol	60	4.0 (0.46)	89.8ab (65.8–124.7)	233.6ab (158.4–541.4)	37.4	60	3.6 (0.40)	221.3b (187.2–263.7)	631.9b (491.7–904)	35.1
Carvacrol	60	6.2 (0.61)	111.6b (101.3–123.0)	205.4a (179.9–245.7)	16.3	60	5.6 (0.60)	164.2c (147.5–183.0)	321.0a (275.5–397.3)	21.0
Linalool	60	5.6 (0.58)	124.2bc (102.5–151.8)	244.0ab (191.1–379.5)	30.0	60	6.0 (0.61)	154.9c (140.1–171.5)	292.1a (253.7–354.7)	15.8
α-Terpineol	60	4.0 (0.45)	164.5c (129.4–213.9)	427.4bc (305.8–783.4)	41.4	60	6.7 (0.75)	289.8b (253.1–334.3)	508.6b (422.3–703.5)	37.9
Geraniol	60	2.5 (0.31)	213.7c (132.6–392.6)	963.7c (489.0–4,919.9)	73.2	60	–	_b	_b	–
(-)-Carveol	60	–	_b	_b	–	60	–	_b	_b	–
Citronellol	60	–	_b	_b	–	60	–	_b	_b	–
Menthol	60	–	_b	_b	–	60	–	_b	_b	–
Dichlorvos	60	7.2 (0.70)	3.6d (3.0–4.2)	6.1d (5–8.0)	18.8	60	6.8	3.9d (3.5–4.2)	6.8c (6–8)	6.1

KT_{50} = 50% knock-down time; KT_{95} = 95% knock-down time; CL = confidence limit.

[a] Total number of nymphs used for bioassay.

[b] KT_{50} or KT_{95} values were not calculated because <50% of knocked-down nymphs were observed after 7 h of exposure.

Within a column, values followed by the same letter are not significantly different ($P > 0.05$).

Table 4. Summary of results obtained in this work, showing the monoterpenes ordered according to their effects on locomotor activity, their repellency, and knock-down

Hyperactivity				Repellency				Knock-down[a]	
LOEL $\mu g/cm^2$	*R. prolixus*	LOEL $\mu g/cm^2$	*T. infestans*	LOEL $\mu g/cm^2$	*R. prolixus*	LOEL $\mu g/cm^2$	*T. infestans*	*R. prolixus*	*T. infestans*
0.39	Deltamethrin	0.39	Deltamethrin	39	DEET	39	DEET	Dichlorvos	Dichlorvos
					Carvacrol			Thymol	Thymol
					Geraniol			Eugenol	Linalool
39	Carvacrol	390	Linalool		Eugenol	≤39	Geraniol	Carvacrol	Carvacrol
			Menthol				Thymol	Linalool	Eugenol
			α-Terpineol					α-Terpineol	α-Terpineol
390	Linalool		Eugenol	390	(S)-*Cis*-verbenol			Geraniol	
	Citronellol		Thymol		Citronellol	390	(S)-*Cis*-verbenol		
	(S)-*Cis*-verbenol		Geraniol		Linalool		Eugenol		
	α-Terpineol		Carvacrol		(-)-Carveol		Citronellol	NC	Citronellol	NC	Geraniol
	(-)-Carveol		Citronellol		Menthol		Linalool		Menthol		Menthol
	Geraniol		(-)-Carveol				Menthol		(-)Carveol		Citronellol
							Carvacrol				(-)-Carveol
							(-)-Carveol				
3,900	Menthol	NE	(S)-*Cis*-verbenol	3,900	α-Terpineol			NT	(S)-*Cis*-verbenol		
	Thymol									NT	(S)-*Cis*-verbenol
NE	Eugenol			NE	Thymol	3,900	α-Terpineol				

LOEL = lowest observed effect level; N = not observed effects at the concentrations tested; NC = not calculated; NT = not tested.
[a] Monoterpenes are shown in order of increasing KT_{50} values.

insects exposed to DEET. Concentrations higher than 350 $\mu g/cm^2$ of this repellent produced an increase in the locomotor activity of *R. prolixus* (Sfara et al. 2009).

Hyperactivity was observed as a toxicity symptom of essential oils in American cockroaches treated with monoterpene alcohol, although it was not quantified (Enam 2001). It was also reported for menthyl acetate in German cockroaches (Alzogaray et al. 2013). The results presented in this article are of relevance for surveillance and control purposes because they constitute the first report of a quantitative analysis of the hyperactivity effect produced by monoterpenes in Chagas disease vectors. Except (S)-*cis*-verbenol and eugenol, the tested compounds produced hyperactivity on both species, although the concentrations required was at least a 1,000 times higher than that of deltamethrin (positive control).

A repellent is a chemical that causes negative chemotaxis: oriented movement directly away from the chemical source (Dethier et al. 1960). Repellents are used mainly to prevent bites from hematophagous arthropods (Katz et al. 2008) and also in food packaging materials as a strategy for keeping insects away from the products (Wong et al. 2005).

Monoterpenes with repellent properties are generally less effective than DEET, either because of their lower repellent activity per se or because of their shorter effective time (Omolo et al. 2004, Isman 2006, Moore et al. 2007). All the monoterpenes studied in this work showed repellency on several species of mosquitoes and sandflies (Ansari et al. 2000; Choi et al. 2001; Kumar et al. 2011; Omolo et al. 2004; Odalo et al. 2005; Müller et al. 2008, 2009; Qualls and Xue 2009; Weldon et al. 2010). Geraniol has also shown repellence against ticks (Tunón et al. 2006) and fifth-instar nymphs of *R. prolixus* (Sfara et al. 2009); menthol and thymol against *Pediculus humanus capitis* (De Geer) (Toloza et al. 2006); and α-terpineol against *Tribolium castaneum* (Herbst) (García et al. 2005). There are even commercial repellents available nowadays, containing geraniol as active ingredient (Chen and Viljoen 2010).

This study constitutes the first report of repellency on Chagas vectors for carvacrol, (-)-carveol, citronellol, eugenol, linalool, menthol, α-terpineol, thymol, and (S)-*cis*-verbenol. Although applications of repellency data are less straightforward and require deeper work in chemical, biological, and safety issues, the results of repellency bioassays are interesting. Carvacrol, eugenol, and geraniol resulted as repellent as DEET for *R. prolixus*, whereas almost every monoterpene had the same result for *T. infestans.*

Repellents are not usually used as a personal protective measure to interrupt transmission of Chagas disease. This may be due largely to the lack of studies and demonstrations of their efficacy for this purpose. However, repellents may be useful to protect the residents of homes infested with Chagas vectors, health workers, and temporary workers who live part of the year in areas affected by this disease. Furthermore, in regions where Chagas is endemic, other diseases vectors exist (i.e., mosquitoes and hematopha-

gous flies), so the use of a broad-spectrum repellents could provide general protection against various insects that threaten human health.

Monoterpenes vapors are much less toxic than other fumigant insecticides such as phosphine, ethyl bromide, and the organophosphate dichlorvos (Rice and Coats 1994a,b; Rajendran and Sriranjini 2008; Lucía et al. 2009; Alzogaray et al. 2011). The lethal concentration 50% values of 22 monoterpenes varied between 2 and >2,500 $\mu g/cm^3$ in *Musca domestica* (L.), whereas the lethal concentration 50% of dichlorvos was 0.01 $\mu g/cm^3$ (Rice and Coats 1994a). In the same study, a similar tendency was observed in *T. castaneum*, where the monoterpenes applied were between 9 and >175.6 times less toxic than dichlorvos. However, there were exceptions: pulegone showed the same toxicity as dichlorvos, and *l*-fenchone resulted in only 1.3 times less toxicity. The KT_{50} values of monoterpenes evaluated here were higher than those for dichlorvos, showing a knock-down time that is, as a first approach, too slow to be of practical use.

Jang et al. 2005 evaluated fumigant toxicity by using the same technique as ours (in closed recipients with impregnated papers) on *Blattella germanica* (L.) and pointed out that degrees of saturation, types of functional groups, hydrophobicity, and vapor pressure parameters appear to play a role in determining the monoterpenoids' toxicity. However, even though their results indicate that the mode of delivery of linalool, α-terpineol, and thymol was likely by vapor action via the respiratory system, neither hydrophobicity nor vapor pressure parameters appeared significantly related to the observed toxicities. Convergently, we did not find any correlation between vapor pressure values or octanol–water partition coefficients and the toxicity of the monoterpenes in knock-down bioassays (results not shown).

In conclusion, we suggest that some of the monoterpenes evaluated in this work could be used as tools for controlling triatomines. In particular, even though the hyperactivation effect was significantly lower than that produced by deltamethrin, the onset of the hyperactivity effect by most of the monoterpenes is encouraging for a possible use as flushing-out agents. We plan to perform experiments for determining the hyperactivant effect of monoterpene alcohols in other triatomine stages (late nymphs and adults). Finally, we will evaluate the flushing-out effect of these compounds applied as aerosol formulations in laboratory and field assays.

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