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Orientation Behaviour of the Blood-sucking Bug *Triatoma infestans* to Short-chain Fatty Acids: Synergistic Effect of L-Lactic Acid and Carbon Dioxide

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Abstract

The role of short-chain fatty acids in the host-seeking behaviour of *Triatoma infestans* larvae was investigated using a locomotion compensator. Several short-chain fatty acids were tested alone over a wide range of doses, or in combination with L-lactic acid (L-LA; 100 μg). Bugs showed no attractive response to single carboxylic acids, but when L-LA was added to airstreams carrying specific intensities of either propionic (C3; 100 μg), butyric (C4; 1 μg) or valeric acid (C5; 1 μg), these mixtures elicited an attractive response, evincing a synergistic effect. No orientation response was observed when caproic acid (C6) was offered with L-LA at the doses tested. Two blends were created: (1) C3, C4 and C5 combined at the effective doses when added with L-LA [C3C4C5 (1)], and (2) C3, C4 and C5 combined at a third of those intensities [C3C4C5 (2)]. Both blends were tested alone, with L-LA (100 μg), with a sub-threshold concentration of CO_2 (300 p.p.m. above the ambient level), and combined with both compounds together. Oriented responses of bugs were only observed with the blend (2) added with L-LA and with the combination of this lure with CO_2. This last combination evoked a behavioural response similar in intensity to that induced by a live mouse.

Key words: carbon dioxide, host orientation, L-lactic acid, short-chain fatty acids, synergism, *Triatoma infestans*

Introduction

Triatomines are haematophagous bugs (Heteroptera: Reduviidae: Triatominae) that are vectors of the flagellated parasite *Trypanosoma cruzi*, the causative agent of Chagas' disease. This trypanosomiasis constitutes one of the most serious sanitary problems in Latin America, and has an important social and economic impact on the region (Dias and Schofield, 1999). Currently, ~20 million people are infected with this disease and more than 100 million are at risk of becoming infected, i.e. ~25% of the population of Latin America (WHO, 2000). The vectors live in many different natural habitats in contact with birds, mammals and reptiles. The adaptation of several species of triatomines to human habitats, which offer abundant food (blood of humans, domestic animals, associated rodents, etc.) and places that are easy to colonize (e.g. cracks and crevices in walls made of dried mud and thatched roofs), define, among other features, the vectorial importance of these bugs. For example, colonies of *Triatoma infestans* are almost exclusively established inside human dwellings and/or peridomestic structures, and this insect is the main vector of the Chagas' disease in southern South America.

Arthropod disease vectors exploit different sensory cues to locate a blood source. Among them, host-emitted odours constitute reliable cues for insects in their search for food. Human breath contains high levels of carbon dioxide (~45 000 p.p.m.) compared with normal ambient values (~300–400 p.p.m.). This gas is a powerful and conservative attractant and activator for most blood-sucking insects (Lehane, 1991). Around 350 substances of diverse chemical composition have been identified in the human skin, including L-lactic acid (L-LA), short- and long-chain fatty acids, aldehydes, alcohols, aromatic compounds, amines, acetates and ketones (Zeng *et al.*, 1991; Bernier *et al.*, 2000). Many of these substances, the product of endogenous or exogenous metabolism, confer a unique 'chemical signature' on vertebrates (Nicolaides, 1974), possibly playing an important role as kairomones for haematophagous arthropods. In particular, L-LA and fatty acids are distinctive volatile substances of the human skin (Acree *et al.*, 1968; Cork and Park, 1996).

Heat, water vapour, CO_2 and other chemical volatiles have been associated with the host-seeking behaviour of *T. infestans* and other related species, such as *Rhodnius prolixus* (Núñez, 1982; Lazzari and Núñez, 1989; Taneja and Guerin, 1995, 1997; Flores and Lazzari, 1996; Guerenstein and Guerin, 2001; Barrozo *et al.*, 2003; Barrozo and Lazzari, 2004). Núñez (1982) observed that *R. prolixus* was able to orient towards odours emitted by a human arm, a mouse, odours released by a cage previously occupied by a mouse, and to CO_2. Likewise, Taneja and Guerin (1995) observed upwind anaemotaxis in *R. prolixus* and *T. infestans* towards airstreams transporting either CO_2, mouse odours or volatiles from rabbit urine. Barrozo and Lazzari (2004) analysed the behavioural response of *T. infestans* to CO_2, and determined that the threshold of responsiveness to this gas is between 300 and 400 p.p.m. over the ambient levels. These authors also established that L-LA, as a sole stimulus on an airstream, is not able to evoke an oriented response in *T. infestans*. However, a marked attractive response became evident in these bugs when L-LA was offered together with subthreshold amounts of CO_2, evincing a synergistic effect between both odours. Moreover, the sensitivity threshold to CO_2 decreased to a level between 75 and 150 p.p.m. in the presence of L-LA (Barrozo and Lazzari, 2004).

In this work we analysed the role played by short-chain fatty acids in the host-searching behaviour of *T. infestans*. Using a locomotion-compensating device, we investigated the orientation of bugs towards airstreams loaded with different doses of propionic (C3), butyric (C4), valeric (C5) and caproic (C6) acids as single stimuli, and with combinations of these carboxylic acids, L-LA and CO_2.

Material and methods

Insects

Fourth-instar larvae of *Triatoma infestans* were used throughout the experiments. Insects were provided as third-instar larvae by the Servicio Nacional de Chagas (Córdoba, Argentina) and maintained in our laboratory under a 12/12 h light/dark illumination regime, with constant temperature (28°C) and ambient relative humidity (30–50%). Third-instar bugs were fed weekly on hens until moulting. The fourth-instar larvae were then starved for 20–30 days, after which they were ready for the experiments.

Recording of walking pathways

A locomotion compensator was used to study the orientation behaviour of *T. infestans* (Barrozo and Lazzari, 2004). Briefly, the insect was tethered by its dorsal thorax and abdomen to a freely rotating stiff steel wire centred on top of a Styrofoam sphere (97 mm diameter, 2.5 g weight), which was suspended by a vertical airstream. When it contacted the surface of the sphere, the animal started to walk, displacing the sphere with its legs. The insect on the locomotion compensator could walk and rotate freely, changing its direction of locomotion without modifying its distance from the stimulus location, i.e. in an open-loop condition for translation, but allowing free rotation. An optic sensor detected the movements of the sphere and sent the signals to a computer every 0.2 s as *x*,*y*-coordinates with the aid of specially written software (Diego Anfossi, unpublished). The walking paths of the bugs were reconstructed and analysed in terms of their spatiotemporal components.

Stimulus delivery

In the same way as previously described (Barrozo and Lazzari, 2004), the insects were confronted with two opposite horizontal charcoal-filtered airstreams (180°), i.e. a simultaneous-discrimination bioassay. In this way, one airstream transported the stimuli (test airstream) and the other was kept clean or loaded with the solvent (control airstream). Each bug could choose to walk towards one of the two streams or could exhibit a non-oriented behaviour, i.e. walk randomly.

Both airstreams, being of constant temperature (26 ± 1°C) and relative humidity (85 ± 2%), passed through two glass bottles arranged in series via silicone tubing (0.4 cm i.d.), connecting ultimately with a glass tube (0.67 cm i.d., 5 cm long). Different odours were placed inside each glass bottle (for further details see below), in order to load the passing airstream. The velocity of the air measured at the exit of the glass tube was set to 6 cm/s. The exit of the glass tube was positioned 3 cm away from the insect position over the sphere.

The synthetic chemicals tested as stimuli were L-(+)-lactic acid (>99% purity), propionic acid (C3), butyric acid (C4), valeric acid (C5) and caproic acid (C6) (>98% purity; Sigma and Fluka Chemie GmbH). Different solutions (0.01, 0.1, 0.33, 1, 10, 33, 100, 1000 μg/50 μl) of the carboxylic acids were prepared with dichloromethane (Merck). L-LA was diluted in distilled water to a final concentration of 100 μg/50 μl. This dose represents an attractive stimulus for *T. infestans*, but only when offered together with CO_2 (Barrozo and Lazzari, 2004). Filter paper strips (2.5 cm^2) were loaded with 50 μl of the test solution or with 50 μl of the corresponding solvent, and, after waiting for 20 s to allow the evaporation of the dichloromethane, were placed inside the test and control flasks respectively (first glass bottles of the series, volume 20 ml). When a blend was used as a stimulus source, the compounds were loaded onto different strips and positioned inside the test flask. Similarly, strips loaded with the corresponding solvents were placed inside the control flask. The evaporated vapours offered to the bugs were left to equilibrate in the glass bottle for 5 min prior to the assay. Filter papers for both control and test glass bottles were replaced after each assay. To prevent contamination, the containers and the connecting tubing used to deliver the stimulus sources were replaced every time a different synthetic compound was tested.

In the second glass bottle of the series (volume 250 ml), the passing airstream was loaded with CO_2, which was chemically generated inside this receptacle by means of the following reaction: $Na_2CO_3 + H_2SO_4 \rightarrow CO_2 \text{ (gas)} + H_2O + Na_2SO_4$. A solution of 0.04 M Na_2CO_3 was injected with a synchronic-motor driven syringe at a constant flow rate (0.08 ml/min) into the receptacle that already contained 100 ml of 0.01 M H_2SO_4. The reaction fluid was continuously stirred to ensure an homogenous and a stable CO_2 production (see Barrozo and Lazzari, 2004). In this way, a CO_2 concentration of 300 p.p.m. over ambient levels (400 ± 20 p.p.m.) was generated. To measure the CO_2 concentration in the air, a non-dispersive infrared sensor (PP Systems, model EGM-3, range 0–5000 p.p.m., accuracy 0.5%) was used. The control airstream passed through an identical glass bottle (250 ml) containing 100 ml of 0.01 M H_2SO_4. This arrangement was set up to create identical conditions (i.e. air speed, relative humidity and temperature) in both currents.

In addition, to compare the efficiency of the different experimental situations with a previously well-established attractant, we tested the orientation behaviour of these bugs when a live mouse was used as source of stimuli (taken as maximal stimulation). The animal was placed inside the 250 ml glass bottle in the series, and the passing airstream was simultaneously loaded with many physical and chemical cues (i.e. heat, water vapour, odours). The experiments were conducted in accordance with the highest standards of animal care as outlined in the 'Guide for the Care and Use of Laboratory Animals' published in 1996 by the National Institute of Health.

To avoid eventual environmental biases, the opposite test and control currents were interchanged between assays in a random fashion.

Bioassay protocol

All assays were conducted in an experimental room, kept at 26 ± 1°C and functionally dark for the insects (i.e. infrared light >900 nm). Animals were monitored from outside of the room by means of an infrared-sensitive video-camera. The orientation of the bugs was evaluated only during the first hours of the scotophase, given that *T. infestans* displays a daily rhythm of orientation towards host odours (i.e. CO_2), with a peak of attractiveness at the beginning of the night (Barrozo *et al.*, 2004).

Each insect was left over the sphere for 120 s to habituate to the experimental situation without stimulation (in still air), after which both airstreams were delivered towards the insect for 180 s. During this time the orientation behaviour of the bugs was registered.

Larvae were exposed to two opposite and simultaneous airstreams carrying different combinations of stimuli, i.e. control (solvent) versus control (solvent), a single fatty acid versus control (solvent), a single fatty acid + L-LA versus control (corresponding solvents), mixture of fatty acids versus control (solvent), mixture of fatty acids + CO_2 versus control (solvent plus 100 ml H_2SO_4), mixture of fatty acids + L-LA versus control (corresponding solvents), L-LA + CO_2 versus control (solvent plus 100 ml H_2SO_4), mixture of fatty acids + L-LA + CO_2 versus control (corresponding solvents plus 100 ml H_2SO_4), live mouse versus control (empty bottle).

Each insect was assayed only once and discarded afterwards.

Data analysis

The walking paths of insects were analysed by means of circular statistics. The mean walking angle (α_i) of each insect was computed, from which the mean angle (α_m) and the length of the resultant vector (r) were calculated for every experimental group. The mean angle ranges from 0° to 360°, and r varies between 0 and 1, where 0 indicates a non-defined mean direction and 1 denotes a straight path to a given direction. The position of the test current was conventionally designated at 0° and the control stream at 180°. The statistical evidence of a uniform distribution around a circle was tested following the Rayleigh test (*Ho*: the data are uniformly distributed, i.e. no orientation) (Batschelet, 1965; Zar, 1984). When the data did deviate significantly from uniformity, the *V*-test was carried out to assess whether the α_m calculated from the sample was statistically distant to the test stream position (*Ho*: α_m is distant from 0°) (Zar, 1984).

To compare the deviation of insect's paths from the test-current position (0°) among the experimental groups, the angular distances were computed by calculating the difference between α_i and 0° (test airstream position). Differences were statistically evaluated by the Kruskal–Wallis test (critical value approximated to a chi-squared distribution), followed by non-parametric Newman–Keuls *post-hoc* comparisons (Zar, 1984).

In addition, an orientation index (OI) was calculated as the cosine of α multiplied by r [i.e. $\cos(\alpha_m) \times r$]. The OI varies between –1 and 1, indicating orientation against and towards the stimulus location respectively.

Results

Response to single short-chain fatty acids

Triatoma infestans larvae stimulated with two opposite odourless airstreams (control versus control) exhibited a non-oriented behaviour on the locomotion compensator, i.e. displayed a random walk on the sphere (OI = 0.09; $n = 30$; Rayleigh test, NS). When one of the two air-currents was loaded with either propionic acid (C3), butyric acid (C4), valeric acid (C5) or caproic acid (C6), insects exhibited a similar non-oriented behaviour, even though a wide range of doses was tested (in all cases, Rayleigh test, NS) (Figure 1).

Response to single short-chain fatty acids and L-lactic acid

Based on previous knowledge that L-LA as a sole stimulus is not attractive, but when combined with CO_2 elicits a synergistic effect on the behaviour of *T. infestans* (see Introduction), the orientation response of these bugs to combinations of short-chain fatty acids and L-LA was investigated. Thus, in the following assays we analysed whether the null attractive effect of both groups of compounds (i.e. short-chain fatty acids and L-LA) as separate stimuli could be reverted by presenting them simultaneously via an airstream (Figure 2).

Larvae exhibited an oriented response towards combinations of doses of C3, C4 and C5 and 100 µg/50 µl of L-LA (Figure 2). Therefore, the existence of an oriented response shows that these volatiles effectively elicit a synergistic effect in the orientation of *T. infestans*. However, only one dose of these short-chain fatty acids combined with L-LA was able to attract the bugs. The only attracting combinations were the ones that retain doses of 100 µg of C3, 1 µg of C4 and 1 µg of C5 [*V*-test, C3 + L-LA, $u(30) = 2.82$, $P < 0.005$; for C4 + L-LA, $u(20) = 2.92$, $P < 0.005$; and for C5 + L-LA, $u(30) = 3.2$, $P < 0.0005$]. Conversely, no orientation behaviour towards the stream loaded with any intensity of C6 and L-LA was observed (Rayleigh test, NS) (Figure 2).

Responses to mixtures of short-chain fatty acids, L-LA and CO_2

To evaluate possible combinatorial effects among the effective carboxylic acids (C3, C4 and C5), the behaviour of insects exposed to air-currents carrying mixtures of these acids added at different doses, in the presence or absence of L-LA and/or CO_2, were tested (Figure 3). Two main odour blends were offered to the animals: (1) [C3C4C5 (1)] propionic, butyric and valeric acids were loaded onto the air-current at the intensity that attracted bugs when presented together with L-LA (i.e. C3: 100 µg, C4: 1 µg and C5: 1 µg) (Figure 2), and (2) [C3C4C5 (2)] each carboxylic acid was loaded at a third of the same intensity (i.e. C3: 33 µg, C4: 0.33 µg and C5: 0.33 µg).

Larvae displayed a non-oriented behaviour towards air-currents enriched with either blend [C3C4C5 (1) or C3C4C5 (2); in both cases, Rayleigh test, NS] (Figure 3). Subsequently, we evaluated whether the addition of 300 p.p.m. of CO_2 to the stream (over the ambient level) (i.e. a subthreshold dose of CO_2 that evokes no oriented response in these bugs; see Barrozo and Lazzari, 2004) could modify the attractiveness of these blends. No oriented responses were observed towards the blends enriched with CO_2 (Rayleigh test, NS)

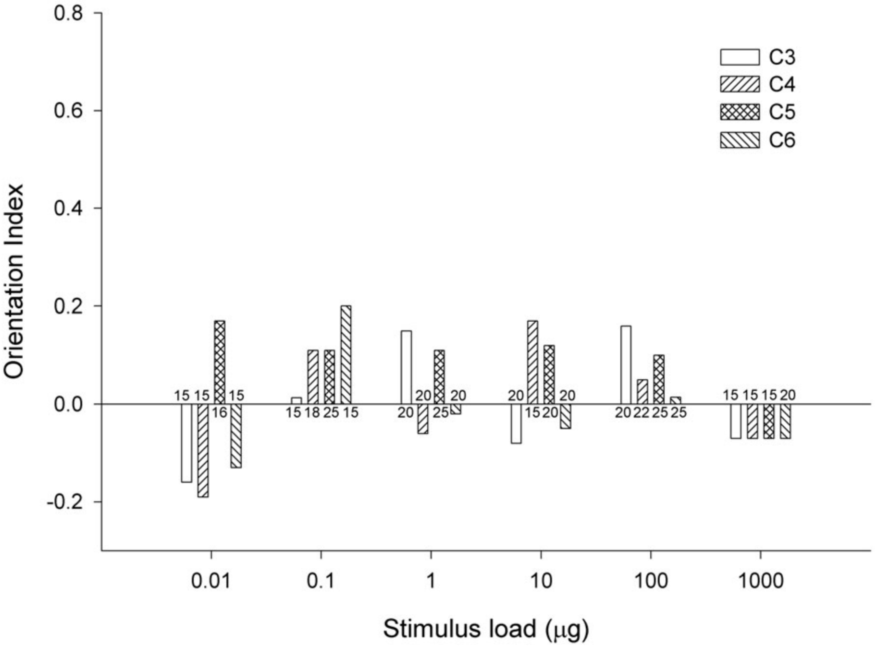

Figure 1 Effect of short-chain fatty acids on the orientation behaviour of *Triatoma infestans* larvae. Bars represent the orientation index [i.e. cos (α_m) × r] of bugs stimulated with different doses of propionic acid (C3), butyric acid (C4), valeric acid (C5) and caproic acid (C6) offered as single stimulus. The test airstream was loaded with a single fatty acid and the control stream contained a filter paper impregnated with dichloromethane. Number of insects tested shown above/below the bars.

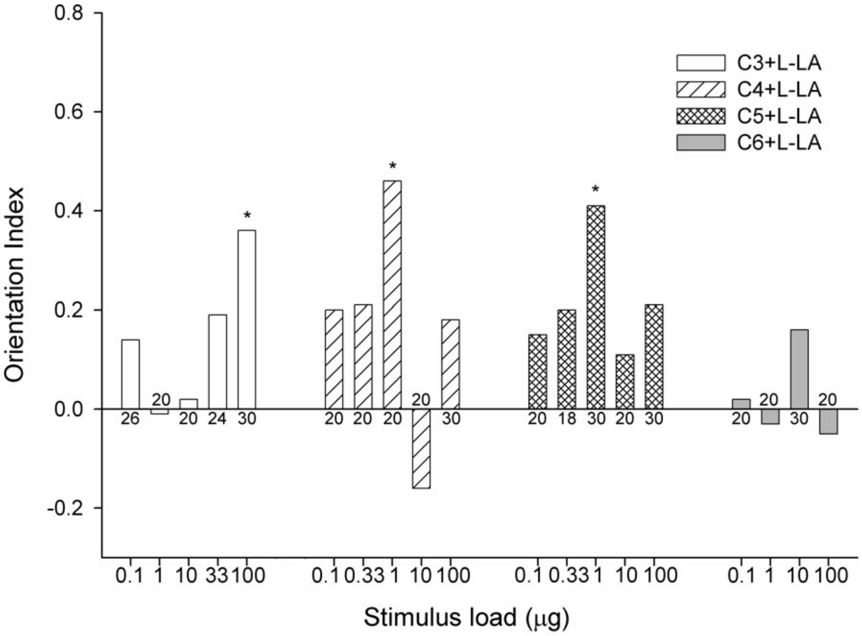

Figure 2 Effect of short-chain fatty acids combined with L-lactic acid (L-LA) on the orientation response of *T. infestans.* The bars denote the orientation index of insects stimulated with different doses of C3, C4, C5, C6 and 100 μg/50 μl of L-LA. The test airstream was loaded with the fatty acid and L-LA and the control stream contained filter papers impregnated with dichloromethane and distilled water (solvents). The asterisk denotes both, the statistically significant differences ($P < 0.05$, see text for more statistical details) from a uniform distribution and a significant mean direction around stimuli location (0°). Number of insects tested shown above/below the bars.

(Figure 3). Insects were significantly attracted to the stream carrying [C3C4C5 (2)] + L-LA [*V*-test, $u(20) = 2.98$, $P < 0.005$], but not to the one that transported the combination [C3C4C5 (1)] + L-LA (Rayleigh test, NS).

In order to determine whether the combinations of the blends [1] and [2] + L-LA could reach higher levels of attractiveness, 300 p.p.m. of CO_2 was added to each mixture. A significantly oriented response of insects towards both blends was observed ([C3C4C5 (1)] + L-LA + CO_2, *V*-test, $u(20) = 2.7$, $P < 0.005$; [C3C4C5 (2)] + L-LA + CO_2, *V*-test, $u(20) = 5.5$, $P < 0.0005$). However, the intensity of the oriented response exhibited by insects towards the blend [1] + L-LA + CO_2 was lower than to the blend [2] + L-LA + CO_2 (i.e. $OI_{\text{blend [1] + L-LA + CO2}} = 0.42$ versus $OI_{\text{blend [2] + L-LA + CO2}} = 0.87$). Moreover, the latter odour stimulation caused bugs to display a higher orientation index than the blend without CO_2, and similar to the response shown by insects towards a live mouse [*V*-test, $u(20) = 5.3$, $P < 0.0005$] (Figure 3). Furthermore, this lure was more attractive for bugs than the combination of L-LA + CO_2 (Figure 3). The orientation response of larvae stimulated with physical and chemical signals from a live host was considered as a reference of maximal attractiveness. It is worth mentioning that a living mouse constitutes a complex source of sensory stimuli, including heat, water vapour and a wide diversity of chemical cues.

Table 1 Angular deviations of the trajectories expressed by *T. infestans* larvae under different stimulations

Stimuli	Angular deviation
(1)[C3C4C5 (2)] + L-LA	54° b
(2)[C3C4C5 (1)] + L-LA + CO_2	63° b
(3)[C3C4C5 (2)] + L-LA + CO_2	23.5° a
(4) L-LA + CO_2	56° b
(5)Mouse	22.1° a

Different letters denote statistically significant differences (Kruskal–Wallis test, $P < 0.05$). Multiple comparisons Newman–Keuls test, (1) versus (3) $Q_{(\infty, 3)} = 5$; (4) versus (3) $Q_{(\infty, 4)} = 4.2$; (4) versus (5) $Q_{(\infty, 3)} = 5.5$; (5) versus (1) $Q_{(\infty, 2)} = 7.3$; (5) versus (2) $Q_{(\infty, 4)} = 4.7$; (3) versus (2) $Q_{(\infty, 5)} = 3.9$.

Finally, we analysed the angular deviations from the stimulus sources of insects exposed to the mixtures that proved attractive (denoted with an asterisk in Figure 3). The results, summarized in Table 1, showed statistically different angular dispersions among the stimulation conditions, demonstrating that insects approached the different odour sources in different ways [Kruskal–Wallis test, $H(4,100) = 14$, $P = 0.01$]. Larvae exposed to the mixture [C3C4C5 (2)] + L-LA + CO_2 or to a live mouse showed trajectories exhibiting angular dispersions significantly smaller than those with the other lures tested, i.e. [C3C4C5 (2)] + L-LA, [C3C4C5 (1)] + L-LA + CO_2 or the combination CO_2 + L-LA (Newman–Keuls test *post-hoc* comparisons, $P < 0.05$).

Discussion

Our results demonstrate that the short-chain carboxylic acids we tested evoke an attractive response in *T. infestans*

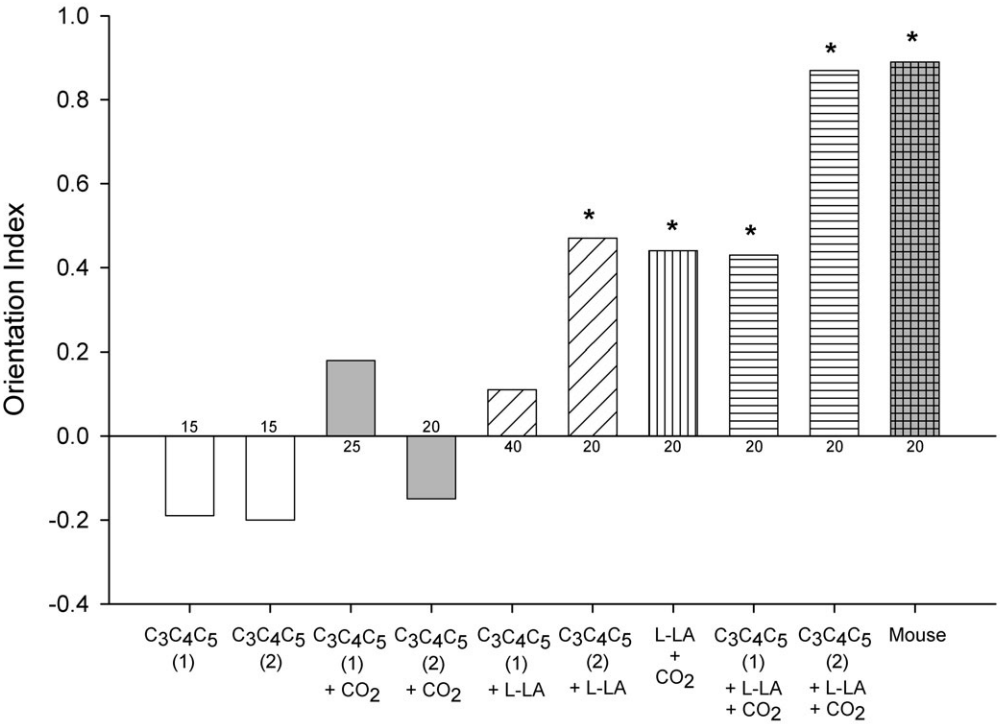

Figure 3 Effect of short-chain fatty acids mixtures combined or not with L-lactic acid (L-LA) and/or carbon dioxide on the orientation response of *T. infestans.* The bars denote the orientation index of insects stimulated with different mixtures of C3, C4, C5, L-LA, CO_2 and a live host. The blend [C3C4C5 (1)] was composed by 100 µg of C3 and 1 µg of C4 and C5, and the mixture [C3C4C5 (2)] contained 33 µg of C3 and 0.33 µg of C4 and C5. Both blends were presented alone or added with L-LA (100 µg/50 µl) or CO_2 (300 p.p.m. above the ambient levels) and together with L-LA and CO_2. The behavioural response of bugs towards a live mouse is shown. The asterisk denotes both, the statistically significant differences ($P < 0.05$) from a uniform distribution and a significant mean direction around stimuli location (0°). Number of insects tested shown above/below the bars.

larvae only when combined with L-LA or in a specific combination in a blend. The oriented behavioural response induced by propionic, butyric and valeric acid was only apparent when added with L-LA, and disappeared when each compound was offered alone via an airstream. Moreover, since L-LA, as a single stimulus, evokes no oriented response of these bugs over a wide range of intensities (Barrozo and Lazzari, 2004), a synergistic effect was evinced.

Human emanations originate from skin glands or from skin microflora, or both. Apocrine glands produce lipids, proteins and steroids, which are initially odourless but are modified to odoriferous compounds— the main components of which are long and short-chain fatty acids—by micro-organisms (Noble, 1993; James *et al.*, 2004). L-Lactic acid is the only major volatile component of eccrine secretions (Acree *et al.*, 1968). Knols and De Jong (1996) pointed out that the fatty acids could represent a group of kairomones for haematophagous insects in relation to their host-finding behaviour. For example, the mosquito *Anopheles gambiae* Giles was attracted to a blend of 18 carboxylic acids (also present in the odour blend of Limburger cheese) at low concentrations (Knols *et al.*, 1997). Coincidently, the microflora of Limburger cheese and the human feet produce similar volatiles (Knols and De Jong, 1996). In addition, *Aedes aegypti* (L) discriminates between carboxylic acids of distinct chain length in the presence of lactic acid, revealing a maximal attraction for the mixtures of formic acid, acetic acid and propionic acid or of valeric acid, caproic acid, heptanoic acid and octanoic acid (Bosch *et al.*, 2000). In addition, Osterkamp *et al.* (1999) identified several carboxylic acids that stimulated the haematophagous ticks *Boophilus microplus* and *Ixodes ricinus* at high doses in olfactometry bioassays. To corroborate the role of short-chain fatty acids in the behaviour of haematophagous insects, the present work demonstrates the response towards C3, C4, C5 and C6 alone or combined with other compounds in a blend in the host-seeking behaviour of a triatomine bug.

The behavioural perception of short-chain carboxylic acids by blood-sucking arthropods correlates with the sensitivity observed by the chemoreceptors involved in the reception of these chemical signals as revealed by several electrophysiological studies (Meijerink and Van Loon, 1999; Van der Broek and Den Otter, 1999; Costantini *et al.*, 2001). *T. infestans* was also shown to exhibit a sensillum basiconica that is sensitive to short-chain fatty acids like isovaleric, butyric, isobutyric and 2-methyl butyric acids (Guerenstein and Guerin, 2001). In the latter work, propionic and isobutyric acids were identified, by means of gas chromatographic analysis, in the headspace of a rabbit. From electroantennogram studies it was determined that the antennae of *T. infestans* also respond to propionic, valeric and caproic acids (Barrozo, 2003).

Although the antenna of *T. infestans* is able to detect low intensities of caproic acid (Barrozo, 2003), the animals showed no behavioural response towards this acid alone or mixed with L-LA under our experimental conditions. Therefore, it is possible that the caproic acid is not an important cue in the host-seeking context of these bugs, although it could be important in other behavioural contexts, and therefore the existence of receptor cells sensitive to C6 would be explained. However, another possibility would be that the experimental conditions (i.e. set-up, chemical doses) used here were inadequate to register a behavioural response to C6. In order to elucidate this subject, further investigations are required. It is worth noting that several host-associated volatiles, which evoke responses at the reception and/or the perception level in bugs, have also been described as components of odour blends involved in triatomines' intraspecific communication. For instance, several carboxylic acids like acetic, propionic, butyric, isobutyric, valeric, isovaleric acids, as well as diverse acetates and alcohols, identified in vertebrate odours (Preti *et al.*, 1976; Cork and Park, 1996; Bernier *et al.*, 2000), were also found as components of triatomines' gland secretions released under different contexts, e.g. after disturbance (Cruz-López *et al.*, 1995; Rojas *et al.*, 2002; Guerenstein and Guerin, 2004) or during mating (Fontan *et al.*, 2002). Therefore, it is possible that the sensitive and specific response to blends of chemical stimuli by triatomines not only increases the insects' ability to locate a food source, but also defines a specific behavioural context, avoiding the ambiguity generated by isolated compounds. The parsimonious use and dependence on the context of orientation signals would allow a more efficient exploitation of the sensory system, since fewer types of receptors would be necessary. Nevertheless, the nervous system is the site for the recognition and gives a meaning to a particular signal, by means of multimodal integration at the central level (Reisenman *et al.*, 1998, 2002).

Interestingly, larvae were unresponsive towards the blend [C3C4C5 (1)] + L-LA, constituted by the doses of C3, C4, C5 that evoked an oriented behaviour when combined separately with L-LA. On the other hand, the diluted blend of [C3C4C5 (2)] + L-LA, which was composed of a similar ratio of odours but of different doses, strongly attracted the insects. Therefore, the stimulus doses in the blend apparently seem to be more relevant than the ratio in the host-seeking behaviour of these bugs. Even so, we cannot truly ascertain whether these insects are able to discriminate among the three short-chain fatty acids, either at the reception or at the perception level. Osterkamp *et al.* (1999) observed a similar phenomenon in ticks, where a blend of seven substances (constituted of several short-chain carboxylic acids including pyruvic acid, ethyl hexanoic acid, caproic acid and valeric acid) was capable of inducing an oriented response similar to that of an extract of bovine skin. However, when each compound was presented individually, a 10^4 times higher dose of each odour was necessary to induce a behavioural response similar to the mixture of seven compounds (Osterkamp *et al.*, 1999). Similarly, our results showed that higher doses of each single carboxylic acid (plus L-LA, Figure 2) are necessary to cause an attractive response in the insects of a comparable magnitude to the blend (2). Therefore, we could hypothesize that the elements of the attractive blend could produce a synergistic effect when added together, but only at an adequate concentration in the blend.

L-Lactic acid has been identified as the major component of human skin (Acree *et al.*, 1968; Geier *et al.*, 1996). Considering the evidence from this work and information previously obtained by us (Barrozo and Lazzari, 2004), it is evident that L-LA has a relevant role as a potent synergist in the orientation behaviour of *T. infestans*, given that the attractiveness observed towards CO_2, C3, C4 and C5, and a specific mixture of them was possible by adding L-LA to the stream. Similar results were observed in other haematophagous insects, e.g. mosquitoes, where lactic acid has been described to increase the attractiveness to CO_2 (Acree *et al.*, 1968; Eiras and Jepson, 1991), ammonia (Geier *et al.*, 1999), carboxylic acids (Bosch *et al.*, 2000), skin odour and skin-rubbing extracts from humans and other vertebrates (Dekker *et al.*, 2002).

Notwithstanding that *T. infestans* larvae expressed a significant oriented behaviour towards the blend of [C3C4C5 (1)] + L-LA + CO_2, this behaviour could be also explained as simply an effect induced by the presence of L-LA + CO_2 in the mentioned mixture, given that both lures evoked similar orientation indexes. On the other hand, the addition of 300 p.p.m. of CO_2 to the blend [C3C4C5 (2)] + L-LA revealed a level of responsiveness in the insects similar to that displayed towards a live mouse, i.e. the highest observed. It is thus possible to speculate that CO_2 could potentiate the behavioural response of the other elements of the blend. However, the effect of CO_2 would be only evident in presence of L-LA and a specific combination of C3, C4 and C5. Therefore, the CO_2 could not replace L-LA as synergist in the orientation behaviour of *T. infestans.* Furthermore, this attractive blend of short-chain fatty acids (2) plus L-LA and CO_2, evoked walking trajectories in bugs holding smaller angular dispersions from the stimuli position than the other odour combinations, and once more, similar to the deviations provoked by the mouse-emitted cues. Similarly, Barrozo and Lazzari (2004) previously demonstrated that the approaching path displayed by these bugs could be modulated by the stimulus. The authors showed that *T. infestans* larvae exhibited an angular deviation whose magnitude depended on the stimulus dose of CO_2, i.e. varying inversely with the CO_2 concentration. Although the artificial blend, constituted by the five components, was highly attractive to *T. infestans* larvae and evoked minimal angular dispersions as towards a living host, we must be cautious since other sensory cues (i.e. including heat and water vapour and other chemical cues) could be also involved in the orientation mechanism of triatomine bugs towards their food source. Different host-emitted components could give the animals spatial information about the food-source location. Thus, the unimodal and multimodal convergence of signals could be important to define a particular behavioural context and/or serve as an 'identification mark' of a specific host. Then, the presence of multiple signals could increase the certainty of the presence and nature of a host, whereas only one or a few stimuli could represent insufficient or ambiguous information.

We studied the orientation behaviour of *T. infestans* to short-chain fatty acids alone or in combination with other volatiles. Although we identified for the first time a synthetic odour blend that provokes an oriented response comparable to a live mouse, it is still evident that further investigations are required to assess the performance of our blend *vis-à-vis* the competence with living hosts in nature. Finally, despite the wide range of short-chain fatty acids doses tested in this work, other putative attracting intensities could be possible.

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