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Appetence behaviours of the triatomine bug *Rhodnius prolixus* on a servosphere in response to the host metabolites carbon dioxide and ammonia

Abstract A combination of 1,000 ppm CO_2 plus 30–40 ppb NH_3 in an air stream induced *Rhodnius prolixus* nymphs walking on a servosphere to perform a series of appetence behaviours. Shortly after the onset of stimulation the nymphs turned sharply upwind towards the source of the chemostimuli (within 13 ± 9 s) from mostly downwind and crosswind walks in the air stream alone. The mean vector angles of these upwind tracks were concentrated in a cone 60° either side of due upwind. The upwind walking bugs stopped more frequently but for a shorter duration and walked at a higher speed than before stimulation. During stops in the presence of the chemostimuli the bugs frequently corrected their course angles and extended their forelegs to reach higher with their antennae in the air. In the air stream alone, *R. prolixus* nymphs frequently sampled the sphere surface with the antennae and cleaned their antennae with the foreleg tarsi. However, the nymphs only briefly tapped the left or right antennal flagellum on the corresponding first leg tarsus and never touched the servosphere surface in the presence of the chemostimuli. After chemostimulus removal from the air stream the bugs continued to respond with the same appetence responses as during stimulation, but walked more tortuously in a crosswind direction in an effort to regain contact with the chemostimuli.

Keywords *Rhodnius prolixus* · Triatomine bug · Blood-sucking · Behaviour · Servosphere

Introduction

The triatomine bug *Rhodnius prolixus* Stål, 1859 (Hemiptera: Reduviidae), referred to hereafter as *Rhod-*

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nius, is an important vector of *Trypanosoma cruzi*, the causative agent of Chagas' disease in Central and South America (Lent and Wygodzinsky 1979). This species, as virtually all members of the tribe Rhodniini, is primarily associated with palm tree habitats and has a wide range of hosts including birds, rodents, marsupials, sloths and reptiles (Lent and Wygodzinsky 1979). *Rhodnius* colonized human dwellings due to the use of palm trees in the construction of houses in rural areas, providing the bugs with a stable niche with a constant food supply. Furthermore, this particular species has been used to study insect orientation behaviours since the beginning of the last century (Wigglesworth and Gillett 1934) and has long since served as a model species in insect physiology (Wigglesworth 1984).

There seems to be an olfactory component to host finding in virtually all blood-sucking insects (Lehane 1991; Guerin et al. 2000). Triatomines use different sensory cues to locate hosts and refugia, but volatile chemostimuli alone can induce appetence behaviours (Taneja and Guerin 1995). CO_2, with a very constant concentration in mammalian breath (4.5%; Lindstedt and Thomas 1994) is generally accepted to be a dominant cue that serves as both an activation and orientation cue for all blood-sucking insects including triatomines (Wiesinger 1956; Mayer 1968; Nuñes 1987; Taneja and Guerin 1995). NH_3 is present in triatomine (Harington 1961; Taneja and Guerin 1997) and host animal excretions (Lentner 1981; Albone 1984) and as such occurs in both refugia used by triatomines as well as in host dwellings. Furthermore, NH_3-sensitive receptor cells occur in basiconic grooved-peg sensilla of *Triatoma infestans* (Diehl et al. 2003) that respond to levels as low as 2 ppb (Taneja and Guerin 1997). The NH_3 concentration in human breath varies between 120 to 1,280 ppb (Norwood et al. 1992). As both CO_2 and NH_3 alone attract *Rhodnius* and *T. infestans* on a servosphere (Taneja and Guerin 1995, 1997; Guerin et al. 2000), we use here a combination of these products to study the appetence responses of *Rhodnius* on a servosphere, we describe the behaviours contributing to such responses and the strategies used by these bugs to try to

recontact the chemostimuli after their removal from an air stream.

Materials and methods

Insects

A colony of *Rhodnius* is maintained at 28°C, 80% RH and 12:12 h L:D cycle in a climate chamber. The bugs are fed on chickens once a month. Experiments were made with fifth-instar nymphs starved for 7–8 weeks after moulting.

Behavioural recordings

A servosphere (Kramer 1976) was used to record the walks of *Rhodnius* nymphs. The servosphere is an open loop set up, i.e. it records behaviours the bugs employ to approach a goal but without reaching it. This design satisfies the minimum of experimental conditions required to study a walking arthropod's behaviour (Kennedy 1977). The apparatus allows a walking untethered triatomine liberty to move unimpeded in all directions at the apex of a sphere (50 cm diameter) in an experimental set up where sensory modalities other than chemostimuli are excluded (Taneja and Guerin 1995). The co-ordinates of displacements of the bug on the servosphere are supplied to a computer (SAMII 68 K; WWS, Ettlingen, Germany) by two incremental pulse-generators mounted on the sphere's equator, both working at a resolution of 0.1 mm and recording at 0.1-s intervals. Subsequent analysis of these records was performed using in-house developed track analysis software (sLocTrack) running under Linux. Each bug was allowed a 2-min period of acclimatisation before recording started. The responses of 24 bugs were recorded in three consecutive 2-min periods: in the air stream alone (C1), the air stream plus chemostimuli (T) and the air stream after removal of the chemostimuli (C2). To asses the effect of the air stream alone on the behaviour of the triatomines, the behaviours of 21 other bugs were observed for 2 min each in still air.

All behaviours on the servosphere were also recorded on video tape (Panasonic VCR NV-180) using two infrared-sensitive video cameras (Canon Ci 20PR, Japan and PCO Computer Optics 77CE, Germany), one positioned at 70° to the apex of the sphere over the air delivery tube and the second placed tangentially to the apex of the sphere from the downwind position. Both views were observed simultaneously using a screen splitter (Panasonic production mixer, WJ-MX12, Japan).

Chemostimuli and their delivery

The stimulus delivery system was as described in Taneja and Guerin (1995). This consisted of a charcoal-filtered air-stream (15 cm s^{-1} as measured by a hot-wire ane-mometer, ThermoAir2, Schiltknecht Messtechnik, Switzerland, accuracy ± 0.01 m s^{-1}, rise time < 1 s) maintained at 24°C and 80% RH (as measured by a dew point termohygrometer, MTR 5.3, DMP, Switzerland, accurancy ± 0.5 K, rise time < 2 min) directed to the apex of the servosphere into which a mixture of 1,000 ppm CO_2 and 30–40 ppb NH_3 were injected as chemostimuli (Taneja and Guerin 1995, 1997). The onset and cessation of stimulus delivery to the bug was controlled automatically (Taneja and Guerin 1995). Carbon dioxide came from a pressured cylinder containing 2% CO_2 in O_2 injected at 50 ml min^{-1} into the air stream. Ammonia came from a gas-wash bottle containing a filter paper disk (12.5 cm diameter) wetted with 0.2 ml of diluted NH_4OH (Fluka, Switzerland) in nanopure water (71.43 mmol^{-1}). The air passing over this filter paper disk was injected at 160 ml min^{-1} into the air stream. The filter paper was treated once for tests with 4–5 bugs. The controls consisted of 50 ml/min charcoal-filtered air alone for CO_2 and 160 ml min^{-1} charcoal-filtered air passing through a gas-wash bottle with 0.2 ml water on filter paper for NH_3. The concentrations of CO_2 in the air at the apex of the sphere was measured with an infrared gas analyser (LI-COR LI-820, Nebraska, USA, accuracy $\pm 3\%$, rise time 5 ppm in ≤ 20 s) and the concentration of NH_3 was measured with an electrochemical sensor (DrägerSensor Pac III S, Lübeck, Germany, accuracy $\pm 1\%$, rise time 1 ppm in ≤ 20 s).

Behaviour analysis

As the mean length of a *Rhodnius* fifth-instar nymph is 1.5 cm and its average walking speed is approximately 30 mm s^{-1}, the basic track recording intervals of 0.1 s were merged to provide intervals of 0.3 s, i.e. allowing the bug to move at least 60% of its length before recording its next position. Subsequently, a running mean was calculated over five successive (0.3 s) intervals to smoothen the data in order to remove some noise related to the insect's gait that was picked up by the servosphere. To identify stops, 3 mm/s was considered the minimum speed the animal had to achieve to be considered walking; all displacements slower than this were discarded.

From these modified track recordings the following instantaneous statistics were calculated; direction (ϕ) with respect to wind (0°), instantaneous speed (v) and angular velocity (ω). The instantaneous walking speeds of the bugs (v) in each experimental period were compared using Student's t-test; compliance to normal distribution (Shapiro-Wilk test for normality and QQ normal plot method) and equality of variances (Leven's test) were tested. Parameters, termed summary statistics, were calculated for each period of each track (C1, T and C2). These summary statistics were overall displacement (D), number of stops (NS), stop duration (SD), mean direction of the track (Φ) and path straightness r (mean

Fig. 1 Angular velocity in degree per 0.3 s made up by *Rhonius* nymphs walking on the servosphere. By designating left turning angles as (−) and right turning angles as (+) and applying a running mean over five consecutive 0.3-s intervals, regular oscillations left and right of a relatively straight course are apparent
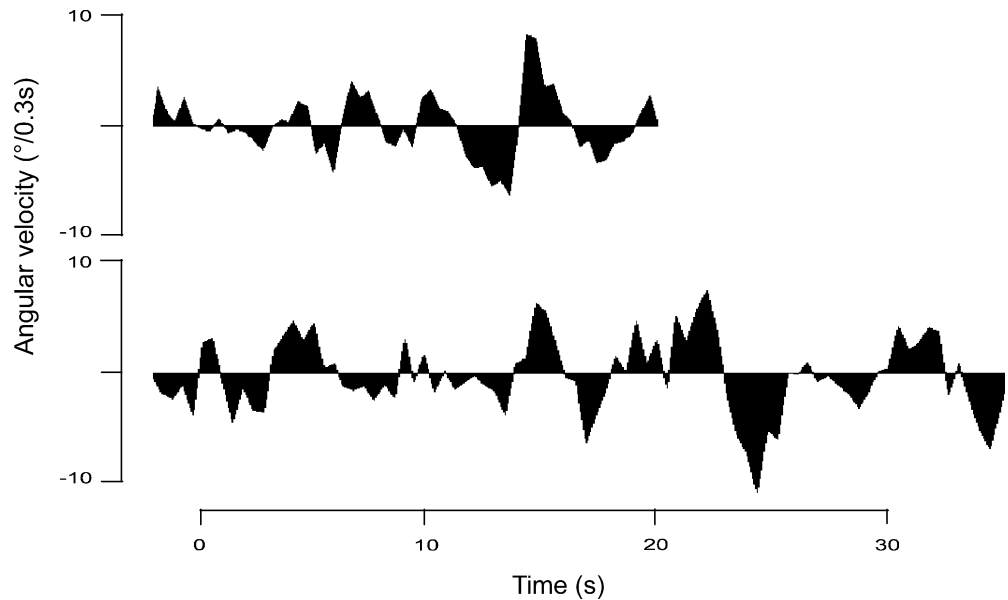
vector length, Batschelet 1981), relative upwind displacement (RUD, i.e. walking distance in a cone 60° either side due upwind) and target vector (TV, calculated by multiplying the cosine of the mean direction of the track Φ by the path straightness r, Jones 1977). All summary statistics except Φ were compared using the two-tailed Wilcoxon paired signed rank test. Rao's spacing test (Batschelet 1981) was applied to determine whether the distributions of Φ in a given period differed from uniformity. In periods where a particular orientation angle was expected, the data were subjected to a modified Rayleigh V-test (Batschelet 1981). Since the mean vector angles of the tracks were mostly distributed across the air stream after removal of the chemostimuli, the corresponding mean angles were doubled (Batschelet 1981) before applying the modified Rayleigh V-test. The dispersion, in degrees, of the mean track directions (Φ) for all 24 bugs was estimated as the mean angular deviation s (Batschelet 1981). In addition, major changes in walking direction that occur after onset of chemostimulation were analysed from plots of the tracks and by replaying the video recordings at a resolution of 1 frame/12.5 s (Panasonic VCR AG-7350, Japan).

As bugs stop frequently during walks, criteria for automatically identifying a walking bout were established in our software in order to examine if the mean direction of such walking bouts was modified in response to exposure of the bugs to the chemostimuli. This was done as follows: if the bug's speed increases above the minimum speed, start a new walking bout and if it drops below the minimum speed interrupt the walking bout; if the walking bout duration is < 0.5 s (minimum walking bout duration) then discard the bout; if a walking bout starts during one experimental period and finishes during the following one, it is divided into two walking bouts in the corresponding periods. Summary statistics for walking bouts were calculated and analysed as described above. All statistical analyses were per-

formed using R for Linux version 1.6–24, Vienna, Austria (Ihaka and Gentleman 1996).

Results

Rhodnius nymph behaviours in still air

Triatomine behaviour in still air is firstly described here as an air stream was used to deliver the chemostimuli. *Rhodnius* nymphs walked in straight bouts (median $r = 0.996$, IQR[1] 0.004; median length of a bout 85.07 mm, IQR 162.71, $n = 321$) punctuated by stops (median 3.2 s, IQR 4.1 s, $n = 335$ stops). The median distance walked during the 2-min recording was 1,924 mm (IQR 1,498 mm, $n = 21$) and the median number of stops/bug was 19 (IQR 25). The bugs began a walking bout at a high speed (mean 45 mm s^{-1}) that gradually decreased by some 20% to end in an abrupt stop. The mean instantaneous velocity during these walks was 27.89 mm s^{-1}. These walking bouts also included intervals (< 1 s) at relatively low speed (mean 11 mm s^{-1}). The bugs walked by turning successively right and left of course in a zigzag path (Fig. 1). The median angular velocity was 8.8°/s (IQR 11.6° s^{-1}). No bug turned at an angular velocity higher than 61.2° s^{-1} (that occurred only at low walking speeds of < 10 mm s^{-1}); at peak walking speeds (> 40 mm s^{-1}) angular velocities only reached 14.3° s^{-1}.

During walking bouts the bug periodically scanned the air by moving their antennae up and down either simultaneously or alternatively with the left or right antenna ($\pm 30°$ with respect to the body long axis) ca. every 2 s, irregularly touched the sphere surface with their antennae and eventually undertook a change in

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[1]IQR: interquartile range, i.e. the difference between the 75th and 25th percentiles.

direction. During stops, the bugs either changed direction by rotating the body and/or scanned the air by moving the antennae (maintaining the thorax–abdomen axis parallel to the ground), or undertook cleaning of the antennae (14 out of 21 nymphs did so). Antennal scanning of the air during stops was performed by moving both antennae simultaneously or alternatively up and down, and laterally. Antennal cleaning is a stereotyped behaviour: the bugs lift and join both first leg tarsi over one antenna and run them distally along the flagellum (through a small comb on the inner side of the fore tibia as described by Campanucci et al. 1998) while slightly lifting the head in the opposite direction. This is followed by rubbing the tarsi against one another and subsequent preening of the second antenna. The entire process lasts approximately 1 min 20 s if uninterrupted.

Of the 21 insects recorded while walking in still air, 8 made consecutive clockwise or counter-clockwise circles while walking. Circling was not continuous but was interrupted by stops. The fraction of the track walked in circles was very variable; seven bugs made from 2 to 5 circles of variable size, shape and sense of rotation, accounting for 22.2% of the track, and one individual made 32 consecutive very regular clockwise circles (ca. 12 cm diameter) throughout its walk. The tracks of nymphs that did not circle were not very straight (median $r = 0.36$, IQR 0.28, $n = 13$), nevertheless the mean vector angles describing these walks showed no bias for a given direction in still air ($p > 0.8$ Rao's spacing test).

Behaviours in the air stream alone

In contrast to the situation in still air, nymphs in the air stream without the chemostimuli walked relatively straight (median $r = 0.75$, IQR 0.39, $n = 24$) but no bugs circled. The mean vector angles of the tracks were either downwind or crosswind ($P < 0.005$ Rao's spacing test, $s = 59°$, Fig. 3a). In the air stream, the nymphs walked less (median 936 mm, IQR 823 mm, $n = 24$, $P < 0.001$), stopped less (median 7, IQR 11, $P < 0.01$) but for a longer duration (median 11.14 s, IQR 6 s, $P < 0.001$) than in still air. The bugs' mean instantaneous walking velocity in the air stream (24.06 mm s^{-1}) was lower compared to still air ($P < 0.0001$). Fifteen of the 24 nymphs cleaned their antennae during stops (3 only cleaned one antenna).

Responses to CO$_2$ plus NH$_3$ in the air stream

The addition of CO$_2$ and NH$_3$ to the air stream induced 22 of the 24 nymphs tested to turn sharply upwind (median of these upwind turns 112°, IQR 43°) shortly after the onset of stimulation (median time to response 13 s, IQR 9 s, maximum 34 s, Fig. 2). The straightness of the walks (median $r = 0.57$, IQR 0.37) did not diminish significantly ($p > 0.152$) compared to walks made in the air stream alone, but the mean vector angles
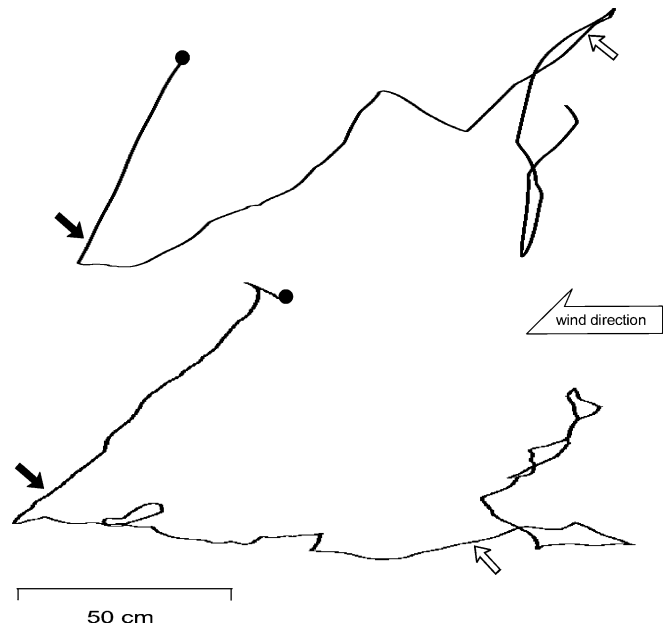
Fig. 2 Tracks made by *Rhodnius* nymphs on the servosphere depicting responses to 1,000 ppm CO$_2$ plus 30–40 ppb NH$_3$ in the air stream (*arrow*). The tracks started (*filled circle*) with the bugs walking crosswind or downwind in the air stream alone (15 cm s^{-1}). After the chemostimuli were added to the air stream (*bold arrows* on the tracks), the bugs turned to walk upwind. After removal of the chemostimuli from the air stream (*empty arrows*) the bugs at first continued to walk upwind but thereafter walked mainly crosswind

of the tracks were concentrated in a cone 60° either side of due upwind ($P < 10^{-7}$ modified Rayleigh *V*-test; angular deviation 30°, Fig. 3b). The bugs still continued to walk in bouts but the distribution of these walking bouts shifted from downwind and crosswind runs in the air stream alone to upwind runs following stimulus delivery (modified Rayleigh *V*-test, $P < 0.001$, Fig. 4). There is, however, a deviation of the running bouts to one side of upwind due to a slight asymmetry in the airflow, detected after the experiments were completed. The median proportion of the distance walked upwind during the 2-min stimulation period reached 63% as compared to 7% in the air stream alone (Fig. 5a). The target vector values increased to 0.48 (IQR 0.27) indicating the efficiency with which the triatomines responded to the chemostimuli (Fig. 5b).

Although *Rhodnius* nymphs stopped more frequently during stimulation (median 15, IQR 12) they walked farther (median 1,614 mm, IQR 781 mm) than in the air stream alone (Fig. 6). This was because the median duration of stops was shorter (3.95 s, IQR 3.2 s, $P < 0.0005$) during exposure to chemostimuli and that the bugs' mean instantaneous velocity (24.77 mm s^{-1}) increased compared to that in the air stream alone ($p < 0.05$). During stops, the bugs frequently lifted the anterior part of their body ($\sim$40°) through extension of their fore legs in order to reach higher with their antennae held up (ca. 60° with respect to ground), a behaviour not seen in still air or in the air stream alone and referred to here as antennal
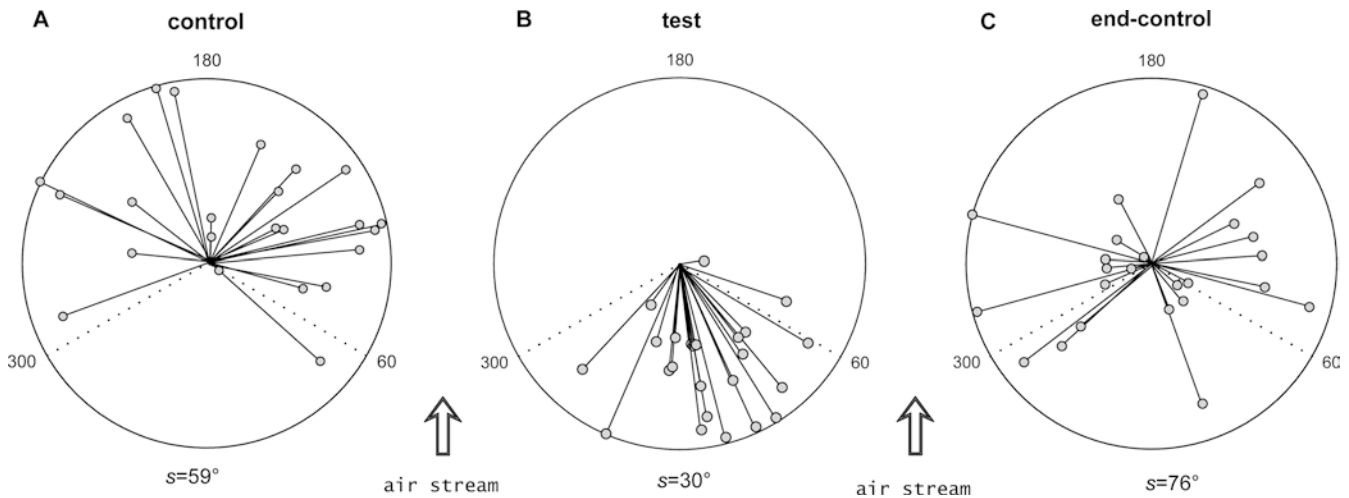
Fig. 3a–c Polar plots of the distribution of the mean vector angles of tracks described by *Rhodnius* fifth instar nymphs ($n = 24$) on the servosphere during consecutive 2-min periods: in the air stream alone (**a**), air stream with 1,000 ppm CO_2 plus 30–40 ppb NH_3 (**b**) and the air stream alone again (**c**). The mean vectors of the tracks shifted from crosswind and downwind in the initial control period to the upwind cone (60° either side of due upwind) during exposure to CO_2 plus NH_3 (test), and to crosswind after the chemostimuli were removed from the air stream (end-control). The nearer the vector weighting to the perimeter the straighter the walk in that direction. The mean angular deviation (*s*) below each plot is a measure of the dispersion of the directions (Batschelet 1981) taken by bugs in each period. The bug walked more crosswind but in a more tortuous manner after treatment removal from the air stream (end-control)

reaching. During exposure to the chemostimuli the bugs did not touch the sphere surface with their antennae. Rather than cleaning the antennae with the foreleg tarsi,

Fig. 4 Histograms of the mean vector angles of subtracks made by 24 *Rhodnius* nymphs during successive 2-min periods in an air stream (upwind 0°; **a**), in an air stream with a mixture of 1,000 ppm CO_2 plus 30–40 ppb NH_3 added (**b**) and in the air stream alone again (**c**). A subtrack is a walking bout of 0.5 s or more with a minimun speed of 3 mm s^{-1}, and the subtrack vector angle is the circular mean (Batschelet 1981) of the instantaneous course angles recorded for each walking bout. The bugs walked essentially downwind and crosswind during the initial period in the air stream alone (control). There was a shift ($P < 10^{-16}$; Wilcoxon unpaired test) to a higher proportion of subtracks in the upwind direction during exposure to CO_2 plus NH_3 (test) but this disappeared after removal of the chemostimuli (end-control)
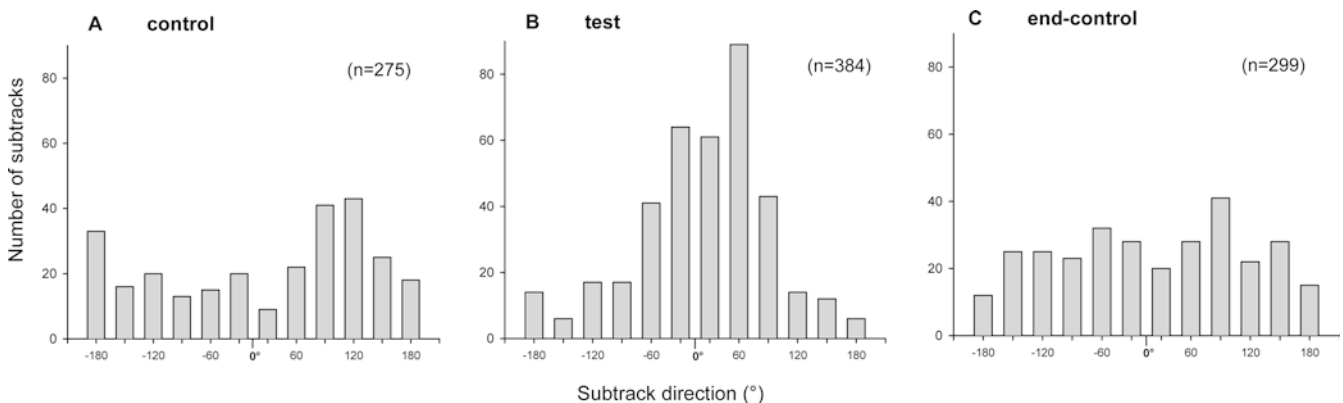
as in still air and in the air stream alone, the bugs exposed to the chemostimuli briefly tapped (< 1 s) the left or right flagellum on the corresponding first leg tarsus, a behaviour referred to hereafter as antennal tapping. Of the 24 bugs tested, 11 exhibited this behaviour while walking or during brief stops.

Response to removal of the chemostimuli from the air stream

The bugs continued to respond with an upwind walk for some time after removal of the chemostimuli (median 16 s, IQR 9 s, $n = 24$). Thereafter, they walked more crosswind ($p < 10^{-8}$ modified Rayleigh *V*-test) as indicated by the mean direction (Φ) of the tracks (Fig. 3c), and significantly less straight (median $r = 0.47$, IQR 0.48, $P < 0.05$, $n = 24$) compared to the initial clean air stream situation. The bugs' instantaneous velocity increased after stimulus removal (mean 26.26 mm s^{-1}, $P < 0.0001$) but the frequency of stops and the distance walked remained the same (Fig. 6). The triatomines continued to scan the air with their antennae by lifting the thorax during stops and did not touch the ground with the antennae. They did not clean the antennae with the forelegs but antennal tapping was observed in 13 of the 24 records after stimulus removal.
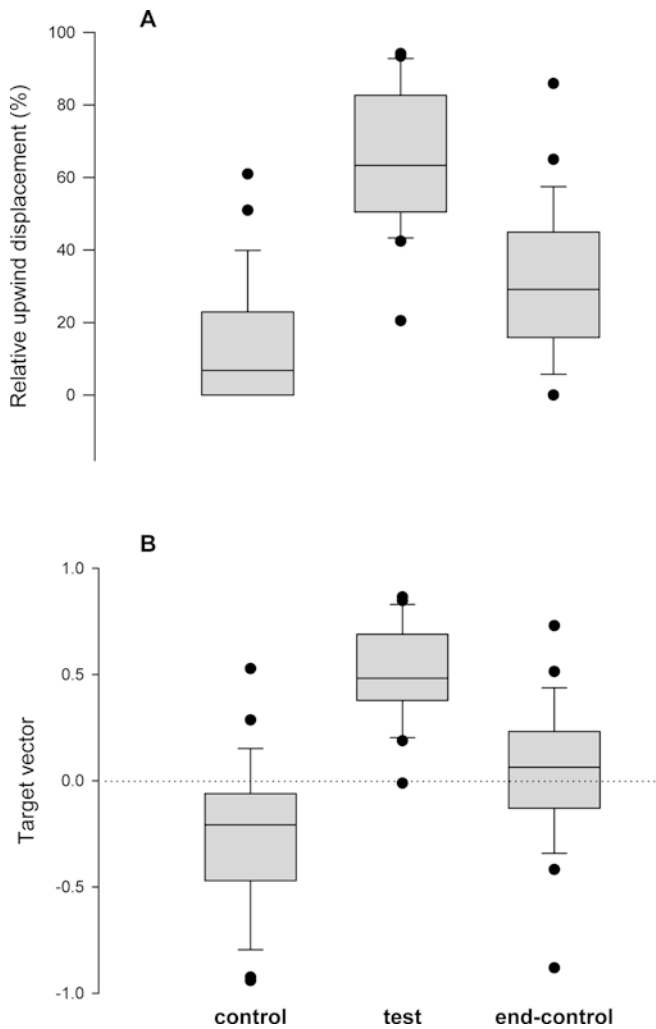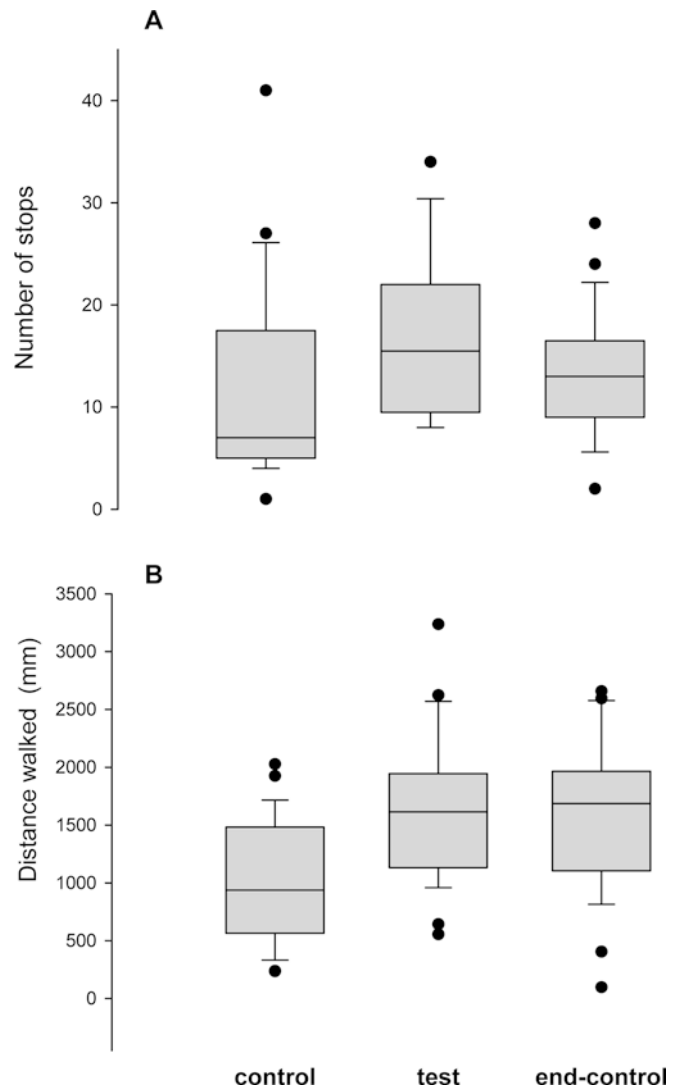
Fig. 5 Responses elicited by a mixture of 1,000 ppm CO_2 plus 30–40 ppb NH_3 in fifth instar *Rhodnius* nymphs on the servosphere were assessed using upwind displacement, i.e. the relative distance walked in a cone 60° either side of due upwind (**a**), and target vector (**b**; Jones 1977, see Materials and methods). **a** The relative upwind displacement during the initial period in the air stream alone (6.8%; control) increased significantly during exposure to CO_2 plus NH_3 (63%, $P < 10^{-7}$; test) and decreased again after removal of the chemostimuli (29%, $P < 10^{-5}$; end-control) but to a level higher than in the initial control period ($P < 0.01$). **b** Target vector was highest during exposure to the chemostimuli ($P < 10^{-5}$; Wilcoxon paired test). In the box plots, the *lines* within a box mark the median, the *lower and upper boundary lines* of a box indicate the 25th and 75th percentiles, *bars* below and above indicate the 10th and 90th percentiles, respectively, and the points represent data beyond these limits

Fig. 6 *Rhodnius* fifth instar nymphs exposed to a mixture of 1,000 ppm CO_2 plus 30–40 ppb NH_3 on the servosphere stopped more frequently (**a**) but walked more (**b**) than in the air stream alone. **a** The number of stops increased on exposure to the treatment ($P < 0.05$; test) and remained higher after removal of CO_2 plus NH_3 from the air stream (end-control) compared to the initial period ($P < 0.01$; control). **b** The distance walked also increased during the test period compared to the initial control period ($P < 10^{-4}$) and remained higher than for this initial period even after removal of the test molecules from the air stream ($P < 0.005$). For boxplot explanations and statistics see legend to Fig. 5

Discussion

Mechanisms of orientation without chemostimuli in the air stream

In still air the starved triatomines, driven either by hunger or need for a shelter, indulge in non-oriented behaviour or ranging (Dusenbery 1992) likely to bring them into contact with a stimulus. Some individuals deprived of chemical, auditory, visual or mechanore- ceptive information from an air stream make irregular paths containing many circular elements as has been reported for other arthropods (Bell and Kramer 1979; Heinzel and Böhm 1989; Lönnendonker and Scharstein 1991; McMahon and Guerin 2000). This may be due to the incapacity of the bugs to maintain a straight course in the absence of a "collimating" stimulus, i.e. one unrelated to a particular goal that serves the propose of maintaining a straight course (Dusenbery 1992). On the other hand, *Rhodnius* in an air current is provided with such a collimating stimulus inducing the bug to walk relatively straight downwind or crosswind.

Mechanism of orientation to the chemostimuli

The combination of CO_2 and NH_3, two small molecules, induces goal oriented behaviours by *Rhodnius* on the servosphere. The triatomines switch to a series of endogenously driven behaviour patterns that reflects their appetence (*sensu lato*). Two different goal oriented behaviours can be distinguished as in other species: guiding (Dusenbery 1992) and casting (Kennedy and Marsh 1974; Dusenbery 1992). Guiding behaviours include the mechanisms the bugs employ to approach the resource of the chemostimuli in the air stream, and casting behaviours (treated below), are those undertaken to regain contact with the chemostimuli after loss (David et al. 1983).

Firstly, upon delivery of CO_2 and NH_3 into the air stream the triatomines turn to walk upwind, i.e. chemoanemotaxis. This certainly entails some mechanosensory input in response to the air current direction. The bugs walk in the direction of the source of the chemostimuli while stimulation persists, making sequential corrections in the course angle during stops and walking bouts vis-à-vis the air stream. The increase in walking speed, upwind displacement, increased number of stops of shorter duration and antennal reaching observed during exposure to the chemostimuli all demonstrate the appetence of the bugs. Antennal reaching in the air stream during stops may, under the open loop conditions on the servosphere, represent yet another behavioural adaptation: as foreleg extension accompanies antennal reaching this could also be interpreted as an attempt by the bug to reach on to a close by host. Antennal tapping in the presence of the chemostimuli is a less time consuming alternative to the antennal cleaning behaviour observed in the air stream alone, thus improving the efficiency of the bug's guiding response. Furthermore, alternation between walks and pauses where antennal scanning occurs has been referred to as saltatory search (O'Brien et al. 1990), i.e. a searching cycle that functions to improve efficiency of the foraging tactic of a species like a triatomine that requires an isolated resource. Bilateral antennal sensing of the air current containing the chemostimuli during each stop plays a crucial role in the directed walk towards the stimulus source.

Behaviours in the air stream after stimulus removal

The more crosswind and tortuous walks performed by the bugs after treatment removal from the air stream represent an effort by the bugs to regain contact with the chemostimuli. Crosswind displacement on loss of the chemostimuli has already been observed for triatomines (Taneja and Guerin 1995) and for flying moths (Kennedy and Marsh 1974; David et al. 1983). Geometrical analysis of casting behaviours (Dusenbery 1992) has shown that crosswind walking increases the probability of encounter with a chemostimulus over upwind,

downwind or a random search strategy. In addition, the relative high speed, more frequent and shorter stops and antennal reaching and tapping observed in the presence of the chemostimuli persist after cessation of stimulation.

The observed appetence behaviour of *Rhodnius* in the presence of NH_3 plus CO_2, two simple and ubiquitous molecules, is interesting from a biological and epidemiological point of view. Rose (1998) working under semi-natural conditions has shown that ammonia alone can activate *T. infestans* (i.e. induce exit from a refuge) but is not enough to recruit the bugs to the source. Furthermore, this author found that even though CO_2 alone under the same conditions could attract *T. infestans* to the source, addition of NH_3 in the air produces an effect greater than the sum of the individual effects of these two molecules in terms of activation and recruitment. The behavioural effect of these molecules on triatomines is not surprising as both are central catabolic products associated with respiration and excretion in animals. As such, the mixture of CO_2 and NH_3 may prove practical in developing a lure to monitor presence of these disease vectors. Both to the experiments of Rose (1998) and those detailed here lend us to conclude that *Rhodnius* may use the combination of CO_2 plus NH_3 to find either a host or a refuge.

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