0014-4754/95/080834-04$1.50 + 0.20

Baker's yeast, an attractant for baiting traps for Chagas' disease vectors

P. G. Guerenstein*, M. G. Lorenzo, J. A. Núñez and C. R. Lazzari**

Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, (1428) Buenos Aires (Argentina), Fax +54 1 541 0625
Received 4 July 1994; received after revision 25 November 1994; accepted 10 January 1995

Abstract. We tested the attraction of volatile compounds, produced by the aerobic growth of *Saccharomyces cerevisiae* on saccharose for *Triatoma infestans*. For these tests, we exploited the behavioural characteristic of these haematophagous insects of dropping when searching for food. In olfactometer assays, yeast cultures activated and attracted bugs as effectively as a mouse. The attraction of the cultures was significantly reduced when the carbon dioxide released was partially eliminated using potassium hydroxide. Yeast cultures were also tested as lures in a novel trap device. A baited device for trapping Chagas' disease vectors using the behavioural peculiarities of *T. infestans* and this simple attractant is described.
Key words. Chagas' disease; Triatominae; lures; baited traps; yeast; carbon dioxide; attractant.

Chagas' disease is a major health problem in Latin America, affecting more than 16 million people. This disease is caused by the flagellate parasite *Trypanosoma cruzi* Chagas, whose main vector is the haematophagous bug *Triatoma infestans* Klug. Adults and larvae of this insect are predominantly domestic in habitat. They assemble in wall crevices inside houses during daylight hours and become active at night.

It is well known that when searching for food, triatomine bugs are attracted both by body heat and odours (e.g. CO_2, lactic acid) released by warm-blooded potential hosts[1,2]. Nevertheless, such knowledge has not yet been applied to the control of triatomines.

At present, cardboard boxes offering a refuge are employed for monitoring triatomine populations[3,4]. They represent a very useful tool for the early detection of bugs[5], which is a fundamental requirement for control programs against Chagas' disease vectors. In these devices, insects are free to enter and leave since these boxes are designed for the detection of any sign of the vector's presence (e.g. insects, eggs, excrement, exuviae, etc.), but not for trapping bugs. However, these boxes only offer shelter to the bugs that find them by chance. A trap with an attractant that actively draws the insects in and then captures them would be preferable, and would constitute a more reliable detector for bugs. Moreover, it might reduce the number of parasite-host contacts by reducing the vector population.

It is generally accepted that carbon dioxide serves as an olfactory cue for virtually all blood-sucking insects[6]. It has been used as a bait in traps for mosquitoes[7] and tse tse flies[8]. However, the usual delivery methods for CO_2 are too expensive and impractical for control purposes in the field[9,10]. Since CO_2 is the main product released by cultures of *Saccharomyces cerevisiae* Hansen in aerobiosis, and given that yeast is quite inexpensive, such cultures deserve to be tested as potential attractants for haematophagous bugs.

In this work, we analysed the response of *T. infestans* to volatiles released from the aerobic growth of baker's yeast *S. cerevisiae*. Furthermore, we developed and tested a novel trap device for triatomines that incorporates this product as a lure.

The rationale of our experiments is a characteristic behaviour of *T. infestans*: bugs let themselves fall from ceilings while searching for food (in fact, its Spanish name *vinchuca* is derived from the Quechua word for 'falling bugs'). This particular behaviour pattern was exploited by us to capture bugs and, in this manner, to quantify the attractiveness of different stimuli.

Materials and methods

Experimental animals were larvae of *T. infestans* reared in the laboratory, and fed on heparinized goat blood by means of an artificial feeder[11]. Starvation time for the insects used in assays ranged from 15 to 60 days post-ecdysis. No differential responses in the behavioural parameters measured for this work were observed among insects within this starvation interval (unpubl. observ.).

Olfactometer tests. The response of the insects was studied in a dual-choice situation, using a simultaneous discrimination olfactometer (fig. 1). It was designed taking into account the behavioural particularities of *T. infestans*, and that CO_2 has been shown to attract triatomines in a low-speed wind-tunnel[2,12]. When compared with other designs previously employed for this species[2,12,13], the novel design has the following advantages: 1) it exploits the tendency of bugs to let them-

* Present address: Institute of Zoology, University of Neuchâtel, Rue Emile-Argand 11, CH-2007 Neuchâtel (Switzerland).
** To whom all correspondence should be sent.

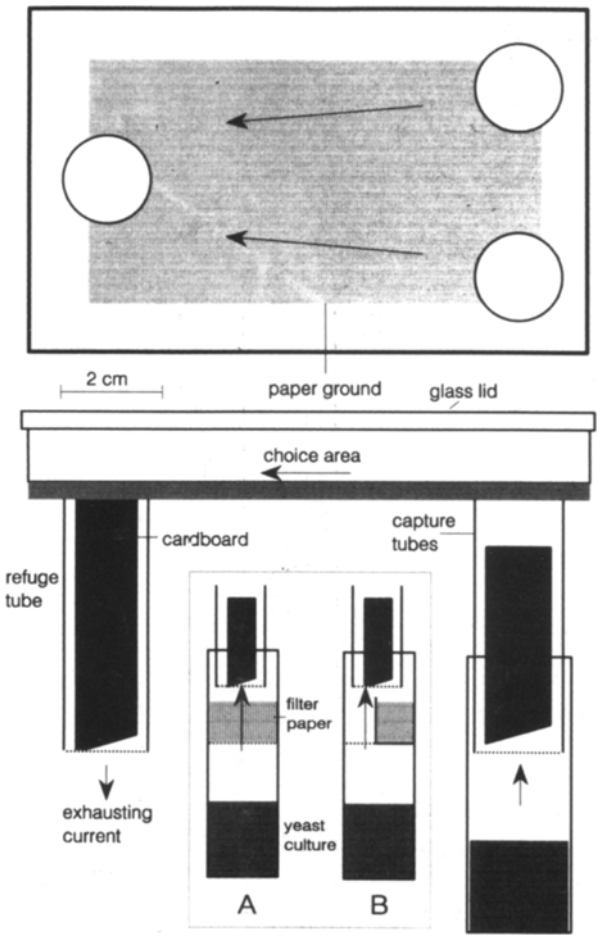

Figure 1. Olfactometer used to test the response of larvae of *T. infestans* to volatiles released from a live mouse or from the aerobic growth of baker's yeast on saccharose. Top: view from above. Bottom: schematic side view of the whole device. Arrows indicate the direction of the air flow. Inset: culture tubes containing KOH-impregnated papers to absorb the CO_2 emitted by yeast (*A*) and with the path of the volatiles separated from the CO_2 absorbent (*B*).

selves fall, 2) it prevents the insects from getting out of the chosen tube and returning to the start point, 3) it does not require the injection of identical air currents at both election arms. The apparatus consisted of a closed rectangular choice arena made of Plexiglas with three plastic tubes connected to it. One tube acted as refuge and starting place; bugs could leave this tube by climbing onto a piece of cardboard that allowed them to reach the arena surface. The other two acted as test and control capture-tubes, associated with a stimulus source (i.e., mouse or yeast culture) to be tested, or its corresponding control. Bugs could drop into the capture-tubes, but could not escape from them (fig. 1). An exhaust fan connected to the inferior end of the refuge tube extracted 5 ml of air per min, i.e. the air speed was of 35 mm/min in the starting tube and about the half of this at each capture-tube. Previous experiments showed that this low air-flow was enough to evoke the response of the bugs, whereas higher currents made the insects to become reluctant to leave the refuge tube.

Ten larvae were used in each assay. They were gently placed in the starting tube and the air flow was turned on after 10 min. Assays were conducted at 24 ± 2 °C in darkness, starting at 21.00 h and finishing at 10.00 h. During this period, insects freely left the starting tube. In the morning, the number of insects in the test and control capture-tubes was counted. Control assays were performed to quantify the basic activity of the insects in the absence of test stimuli.

From the number of captured insects, the attraction of and activation induced by the stimulus were established. Activation was defined as the percentage of insects caught in both capture-tubes relative to the total number of insects in the experiment. Attraction was defined as the percentage of insects caught in the test tube relative to the number caught in total. Positional controls were performed for all olfactometer tests, but no significant asymmetry was detected. Room temperature and that of the stimulus source were measured by means of a thermistor thermometer (accuracy 0.1 °C).

In a first experimental series, the response of *T. infestans* to volatiles released by yeast cultures was studied. Fourth instar larvae were tested with each of the following stimuli separately: 1) a living mouse; 2) a culture of fresh yeast (CALSA, Argentina) (6 g yeast + 4 g saccharose + 30 ml water at room temperature); and 3) a culture of dehydrated yeast (LEVEX®, Mexico) (1 g dry yeast + 4 g saccharose + 10 ml water at room temperature). The corresponding controls were an empty container for 1), and the respective saccharose solutions without yeast for 2) and 3).

In a second experimental series, a similar method was used to test whether the carbon dioxide released by yeast cultures is involved in the response of the bugs. A piece of filter paper 60×3 cm was rolled and soaked with 2.5 ml of a 25% w/w aqueous solution of KOH. This was placed in the upper half of the culture tube, and held in place by a plastic mesh (fig. 1, inset A). In this way, yeast volatiles passed in close proximity to a CO_2-absorbing agent. The simultaneous control consisted of an identical culture tube, but with papers impregnated with distilled water.

In order to control for possible interference due to odours released by the KOH, the experiment was repeated, but with the path of yeast volatiles separated from the impregnated papers (fig. 1, inset B). In this way, the KOH solution was still present, but the absorption of CO_2 was impaired.

Statistical significance of the results was analysed from transformed data[14]. The following tests were used: 1) paired-samples *t*-tests, for comparing attraction between test and control tubes; 2) *t*-test for independent samples, for comparing activation between test and the respective control situations; 3) one-way ANOVA fol-

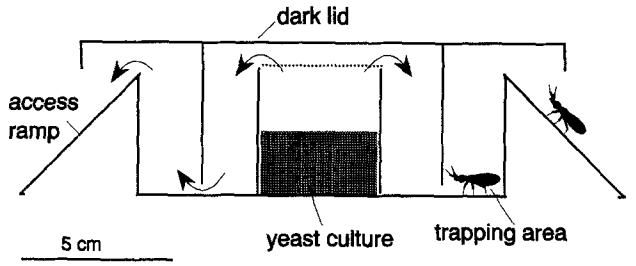

Figure 2. Baited trap for triatomine bugs. The device consists of a circular access ramp from which the bugs can drop into a plastic container from which they cannot escape. In the centre is a plastic receptacle containing the yeast culture, from which volatiles can diffuse through a pierced cover (arrows). These substances descend to the floor of the trap where they reach a series of exit slits in the rim of an inverted cup. The substances then diffuse up to the dark cover of the trap device. When wandering insects reach the upper edge of the ramp, they perceive the odours as coming from below. Preliminary experiments revealed that bugs only drop in when volatiles reach them from below.

lowed by multiple comparisons of the means, for comparing activation and attraction between test stimuli.

Baited trap tests. After promising olfactometer studies, yeast was tested for its usefulness as a lure for baited traps. A trap device was developed, based on the same principle as the olfactometers, i.e. the bugs can drop into it, but they cannot escape (fig. 2).

Experiments were conducted in an open experimental arena 40 × 100 cm, with a paper ground and non-climbable glass walls. Two traps were placed at opposite ends of the arena (10 cm away from the edges of the arena), one containing fresh or dehydrated yeast, as used in the olfactometer experiments, and the other the saccharose solution used as culture medium. In the middle of the arena, 20 larvae of the 4th (fresh yeast tests) or 28 of the 5th instar (dehydrated yeast tests) were confined in a Petri dish. After 15 min, the dish was gently opened and the insects were allowed to leave it. As in the olfactometer studies, experiments were conducted overnight, starting after dusk (19.00 h). Next morning (10.00 h), the number of insects caught by each trap was counted. Natural illumination was provided by locating the arena 1 m away from a large window. In order to compensate for external asymmetries, the position of the control and experimental traps in the arena was changed in successive trials.

The same statistical procedures as those employed for olfactometer results were applied.

Results

In the first series of olfactometer assays, a mouse and fresh or dehydrated yeast exhibited similar activation effects (ANOVA, n.s.). Significantly more insects were found in both capture-tubes, test and control, in the assays performed in the presence of a mouse or yeast, than in those where these stimuli were absent (table 1). Furthermore, the tube containing each of the test stimuli always caught significantly more bugs than the control (table 1). The attractiveness did not significantly differ between mouse, fresh or dehydrated yeast (ANOVA, n.s.).

The temperature of the saccharose solutions was not different from ambient, but the yeast culture was about 1 °C warmer. However, no difference was detected between the temperature of the air mass immediately above the cultures and the ambient temperature.

In the olfactometer assays testing the role of CO_2, significantly fewer insects were attracted by cultures containing KOH-impregnated papers than by those with papers impregnated with water only (mean ± SE, 36.8 ± 5.2%, n = 13, $t = 2.1$, p = 0.028). However, when the vapour path was separated from the papers, a similar attractive performance was found (50.0 ± 8.4%, n = 9, $t = 0.7$, n.s.), indicating no effect of the KOH by itself on the response of the bugs.

Our trap proved to be effective for catching bugs in the open arena experiments and the addition of yeast considerably improved its performance. On average, more than 50% of the insects present in the arena were caught by both traps in a single night (table 2). The number of insects captured by the baited trap was significantly higher than that of the control (table 2). No significant effect of the position of experimental and control traps was detected. Fresh and dehydrated yeast exhibited no significant difference in their attractiveness ($t = 0.1$, n.s.).

Table 1. Olfactometer results (first series) presented in terms of activation and attraction.

	Activation		Attraction
	control	experimental	
Mouse	35.0 ± 5.0% (2)	83.3 ± 10.9% (6) [$t = 2.26$, p = 0.03]	87.4 ± 5.9% (6) [$t = 4.4$, p = 0.003]
Fresh yeast	40.0 ± 10.0% (2)	85.0 ± 2.2% (6) [$t = 6.56$, p = 0.003]	75.9 ± 4.8% (6) [$t = 4.85$, p = 0.002]
Dehydrated yeast	20.0 ± 10.0% (2)	68.3 ± 8.3% (6) [$t = 2.44$, p = 0.02]	81.9 ± 5.5% (6) [$t = 4.19$, p = 0.004]

Activation: proportion of all insects tested which dropped into both the test and control capture tubes. Attraction: proportion of captured insects found in the test capture-tube. Number of replicates are in parentheses.

Table 2. Arena results.

	Trapping	Attraction
Fresh yeast	51.7 ± 9.5% (6)	86.4 ± 5.5% (6) [$t = 4.52$, $p = 0.003$]
Dehydrated yeast	66.1 ± 4.2% (2)	89.2 ± 0.3% (2) [$t = 95.6$, $p = 0.003$]

Trapping: proportion of all insects tested which were trapped by both control and test traps in one-night assays. Attraction: proportion of trapped insects found in the baited trap. Number of replicates are in parentheses.

Discussion

Both the olfactometer assays and those made in the arena with baited traps showed that volatiles released by yeast cultures in aerobic growth constitute powerful attractants for *T. infestans*. Their attractiveness proved to be as high as that of a mouse.

The behavioural evidence so far presented suggests that the response of the bugs is due to the effect of a volatile(s) released by the yeast culture. The results of the second series of olfactometer assays show that some volatile(s) captured by the KOH solution, probably CO_2, plays an important role as an attractant. Comparison of the list of volatiles that are produced by the aerobic growth of *S. cerevisiae*[15] with the group of compounds that are known to attract haematophagous insects showed that CO_2 is the only common factor. In view of the high sensitivity of triatomines to CO_2[2], we may postulate that CO_2 is responsible for the attractiveness of yeast. Although yeast cultures were about 1 °C warmer than ambient air, the KOH experiments indicate that thermal effects are irrelevant because in such assays, both capture-tubes were associated with yeast cultures, i.e. they were at the same temperature, but their differences in attractiveness were maintained.

This paper is the first report on the utilisation of a natural attractant as lure for trapping triatomine bugs. Yeast is a very cheap and widely-available material which can readily be transported and prepared in the field. The dehydrated form is especially suitable because it does not require cold storage and as such may provide a useful tool for control of Chagas' disease vectors. In addition, the attractiveness of yeast for other species of haematophagous insects deserves testing.

We describe here a catching device for triatomine bugs which behaved efficiently in the laboratory. In the experimental arena it was possible to trap more than 50% of the insects present in a single night. Its effectiveness in the field remains to be compared with that of monitoring boxes currently used for population census. These tests should assist in adapting the trap to the particular needs of different species of triatomine, as well as to the diverse locations that these insects inhabit (domestic, peridomestic, stables, or other).

Other substances such as those present in faeces, which induce aggregation[13,16], could be exploited as lures for baited traps. Preliminary experiments carried out in our laboratory testing faeces as a lure in our traps failed to capture many bugs. Despite its well established attractiveness for *T. infestans*, excrement did not induce the bugs to drop down into the traps. It is worth mentioning that we determined that bugs are induced to drop when they are seeking a host. Faeces, however, seem to be used as a signal for the orientation to a refuge[17]. The failure of faeces to increase trap efficiency stresses the importance of fitting the design of the trap to the behavioural context in which each attractant works.

Further laboratory and field tests should help to improve our design until an efficient baited trap is available, useful for population monitoring or even population reduction through trapping out of Chagas' disease vectors in their natural environment.

Acknowledgements. Thanks are given to W. Farina, G. Flores, M. Giurfa, A. Lorenzo, G. Manrique and F. Roces for many fruitful discussions. We are indebted to P. Guerin and two anonymous referees for helpful criticism and many improvements on the English. The work was supported by grants from the CONICET (Argentina) and the Universidad de Buenos Aires.

1 Lazzari, C. R., and Núñez, J. A., J. Insect Physiol. *35* (1989) 525.
2 Núñez J. A., in: Chagas' Disease Vectors, vol. II, p. 1. Eds R. R. Brenner and A. M. Stoka. CRC Press, Florida 1987.
3 Gómez Núñez, J. C., Acta cient. venez. *16* (1965) 26.
4 Wisnivesky-Colli, C., Paulone, I., Perez, A., Chuit, R., Gualtieri, J., Solarz, N., Smith, A., and Segura, E., Medicina, B. Aires *47* (1987) 45.
5 TDR News, N°40, p. 4., WHO, Geneva 1992.
6 Lehane, M. J., Biology of Blood-sucking Insects. Harper Collins Academic. The University Press, Cambridge 1991.
7 van Essen, P. H. A., Kemme, J. A., Ritchie, S. A., and Kay, B. H., Med. and Vet. Entomol. *8* (1994) 63.
8 Vale, G. A., Bull. ent. Res. *70* (1980) 563.
9 Vale, G. A., Flint, S., and Hall, D. R., Bull. ent. Res. *76* (1986) 685.
10 Green, C. H., Bull. ent. Res. *83* (1993) 553.
11 Núñez, J. A., and Lazzari, C. R., J. appl. Ent. *109* (1990) 87.
12 Nuñez, J. A., Bull. ent. Res. *72* (1982) 252.
13 Lorenzo, F. A. N., Kenigsten, A., and Lazzari, C. R., J. Insect Physiol. *40* (1994) 311.
14 Zar, J. H., Statistical Analysis. Prentice Hall Inc., New Jersey 1984.
15 Drawert, F., and Klisch, W., in: Fundamentals of Biotechnology, p. 382. Eds. P. Präve, V. Faust, W. Sittig and D. Sukatsch, VCH Verlagsgesellschaft, Weinheim 1987.
16 Schofield, C. J., and Patterson, J. W., J. med. Ent. *13* (1977) 727.
17 Lorenzo, M. G., and Lazzari, C. R., Mems Inst. Oswaldo Cruz *89* suppl. I (1994) 198.