Performance of yeast-baited traps with *Triatoma sordida, Triatoma brasiliensis, Triatoma pseudomaculata,* and *Panstrongylus megistus* in laboratory assays

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ABSTRACT *The effectiveness of a trap for triatomines baited with yeast cultures has been previously demonstrated for* Triatoma infestans *in laboratory assays. We report here results from laboratory assays testing yeast traps for* Triatoma sordida, Triatoma brasiliensis, Triatoma pseudomaculata, *and* Panstrongylus megistus. *All assays were conducted in an open experimental arena 100 cm × 100 cm, with two traps placed at opposite sides of the arena. One of the traps contained a yeast culture, and the other trap contained a saccharose solution as a control. Two series of experiments were done, one without a refuge for the insects and one with a refuge. The results obtained clearly demonstrated that the yeast-baited traps were effective in the laboratory in capturing both* T. sordida *and* P. megistus. *For* T. sordida*, yeast-baited traps captured significantly more bugs than did the control traps (*t *test* P *value = 0.03). For* P. megistus, *when a refuge was provided during the assay, yeast-baited traps also captured significantly more bugs than did the control traps (*t *test* P *value = 0.006). In the experiments with* T. brasiliensis *and* T. pseudomaculata*, both traps captured some insects, but the yeast traps captured many fewer bugs than was true with the* T. sordida *and* P. megistus *bugs. These results indicate that, in the laboratory, yeast traps can capture considerable numbers of* T. sordida *and* P. megistus *in one night. We discuss the potential use of yeast traps for detecting and capturing both triatomine species.*

Chagas' disease is the third-largest health burden in Latin America. In 1993 it accounted for a loss equivalent to 1.3% of the external debt of all of South America (1). The disease is caused by a flagellate protozoan parasite, *Trypanosoma cruzi* (Chagas, 1909), for which many kinds of wild and domestic animals act as hosts and hence as reservoirs of the disease. Triatomine bugs act as vectors of the parasite, but transmission also happens to a lesser extent via blood transfusions and congenitally. Only the acute stage of the disease can be effectively treated with drugs. Approximately 17 million people are infected with Chagas' disease, and it is estimated that 100 million more people are at risk of infection (2).

The control of Chagas' disease is based on a combination of activities, with vector control being the central one. Vector control tools include insecticides of various formulations, early detection of reinfestations after spraying, housing improvements, and health

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education (1). Early detection is fundamental in order to avoid the establishment of new triatomine colonies. Detection is currently performed with hand collections or by using triatomine sensors (3). Sensors detect the presence or signs of bugs, such as feces, exuviae, or eggs. Triatomine sensors are plain cardboard boxes that allow the insects to enter and leave freely, neither actively attracting the insects nor capturing them (4, 5).

Trap technology is used to detect and control various insect vectors. The use of semiochemicals as baits to improve the attractiveness of traps is frequent (6–8). Using this rationale, Guerenstein et al. (9) designed a trap device for triatomines baited with yeast cultures, and showed that it could attract and capture *Triatoma infestans* (Klug, 1834). Subsequently, Lorenzo et al. (10) reported results from assays testing yeast traps for *T. infestans* performed under natural conditions in experimental chicken coops colonized by bugs. Those results demonstrated clearly that yeast-baited traps are effective for capturing triatomine bugs under the cited conditions. Furthermore, using electroantennogram techniques with *Rhodnius prolixus*, Lorenzo et al. (11) demonstrated that the antennae of this insect sense the yeast volatiles. In the same work, these authors showed that *R. prolixus* can be captured by a slightly modified version of that yeast-baited trap. Recently, a work by Noireau et al. (12) successfully tested another trap design for triatomines, baited with a live host.

CO_2 acts as a host attractant for virtually all bloodsucking arthropods (13). The attractivity of yeast cultures appears to be due to their intense production of this substance (9). In the present work, we tested the attractive effect of yeast cultures for *Triatoma sordida* (Stal, 1859), *Triatoma brasiliensis* (Neiva, 1911), *Triatoma pseudomaculata* (Correa & Espínola, 1964), and *Panstrongylus megistus* (Burmeister, 1835) in laboratory assays. We also tested the capture capability of the trap designed by Guerenstein et al. (9) for the same species.

MATERIALS AND METHODS

All the assays were done in October and November 1997 at the Triatomine Laboratory of the René Rachou Research Center of the Oswaldo Cruz Foundation, Belo Horizonte, Minas Gerais, Brazil. The assays were done in square experimental arenas that were 100 cm $\times$ 100 cm and that had glass walls 20 cm high around them. The arenas did not have a cover, and plain brown wrapping paper, changed between assays, was used for flooring. Insects were gently released in the center of the arena, unless otherwise stated, by means of remotely lifting an inverted container in which they had been previously placed. The animals used had been starved for between 15 and 30 days after ecdysis, and came from a laboratory colony reared on chickens and maintained at a natural illumination cycle, with light coming from an adjacent window. Assays started at dusk, and the counts of captured insects were done the following morning. In all the experiments the temperature of the experimental room was controlled at 30 °C ± 2 °C, and the illumination regime consisted of 12 hours of light and 12 hours of darkness. In all cases, two traps using the design of Guerenstein et al. (9) were simultaneously assayed, placed on opposite sides of the arena. One trap presented a yeast culture inside, while the other only had a flask with saccharose solution as a control.

We performed two series of experiments, one without a refuge for the insects and another with a refuge. As stated below, the second series was performed for those species that did not show an intense response to yeast volatiles in the first series, in order to try to improve the sensitivity of the experiment. In that second series, bugs were offered an artificial refuge that consisted of a piece of corrugated cardboard of 10 $\times$ 20 cm, folded in half, to create a shelter of 10 $\times$ 10 cm with two entryways. This refuge was put in the center of the arena.

In order to evaluate the attractive effect of yeast culture volatiles, we compared the numbers of bugs captured in control traps and experimental traps in all assays of each experiment, by means of a one-tailed *t* test for paired samples (14).

First experiments testing yeast traps, without refuges

For our first series of experiments we did not place refuges in the arenas. For the experiments with *T. brasiliensis*, we used 120 adults total in four assays and 160 fifth instar larvae total in four assays. We used only fifth instar larvae in the experiments with *P. megistus* (166 larvae total in six assays), *T. pseudomaculata* (160 larvae total in four assays), and *T. sordida* (176 larvae total in six assays).

Second experiments, with bugs hidden in refuges

In those experiments where the bugs' response to the experimental trap was not high, we decided to test the potential attraction of yeast in a "richer" context. To do that, we placed a refuge in the center of the arena and performed the trap-preference experiment on the third night, that is, after the bugs had stayed in the arena for three days and had become accustomed to the conditions (15).

This experimental design had the objective of further testing whether or not the yeast had an attractive effect, by only investigating the behavior of those bugs that left the shelter, possibly searching for food. In this way, we excluded the participation of bugs searching for refuge and not for food.

In this section, we performed three series of assays. The first was done with 152 fifth instar larvae of *T. brasiliensis*, the second with 72 fifth instar larvae of *T. pseudomaculata*, and the third with 118 fourth instar larvae of *P. megistus*.

RESULTS

Experiments without refuges

For the experiments without refuges, for all the species studied, both

TABLE 1. Captures of bugs with control traps and yeast-baited traps in laboratory experiments without refuges, Belo Horizonte, Brazil, October and November 1997

Species (instar)	No. of assays	Total no. of bugs tested	Percentage of bugs captured by both traps	Percentage of all bugs captured that were captured in the yeast trap	Total number of bugs captured in the yeast trap	P^a
T. brasiliensis (adults)	4	120	14.2	88	15	0.006
T. brasiliensis (5th instar)	4	160	16.3	73.1	19	0.2
T. sordida (5th instar)	6	176	54	72.6	69	0.03
T. pseudomaculata (5th instar)	4	160	10.6	94.1	16	0.013
P. megistus (5th instar)	6	166	46.4	50.7	39	0.39

[a] *P* value shown is the statistical significance of attraction to yeast traps, calculated from a one-tailed paired *t* test. Using this test, we compared the numbers of insects captured in the control traps and in the experimental traps for all assays in each experiment.

types of traps captured bugs (Table 1). For three of the four species, the yeast-baited traps captured significantly more bugs than did the control traps.

In the assays with *T. sordida* the proportion of all the bugs captured by both traps—a parameter that shows the strength of the capture capacity of traps—was similar to that obtained for *T. infestans* in a previous work (9). With the *T. sordida* the yeast-associated traps captured significantly more bugs than did the control traps ($P = 0.03$).

In the assays with *T. brasiliensis*, we found a significant difference in the captures obtained with adult bugs ($P = 0.006$) but not with the larvae ($P = 0.2$). In both these cases the proportion of bugs that both traps captured was extremely low.

In the series with *T. pseudomaculata*, the yeast-associated traps captured significantly more bugs than did the control traps ($P = 0.013$).

In the series with *P. megistus* the proportion of all insects captured by the two traps was similar to that observed with *T. sordida*, but the *P. megistus* preference for the yeast-baited traps was not statistically significant ($P = 0.39$).

Experiments with refuges

In the assays with *T. brasiliensis* bugs with refuges placed in the arena, the yeast-baited traps captured more insects than did the control traps (Table 2). Nevertheless, the proportion of all *T. brasiliensis* bugs captured by the two traps was noticeably lower than that observed for *T. sordida* in the first series, without the refuge.

The assays performed with *T. pseudomaculata* larvae showed that the yeast-associated traps did not attract these insects when the bugs were hidden in refuges.

In the assays performed with *P. megistus* the yeast traps captured significantly more bugs than did the control traps ($P = 0.006$). Also, the proportion of all *P. megistus* insects captured by the two traps was high, reaching a similar level to that obtained with *T. sordida* in the first section.

DISCUSSION

Our experiments showed that the trap designed by Guerenstein et al. (9) can capture all four triatomine species tested in this work. In addition, yeast proved highly attractive for *T. sordida* and *P. megistus*, capturing considerable numbers of bugs from both species in one-night assays. The experiments done with *T. brasiliensis* and *T. pseudomaculata* showed that the yeast was only slightly attractive for these species.

TABLE 2. Captures of bugs with control traps and yeast traps in laboratory experiments with bugs hidden in refuges, Belo Horizonte, Brazil, October and November 1997

Species (instar)	No. of assays	Total no. of bugs tested	Percentage of bugs captured by both traps	Percentage of all bugs captured that were captured in the yeast trap	Total number of bugs captured in the yeast trap	P^a
T. brasiliensis (5th instar)	4	152	11.8	83.3	15	0.012
T. pseudomaculata (5th instar)	2	72	0	0	0	—
P. megistus (4th instar)	4	118	41	94	45	0.006

[a] *P* value shown is the statistical significance of attraction to yeast traps, calculated from a one-tailed paired *t* test. Using this test, we compared the numbers of insects captured in the control traps and in the experimental traps for all assays in each experiment.

Both *T. sordida* and *P. megistus* are likely to invade houses or peridomestic areas after spraying with insecticides, and thus their detection or capture is a goal of control campaigns. A trap that attracts and captures these insects could be advantageous for such control efforts. Therefore, yeast-baited traps are an alternative to current detection methods and deserve to be tested in field trials.

Yeast-baited traps were not effective with *T. brasiliensis* and *T. pseudomaculata*. This may indicate that a more potent host-related cue is required. The chemical ecology of triatomines is still poorly understood and compounds yet to be discovered may prove more effective. As Guerenstein et al. (9) have shown, triatomine feces cannot trigger the behavior that is crucial for the correct performance of these traps, that is, stimulating the insects to drop down inside the traps in search of an odor. This is in spite of the role that feces play in triatomine communication (16–19). Cardboard boxes are used for bug detection in some control campaigns, but these boxes do not capture the insects nor attract them by means of chemical compounds. Therefore, we suggest that control campaigns could benefit considerably by using attractants and traps to detect triatomine bugs.

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RESUMEN

Rendimiento de trampas cebadas con levaduras en la captura de *Triatoma sordida, Triatoma brasiliensis, Triatoma pseudomaculata* y *Panstrongylus megistus* en experimentos de laboratorio

Con anterioridad se ha demostrado la eficacia de las trampas cebadas con cultivos de levaduras (TCL) para capturar *Triatoma infestans* en experimentos de laboratorio. En el presente estudio se describen los resultados obtenidos con estas trampas para capturar *T. sordida, T. brasiliensis, T. pseudomaculata* y *Panstrongylus megistus*, también en experimentos de laboratorio. Todos los experimentos fueron realizados en un recinto experimental abierto de 100 cm por 100 cm, con dos trampas colocadas en sitios opuestos. Una de ellas contenía un cultivo de levaduras, mientras que la otra contenía una solución de sacarosa como control. Se realizaron dos series de experimentos: una con y la otra sin un refugio para los insectos. Los resultados obtenidos demostraron claramente la eficacia de las TCL para capturar tanto *T. sordida* como *P. megistus* en el laboratorio. Las TCL capturaron un número significativamente mayor de *T. sordida* que las trampas de control (*t* de Student; $P = 0{,}03$). Cuando se les proporcionó un refugio a los insectos, las TCL también capturaron un número significativamente mayor de *P. megistus* que las trampas de control (*t* de Student; $P = 0{,}006$). Ambos tipos de trampas capturaron algunos ejemplares de *T. brasiliensis* y *T. pseudomaculata*, aunque el número capturado en una noche con las TCL fue muy inferior al de *T. sordida* y *P. megistus*. Estos resultados indican que, en el laboratorio, las TCL pueden capturar un número considerable de *T. sordida* y *P. megistus* en una noche. Se analiza la posible utilización de las TCL para detectar y capturar estas dos especies de triatominos.