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A multimodal bait for trapping blood-sucking arthropods

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ABSTRACT

Artificial baits constitute important tools for the detection and sampling of blood-sucking arthropods, in particular those that are vectors of parasites affecting human health. At present, many different devices have been proposed to attract blood-sucking arthropods, mostly based on the attractiveness of particular chemicals or blends. However, most of them revealed themselves as unpractical (e.g. they require an electrical supply), expensive (e.g. gas bottles) or not efficient enough. On the other hand, the use of living baits is as effective but it has practical constraints and/or raises ethical questions. We present here a multimodal lure to attract blood-sucking arthropods designed taking into account both practical constraints and costs. The main characteristics of our bait are: (1) artificiality (no living-host); (2) multimodality (it associates heat, carbon dioxide and chemical attractants); (3) independency from any energy source; (4) no need for gas bottles; (5) easy to prepare and use in the field; (6) low cost. We tested the ability of the bait to attract blood-sucking arthropods in the laboratory and in the field, using capture sticky-traps. Our bait evinced to be almost as efficient as live hosts (mice) for the capture of Chagas disease and *Borrelia* vectors in Bolivia. The multimodal lure here presented is a generalist bait, i.e. effective for attracting different haematophagous species.

1. Introduction

The detection and capture of disease vectors in both, domestic and wild habitats, constitute main strategies for the study and control of the diseases that they transmit.

Different methods based on the exploitation of behavioural responses of blood-sucking insects are being used for a long time ago (Lumsden, 1958). Some of them are physical devices providing refuge (e.g. Gomez-Nuñez, 1965; Wisnivesky-Colli et al., 1987; Vazquez-Prokopec et al., 2002), whereas others employ different types of lures, either living or artificial (e.g. Guerenstein et al., 1995; Lorenzo et al., 1998; Noireau et al., 1999; Lourenço-de-Oliveira et al., 2008; Anderson et al., 2009). Refuge-like sensors reveal as very useful for long-term surveillance and do not need any kind of maintenance. Insects are not attracted, but when they encounter the device, they may use it as a refuge or leave traces of their passage inside (e.g. excrements, exhuviae). Baited devices are more useful for rapid detection, due to their ability to attract insects by means of chemical or physical lures, or a combination of both. They are usually more complex than the first one; their source of attractants remains active for relatively shorter periods and they need in some cases an energy source.

Triatoma infestans and *Rhodnius prolixus* are the main vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease, in Latin America. To capture them, different types of traps are used, in some cases including live hosts, e.g. mice, rats, rabbits, guinea pigs, chicks, hens and chickens, as bait (Rabinovich et al., 1976; Tonn et al., 1976; Carcavallo, 1985; Noireau et al., 1999). Numerous studies showed that haematophagous arthropods, including triatomine bugs, find their hosts detecting the emission of heat, carbon dioxide and odours emitted by their bodies (see reviews by Guerenstein and Lazzari, 2009 and Lazzari, 2009). In the present work, we describe a multimodal bait, delivering different potentially attractive signals for haematophagous arthropods. In order to assess the effectiveness of this bait, we tested its ability to capture triatomine bugs, which are vectors of Chagas disease, in the laboratory and in wild natural environment.

2. Materials and methods

2.1. Lure description

The lure consisted on a combination of sources of heat, carbon dioxide and volatiles. Heat production was obtained by means of iron oxidation (exothermic reaction) by mixing 5 g of iron powder,

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steel wool or workshop sawdust (oxidation substrate), 1 g activated charcoal (to increase the oxidation surface), 5 g vermiculite or sand (heat conservation), 3 g NaCl (table salt, to slow down oxidation) and 2.5 g tap water. Five grams of the mixture were put into a pocket made of aluminium foil, which constituted the heat source. By making a few holes with a needle in the aluminium foil just before use, air entry initiated iron oxidation. The temperature and duration of heat production could be adjusted by changing the total amount and the proportion of each individual component, as well as their granulometry. Provided that the adequate amounts depend on the employed components, users should conduct some tests to find out the right proportions.

The CO_2 was produced by means of a simple acid–base reaction combining citric acid and sodium bicarbonate in a proportion of 1:3, in the presence of water. A soaked piece of filter paper was separated from the powder by an aluminium foil previously pierced with a needle. This provides a humid environment for the reaction to occur, but assure a slow CO_2 release. The concentration of CO_2 was maintained above the perception threshold of triatomines, which is 300–400 ppm over the environment (Barrozo and Lazzari, 2004a).

As volatiles, we employed a compound mixture, which combined with CO_2 revealed as highly attractive for triatomines (composition in Barrozo and Lazzari, 2004b). The mixture included L-lactic, valeric, butyric and propionic acid. Three ml of the mixture were put inside a 15 ml glass recipient, which was closed with a small cotton ball to allow a slow evaporation of volatiles for many hours.

Exact amounts can be adjusted according to the characteristics of the products employed (i.e. powders granulometry, volatile concentration), as well as the environmental temperature, in order to obtain the desired delivery period.

2.2. Traps

The traps used for testing the bait were the same described by Noireau et al. (1999), associated either with a living mouse, or with the artificial bait. In some experiments, empty traps (no bait) served as negative controls.

2.3. Laboratory tests

Two species of triatomine bugs were used. *Rhodnius prolixus* were maintained and tested in our laboratory in Tours, France, under a 12:12 h L:D illumination regime, at 26 °C and 30–50% RH. Bugs were fed weekly on heparinised sheep blood using an artificial feeder (Núñez and Lazzari, 1990; Núñez et al., 1996). *Triatoma infestans* were reared and tested in Cochabamba, Bolivia, under a 12:12 h L:D illumination regime, at 25 °C and 30–50% RH, and fed weekly on live hens.

Tests on *R. prolixus* were conducted in an experimental arena (100 length × 60 wide × 20 cm height), in a room kept at 25 ± 1 °C under darkness.

In order to better simulate a natural situation, fifth-instar larvae and adults from both sexes were used in each tests. Eight bugs were placed in an artificial shelter made of cardboard in the centre of the arena and left 24 h for familiarization. After this period, two traps were placed at opposite ends of the arena at the evening. The experiment lasted for one night and the number of bugs captured by each trap recorded. Every experiment was repeated 10 times ($n = 8$; $k = 10$; $N = 80$). Three experimental series were done: (1) Living bait vs. Control; (2) Artificial bait vs. Control; and (3) Living vs. Artificial bait.

Laboratory tests on *T. infestans* were conducted in a similar way, but the experimental arena consisted in a 50 cm side cube made of plastic supports and tissue walls. A total of 11 replications using 8 bugs at a time ($n = 8$; $k = 11$; $N = 88$). Again, to match natural conditions, different larval instars and adults of both sexes were tested together. One experimental series was performed, comparing Artificial bait vs. Control.

2.4. Field tests

2.4.1. Sites

The two study areas were rocky outcrops made of large blocks located in the Cochabamba valley in the central zone of the eastern cordillera in the Department of Cochabamba (Bolivia). The first, situated some 100 m from the nearest house, is named "Inca wall" by the local population after its resemblance to human constructions. The second study area, which was quite close to a house, is named here as "peridomestic outcrop". These two zones were described by Cortez et al. (2007) as areas of high *T. infestans* density (Inca wall: summer: 45.1%, winter: 20.5% of positive mouse baited traps; and peridomestic zone: summer: 69.7%, winter: 29.4%). All the assays were conducted during May 2009 (beginning of the winter).

We placed two types of traps, i.e. baited either with our artificial lure or with a living mouse. The traps were installed around 06:00 pm (early night) and recovered at the following morning, around 09:00 am. The traps were deployed at random, in potential *T. infestans* habitats, in pairs (mouse, artificial bait) per type of environment, but not side by side.

2.5. Statistical analyses

Numbers of bugs captured in the experimental arenas were compared by means of a Wilcoxon test in paired experiments or with Fisher's exact test of proportion for independent samples.

Field captures by the different types of baits were compared by means of a Mann–Whitney test.

3. Results

3.1. The artificial bait

By adjusting the proportions of the different components, we have obtained a production of heat for many hours. The thermal pad containing the mixture had a size similar to that of a mouse and kept a constant temperature of around 30 °C for at least 15 h (Fig. 1(a)). Changes in the proportions of the components allowed for a variation of both, the temperature and the duration of heat production. With this procedure, temperatures up to 90 °C could be obtained, but the higher the temperature, the shorter the duration.

Concerning carbon dioxide production, the amount produced and the duration were adjusted by modifying, in a similar way as for heat production, the composition and the mixture humidity. Concentrations above the triatomines detection threshold (300–400 ppm over the environmental background) could be maintained for at least 9 h (Fig. 1(b)).

The release of volatiles was not measured; we just incorporated the attracting cues such as previously described (Barrozo and Lazzari, 2004b).

3.2. Laboratory experiments

We found significantly more bugs captured by baited (living or artificial) than by control traps in the experimental arenas (Figs. 2 and 3) for the two species tested, *T. infestans* (Fig. 2) and *R. prolixus* (Fig. 3(b) and (c)) (Wilcoxon test $p < 0.01$ in all cases). When both living and artificial baits were presented simultaneously, more bugs were captured by the living-baited trap than by the artificially-baited trap (Fig. 3(a), Wilcoxon test, $p < 0.05$). However, when tested