Different methods of using attractive sugar baits (ATSB) for the control of Phlebotomus papatasi

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ABSTRACT: We have previously shown that fermented ripe fruit is a strong attractant for several mosquito species, and when mixed with oral insecticide these attractive toxic sugar baits (ATSB) were highly effective for local mosquito control. In the present study, we compared the effects of ATSB presented in different ways on isolated populations of Phlebotomus papatasi Scopoli. Experiments were carried out in the arid habitat of the Jordan valley, Israel where the effectiveness of three methods was compared: ATSB sprayed on patches of vegetation, net fence coated with ATSB, and bait stations soaked with ATSB. Spraying ATSB reduced the population to about 5% of the control area population. Barrier ATSB coated fences, had a similar effect decreasing the population to about 12% of the concurrent catch in the control site. The effect of ATSB presented on bait stations was much smaller and compared to the control, only caused the population to be reduced to 40%. In the control areas where only food dye marker was used, the solution presented on bait stations only marked an average of 22.3% of female sand flies while spraying vegetation and using barrier fences in the two other experiments marked about 60% of the females. Our experiments show that ATSB either sprayed on the vegetation or on barrier fences is an effective means against sand flies at least in arid areas where attractive plants are scarce or absent. Journal of Vector Ecology 36 (Supplement 1): S64-S70. 2011.

Keyword Index: Sand flies, Phlebotomus papatasi, attractive toxic sugar baits, ATSB, control, Israel.

INTRODUCTION

The sand flies, Leishmania major, Yakimoff and Schokhor, 1914, and L. tropica, Wright, 1903 (Kinetoplastida: Trypanosomatidae) are endemic to Israel and the Palestinian Authority where they cause cutaneous leishmaniasis (CL) in humans. Until recently, L. tropica was relatively rare and the prevalent pathogen was L. major that occurred mostly in the deserts of the Jordan Valley, the Negev, and the Arava while L. tropica was found in the central mountain range (Greenblatt et al. 1985, Sawalha et al. 2003). This situation is changing and both parasites are expanding into the north of Israel and into the Palestinian West Bank (Baneth et al. 1998, Abdeen et al. 2002, Jacobson et al. 2003, Jaffe et al. 2004, Svorodova et al. 2006).

Sugars of plant origin are an essential diet of sand fly vectors of Leishmania. In the wild, they obtain sugar meals from honeydews excreted on plants by aphids and coccids (Moore et al. 1987, MacVicker et al. 1990, Wallbanks et al. 1991), by feeding directly on tissues of plants (Schlein and Jacobson 1994, Schlein and Müller 1995), and from floral nectar (Müller and Schlein 2004). A sand fly’s search for sugar sources is apparently guided by order of preferences to attractants (Schlein and Yuval 1987, Müller and Schlein 2004).

We have shown that fermented juice of ripe fruit is a strong attractant for a range of different mosquito species, and that mixed with oral insecticides these attractive toxic sugar baits (ATS) presented in bait stations can be highly effective for local mosquito control (Müller et al. 2008, Müller and Schlein 2008). The same ATS was used successfully against sand flies when sprayed on patches of the vegetation in sand fly habitats in open areas and around houses, in a village near Jerusalem (Schlein and Müller 2010). In the present study in the Jordan Valley, we compared the effects of ATS presented in different ways on isolated populations of Phlebotomus papatasi Scopoli. Experiments were carried out with a low fence of net coated with ATS, bait stations soaked with ATS, and ATS sprayed on patches of the vegetation.

MATERIALS AND METHODS

Study sites

The study was carried out in the summer in the central Jordan Valley desert where large populations of Ph. papatasi are found in the colonies of the prevalent sand rats Psammomys obesus (Cretzschmar), the rodent reservoirs of L. major. The area is characterized by a warm and dry climate, with an average annual rainfall of 50 to 100 mm and summer temperatures that can exceed 45° C (Zohari, 1982). The annual winter vegetation was dry and the remaining vegetation consisted mostly of chenopod bushes of Atriplex halimus (L), Suaeda asphaltica (Boissier) and S. fruticosa (Gmelin). P. obesus colonies were found under and between these bushes. Experiments were carried out in several similar, separate, small shallow ravines with sand rats’ colonies. Experimental and control sites were each about 300 m long, 20-30 m wide and separated by about 1 km from each other.
Monitoring sand flies

On monitoring days, sand flies were caught overnight at each site with six non-baited, CDC-like miniature light traps (Model 512, John W. Hock, Gainesville, FL, USA) in fixed positions. Traps were hung on bamboo tripods with openings approximately 30 cm from the ground. Sand flies in trap nets were kept at 0-5° C for one to two h while being transported to the laboratory. The flies were than anesthetized with CO₂ to be processed immediately or were stored at -70° C. For the observation of food dye in the gut, the flies were immersed in saline solution with a few drops of detergent and examined under a dissection microscope. Experiments were conducted from early June to the end of August. Pre-treatment, the sites were monitored every second day for ten days (five times) and after the treatment, on every fifth day (15 times).

Bait solution

Bait solution was prepared as described by Müller et al. (2008). For the control sites, it consisted of 95% juice of over-ripe nectarines (Prunus persica var. nectarina : Rosacea), 5% wine (“Binyamina”- dry red wine, Binyamina Winery, Binyamina, Israel), 10% w/v brown sugar (“Nature Sugar” brown, Louis Dreyfus, Israel), 0.5% w/v red food dye (Carmoisine E122, Stern, Natanya, Israel) for marking the fed sand flies (Schlein 1987) and 10% of a mixture of slow-release substances and preservatives (BaitStabH; Westham Ltd., Tel Aviv, Israel). The solution was ripened for 48 hrs, in covered buckets, outdoors in the sun where daily temperatures reached an average of 30° C. Boric acid (Xue and Barnard 2003) 1.0% w/v and 0.04% w/v spinosad (Tracer®; Dow Agrosciences, Toronto, Ontario, Canada), were added as oral toxins to the solution used in the experimental sites.

Presentation of ATSB at different sites

Six sites were chosen in the general study area; for each of the three types of ATSB presentation, an adjacent site was chosen to serve as an untreated control for the duration of the experiment. Sites were chosen for their similarities in numbers of sand flies and vegetation type and cover. Solution with toxin was used for the experimental sites and without toxins for the control sites.

Spraying ATSB on vegetation

At the first pair of sites, ATSB solution was sprayed using a 7 liter hand sprayer (Killaspray, Model 4005, Hozelock-ASL, Birmingham, England) in patches of 0.5 m³ on about every fifth thicket or bush thus treating about 10 to 20% of the vegetation (Figure 1).

ATSB treated barrier fence

At the second pair of sites, rolls of semi rigid plastic net 50 cm wide and 20 m long, with thickness 1x2 mm and openings of 50x50 mm, was used for building a bait barrier fence. Strips of cotton cloth 5x60 cm were connected transversally to the net and their ends were folded around the margins and stapled. The strips were thus fixed to the net at intervals of 20 cm. After fixing the cloth strips, the net was rolled into a cylinder that was dipped into a bucket containing ATSB solution or solution without toxins. The experimental and control sites were surrounded by wooden sticks 5 x 5 cm in transversal section and 90 cm long that were driven 30 cm deep into the ground at distances of 5 m. The ‘bait barrier’ of treated net was stapled to the sticks at a height of 10 cm above ground to allow passage of small animals. A gap of 1.5 m was left every 25 m to allow the passage of larger animals (Figure 2).

ATSB bait stations

The third pair of sites used bait stations as the method of ATSB presentation. The bait stations were cylindrical constructions made of the same components as the barrier fence including plastic net 50 cm wide and 20 m long with a thickness of 1 x 2 mm and openings of 50x50 mm with vertically interwoven and stapled strips of cotton cloth (5x60 cm) at 10 cm intervals. Pieces of 50 cm long net were cut, rolled to form cylinders and the cylinder edges were fixed together with staples. One row of wooden sticks had been previously prepared surrounding the sites (Figure 3).
yield of 76.55±SE 12.98 females per trap (Figure 5). Food dye marker of the bait was observed in an average of 61.2% of the females and 68.5% of the males in the control site.

**ATSB bait stations**

Bait stations surrounding two similar experimental and control sites at distances of 10 m were less effective. Pre-treatment catches in the experimental area were 31.00±SE 4.90 females per trap, while in the control area there were 31.82±SE 2.68 females per trap. After the treatment during July and August, the catch in the treated area was 22.24±SE 2.64 females per trap and in the control area the average was 57.35±SE 4.23 females per trap (Figure 6). Thus, although the effect of this treatment was significant (t = 5.969, df = 56, P<0.05), it caused a comparatively small decrease of only about 60% in the female population (Figure 6). The food dye in the bait solution marked an average of only 22.3% of the females and 35.3% of the males in the control site.

**DISCUSSION**

Most control efforts against sand flies are aimed at interrupting contact between female sand flies and humans. Sand flies generally are highly susceptible to insecticides but it depends on the manner of exposure and contact (Alexander et al. 1995, Alexander and Maroli 2003, Wilamowski and Pener 2003, Orshan et al. 2006). Residual formulations of DDT and pyrethroids have been used to control sand flies both in the old world and the Neotropics (Hertig and Fisher 1945, Hertig and Fairchild 1948, Hertig 1949, Le Pont et al. 1989, Marcondes and Nascimento 1993). Residual insecticide house-spraying has been used successfully against endophilic species, mostly in Latin America (Alexander et al. 1995, Vieira and Coelho 1998). Insecticide-impregnated curtains, bed nets, or bed covers were also used but with limited success against sand flies (Basimike and Mutinga 1995, Elnaiem et al. 1999, Kroeger et al. 2002, Courtenay et al. 2007). Barriers may be useful to stop incoming sand flies.

In French Guiana, clearing the forest around a village, and fogging with insecticide to a radius of 400 m, reduced the sand fly density and the incidence of leishmaniasis significantly but it is not an acceptable method since it causes considerable damage to the environment (Esterre et al. 1986, Alexander and Maroli 2003). In a similar way, sand fly populations were reduced in Guatemala by spraying pyrethroids on ground vegetation and tree trunks in a 100 m radius (Perich et al. 1995). A horizontal plastic belt barrier sprayed with DDT was tested, and failed to prevent the entrance of sand flies to a village in the eastern Judean Mountains (Orshan et al. 2006). In general, contact insecticides such as these should be non-repellent and regularly encountered in the normal course of sand fly movements. Even then, the sand fly-toxin contact may be insufficient. However, when sand flies are attracted to land on ATSB, their tarsal response to contact with the sugar leads to sugar feeding (Fringis and Hamrum 1958, Papas and Larsen 1978) and thereby to ingestion of the oral toxin. In this study, we treated only limited amounts of the total
Figure 4. Average catch of *P. papatasi* females per trap before and after spraying baits on vegetation (treatment). The experimental population was exposed to toxic (ATSB) baits while the control population was exposed to baits without toxin (ASB).

Figure 5. Average catch of *P. papatasi* females per trap before and after application of barrier fences treated with baits. The experimental population was exposed to toxic (ATSB) baits while the control population was exposed to baits without toxin (ASB).
landscape area and this was done because the ATSB should serve to attract the sand flies to concentrated areas where they feed on toxic bait and are killed. This is advantageous because the sand flies do not need to land on, or be sprayed with, a specific contact-insecticide.

We recently carried out two successful experiments in which patchy spraying of vegetation with ATSB caused drastic reduction of sand flies. In one experiment, *P. papatasi* populations were reduced to less than 5% in an experiment in the Jordan Valley in natural habitats (Schlein and Müller 2010) while in the other experiment *P. syriacus, P. sergenti, P. tobbi* and *P. papatasi* were controlled successfully with repetitive treatments in an urban setting over a complete sand fly season. In continuation of this study, we wanted to evaluate other ways of using ATSB against sand flies and so we compared the efficiency of ATSB coated barrier fence, a line of bait stations, and spraying of vegetation, which is a repetition of the first successful attempt of treating vegetation as described by Schlein and Müller (2010). In the latter experiment, the spraying of ATSB reduced the population to about 5% of the control area population (Figure 4). Barrier ATSB coated fences, had a similar effect, reducing the number of females to about 12 % of the concurrent catch in the control site (Figure 5). The effect of ATSB presented on the vegetation was much smaller and compared to the control, only caused a female population decrease of about 60% (Figure 6). In the control area, food dye in the bait solution marked an average of 22.3% of the females while in the two other experiments the marker was observed in about 60% of the females. Our experiments show that ATSB either sprayed on the vegetation or on barrier fences is an effective means against sand flies at least in arid areas where attractive flowering plants are scarce or absent.

The use ATSB has an advantage over most of the customary sand fly control methods. In particular, it is more selective, environmentally friendly and less expensive than large scale use of insecticides.

The association between sand flies and the vegetation they feed on is a relatively neglected field and, even more so, are the practical implications of such observations to sand fly control. In this direction, further research is required to identify better attractants and ways of presentation that will improve the ATSB control method.

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Figure 6. Average catch of *P. papatasi* females per trap before and after application of bait stations. The experimental population was exposed to toxic (ATSB) baits while the control population was exposed to baits without toxin (ASB).


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