

Control of *Culex quinquefasciatus* in a storm drain system in Florida using attractive toxic sugar baits

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Abstract. Attractive toxic sugar baits (ATSBs) were used to control mosquitoes in the storm drains of a residential area on the outskirts of St Augustine, Florida. The drainage system was newly constructed and no mosquitoes were breeding inside it. The area covered by the storm drains was divided in half; 10 drains served as control drains and 16 drains served as experimental drains. The baits, which consisted of a mixture of brown sugar, fruit juice, green dye marker and boric acid, were presented at the entrances of the treated drains and exit traps were positioned over the drain openings and the connecting tubes leading to retention ponds. Similar baits with orange dye and without toxin were presented at the entrances of control drains. A total of 220 pupae of *Culex quinquefasciatus* (Diptera: Culicidae) were released in each control and toxin-treated drain, and the numbers of recovered mosquitoes were examined to determine the effectiveness of ATSBs in the storm drain system. An average of 178.2 mosquitoes exited each drain in the control area; 87.0% of these had fed on the baits and were stained orange, whereas 13.0% were unstained. In the toxin-treated drains, 83.7% of hatched females and 86.6% of hatched males were controlled by the baits.

Key words. *Culex quinquefasciatus*, attractive toxic sugar baits (ATSBs), mosquito control, storm drains, underground breeding.

Introduction

Underground storm drain systems in urban areas can consist of thousands of miles of gutters and underground pipes (Su *et al.*, 2003), as well as countless numbers of catch basins and manhole chambers, which serve to drain run-off water from homes, businesses and streets. These networks represent an important urban habitat for mosquito resting and breeding (Dhillon *et al.*, 1985; Strickman & Lang, 1986). Various methods have been tested to control 'catch basin breeders', including the application of oils, insecticides, growth regulators or bacterial agents (Stewart, 1977; Pfunter, 1978; Mulligan

et al., 1981; Klueh *et al.*, 2006), physical flushing of the systems with water (Schoeppner, 1977) and the use of barriers to shut mosquitoes out (Mulligan & Schaefer, 1982a). However, the scale of some drainage systems makes most approaches very expensive and often impracticable. These methods can be damaging to the environment and the proposed treatment may not even reach the target area (Su *et al.*, 2003).

Mosquitoes of the genus *Culex* (Diptera: Culicidae) are known to either develop or find shelter in storm drain systems (Hazelrigg & Pelsue, 1980; Marfin *et al.*, 1993; Kay *et al.*, 2000). These species are a notorious biting nuisance and an important vector of diseases, including lymphatic filariasis

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Fig. 1. Aerial view of Old Sebastian Point showing the locations of control drain baits, toxic baits and drain outlets to the retention ponds.

and several arbovirus diseases, such as Japanese encephalitis, St Louis encephalitis, Rift Valley fever and West Nile fever (Vinogradova, 2000). Mosquitoes of the *Culex pipiens* complex were also the principal vectors in the outbreak of West Nile fever that occurred in North America in 1999 and spread across the continent and into Central America (Kilpatrick *et al.*, 2005).

The use of attractive toxic sugar baits (ATSBs) is a promising, yet under-explored, control method that exploits the dietary staples used to sustain the daily activities of mosquitoes. Female and male mosquitoes obtain their essential sugar diet mostly from floral nectar, but also from nectaries on leaves and stems and from honeydew excreted by homopterans (see reviews by Yuval, 1992; Foster, 1995). In a previous study, we showed that some flowering trees are extreme attractants to nectar-searching mosquitoes (Müller & Schlein, 2006) and can be used for their control. Furthermore, ripe fruits can also attract mosquitoes (Joseph, 1970) and solutions containing these products have been blended with oral toxin and presented in bait stations to control the cistern-breeding urban malaria vector *Anopheles claviger* (Diptera: Culicidae) in Israel (Müller *et al.*, 2008). In this study, we constructed simple bait stations filled with ATSBs and tested their effect on a *Culex quinquefasciatus* (Diptera: Culicidae) population in a storm drain system.

Materials and methods

Study site

The study was conducted in the spring of 2008 at Old Sebastian's Point, a recently developed area north of St Augustine,

Florida, U.S.A., which has a finished road and storm drain system. At the time of the experiments, the area was without houses. This 17-ha area, which is situated near marshland, is divided into 85 plots for houses and contains three retention ponds and some parkland. The infrastructure consists of 1500 m of roads drained by 26 underground, interconnected storm drains that empty into 3300 m of drainage canals (also underground) with 11 outlets into three retention ponds. The drains vary in depth from about 1.2 m to 2.0 m; some of them are naturally wetted, and all of them are covered with a metal grid that has an additional opening on the curb. At the time of the experiments, no mosquitoes were breeding in the underground storm drain system because the drains were covered with heavy felt blankets to prevent silt washing into them. Ten storm drains at the west end of the site were used as controls and 16 drains at the east side were used as the experimental drains (Fig. 1).

Attractive toxic sugar baits and control solution

Bait solutions for the experimental site consisted of the following solution: ~95% juice of over-ripe to rotting plums (*Prunus americana* Marshall: Rosacea), 5% v/v red wine (Veo Grande Cabernet Sauvignon; Viñedos Errázuriz Ovalle SA, Santiago, Chile). To this solution was added 10% w/v brown sugar (Nature Sugar, brown; Louis Dreyfus Commodities, Ashdod, Israel), 10% w/v of a mixture of slow-release substances and preservatives (BaitStab™; Westham Ltd, Tel Aviv, Israel), 0.5% w/v green food dye (Tartrazine 19140; Stern Inc., Natanya, Israel) and 1.0% w/v oral insecticide (boric acid). The entire solution was ripened for 48 h in covered buckets which were left outdoors in the sun in temperatures of up to ~30°C.



Fig. 2. Non-toxic orange dye bait station constructed for this study with a square hook for hanging on storm drain grates. For a full description, see Materials and methods.

A similarly prepared solution containing identical components except for the insecticide, and including orange food dye (Carmoisine E122; Stern Inc.) instead of green, was used for the control site.

Plastic soft drink bottles (0.5 L), with a ~2-cm hole about two-thirds of the way up, were prepared. Cotton wicks were inserted through the holes so that both ends of the wick reached the bottom of the bottle. The bottles were then inserted, bottom first, into large, light-coloured, cotton flannel socks that had been thoroughly washed in water and dried. The socks were then wetted by dipping them into the solution and 0.3 L of solution was poured into each bottle. Thus, fluid from inside the bottle was sucked out by the wick as the external layer of flannel dried. Each bottle was covered with an 18-cm diameter, bowl-shaped plastic cover. Each bait station (Fig. 2) was hung at the opening of a storm drain and the normal covers of these structures were then replaced.

Experimental set-up

The dry storm drains were wetted with about 38 L of local tap water to create favourably humid conditions for mosquitoes. In each of the drains, including both wetted and naturally flooded drains, we placed a single 500-mL container holding 220 pupae of *Cx. quinquefasciatus*. In the first 10

drains (control area), we hung bait stations soaked and filled with attractive sugar solution and orange food dye. In the remaining 16 drains (treated area), we placed toxic bait stations soaked and filled with attractive sugar solution, green food dye and 1% boric acid (Fig. 2). On each prepared drain, the horizontal opening on the street surface was three-quarters covered with roofing tiles; the remaining quarter was covered with a conical exit trap and the vertical opening along the curb was covered with a heavy blanket. The rim of the exit trap was filled with 0.5 L of local tap water to provide moisture to the emerging mosquitoes. Connecting pipes to the retention ponds were blocked with cylindrical fish basket-like conical exit traps.

For the following 8 days, the mosquitoes were recovered daily from both types of exit trap; they were counted, sexed and checked for food dye in the guts. As a control, six additional beakers were kept in a storm drain close to the experimental site in separate cage-like containers (mosquito breeder model 1425; BioQuip Products, Inc., Rancho Dominguez, CA, U.S.A.) and hatching mosquitoes were counted and sexed on a daily basis. The mosquitoes used in the experiments were reared under standard conditions in the insectaries of the United States Department of Agriculture (USDA), Gainesville, Florida.

Statistical analysis

All statistical analyses were performed with JMP Version 5.0.1 (SAS Institute, Inc., Cary, NC, U.S.A.). Data distributions were tested for normality (Shapiro–Wilk W test). As normal distribution equivalence was not achieved in any of the samples and the coefficient of variation was large, non-parametric Kruskal–Wallis/Wilcoxon rank tests with chi-squared approximation with untransformed data were used for sample comparison and significance was taken at $P < 0.01$.

Results

Overall, 93.5% of the six control samples of pupae hatched (51.9% of all the mosquitoes to emerge were male and 48.1% were female). In the experimental and untreated control storm drains, a total of 5720 pupae were released, of which, according to the results of the hatching control, about 93.5% should have hatched. This would amount to approximately 2057 mosquitoes (989 females, 1068 males) in the 10 untreated control storm drains and to 3291 mosquitoes (1582 females, 1709 males) in the 16 ATSB-treated drains.

The first mosquitoes caught in the exit traps in the field were collected one night after the mosquitoes in the control samples had hatched. In the 10 untreated control storm drains, 704 female and 748 male orange-stained mosquitoes, no green-stained mosquitoes, and 107 female and 111 male unstained mosquitoes were caught. Additionally, in the seven tubes connecting to the retention ponds, 78 female and 26 male orange-stained mosquitoes, as well as six female and two male unstained mosquitoes, were recovered. Furthermore, another 61 female and 18 male orange-stained mosquitoes penetrated storm drains neighbouring the treated site.

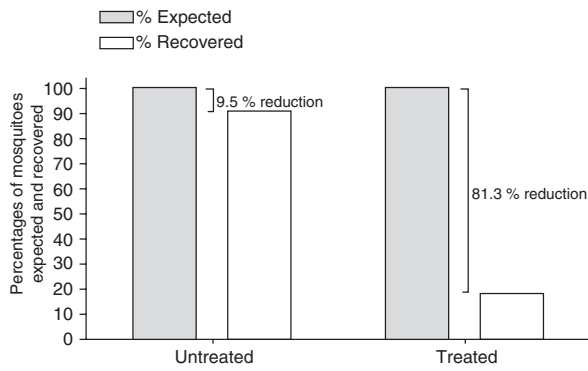


Fig. 3. Expected and actual percentages of mosquitoes recovered at the treated and untreated sites. The total numbers of mosquitoes are expressed as percentages of the numbers expected from hatching in control experiments.

The total number of mosquitoes recovered from these untreated drains (including the orange-stained mosquitoes that penetrated the treated area) amounted to 956 females and 905 males; 88.2% of the females and 87.5% of the males were stained orange. There was no significant difference between the numbers of orange-stained males and females ($\chi^2 = 1.03$, d.f. = 1, $P = 0.31$).

In the 16 treated storm drains, 77 females and 51 males with green stain and 248 females and 222 males without stain were caught. Additionally, in the four tubes connecting to the retention ponds, two female green-stained, and 10 female and seven male unstained mosquitoes were recovered. No green-stained mosquitoes were found penetrating drains neighbouring the control site. In the treated site, 83.7% of the hatched females and 86.6% of the males were either not recovered or were stained green from the toxic bait. Again, there was no significant difference by sex in the numbers of mosquitoes recovered from the treated drains ($\chi^2 = 0.84$, d.f. = 1, $P = 0.36$).

In general, the number of mosquitoes that fed on the two types of bait, orange and green stains combined, was significantly higher than the number that did not feed on baits ($\chi^2 = 98.16$, d.f. = 1, $P < 0.0001$). In addition, the number of orange-stained mosquitoes greatly outnumbered the number of green-stained mosquitoes ($\chi^2 = 56.78$, d.f. = 1, $P < 0.0001$). Overall, 90.5% of the expected number of mosquitoes were recovered from the 10 control drains, but only 18.7% of the expected number of mosquitoes were recovered from the 16 treated drains (Fig. 3).

Discussion

The first mosquitoes to be recovered from the exit traps in the field were collected one night after the mosquitoes in the control samples had hatched. Given the hatching time of the mosquitoes in the control samples and the later appearance of mosquitoes in the exit traps in the field, most *Cx. quinquefasciatus* appeared to have been resting on the

night of emergence and during the following day in the storm drains. It appears that many mosquito species typically feed on sugar before seeking a bloodmeal (Vargo & Foster, 1984; Smith & Gadawski, 1994; Schlein & Müller, 2008). It is not clear if the delayed emergence from the storm drains reflects a general behaviour of *Cx. quinquefasciatus* or if the easy availability of a sugarmeal delayed the dispersal of the mosquitoes. Regardless, this delayed dispersal probably contributed significantly to the positive outcome of the trial (Fig. 3).

Boric acid is an effective stomach poison for insects (Habes *et al.*, 2006). In previous studies, it was successfully blended with non-attractive sugar baits that were sprayed directly onto plant foliage for barrier treatment (Xue & Barnard, 2003; Xue *et al.*, 2006). In our study, the available sugar was in either the toxic or the non-toxic control solution and thus, in the experimental site, most mosquitoes were killed before they left the storm drain system, whereas in the control drains the bulk of the emerging mosquitoes were stained with colour. At least 10% of the recovered control population dispersed in neighbouring drains and connecting pipes, in some cases to distances of up to 100 m. By contrast, it appears that mosquitoes exposed at the treated site to toxic baits were less mobile; none or a very small percentage of the population penetrated the neighbouring control site and the pipes connecting to the retention ponds. Although boric acid can cause immediate death to mosquitoes, sub-lethal doses can reduce survival time, as well as host-seeking, blood-feeding and fecundity rates in a mosquito population (Ali *et al.*, 2006).

Although numerous species breed and rest in subterranean habitats, and especially in storm drains, this study was restricted to the most common species, *Cx. quinquefasciatus* (Smith & Shisler, 1981; Mulligan & Schaefer, 1982b; Rey *et al.*, 2006). Nevertheless, it is reasonable to assume that other species and genera found in storm drains are also vulnerable to ATSBs, as has been shown in previous studies conducted in Israel in different types of habitat (Müller *et al.*, 2008; Schlein & Müller, 2008). The stable microclimatic conditions of storm drain systems offer mosquitoes an ideal habitat for breeding and resting and are a major source of urban mosquito production (Chanda & Shisler, 1980; Dhillon & Mulla, 1982; Mulligan & Schaefer, 1982b; Su *et al.*, 2003).

It is extremely difficult to manage these subterranean systems (Hazelrigg & Pelsue, 1980) and various chemical and biological approaches to control have been developed, including the application of oils (Kimball & Perruzzi, 1970; Pfunter, 1978), traditional insecticides (Dill & Roberts, 1975; Klueh *et al.*, 2006), insect growth regulators (Stewart, 1977), bacterial agents (Mulligan *et al.*, 1981) and the use of mosquito fish (Farley & Caton, 1982). Physical methods include flushing the subterranean systems with water (Schoeppner, 1977) and shutting mosquitoes out of these habitats with barriers (Mulligan & Schaefer, 1982a).

Chemical treatments for fast subterranean systems are often laborious, expensive and, from an environmental point of view, problematic. Given the limitations of traditional methods in storm drains, there is a need for the further development of innovative control strategies (Dhillon *et al.*, 1984; Metzger *et al.*, 2008).

The application of ATSBs may provide an effective and economical alternative to ground application of larvicides. The spot treatments that aim to attract and kill were not only very cheap (< US\$10 in materials for 26 drain openings), but the procedure was also very environmentally friendly because the baits were retained inside the bait station units and did not contaminate either the water or the environment. Our experiments required us to open the very heavy storm drain grates because of the size and shape of the bait stations. For daily control operations, specially designed bait stations could be applied through the grids covering the storm drains to save time and labour. Baits could possibly be applied directly onto concrete walls by simple spraying or painting.

The results of this study suggest that, at least for *Cx. quinquefasciatus*, attractive toxic baits may represent an effective, inexpensive and environmentally friendly tool for integrated mosquito management programmes.

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