Medical and Veterinary Entomology

Medical and Veterinary Entomology (2011), doi: 10.1111/j.1365-2915.2011.00970.x

An autodissemination station for the transfer of an insect growth regulator to mosquito oviposition sites

R. GAUGLER, D. SUMAN and Y. WANG

Center for Vector Biology, Rutgers University, New Brunswick, NJ, U.S.A.

Abstract. A prototype autodissemination station to topically contaminate oviposition-seeking container-dwelling mosquitoes with the insect growth regulator, pyriproxyfen, was developed and tested in the laboratory. Our test subject was the Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae), an urban species that colonizes small-volume cryptic larval habitats and is a skip ovipositor that visits multiple containers. The station consists of a water reservoir to attract gravid females, which is joined to a transfer chamber designed to contaminate visiting mosquitoes. The unit is easily constructed by moulding wet shredded cardboard using corn starch as a binder. The essential criteria that must be met to prove the efficacy of an autodissemination station require it to demonstrate effectiveness in attracting the target insect, in transferring the toxicant to the insect that will disperse the agent, and in facilitating the subsequent transfer of the toxicant from the insect to target habitats at a lethal concentration. Cage experiments demonstrated that the unit was readily accepted by gravid females as an oviposition site. A powder formulation of pyriproxyfen-impregnated silica particles adhered to visiting *Ae. albopictus* females (mean: 66 particles/female), although particles were lost over time. In cage (2.2 m^3) trials, pyriproxyfen-charged stations resulted in 100% inhibition of adult emergence, whereas in small-room (31.1 m^3) trials, 81% emergence inhibition was recorded. The venereal transfer of pyriproxyfen from contaminated males to virgin females was also observed, and pyriproxyfen was subsequently transferred to water-holding containers at concentrations that inhibited emergence. Key autodissemination station features include lack of maintenance requirements, biodegradable construction, low cost and low risk.

Key words. *Aedes albopictus*, autodissemination, insect growth regulator, larval control, larval habitat, pyriproxyfen, urban mosquitoes, venereal transfer.

Introduction

Autodissemination is a pest management method in which insects contaminated with a biological or chemical insecticide transfer lethal concentrations horizontally or vertically to other insects via mating, oviposition, aggregation and other behaviours. Autodissemination is a 'pull' (attraction and transfer) and 'push' (dispersal and transfer to target habitats) technology. This approach offers the potential for economic (savings in product and labour) and environmental (less active ingredient/ha as a result of accurate targeting afforded by exploiting insect behaviour) benefits relative to broadcast spray applications.

Autodissemination is particularly well suited to use with insect pathogens because of the small size and host replication ability that are characteristic of infectious microbes and, accordingly, insect pathologists have pioneered this area of investigation. Most studies have examined the dissemination of entomopathogenic fungi and baculoviruses (Soper, 1978; Yu & Brown, 1997; Klein & Lacey, 1999), but even nematode

Correspondence: Randy Gaugler, Center for Vector Biology, 180 Jones Avenue, Rutgers University, New Brunswick, NJ 08901-8536, U.S.A. Tel.: +1 732 932 9341; Fax: +1 888 504 2379; E-mail: gaugler@rci.rutgers.edu

autodissemination has been considered (Lacey *et al*., 1993). These strategies tend to centre on the use of a pheromone lure to bring insects into a contamination station in which they are topically contaminated with a pathogen before they exit the station. The autoinoculated insects serve as carriers to disperse the agent to other habitats. For example, Dowd & Vega (1995, 2003) described a pheromone-baited device for contaminating sap beetles with spores of an entomopathogenic fungus for transference to adult overwintering sites.

Less consideration has been given to deploying chemical insecticides in autodissemination. Chemicals would seem to be poorly suited to autodissemination because, in addition to being unable to replicate, they tend to kill or incapacitate contaminated individuals, which greatly restricts dispersal. Itoh (1994), however, recognized a unique opportunity to use the insect growth regulator (IGR), pyriproxyfen, in an autodissemination strategy against mosquitoes. He noted that pyriproxyfen is a mosquito larvicide that does not impair adult activity (Kawada *et al*., 1993), is active at extraordinarily low concentrations (LC_{50} in *Aedes aegypti* L. is 0.023 p.p.b., which is equivalent to 200× the activity of temephos), and that females might serve as vehicles for transferring insecticides to other larval habitats (Schlein & Pener, 1990). Itoh (1994) exposed blood-fed *Ae. aegypti* in the laboratory to surfaces treated with pyriproxyfen and then allowed the females to oviposit in cups of water seeded with larvae, after which he recorded pyriproxyfen transfer from the females to the water and inhibition of adult emergence. Subsequent tests were conducted in a small house in Thailand by treating nylon netting in resting boxes and positioning sentinel ovicups within the house; these were later inoculated with *Ae. aegypti* larvae. Bioassays indicated that adult *Ae. aegypti* that contacted the treated netting carried pyriproxyfen into several of the ovicups. Chism & Apperson (2003) initiated similar small-cage experiments by forcing females to walk on pyriproxyfen-treated paper and demonstrated that sufficient toxicant was subsequently transferred to ovicups to inhibit up to 70% of *Ochlerotatus* (= *Aedes*) *triseriatus* (Say) and 59–73% of *Aedes albopictus* (Skuse) adult emergence.

Devine *et al*. (2009) extended Itoh's (1994) concept to the field, conclusively demonstrating that gravid female *Ae. aegypti* contaminated with pyriproxyfen transferred lethal concentrations to larval habitats. In this experiment, dissemination stations were constructed from plastic pots holding 200 mL of water, lined with cloth, and placed in a cemetery in Peru. The cloths were dusted with pyriproxyfen granules that had been crushed into a fine powder. The powder adhered to the cloth lining, providing a treated surface for the transfer of the IGR powder to visiting females. Sentinel stations (ovicups of water serving as oviposition sites) holding larvae were placed near the dissemination stations. Nearly all of the sentinel stations were visited by contaminated adults and subsequent adult emergence was reduced by 42–98% in replicated experiments. Thus, high coverage of larval habitats was achieved by treating an extremely small area. In short, Devine *et al*. (2009) exploited female oviposition behaviour to demonstrate pyriproxyfen autodissemination as a possible new approach to the management of container-dwelling mosquitoes.

We describe efforts to develop a prototype device for the autodissemination of pyriproxyfen in container-dwelling mosquitoes that is standardized, inexpensive, biodegradable, free of maintenance requirements, offers extended toxicant activity and maximizes the topical transfer of the toxicant by manipulating the search behaviour of gravid females. Our test subject was the Asian tiger mosquito, *Ae. albopictus*, which, like *Ae. aegypti*, is a highly urbanized container-dwelling species, colonizes small-volume cryptic larval habitats, and is a skip ovipositor that distributes eggs among multiple water-holding containers (Trexler *et al*., 1998). These are highly favourable traits for supporting autodissemination to mosquito oviposition sites.

Materials and methods

Station design

A prototype autodissemination station to topically contaminate oviposition-seeking mosquitoes was developed. This consists of two main sections comprising a water reservoir at the bottom, above which is a transfer chamber (Fig. 1). The sections are constructed by moulding wet shredded cardboard using corn starch as a binder, a technology commonly used in the manufacture of disposable plant containers. Peat moss, egg cartons, rice hulls, newspaper, straw, natural polymers, and other materials and binders can also be used.

The reservoir chamber is flask-shaped to maximize water-holding capacity (600 mL). The reservoir's role is to present a lure (water) to attract gravid females. The reservoir also holds an organic food substrate (e.g. dried shredded oak leaves, yeast, rabbit pellets, etc.) for bacteria that produce volatile cues used by female mosquitoes in the selection of oviposition sites (Ponnusamy *et al*., 2008). The food substrate is secured within a bag constructed from tubular cotton gauze (Surgitube®; Surgitube Corp., New York, NY, U.S.A.) and the bag is fixed with paraffin to the reservoir bottom. This bag design prevents food particles from obstructing the overflow holes when the unit is flooded, but does not impede the release of nutrients.

The base of the transfer chamber is designed to friction-fit within the rim of the reservoir. The reservoir rim is slightly larger in diameter and fits over the transfer chamber rim by several millimetres; this difference in diameters creates a shallow gutter between reservoir and chamber. The gutter collects run-off water from the chamber, which is dome-shaped to enhance the capture of rainwater and dew. The bottom edge of the transfer chamber is scalloped to generate multiple holes that drain water from the gutter into the reservoir. End-users may elect to fill the reservoir with tap water when the station is deployed or may alternatively permit the reservoir to be auto-filled with rainfall. The auto-fill feature is projected to increase the duration of attraction. A row of overflow holes near the top edge of the reservoir prevent excess water from breaching the reservoir and entering the transfer chamber.

The transfer chamber holds an insecticide-impregnated cotton mesh fabric (86 cm^2 with 39 openings/cm^2) fashioned into a shallow cone and secured within the chamber base. The mesh is the primary platform for the transfer of pyriproxyfen from the station to searching gravid mosquitoes. The transfer mesh is washed to induce fraying, which increases the surface area

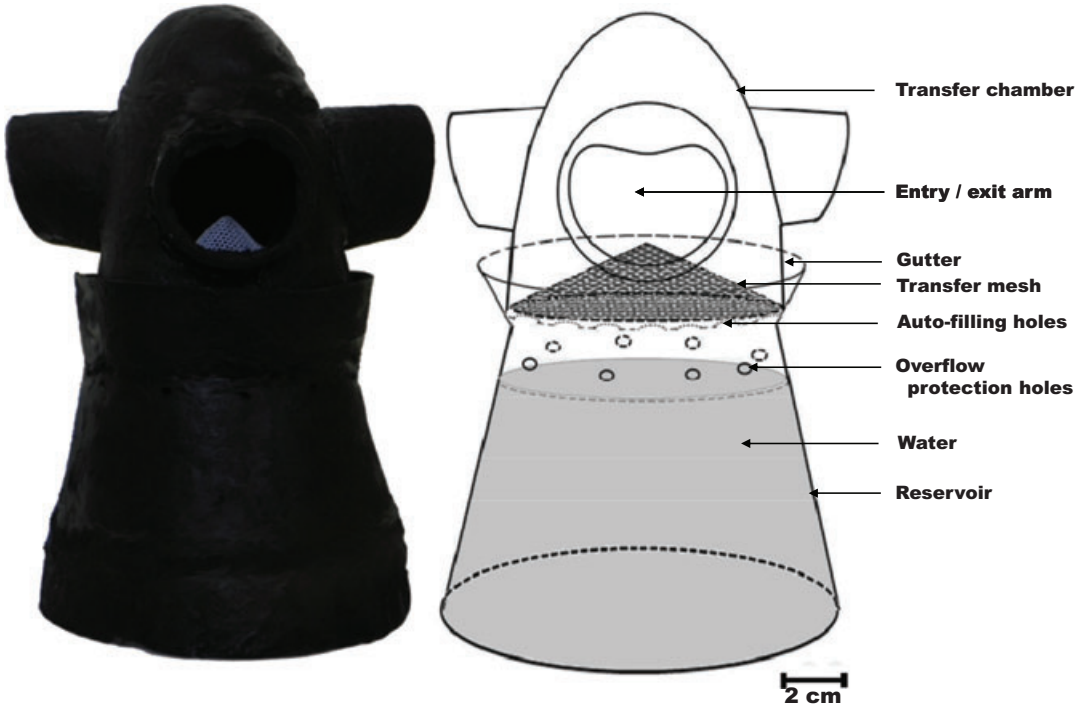

Fig. 1. Image and schematic drawing of prototype autodissemination station.

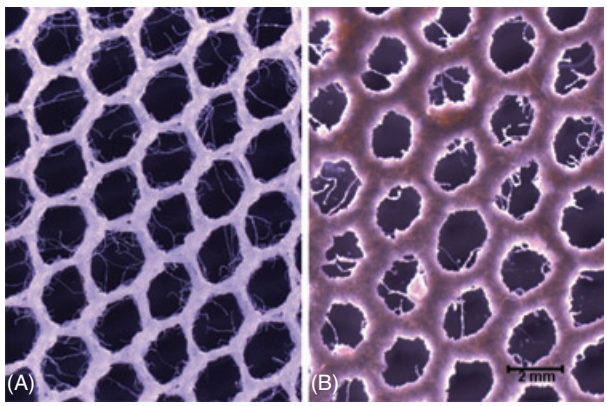

Fig. 2. Mesh serving as a platform for the transfer of toxicant to adult mosquitoes entering the autodissemination station as it appears (A) uncoated and (B) coated with pyriproxyfen-impregnated silica particles.

available for toxicant loading (Fig. 2). A secondary function of the mesh is to act as a barrier to prevent mosquitoes from entering the reservoir. The cone shape of the transfer mesh provides additional surface area for pyriproxyfen loading and mosquito searching; it also reduces the likelihood that mosquitoes will exit the transfer chamber without contacting the mesh. The overflow and gutter auto-fill holes are sufficiently large for water to pass through, but are too small to allow the passage of mosquitoes.

The transfer chamber has three large portals (4 cm in diameter) for the entry and exit of mosquitoes. The extruding shape of the portals buffers the mesh and adhering chemical from abiotic (e.g. rain, wind, light) and biotic (e.g. children, pets, wildlife) contact.

The station is coated with paraffin wax to waterproof it and to extend its life. The unit is designed, however, to break down after a single mosquito season, which, for *Ae. albopictus* in New Jersey, amounts to approximately 4 months. The station's effective lifespan is proscribed by adjusting the thickness of the wall and wax coat. The inner surface of the transfer chamber is not wax-coated. Because a dark, humid interior may be attractive to resting mosquitoes, this inner surface is dusted with pyriproxyfen as a secondary platform for toxicant transfer. The station is dyed dark grey with ink.

Consideration was given to the need for the station to remain stable and upright when challenged by wind or animal or human activity because, if it is knocked over, the lure is purged and the unit inactivated. The unit is best placed in protected, low-traffic areas near vegetation that provides the shade favoured by container-dwelling species in oviposition site selection (Vezzani & Albicocco, 2009). The reservoir has a wide, flat bottom to assist the unit in maintaining an upright position. Sand embedded in the floor adds weight that also assists in stability. A cotton string implanted at the station apex permits the unit to be suspended above ground.

Laboratory colony

Larvae of *Ae. albopictus* for bioassays were obtained from a colony established from eggs collected in Mercer County, NJ, U.S.A. in 2008 and supplemented during 2009 and 2010. The

colony was maintained at 26 °C, at a relative humidity (RH) of 75% under an LD 16 : 8 h photoperiod. Adults were held in 30 × 30 × 30-cm aluminium screen cages and supplied with 10% sucrose solution on cotton wicks. Restrained guinea pigs were used to blood-feed females (Rutgers University Animal Use Protocol #86–129) and subsequent eggs were collected on seed germination paper and stored at 26 °C. Eggs were hatched as needed by flooding egg papers and the larvae held in enamel trays with 1 L of deionized water and 0.15 g of Brewer's yeast (lactalbumin 50 : 50). Third instars were used in all bioassays.

Attraction of gravid females

We tested the attractiveness of our station design against alternative oviposition sites in 1.3 × 1.3 × 1.3-m cages covered with cotton mesh. Each cage held an autodissemination station and two 360-mL black oviposition cups [experimental lethal ovitrap cups lacking toxicant (SpringStar, Inc., Woodinville, WA, U.S.A.)], each containing 200 mL of oak infusion water as a lure to attract gravid females (Trexler *et al*., 1998). The station and ovicups were placed equidistantly apart (80 cm) and a sucrose solution was placed at the cage centre. The inner wall of the station reservoir and the cups were lined with a 5-cm wide strip of Whatman No. 1 filter paper, of which the bottom 3 cm were submerged in the infusion and the upper 2 cm were available for oviposition. The transfer mesh was not inserted into the station in order to allow females to access the oviposition paper. No pyriproxyfen was used. Twenty gravid *Ae. albopictus* were introduced into each cage. After 48 h, the eggs on each paper strip as well as any deposited on the water surface were counted. The experiment was repeated three times with three replicates (cages) per experiment at 21–27 °C, 60–75% RH and LD 16 : 8 h. We compared egg deposition in the station and ovicups using a one-way analysis of variance (ANOVA; $P \leq 0.05$) with the least significant difference (LSD) among the means.

Formulation

Our source of pyriproxyfen for all experiments was a mixture of technical compound and NyGuard® IGR [10% active ingredient (a.i.)] (2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine) obtained from MGK Chemical Co. (Minneapolis, MN, U.S.A.) as an emulsifiable concentrate. The technical compound permitted high loading of active ingredient, whereas the commercial product provided a source of emulsion to aid pyriproxyfen release when contaminated females made contact with water. A formulation was prepared for each station using 500 mg of silica particles (silicon dioxide, 3–30 μm in diameter), 500 mg of technical pyriproxyfen, 200 μL of NyGuard® EC10 and 200 μL of corn oil as a sticking agent. The technical compound was dissolved in 3 mL of reagent grade acetone before being mixed with the other components. The mixture was air-dried at room temperature for 24 h to result in a powder formulation of pyriproxyfen-impregnated particles.

The powder was used to treat the transfer mesh and inner wall of the transfer chamber. To enhance the adherence of the powder, these surfaces were first lightly brushed with corn oil. The mesh was blotted to remove excess oil, but this was unnecessary on the chamber wall because it was absorbent. The mesh was then attached to the chamber, the chamber inverted and the pyriproxyfen powder added. The chamber was sealed with plastic film and then agitated to distribute the powder before the transfer and reservoir chambers were joined. Each station was treated with 520 mg of active ingredient at a concentration of 1.63 mg/cm^2.

Transfer from station to females

Station effectiveness in transferring pyriproxyfen powder to visiting females was tested by counting the particles attached to individual test mosquitoes. Forty gravid females were introduced into a pyriproxyfen-treated station, the entrances blocked and the station placed in a 2.2-m^3 screened cage provisioned with sucrose solution. The station portals were reopened after 30 min to allow the mosquitoes to quickly exit the station and enter the cage. The station was then removed. Five gravid females were individually captured with an aspirator at 0 h, 1 h, 2 h, 6 h, 12 h, 24 h and 48 h, transferred into centrifuge tubes and killed by freezing at −4°C for 4 h. The mosquitoes were placed into individual 200-μL drops of 0.1% detergent (Sparkleen®; Fisher Scientific, Inc., Pittsburgh, PA, U.S.A.) to remove any adhering powder and the retrieved particles were counted. The experiment was replicated three times at environmental conditions of 26–28 °C, 60–75% RH and LD 16 : 8 h. To assess whether the efficacy of transfer from the station would be reduced as the formulation aged as a result of water sorption, the work was repeated with stations placed in a 100% RH environment for 7 days.

Transfer from females to larval habitats

The efficacy of pyriproxyfen-contaminated females in transferring lethal concentrations of the toxicant to water-holding containers (i.e. larval habitats) was tested in cage and small-room experiments. A 10% sucrose solution was provided in all tests.

In the cage experiment, an autodissemination station equipped with a pyriproxyfen-treated transfer mesh and 450 mL of oak leaf infusion water was centred in a 2.2-m^3 cage. A 360-mL plastic cup was placed in each cage corner 15 cm from the walls. Each of the four cups was filled with 200 mL of tap water and lined with oviposition paper strips as described above. Controls used a station with an untreated transfer mesh. Fifty gravid females were then introduced into the cage. After 5 days, the test was terminated, oviposition papers and eggs discarded, the containers removed and the water assessed for pyriproxyfen transfer by larval bioassay (see 'Insecticide bioassays' below). Each experiment used one control and three treatment cages and was repeated three times, providing a total of 36 test and 12 control ovicups for bioassay. Environmental conditions were 21–28 °C, 60–75% RH and LD 16 : 8 h.

In the small-room trial, the test arena was scaled up to a larger area measuring 2.1 m in width, 5.7 m in length and 2.6 m in height (31.1 m^3). A station was placed in the centre of the room and ovicups with oak infusion and ovistrips were positioned at each corner 30 cm from the walls. Fifty gravid females were introduced for a 5-day test. The ovistrips and eggs were discarded and the water from each container was tested for insecticidal activity by larval bioassay; four additional untreated cups were included as controls. The experiment was conducted under environmental conditions of 26–28 °C, 60–75% RH and LD 16 : 8 h. The inhibition of emergence in the cage and room trials was analysed against their respective controls with a *t*-test ($P \leq 0.05$).

Venereal transfer

This experiment determined whether contaminated mosquitoes could transfer pyriproxyfen to uncontaminated individuals via mating behaviours. To ensure their virginity, pupae were individually transferred to 50-mL centrifuge tubes until emergence and then caged by gender before testing at 5–7 days post-emergence. Adult males were exposed to pyriproxyfen-treated filter paper (8 × 10 cm Whatman No. 1) lining a 50-mL test tube. The paper was coated with 1.5 mL of corn oil as a sticking agent, blotted to remove excess oil and then dusted with the pyriproxyfen powder formulation at 0.26 mg/cm^2 of a.i. After 10 min of exposure, males were transferred from the tube by aspirator to mating containers (450 mL, 7.5 cm in depth) covered with mosquito-proof mesh and paired to mate with uncontaminated females. After 48 h, females were collected by aspirator from the cage sides and transferred briefly to a 450-mL plastic holding container before being transferred to individual oviposition chambers (two 450-mL containers joined with tape to form an enclosure) holding 200 mL of tap water and lined with oviposition paper. The upper portion of the oviposition chamber was perforated for aeration and a portal was provided for cotton wicks used in sugar feeding. The dual transfer increased handling but reduced the likelihood of any inadvertent transfer of pyriproxyfen to the chambers. Environmental conditions were 25–29 °C, 60–75% RH and LD 16 : 8 h. Oviposition chamber water was collected after 5 days and assayed for inhibition of adult emergence using methods described below. The experiment was repeated twice with three replicates each time. A *t*-test was used to test for significant differences ($P < 0.05$).

Insecticide bioassays

All insecticidal activity tests were conducted in adult emergence units represented by 450-mL plastic containers, each of which included a screen-covered lid and contained 200 mL of water (water lost via evaporation was replaced before assay). Each container held 20 third-instar *Ae. albopictus*. Test containers were incubated at 26 °C and LD 16 : 8 h. At 2-day intervals, ground rat chow (30 mg/L) was provided as food, water lost to evaporation was replenished, dead and emerged individuals were removed, and successful adult emergence was recorded. Incomplete emergence or adults with attached exuviae were recorded as dead.

Results

The first criterion an autodissemination station must demonstrate to prove its effectiveness is efficacy in luring the target insect. Our earlier designs did not compete effectively with ovicups for the attention of females. Attraction increased, however, with subsequent designs that increasingly simulated tree holes by the use of portals, texture, colour, etc. Our final design was successful in attracting gravid females because in our cage experiment we found no difference in the proportion of eggs recovered from the station [mean ± standard error (SE) 38.25 ± 4.63%] compared with those recovered from two competing ovicups (mean ± SE: 34.04 ± 3.12% and 27.71 ± 4.15%) (d.f. = 2, $F = 1.15$, $P = 0.33$).

The station's efficacy in suppressing container-dwelling mosquitoes rests heavily on its ability to compete with the numerous artificial containers characteristic of urban environments. Our main target, *Ae. albopictus*, is a highly anthropophilic and ecologically flexible species that has adapted from tree holes to colonize discarded tyres, bird baths, flower pots and saucers, vases, rain gutters, tarpaulins, catch basins, cemetery urns, toys, cans and trash (Hawley, 1988).

Although the oak leaf infusion we tested was effective in attracting *Ae. albopictus* females, the response of gravid container-dwelling species to organic infusions varies with the plant species, biomass and duration of fermentation (Ponnusamy *et al*., 2010). Optimal attraction to the station will require species-specific attractants.

The second criterion for effective autodissemination refers to the transfer of toxicant to the target insect that will disperse the agent. Elements of the station were designed to maximize transfer. Whereas the reservoir attracts females, the transfer mesh prevents visiting females from reaching the water and ovipositing. This serves two functions. Most importantly, the fact that visiting females must search without reward for a conduit to the water below will maximize their visit time and contact with the mesh and, presumably, their toxicant loading. Because their egg complement is undiminished, females must visit additional proximal larval habitats, which provides them with more opportunities to transmit the chemical. Further, because oviposition in the station is precluded, the station will not reach a threshold of egg deposition that deters new females.

The powder formulation proved effective in its ability to initially attach to *Ae. albopictus* females. Microscopic examination demonstrated that particles adhered topically to every body part, including the tarsi and tip of the abdomen (Fig. 3). These are the locations most likely to make contact with container water and release the insecticide. A mean ± SE of 65.5 ± 6.92 particles/female were counted on mosquitoes exiting the station and flying to the cage netting before being captured. Powder was lost over time so that the mean ± SE number of particles/female declined to 40.3 ± 3.84, 29.3 ± 4.13, 22.0 ± 1.78, 5.5 ± 0.96, 2.0 ± 0.71 and 2.2 ± 0.67 after

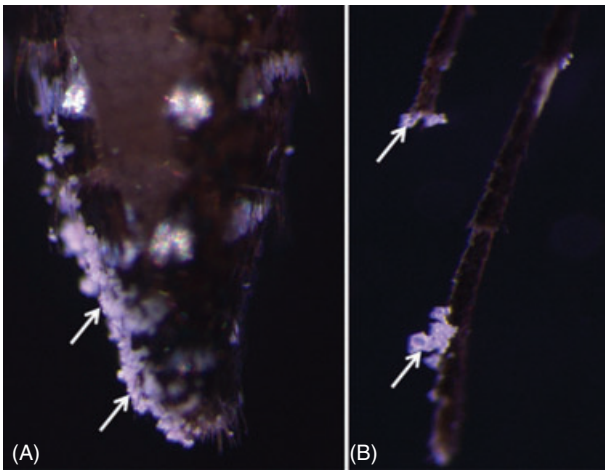

Fig. 3. (A) Abdomen and (B) foreleg tarsi showing adherence of pyriproxyfen-impregnated particles (arrows) to *Aedes albopictus* females.

1 h, 2 h, 6 h, 12 h, 24 h and 48 h, respectively. Losses are attributable to a combination of events, including flight, aspiration and post-capture handling. However, we attributed the loss of particles largely to the grooming behaviours frequently observed in resting females. Walker & Archer (1988) recorded 12 different grooming behaviours in female *Ae. triseriatus* Say in which tibial combs were used for cleaning. Grooming frequency increases when insects are exposed to silica dust (El-Awami & Dent, 1995). Although grooming is generally disadvantageous for autodissemination, in some circumstances it may prove beneficial, notably when resting and oviposition sites converge as they do in tyres.

The exposure of powder to high humidity can result in water sorption and plasticization (Fox & McSweeney, 1998). This may cause 'caking', in which the powder is changed into a solid mass. Caking would interfere with particulate adherence to visiting mosquitoes and may be a legitimate threat to a station in which continuous exposure to high humidity is an intrinsic condition. Maintaining the station with a full reservoir for a week, however, did not reduce the number of silica powder particulates adhering to mosquitoes or result in visible caking. Nevertheless, given the potential significance of caking, factors that impact the water sorption of powders, including formulation components, particle size and exposure duration, must be considered.

The third step in successful autodissemination involves the transfer of toxicant from contaminated individuals to target habitats. In cage experiments, pyriproxyfen-charged stations resulted in 100% inhibition of adult emergence in all 36 ovicups tested, whereas mean control mortality was $6.51 \pm 4.72\%$. The room trial was more challenging because it presented a test arena of 0.62 m^3/female, representing more than 14 times the arena of the cage study. The increase in area from cage to room was reflected in a reduction in the level of inhibition achieved: $81.25 \pm 2.39\%$ compared with $10.0 \pm 2.04\%$ in the control ovicups. All of the experimental ovicups had been visited by females as the smallest reduction in adult emergence was 75%. These results bolster earlier work with *Ae. aegypti* (Itoh, 1994; Chism & Apperson, 2003; Devine *et al*., 2009) in demonstrating the potential of using mosquitoes as vehicles to carry pyriproxyfen to larval habitats, and extends the concept to *Ae. albopictus*.

The transfer of lethal pyriproxyfen concentrations from mosquitoes to water-holding containers was inversely related to time and container volume. Consider time first. Immediately after exiting the station, each female retained an estimated average of 0.524 µg of a.i., which declined to 0.324 µg, 0.234 µg, 0.176 µg, 0.044 µg, 0.016 µg and 0.018 µg after 1 h, 2 h, 6 h, 12 h, 24 h and 48 h, respectively. Because the LC_{50} for pyriproxyfen against *Ae. albopictus* is a mere 0.012 p.p.b. (D. Suman, unpublished data, 2011), a single mosquito could theoretically transfer lethal pyriproxyfen concentrations into most containers colonized by *Ae. albopictus*. Next, consider container volume. The Asian tiger mosquito prefers small to medium-sized containers (Hawley, 1988). Carrieri *et al*. (2003) found *Ae. albopictus* tended to colonize small containers, even those holding just a few millilitres of water; most (80%) containers had a volume of <5 L. Discarded tyres are an important *Ae. albopictus* larval habitat and hold an average of 1 L of water (Schreiber *et al*., 1992). Considering time and volume together, we estimate that if a single contaminated female were to release all adhering pyriproxyfen into a single container, this could induce 50% mortality in containers of 28.3 L up to 6 h after becoming contaminated, declining to 3.5 L after 12 h, and 0.13 L after 24 h. These estimates are useful only in demonstrating that: (a) females contaminated with our current formulation have a limited time-span to transfer lethal pyriproxyfen concentrations to larval habitats, and (b) the concentrations of pyriproxyfen that may potentially be carried into drinking water will not approach the 300 p.p.b. limit recommended by the World Health Organization (2002).

We tested the hypothesis that mating behaviour would result in venereal transfer and visually established the horizontal mechanical transfer of pyriproxyfen powder from contaminated males to virgin females (Fig. 4). Acquired particles were observed clinging to various body regions, including the tarsi, and were often found attached to the adult female's last two segments (7 and 8; i.e. the ovipositor). The male first grasps the female with the tarsi before swinging the abdomen up. The venter-to-venter position is then assumed and the ovipositor is extruded for mating, making these terminal segments particularly vulnerable to mechanical transfer from a contaminated male. The ovipositor is then retracted to its normal condition, which provides a degree of shelter for particulates from grooming. When extruded again, for oviposition site selection and egg deposition, this area can contact water and release the adhering insecticide. In bioassays to assess whether venereally acquired pyriproxyfen could subsequently be transferred to water-holding containers at lethal concentrations, we recorded $48.33 \pm 5.87\%$ inhibition of emergence, a significant difference from control mortality of $5.0 \pm 1.83\%$ (d.f. = 5, t-test value = 7.05, $P = 0.00003$). Venereal transmission of pathogens in mosquitoes, notably infectious viruses including chikungunya (Mavale *et al*., 2010), LaCrosse (Thompson & Beaty, 1978) and Sindbis (Ovenden & Mahon, 1984), is well documented and is believed to play a role in the infection

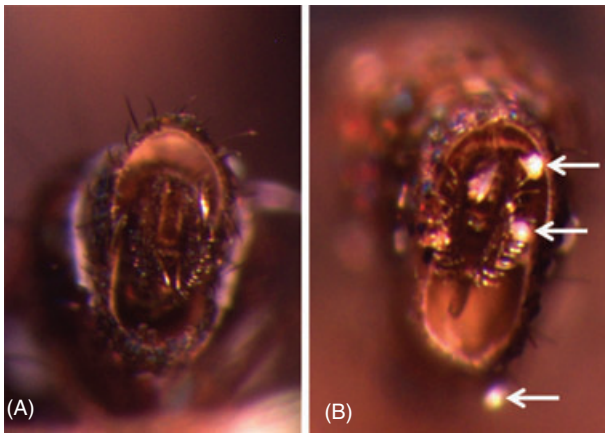

Fig. 4. Distal view of ovipositor of *Aedes albopictus* female (A) before (virginal) and (B) after mating, showing pyriproxyfen-impregnated particles (arrows) transferred by mating from the contaminated male to the female.

cycle. Vail *et al*. (1993) showed how male Indian meal moths contaminated with a powder formulation of a granulosis virus transferred this insect pathogen to adult females during copulation and that larval food subsequently became contaminated during oviposition. Moya *et al*. (2010) reported that male fruit flies fed on the chemosterilant lufenuron subsequently transferred the compound to females. The venereal transference of a chemical insecticide by mechanical means and subsequently to an oviposition site has not been previously reported. The contribution of venereal transmission to the reduction of a mosquito population is unclear but it may play a significant if modest role as we have found males are attracted to oak infusion water, use the station for resting and carry more particles than females (Y. Wang, unpublished data, 2011).

Discussion

Key features of our prototype autodissemination station include its: (a) lack of any maintenance requirements; (b) biodegradability; (c) low cost; (d) low risk; (e) high rate of mosquito attraction; (f) extended attraction, and (g) facility to serve as a lethal ovitrap.

Zero maintenance

Our concept refers to a user-friendly station that, once deployed in the urban environment, requires no further maintenance over the container-dwelling mosquito season. The unit's reservoir will be replenished automatically by rain and dew. Oviposition cannot occur in the station and thus there is no requirement to replace oviposition papers as they fill with eggs [container-dwelling mosquitoes tend to shun sites containing eggs of conspecifics (Chadee *et al*., 1990)]. The toxicant will not have to be refreshed because it shows outstanding persistence under normal field conditions (Sihuincha *et al*., 2005) and will be buffered from damaging environmental extremes including flushing, sunlight and microbial action within the station. Furthermore, there is no need for labour-intensive season-end retrieval because the unit is designed to structurally break down.

Biodegradability

The station is intended for a single season of use. Components are designed thereafter to degrade (resulting in leaking) and disintegrate into the soil, which precludes the unit from becoming a source of mosquito production. A station can also be constructed from non-degradable materials so that it can be reused; however, such a unit would need periodic recharging with insecticide or would become a source of mosquito production.

Low cost

The station must be economical to manufacture so that an adequate number of units can be deployed to compete with natural oviposition sites. A cost-of-goods analysis estimates that, given the inexpensive components required for manufacture, a unit might be produced for <US$1.00.

Low risk

The U.S. Environmental Protection Agency has designated pyriproxyfen a 'low-risk' insecticide that is virtually non-toxic to mammals and birds. Containing the chemical within stations bars access to wildlife, pets and children, which reduces risk further. More significantly, mosquito autodissemination requires a small amount of insecticide which precisely targets water-holding containers and therefore presents less exposure than the broadcast sprays used by homeowners and local mosquito control agencies.

High attraction

Every effort was made to enhance the station's ability to lure gravid females and to compete with alternative containers. The prototype simulates a tree hole, is provisioned with a food substrate for bacterial growth and has a water reservoir. The unit was acceptable to females seeking to oviposit in our laboratory studies.

Extended attraction

The effectiveness of the station depends on its ability to maintain sufficient moisture to attract gravid females for the greatest possible duration. Accordingly, the water reservoir is designed to be replenished by the collection of rain and dew: 5.5 cm of rainfall is sufficient to fill the unit. The design also reduces evaporative loss so that the station retains water for

>7 weeks at 26 °C and 50% RH. These features will assist the unit in bridging periods of low rainfall and magnify its effectiveness in competing with alternative oviposition sites.

Lethal ovitrap

Devine & Killeen (2010) identified the key advantages of a 'lure and disseminate' (i.e. autodissemination) protocol over a 'lure and kill' [i.e. lethal ovitrap method (Perich *et al*., 2003)] system as the potential amplification of coverage at the larval habitat and the use of a chemistry to which resistance has not yet developed. Nevertheless, our station could be modified to kill mosquitoes that attempt to oviposit simply by treating the transfer mesh with an adulticide such as a pyrethroid rather than with an IGR. That is, the station could function as a lethal ovitrap because it offers many of the same features, including the ability to lure gravid females, in addition to new advantages such as extended insecticidal activity, inability to serve as a larval habitat when insecticidal activity fails, the elimination of egg papers, and reduced non-target exposure to insecticides.

The most important prerequisite for field testing the prototype is a formulation that both facilitates cuticular attachment and retention (e.g. is resistant to grooming) and is able to release insecticide effectively in the larval habitat. Current lines of investigation include exploring smaller particle sizes, which are picked up by insects more readily (El-Awami & Dent, 1995) and are more difficult to remove by mechanical means, yet release more effectively in water as a result of their high surface area : volume ratio than our present formulation. Carriers other than silica powder are also being evaluated. Yu & Brown (1997), for example, reported talc to be the most effective among six baculovirus carriers tested as a result of its greater adhesion to beet armyworm adults. We are also exploring formulations based on non-particulates, as well as alternative sticking agents.

Our approach centres on the development of a practical and effective IGR-treated autodissemination station for use by local mosquito control agencies, homeowners, livestock managers, military organizations and the pest control industry. For example, we envision that local control agencies might place stations in or near container-dwelling mosquito focal points (e.g. junkyards, feedlots, poorly maintained yards, etc.) where surveillance indicates a hot spot of vector-borne virus activity or where complaints of persistent nuisance are received from residents. Area-wide control deserves consideration, but the labour involved in deploying numerous stations may limit such strategies. The deployment of autodissemination stations may be a particularly valuable tool when combating a problem that originates on a property to which access is restricted by the resident, a factor that is an everyday impediment to mosquito control personnel.

Acknowledgements

We thank L. McCuiston for laboratory assistance. This work was supported in part by a grant from the Deployed War-Fighter Protection (DWFP) Research Program and funded by the U.S. Department of Defense through the Armed Forces Pest Management Board (AFPMB). Funding was also provided through the U.S. Department of Agriculture Cooperative Agreement USDA-ARS-58-6615-8-105 entitled 'Area-wide Pest Management Program for the Asian Tiger Mosquito in New Jersey' and the New Jersey Agricultural Experiment Station.

References

Carrieri, M., Bacchi, M., Bellini, R. & Maini, S. (2003) On the competition occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. *Environmental Entomology*, **32**, 1313–1321.

Chadee, D.D., Corbet, P.S. & Greenwood, J.J.D. (1990) Egg-laying yellow fever mosquitoes avoid sites containing eggs laid by themselves or by conspecifics. *Entomologia Experimentalis et Applicata*, **57**, 295–298.

Chism, B.D. & Apperson, C.S. (2003) Horizontal transfer of the insect growth regulator pyriproxyfen to larval microcosms by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory. *Medical and Veterinary Entomology*, **17**, 211–220.

Devine, G.J. & Killeen, G.F. (2010) The potential of a new larvicide ing method for the control of malaria vectors. *Malaria Journal*, **9**, 142–145.

Devine, G.J., Perea, E.Z., Killeen, G.F., Stancil, J.D., Clark, S.J. & Morrison, A.C. (2009) Using adult mosquitoes to transfer insecticides to *Aedes aegypti* larval habitats. *Proceedings of the National Academy of Sciences*, **106**, 11530–11534.

Dowd, P.F. & Vega, F.E. (1995) Autoinoculating device for contaminating insects with active agents. U.S. Patent 5 452 540.

Dowd, P.F. & Vega, F.E. (2003) Autodissemination of *Beauveria bassiana* by sap beetles (Coleoptera: Nitidulidae) to overwintering sites. *Biocontrol Science and Technology*, **13**, 65–75.

El-Awami, I.O. & Dent, D.R. (1995) The interaction of surface and dust particle size on the pick-up and grooming behaviour of the German cockroach *Blattella germanica*. *Entomologia Experimentalis et Applicata*, **77**, 81–87.

Fox, P.F. & McSweeney, P.L.H. (1998) *Dairy Chemistry and Biochemistry*. Kluwer Academic/Plenum Publishers, New York, NY.

Hawley, W.A. (1988) The biology of *Aedes albopictus*. *Journal of the American Mosquito Control Association*, **1** (Suppl.), 1–40.

Itoh, T. (1994) Utilization of blood-fed females of *Aedes aegypti* as a vehicle for the transfer of the insect growth regulator, pyriproxyfen, to larval habitats. *Tropical Medicine*, **36**, 243–248.

Kawada, H., Shono, Y., Ito, T. & Abe, Y. (1993) Laboratory evaluation of insect growth regulators against several species of anopheline mosquitoes. *Japanese Journal of Sanitation Zoology*, **44**, 349–353.

Klein, M.G. & Lacey, L.A. (1999) An attractant trap for autodissemination of entomopathogenic fungi into populations of the Japanese beetle *Popillia japonica* (Coleoptera: Scarabaeidae). *Biocontrol Science and Technology*, **9**, 151–158.

Lacey, L., Bettencourt, A., Garrett, F., Simoes, N. & Gaugler, R. (1993) Factors influencing parasitism of adult Japanese beetles by entomopathogenic nematodes. *Entomophaga*, **38**, 501–509.

Mavale, M., Parashar, D., Sudeep, A. *et al*. (2010) Venereal transmission of chikungunya virus by *Aedes aegypti* mosquitoes (Diptera: Culicidae). *American Journal of Tropical Medicine and Hygiene*, **83**, 1242–1244.

Moya, P., Flores, S., Ayala, I., Sanchis, J., Montoya, P. & Primo, J. (2010) Evaluation of lufenuron as a chemosterilant against fruit flies of the genus *Anastrepha* (Diptera: Tephritidae). *Pest Management Science*, **66**, 657–663.

Ovenden, J.R. & Mahon, R.J. (1984) Venereal transmission of Sindbis virus between individuals of *Aedes australis* (Diptera: Culicidae). *Journal of Medical Entomology*, **21**, 292–295.

Perich, M.J., Kardec, A., Braga, I.A. *et al*. (2003) Field evaluation of a lethal ovitrap against dengue vectors in Brazil. *Medical and Veterinary Entomology*, **17**, 205–210.

Ponnusamy, P., Xu, N., Nojima, S., Wesson, D.M., Schal, C. & Apperson, C.S. (2008) Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. *Proceedings of the National Academy of Sciences*, **105**, 9262–9267.

Ponnusamy, L., Xu, N., Böröczky, K., Wesson, D.M., Ayyash, L.A., Schal, C. & Apperson, C.S. (2010) Oviposition responses of the mosquitoes *Aedes aegypti* and *Aedes albopictus* to experimental plant infusions in laboratory bioassays. *Journal of Chemical Ecology*, **36**, 709–719.

Schlein, Y. & Pener, H. (1990) Bait-fed adult *Culex pipiens* carry the larvicide *Bacillus sphaericus* to larval habitat. *Medical and Veterinary Entomology*, **4**, 283–288.

Schreiber, E., Chamberlain, S., Thomas, R., Parsons, R. & Baker, G. (1992) Surveys on artificial container-inhabiting mosquitoes in Sarasota and Tallahassee, Florida. I: characterizations of larval habitats. *Journal of the Florida Mosquito Control Association*, **63**, 7–15.

Sihuincha, M., Zamora-Perea, E., Orellana-Rios, W., Stancil, J.D., Pez-Sifuentes, V.L., Vidal-Ore, C. & Devine, G.J. (2005) Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru. *Journal of Medical Entomology*, **42**, 620–630.

Soper, R. (1978) Autodissemination of entomopathogens: fungi. *Future Strategies in Pest Management Systems* (ed. by G. E. Allen, C. M. Ignoffo & R. P. Jaques), pp. 63–65; *Proceedings of the National Science Foundation*. U.S. Department of Agriculture and the University of Florida Workshop on Microbial Control of Insect Pests, Gainesville, FL.

Thompson, W.H. & Beaty, B.J. (1978) Venereal transmission of LaCrosse virus from male to female *Aedes triseriatus*. *American Journal of Tropical Medicine and Hygiene*, **27**, 187–196.

Trexler, J., Apperson, C.S. & Schal, C. (1998) Laboratory and field evaluations of oviposition responses of *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) to oak leaf infusions. *Journal of Medical Entomology*, **35**, 967–976.

Vail, P.V., Hoffmann, D.F. & Tebbets, J.S. (1993) Autodissemination of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) granulosis virus by healthy adults. *Journal of Stored Products Research*, **29**, 71–74.

Vezzani, D. & Albicocco, A.P. (2009) The effect of shade on the container index and pupal productivity of the mosquitoes *Aedes aegypti* and *Culex pipiens* breeding in artificial containers. *Medical and Veterinary Entomology*, **23**, 78–84.

Walker, E.D. & Archer, W.E. (1988) Sequential organization of grooming behaviours of the mosquito *Aedes triseriatus*. *Journal of Insect Behaviour*, **1**, 97–109.

World Health Organization (2002) *Pyriproxyfen—Guidelines for Drinking-Water Quality*, 3rd edn. WHO, Geneva.

Yu, Z. & Brown, G.C. (1997) Autodissemination of a beet armyworm (Lepidoptera: Noctuidae) baculovirus under laboratory conditions. *Journal of Economic Entomology*, **90**, 1187–1194.

Accepted 7 May 2011