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Point-source and area-wide field studies of pyriproxyfen autodissemination against urban container-inhabiting mosquitoes

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ABSTRACT

Autodissemination of insecticides is a novel strategy for mosquito management. We tested if contaminated *Aedes albopictus* (Skuse) mosquitoes from a small area treated with commercial formulations (79 gm a.i. pyriproxyfen/ha) using conventional techniques, would disseminate pyriproxyfen over a wider area. Pyriproxyfen showed LC_{50} = 0.012 ppb for *Ae. albopictus*. Direct treatment and autodissemination efficacy was measured as a pupal mortality by conducting *Ae. albopictus* larval bioassay. A tire pile (n = 100 tires) treated by backpack sprayer as a point-source treatment showed higher pupal mortality in 2010 (60.8% for week 0–6) than in 2011 (38.3% for week 0–6). The sentinel containers placed for autodissemination in four compass directions out to 200–400 m from the tire pile showed 15.8% pupal mortality (week 1–6) in the first year, and 1.4% pupal mortality in the second year. No significant difference was detected among the distances and direction for pupal mortality. In area-wide treatments, vegetation was sprayed in checkerboard pattern (3.7% of 105 ha) using backpack sprayer in 2010 and in strips (24.8% of 94 ha) using truck-mounted ultra-low volume (ULV) sprayer in 2011. In both years, the area-wide direct treatment efficacy was lower (30.3% during 2010 and 5.3% in 2011) than point-source treatments. Autodissemination in area-wide plots was higher in 2010 (10.3%) than 2011 (2.9%). However, area-wide treatments were ineffective on field populations of *Ae. albopictus* as monitored by using BGS traps. We found accumulation of pyriproxyfen in the week 6 autodissemination containers in both experiments. The differences in autodissemination in 2010 and 2011 can be attributed to higher rainfall in the second year that may have eroded the pyriproxyfen from treatment surfaces and sentinel containers. Our study shows that ULV surface treatments of conventional formulation do not work for autodissemination. The effectiveness of pyriproxyfen in autodissemination may be improved by developing specific formulations to treat vegetation and tires that can load high doses on mosquitoes.

1. Introduction

Pyriproxyfen is a pyridine-based insect growth regulator (IGR) that acts as a juvenile hormone analog, overloading the insect endocrine system and disrupting the normal development of immature stages with lethal results (Ishaaya and Horowitz, 1992). Pyriproxyfen is also considered a 'reduced-risk pesticide' or 'unlikely to present acute hazard' that is virtually non-toxic to birds or animals and is neither carcinogenic or genotoxic and can be safely added to drinking water for mosquito control at a concentration of 0.01 ppm (WHO, 2009). But because some aquatic invertebrates, notably flies and copepods (EPA, 2000), can be affected, direct application to natural water bodies is prohibited in the US.

The field evaluation of pyriproxyfen against mosquito larvae has shown longer persistence and high efficacy (Yapabandara and Curtis, 2002; Nayar et al., 2002; Vythilingam et al., 2005).Despite this exceptional persistence as a larvicide, studies indicate a low potential for the development of resistance. For example, even after *Culex quinquefasciatus* (Say) larvae were pressured with pyriproxyfen exposure for 17 generations, their susceptibility remained

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unchanged (Schaefer and Mulligan, 1991). Yet this chemical has seen almost no use against mosquitoes. We suspect that pyriproxyfen possesses unexploited potential as a mosquito larvicide, particularly in regards to new control approaches such as autodissemination.

Autodissemination is a novel concept that has abruptly altered the mostly academic interest in pyriproxyfen for mosquito control. First demonstrated in laboratory trials by Itoh (1994) and subsequently confirmed by Chism and Apperson (2003), Devine et al. (2009) demonstrated in field experiments in Peru that gravid female *Aedes aegypti* (L.) contaminated with pyriproxyfen transfer lethal concentrations to larval habitats. Devine and his coworkers demonstrated pyriproxyfen autodissemination as a promising new approach to manage container-breeding mosquitoes. In the Devine et al. (2009) study, dissemination stations lined with dusted (crushed pyriproxyfen granules) cloth were used for autodissemination against sentinel ovicups holding *Ae. aegypti* larvae, which produced 42–98% inhibition of adult emergence. Recently, Caputo et al. (2012) achieved considerable dissemination of pyriproxyfen from a station coated with powdered granules under field conditions. Thus, high coverage of larval habitats was achieved by treating a small area with a minute amount of the active ingredient.

Our study was designed to build on the pyriproxyfen autodissemination work of earlier researchers via operational research in which this simple concept might be utilized for area-wide control using conventional insecticide formulations and equipment. Foremost, our goal was to develop a practical application strategy by testing a commercially available product in the USA and delivered via conventional spray equipment by local mosquito control personnel. Second, we aimed to extend the autodissemination concept to additional mosquito species in the field, specifically the important container-inhabiting species *Ae. albopictus*. This mosquito is a peridomestic vector of dengue and chikungunya (Gratz, 2004) with diurnal and exophagic activity (Hawley, 1988) that reduces the outdoor activities of children (Worobey et al., 2013). Similar to *Ae. aegypti*, this container-inhabiting mosquito also exhibits 'skip' oviposition behavior in which multiple larval habitats are visited by the gravid female (Trexler et al., 1998), resulting in increased opportunities for chemical dispersal. Third, whereas Devine et al. (2009) deployed 'stations' to disseminate pyriproxyfen, we conducted broadcast sprays to adult resting and larval habitats. The central hypothesis was that gravid females contaminated either directly from the spray or indirectly by contacting contaminated surfaces would transfer pyriproxyfen to new larval habitats. We tested our hypothesis using commercial product of pyriproxyfen with conventional sprayers in an area-wide treatment of vegetation and a point-source treatment of a tire pile to know the direct impact and autodissemination efficacy over the time and autodissemination distance. Our study showed autodissemination of pyriproxyfen from treated sites to untreated sentinel oviposition sites under field conditions in area-wide and point-source treatment experiments, and pyriproxyfen can be delivered by mosquitoes up to 200 m distances.

2. Materials and methods

2.1. Test chemical and sprayer

We used a commercial pyriproxyfen product labeled for mosquito control in the U.S. (NyGuard® IGR concentrate -MGK® Corp., Minneapolis, MN, USA) containing 10% pyriproxyfen (2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine), formulated as an emulsifiable concentrate. In all point-source as well as area-wide field treatments, pyriproxyfen was applied as per manufacturer's recommendation (789.23 mL/ha).

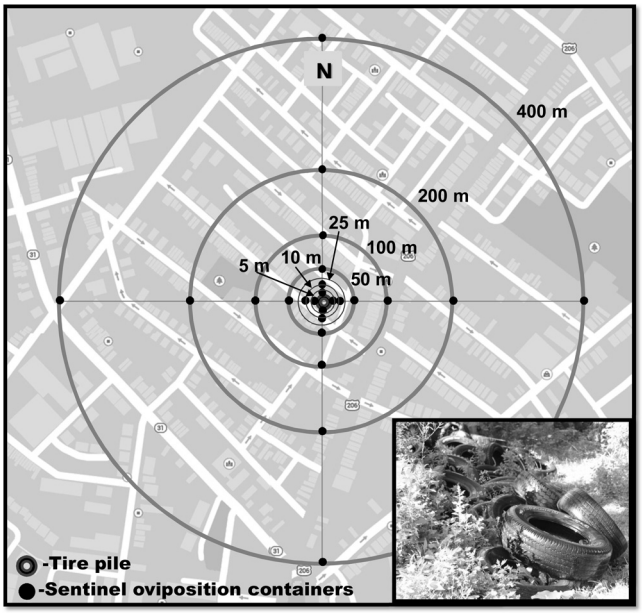

Fig. 1. Point-source treatment showing direct treatment of a tire pile (at the center) using a backpack sprayer, and autodissemination experiment setup at Trenton, NJ, USA. Four directional transects further show the locations of sentinel oviposition containers positioned from 5 to 200 m in 2010 and 25–400 m in 2011 to detect the extent of autodissemination distance. Inset shows tire pile consisting of >100 discarded automobile tires. (Map not to scale).

Stihl SR 420 backpack sprayers (Andreas Stihl Ag & Co. KG, Waiblingen, Germany) equipped with a mist blower conversion kit and tapered baffle screen was used for point-source treatment in 2010 and 2011 and for area-wide treatment in 2010. For point-source applications, the flow rate was 0.86 L/min at setting 3, which provided 84.6 μm VMD (volume median diameter) droplets. For area-wide field applications, the flow rate was 0.44 L/min (setting 2), which provided 74.7 μm VMD droplets. The higher setting for point-source applications was to enhance droplet penetration into stacked tires, whereas the lower setting for area-wide applications was intended to improve coating of surface areas to maximize mosquito–insecticide contact.

During 2011, area-wide pyriproxyfen application was made in a strip pattern with a truck-mounted single nozzle Cougar® ultra-low volume (ULV) cold aerosol sprayer (Clarke Mosquito Control, Roselle, IL). For best results, pyriproxyfen was applied from a vehicle moving at 8 Km/h, approximately perpendicular to the wind direction, using a swath width of 91.4 m. Spray equipment was adjusted for the VMD as 25–50 microns ($25\ \mu \leq Dv0.5 \leq 50\ \mu$) and 90% of the droplets were below 80 microns ($50\ \mu \leq Dv\ 0.9 \leq 80\ \mu$). Recognizing label restrictions, a 50 m buffer was provided to exclude natural water bodies from the treatment area. Only a single application was conducted for the experiment.

2.2. Point-source treatment

2.2.1. Study area

Dissemination of the chemical from a single localized treatment to larval habitats was tested by creating a point-source infestation. In mid-June 2010, a tire pile was constructed at the treatment site by placing 100 discarded vehicle tires in a mound up to a 1.2 m height and encompassing an area of 20.9 m^2 (Fig. 1). Only rainwater was available to provide larval habitat in the tires. Subsequent sampling demonstrated that the tires were soon colonized by *Ae. albopictus*. The point source treatment site was located in an abandoned lot (40.229130 lat., -74.764184 long.) within a residential area in Trenton, NJ. The control site was located in a commercial