EFFICACY OF GROUND ULV APPLICATION OF NYGUARD® (10% PYRIPROXYFEN) AGANIST AEDES ALBOPICTUS LARVAE IN ST. AUGUSTINE, FLORIDA

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ABSTRACT. Semi-field experiments were conducted on the efficacy of ground ultra-low volume (ULV) application of NyGuard Concentrate (10% pyriproxyfen) on adult emergence of *Ae. albopictus* larvae exposed as late third and early fourth instar. Direct contact was evaluated by direct exposure of pyriproxyfen to larvae in water collected when ULV conducted in the streets. Indirect contact was evaluated by removing treated vegetation from the streets and soaking the leaves in water for 24 hours before adding larvae in the laboratory. Both experiments indicated that ground ULV of NyGuard (10% pyriproxyfen) exhibited adult emergence inhibition >85%. Operationally, these results suggest that ground ULV application of 10% pyriproxyfen would suitably inhibit adult emergence of *Ae. albopictus*. We recommend that further testing be conducted so that this insect growth regulator can be labeled for area-wide ULV application as a larvicide.

Key Words:. *Aedes albopictus*, pyriproxyfen, larvicide, emergence inhibition, vegetation

I. INTRODUCTION

Aedes albopictus (Skuse) is an imported and common anthropophilic container inhabiting mosquito (O'Meara 1997) that has been found to be a competent disease vector in laboratory studies of more than 30 viruses including dengue. Because *Ae. albopictus* is a nuisance and competent vector of disease pathogens, control of this species is very important. One effective control method is to remove or empty water holding containers. However, for one reason or another, residents may be unwilling to remove or empty containers from their yards. If treatment with an insecticide is necessary, the abundance and sometimes small nature of individual containers impedes the ability of larvicides to reach every container, thus propagating mosquitoes. Treating containers using a ground ultra low volume (ULV) approach would not only cover more area but has the potential for better protection from nuisance and vector mosquitoes.

Through the years a wide range of larvicidal application methods against *Aedes spp.* have been evaluated (Ali et al. 1995, Sulaiman et al. 1997, Andrighetti et al. 2008, Gomez et al. 2011) including recent studies on the insect growth regulator (IGR) pyriproxyfen (Mulla 1991, Ali et al. 1995, Ali et al. 1997, Kono et al. 1997, Dhadialla et al. 1998, Sullivan 2000, Nayar et al. 2002, Chen et al. 2008, Invest and Lucas 2008). Currently, pyriproxyfen is not labeled for area-wide ULV application for mosquito control. However, this insecticide has been demonstrated to inhibit adult emergence of *Ae. aegypti* (L) after previous exposure as late third and early fourth instars at Anasta-

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sia Mosquito Control District (AMCD), St. Johns County (SJC), Florida our mission is to protect the public from mosquito-borne disease. Detailed evaluation of new and advanced methods of control must be conducted in order to efficiently protect the people of SJC. Here we report on the efficacy of experimental ground ULV applications of NyGuard® (10% pyriproxyfen) to control *Ae. albopictus* by first exposing the larvae to this larvicide in field and laboratory studies.

II. MATERIALS AND METHODS

NyGuard® EC (AI 10% pyriproxyfen) was used in all studies and was provided by the Navy Entomology Center of Excellence (NECE), Jacksonville, FL. Because this product was not labeled for area-wide ULV application against mosquitoes in Florida, this experiment was conducted in tandem with NECE, who received an experimental use permit from the Florida Department of Agricultural and Consumer Services, to apply truck-mounted ULV applications of this chemical. All experiments were conducted in St. Augustine, Florida, during August and September 2012. The treatment and control areas each consisted of forty hectares of residential neighborhood. The control area was 3.22 km away from the treatment site. Both sites represented a typical neighborhood setting with similar vegetation where *Ae. albopictus* is often found by AMCD inspectors. Three houses, in each area, were chosen on separate streets as the sites for the experimental evaluations. Houses were >500 m from each other.

A Cougar truck mounted cold ULV aerosol sprayer (Clarke® Mosquito Control, Roselle, IL) was provided by NECE. Three applications of undiluted pyriproxyfen were conducted by a licensed AMCD applicator. Treatments occurred on 29 August, 7 and 25 September 2012. The first application was made at a rate of 319 ml/min and a speed of 13 km/hour. Two passes were made for the first trial. The second and third trials were dispensed as a single pass at a rate of 532 ml/min and a speed of 8.04 km/hr.

Direct Exposure Study A 946 ml plastic container (Ace Hardware, St. Augustine, FL.), filled with 200 ml of reverse osmosis tap water (GE SmartWater™ Reverse Osmosis Filtration System, Fairfield, CN) and placed at one (i.e. primary) residence and another cup directly across the street and labeled accordingly. Containers were placed in the vicinity of similarly leafed vegetation. Plant leaves were collected in zip lock bags at each site to provide an indication of the effectiveness of the active ingredient on vegetation when it rains as well as runoff into standing water. Though the plant type varied from each of the experimental sites, every effort was made to collect leaves of similar size and shape. No plants species names or leaf surface areas were notated during this experiment.

Indirect Exposure Study Ten leaves (average 2 × 3 cm^2) from the treatment and 10 from control areas were removed and kept separately in zip lock bags and returned to the laboratory where they were immediately placed into 200 ml of reverse osmosis water in plastic containers. Leaves were soaked for 24 hours then removed from containers. Twelve cups were utilized for control and treated areas.

For both studies, immediately after application, treatment and control containers were brought back to the laboratory where ten late third/early fourth instar *Ae. albopictus* with average of 0.0749 g of food were added (Milk Bone™ Mini's Dog Treats, Del Monte Corporation). Larvae were previously reared from *Ae. albopictus* eggs obtained from the USDA-Agricultural Research Service, Center for Agricultural, Medical, and Veterinary Entomology, Gainesville FL in AMCD's insectary (insectary maintained at 25.5 ± 0.5°C, 70-80% RH, and a 16L:8D photoperiod). Adult emergence data was collected for 2 weeks where larvae pupated and emerged, or died in the controls. Thus, adult emergence inhibition was calculated at the conclusion of the experiment, using the following equation:

$$EI\ (\%) = ([A+D]/Total) \times 100$$

(EI = emergence inhibition, A = alive larvae, D = dead larvae, Total = 10 larvae per container). Abbott's correction was

Table 1. Mean percent emergence inhibition for direct and indirect contact of three ULV application sites of pyriproxyfen. Location of placement data corresponds with primary and across the street locations for each application.

	Direct Contact		Indirect Contact	
Placement Location	Mean ± SEM[1]	*P* value[2]	Mean ± SEM[1]	*P* value[2]
836 W 2nd St	90 ± 10.00	0.71	90 ± 5.77	0.50
Across from: 836 W 2nd St	95 ± 05.00		95 ± 05.00	
824 W. 13th St	87 ± 13.33	0.42	90 ± 10.00	0.42
Across from: 824 W. 13th St	100 ± 0.00		100 ± 0.00	
894 South Volusia	77 ± 14.53	0.50	88 ± 06.23	0.20
Across from: 894 South Volusia	79 ± 01.50		97 ± 03.33	

[1]Abbott's correction performed on data to account for emergence inhibition in controls.
[2]Paired t-test.

then performed on all data to account for emergence inhibition in controls (Abbott 1925). Paired t-tests were conducted on data to determine significant differences between location of containers (and associated vegetation) on each side of the street using GraphPad (GraphPad Software, Inc., La Jolla, CA.).

III. RESULTS AND DISCUSSION

At the primary and across the street locations no statistical difference in mean percent emergence inhibition occurred with regard to direct treatment of containers (88.3%) or indirect contact (93.4%) in water from treated vegetation for each neighborhood (Table 1, Figures 1 and 2). The lack of statistical difference could be attributed to an even distribution of pyriproxyfen. However, we note that emergence inhibition was above 85% and may reflect a concentration-dependent factor of our application. Chism et al. (2003) demonstrated, under laboratory conditions, that the EI_{95} for pyriproxyfen was 0.668 ppb, whereas our experiments applied undiluted 10% (AI) pyriproxyfen.

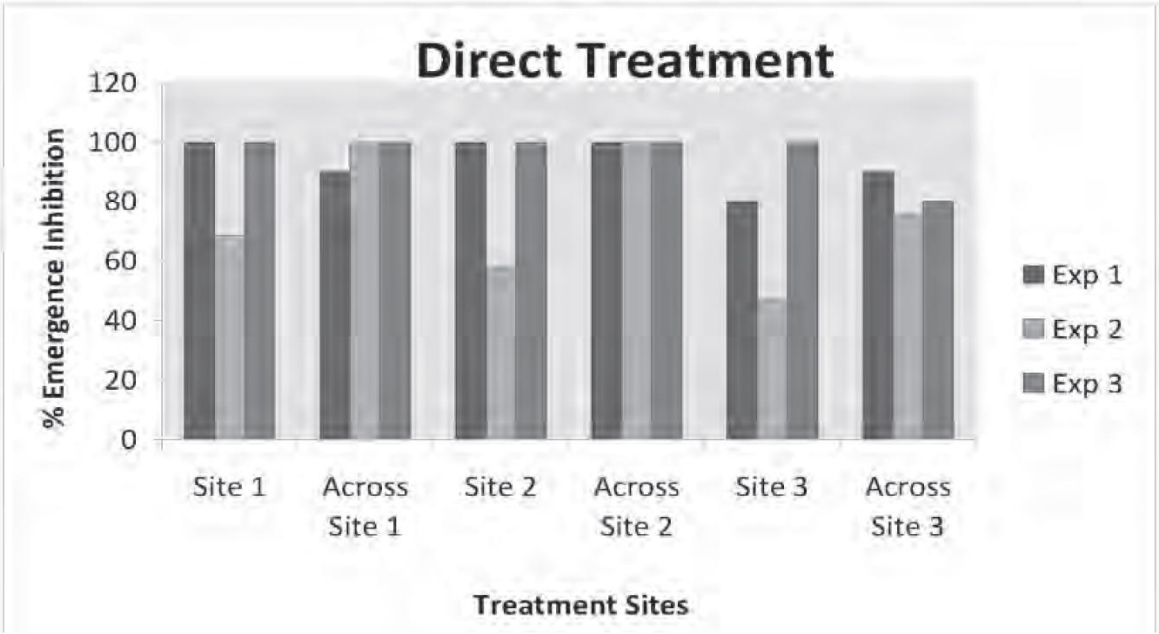

Figure 1. Mean percent emergence inhibition of adult *Ae. albopictus* (showing experimental variation) when directly exposed as 3rd-4th instar to water collected from the field where ground ULV applications of pyriproxyfen were conducted. The three sites coincide with the three houses chosen for the experiments. Abbott's correction performed on data to account for emergence inhibition in controls.

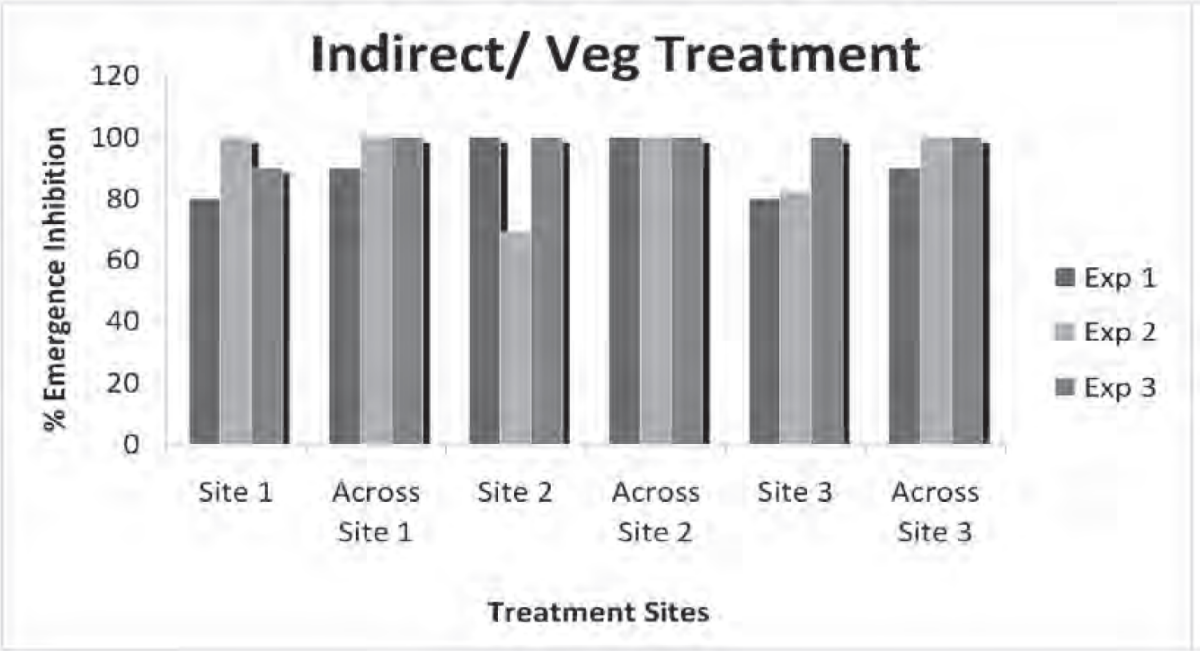

Figure 2. Mean percent emergence inhibition of adult *Ae. albopictus* (showing experimental variation) when exposed to ULV applications of pyriproxyfen as 3rd-4th instar indirectly in water from treated vegetation. The three sites coincide with the three houses chosen for the experiments. Abbott's correction performed on data to account for emergence inhibition in controls.

There are many studies that address the utilization of adult mosquitoes as a carrier of pyriproxyfen via horizontal transfer. Itoh et al. (1994) and Chism et al. (2003) exposed adult mosquitoes to surfaces that allowed them to pick up pyriproxyfen and horizontally transfer it to their next ovipostion point. Later, Gaugler et al. (2012) proposed the use of auto dissemination stations that utilize the ovipostion-seeking strategy of mosquitoes to disseminate pyriproxyfen into larval habitats. Although the study we conducted sought to discover the emergence inhibition efficacy of an ULV application of pyriproxyfen, there are strong implications that this application method could serve as a method to disperse this larvicide to larger areas than previously studied by adult transference. Our results strongly support that further research into ULV application of 10% pyriproxyfen for emergence inhibition of adult *Ae. albopictus* populations should be continued.

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