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IMPACT OF THE INSECT GROWTH REGULATOR PYRIPROXYFEN ON IMMATURE DEVELOPMENT, FECUNDITY, AND FERTILITY OF *AEDES ALBOPICTUS*

MEGAN N. RHYNE AND STEPHANIE L. RICHARDS[1]

Environmental Health Sciences Program, Department of Health Education and Promotion, College of Health and Human Performance, East Carolina University, 3403 Carol Belk Building, 300 Curry Court, Greenville, NC 27858

ABSTRACT. *Aedes albopictus* is a vector of several arboviruses; however, control of this day-active species is difficult with ultra-low-volume insecticide treatments applied at dusk/dawn periods. In the current laboratory study, blood-fed *Ae. albopictus* were exposed to Archer® (insect growth regulator AI: pyriproxyfen) residue in glass bottles (to approximate barrier treatment) and allowed to oviposit. Control mosquitoes were exposed to clean bottles. To evaluate potential dilution effects of water volume, mosquitoes were allowed to oviposit in (relatively) small (59 ml water) or large (177 ml water) containers. The extent to which fecundity (number of eggs laid), fertility rate (number of larvae hatched/number of eggs laid × 100), and emergence rate (number of adults emerged/number of larvae hatched × 100) differed between groups was characterized. In the control group, 18–21 (82–95%) mosquitoes laid eggs, while only 10–11 (45–50%) of mosquitoes in the pyriproxyfen group laid eggs. These sample sizes should be considered when comparing results to other studies. Significantly lower ($P = 0.0008$) fecundity was observed in mosquitoes exposed to pyriproxyfen (mean ± SE) (small container: 25.2 ± 7.1, large container: 24.3 ± 7.1) compared to control mosquitoes (small container: 49.2 ± 7.8, large container: 52.7 ± 5.2). Regardless of treatment, no significant differences in fecundity were observed between mosquitoes allowed to oviposit in different-sized containers. Hatch rate was significantly lower in the pyriproxyfen group and was impacted by container size ($P = 0.032$) and treatment ($P < 0.0001$) (large, control: 61.9% ± 7.8; small, control: 38.0% ± 7.1; large, treated: 10.3% ± 2.4; small, treated: 2.9% ± 1.9). Adult emergence rates were not significantly impacted by treatment or container size. Pyriproxyfen applied as a barrier treatment may be an effective tool for controlling *Ae. albopictus*.

KEY WORDS Insect growth regulator, life table characteristics, pyriproxyfen

INTRODUCTION

Increased insecticide resistance, precipitation, humidity, and elevated temperatures create favorable growth conditions for mosquitoes. Control methods should be evaluated to provide the most efficacious mosquito control. *Aedes albopictus* (Skuse) is an invasive mosquito and competent vector of arboviruses of public health concern (Paupy et al. 2009, Unlu et al. 2013). Reduction of oviposition sites, mosquito control, and avoiding mosquito exposure are the primary means of controlling mosquito-borne diseases (Chandel et al. 2016). Routine oviposition source reduction/removal of water-holding containers (e.g., tires, buckets, tarps, plant pots, bird baths) can help reduce populations of container-ovipositing mosquitoes, such as *Ae. albopictus* (Hawley 1988, Qualls and Mullen 2006, Yee 2008, Yee et al. 2012). Many mosquito control programs do not possess the manpower and/or financial resources to suppress *Ae. albopictus* effectively in peridomestic environments (Faraji and Unlu, 2016).

Insect growth regulators (IGRs) are effective in mosquito control due to their low mammalian toxicity and reduced risks to nontarget species (Mian et al. 2017). Insect growth regulators have modes of action such as juvenile hormone analogs, chitin synthesis inhibitors, and ecdysone agonists (Soin et al. 2010). Pyriproxyfen (IGR) mimics insect hormones to stop maturation; however, it is thought that pyriproxyfen is rarely toxic to adult insects (Hallman et al. 2015). Products containing pyriproxyfen can be mixed with adulticides (e.g., Demand® CS [AI: lambda-cyhalothrin] + IGR Archer® [AI: pyriproxyfen] used in barrier treatments for mosquito control). In a previous study involving *Ae. albopictus*, the efficacy of pyriproxyfen autodissemination stations was assessed in cryptic and open cups (250 ml water; Chandel et al. 2016). Autodissemination stations attract gravid female mosquitoes and subsequently contaminate mosquitoes with pyriproxyfen (Gaugler et al. 2012). As mosquitoes exit autodissemination stations, they transfer the pyriproxyfen to other oviposition sites, thereby providing control in multiple sites (Gaugler et al. 2012).

The central hypothesis in this pilot study is that pyriproxyfen (Archer, a product used in barrier treatments) impacts *Ae. albopictus* fecundity, fertility, and eclosion. The objective of these experiments was to characterize the extent to which this occurs under laboratory conditions. Although previous research has examined variation in oviposition behavior affected by IGRs, little work has evaluated impacts on these characteristics for this AI in *Ae. albopictus*.

[1] To whom correspondence should be addressed.

MATERIALS AND METHODS

Mosquito rearing

The eggs of an existing *Ae. albopictus* (generation F_{30} originally from Louisiana) colony were submerged in 16 plastic pans (24 cm × 36 cm × 5 cm) (to reach the target goal of 1,000 females) each containing 700 ml of dechlorinated water and fed liver powder. Pans were housed in an incubator at 28°C with 14h:10h (light:dark) cycle. Larvae were fed liver powder ad libitum for the duration of their growth cycle. Pupae were transferred to a (150 ml) plastic cup half-filled and placed into metal mosquito cages (30 cm^3) prior to adult emergence. Batches of pupae were transferred in this manner for approximately 5 days until all larvae had developed into pupae. Adults emerging in the cage were provided with a 20% sugar solution ad libitum.

Mosquito blood feeding

Four- to 5-day-old adult female *Ae. albopictus* were transferred to 8, 1-liter cardboard cages (125 mosquitoes/cage) with mesh screen and provided with a 20% sugar solution ad libitum. The sugar solution was removed and replaced with water 24 h before blood feeding to improve feeding rate. Mosquitoes were blood fed with a Hemotek Membrane Feeding System (Hemotek Limited, England) using a BG human scent lure to stimulate feeding. After a 1-h feeding period, mosquitoes were immobilized with cold, and fully engorged females were transferred to 2 separate 1-liter cardboard cages (50 mosquitoes/cage). Mosquitoes were transferred to a 28°C incubator and provided a 20% sugar solution ad libitum until further processing.

Pyriproxyfen exposure of blood-fed mosquitoes

Control (acetone only) and treatment (Archer solution made in acetone: AI pyriproxyfen) solutions were prepared. Field application label recommendations were used to determine the dose (7.49 g/liter) of Archer liquid stock used. Glass Wheaton bottles (250 ml) and lids were completely coated with either 1 ml acetone (control) or 1 ml of Archer stock (treatment). This means that each bottle was coated with 0.00749 g/ml of Archer stock (area inside bottle 99 cm^2, so 0.0000757 g Archer stock/cm^2). Glass bottles with their caps removed were placed on a bottle roller at 33 revolutions per minute until the contents evaporated (1–2 min), leaving a film of pyriproxyfen in the treatment bottles and clean control bottles. Once the bottle-coating procedure was completed, uncapped bottles were placed into a dark drawer to prevent light degradation and were used within 24 h.

Twenty-four hours after blood feeding, blood-fed mosquitoes ($N = 50$ for treatment, $N = 50$ for control) were transferred into prepared glass bottles for a 2-h exposure period. This was to approximate resting behavior after mosquito blood feeding. Twenty-five mosquitoes were placed in each respective bottle. Bottles were rolled 180° every 30 min (total of 3 times) to ensure mosquito tarsi were exposed as they would be on foliage in the environment when resting after a blood meal and/or while sugar feeding.

For both Archer and control groups, either small (59 ml) or large (177 ml) black plastic oviposition cups were hot glued into 1 liter cardboard cages with mesh as follows: 1) small ovicup, Archer, 2) large ovicup, Archer, 3) small ovicup, control, 4) large ovicup, control. A total of 88, 1-liter cardboard cages ($N = 22$/group) were created. Clear plastic ovicups (59 ml or 177 ml) were placed inside the black ovicups for ease of removal and counting larvae later. Ovistrips (dimensions of strip in small ovicup: 13 × 4.3 cm, dimension of strip in large ovicup: 18.8 × 5.4 cm) were placed in ovicups to ensure that roughly half of each strip was submerged in the water and the other half was above the water. Each cage was coded by treatment group and ovicup size. A single blood-fed mosquito was transferred to each cage and provided a 20% sucrose solution ad libitum. Liver powder was provided to larvae ad libitum. Six days after blood feeding, each adult mosquito was removed from its individual cage (noted as dead or alive), and its ovaries were dissected via forceps using a dissecting microscope to enumerate eggs that may have been retained. Egg strips were also retrieved from each cage, and eggs were counted to obtain the fecundity rate for each individual mosquito. Egg strips were dried and then placed back into the same coded (by specific cage number) ovicup and returned to its respective cardboard cage. At 6 and 12 days after egg strips were submerged, larvae were counted in ovicups to track fertility rates (number of larvae hatched/number of eggs laid) × 100. All adults (females and males) that emerged were killed by freezing, counted, and recorded for the duration of the study to examine the emergence rates (number of adults emerged/number of larvae hatched) × 100.

Statistical analyses

Data analyses were conducted using SPSS (IBM SPSS Statistics, Chicago, IL). Descriptive statistics were used to compute means, standard error, and standard deviation of each variable (fecundity, fertility, and eclosion). Analysis of variance (ANOVA) and a test of homogeneity of variances was conducted to determine the extent to which number of mosquitoes alive, fecundity, and fertility of exposed females, as well as adult emergence rates of progeny differed between treatments and ovicup sizes at 6 days posttreatment. A Bonferroni correction was applied to account for multiple comparisons.

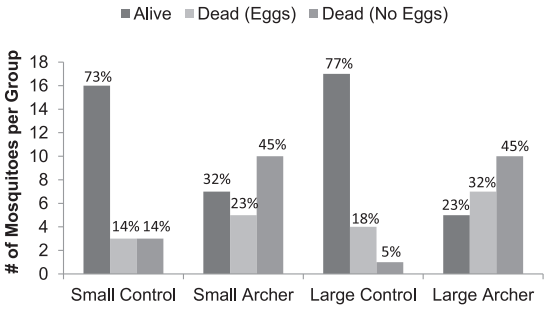

Fig. 1. *Aedes albopictus* adults that laid eggs or did not lay eggs 6 days after exposure to treatment (Archer or control bottles). Percentages shown within each group. Ovaries of mosquitoes (dead or alive) were dissected and eggs noted.

RESULTS

Laboratory simulated barrier spray exposure experiment

In the pyriproxyfen-exposed treatment group, $\leq$ 25% of blood-fed mosquitoes laid at least 1 egg, while $\leq$ 48% of blood-fed mosquitoes in the control group laid at least 1 egg (Fig. 1). Significantly lower ($P = 0.0008$) fecundity was observed in treatment mosquitoes that laid eggs in oviposition containers (mean $\pm$ SE), i.e., (small container: 25.2 $\pm$ 7.1 eggs; large container: 24.3 $\pm$ 7.1 eggs, compared to control mosquitoes (small container: 49.2 $\pm$ 7.8 eggs; large container: 52.7 $\pm$ 5.2 eggs) (Fig. 2). Regardless of treatment, no significant differences ($P > 0.05$) in fecundity were observed between mosquitoes allowed to oviposit in different-sized containers.

Hatch rate was significantly lower in the treatment group (compared to control) and was impacted by size of container ($P = 0.032$) and treatment (df = 3, F = 14.73, $P < 0.0001$) (large, control: 61.9% $\pm$ 7.8; small, control: 38.0% $\pm$ 7.1; small, treated: 2.9% $\pm$ 1.9; large, treated: 10.3% $\pm$ 2.4) (Fig. 3). Adult emergence rates were significantly lower in the treatment group compared to control group (df = 3, $F = 15.58$, $P < 0.0001$) and these rates were also impacted by the size of container and treatment (Fig. 4).

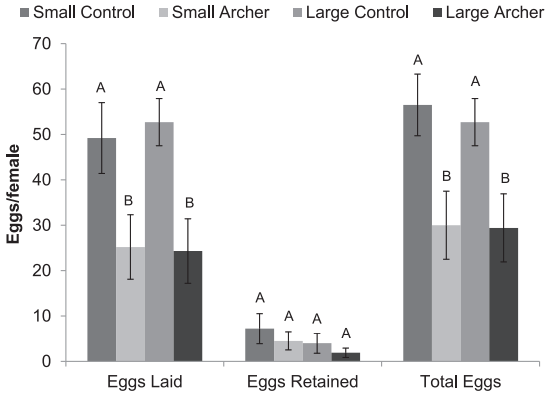

Fig. 2. Mean numbers ($\pm$ standard error) of eggs (fecundity) in *Ae. albopictus* mosquitoes exposed to Archer (AI: pyriproxyfen) compared to control group (acetone). Different letters above bars indicate significant differences within each group. The group noted as "eggs retained" includes eggs counted after ovary dissection.

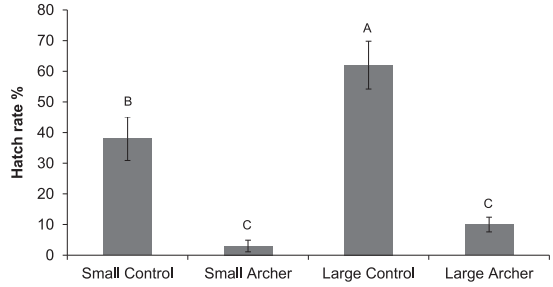

Fig. 3. Hatch rate (% $\pm$ standard error) of *Ae. albopictus* treatment group (Archer; AI: pyriproxyfen) compared to control group (acetone) in small (57 ml) and large (117 ml) containers. Different letters above bars indicate significant differences between groups.

DISCUSSION

Six days after the initial exposure to pyriproxyfen (24 h after blood feeding), more mosquitoes died and/or lived but produced no eggs (retained in ovaries or oviposited) in the treatment compared to control group (Fig. 1). The control group experienced little (25%) adult mortality postexposure, while the treatment group experienced high (73%) mortality. Sample sizes for each group should be considered when interpreting results of this laboratory study. We plan to repeat this experiment using *Ae. albopictus* and other species such as *Culex quinquefasciatus* Say because there will likely be variation between mosquito populations and species. This raises the possibility of using pyriproxyfen to control adult

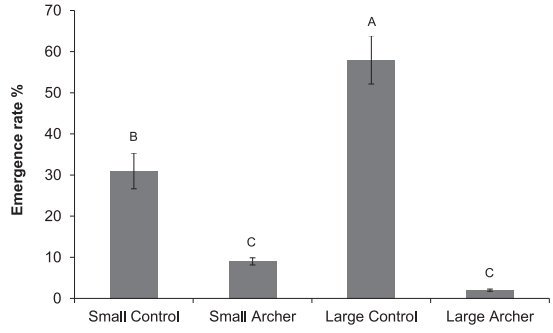

Fig. 4. Adult emergence in offspring of *Ae. albopictus* exposed to treatment (Archer; AI: pyriproxyfen) compared to control (acetone) in small (57 ml) and large (117 ml) containers. Different letters above bars indicate significant differences between groups.

mosquitoes. The impacts of pyriproxyfen exposure should also be tested at different time points before and after blood feeding. A study on *Anopheles arabiensis* Patton exposed mosquitoes to pyriproxyfen for 30 min 3 days after blood feeding, 1 day after blood feeding, 1 day before blood feeding, and 3 days before blood feeding (Harris et al. 2013). For the group (like the exposure timing in our study) exposed to pyriproxyfen 1 day after blood feeding, 100% reduction in fertility was observed (Harris et al. 2013). In the same study, the timing of the other pyriproxyfen treatments in relation to blood feeding did not seem to affect fertility or fecundity in *An. arabiensis*. Products that have varying levels of AI should also be tested.

Fecundity was significantly lower among treatment (exposed to pyriproxyfen) compared to control mosquitoes; i.e., mosquitoes in the control group had almost two times the number of eggs compared to the treatment group. Hence, pyriproxyfen exposure reduced fecundity under the conditions of this test. Fertility rate was significantly lower in the treatment group and was significantly impacted by the size of oviposition container (amount of water) and treatment. Adult emergence rates were higher among the control group compared to the treatment group (mother exposed to pyriproxyfen), and emergence was significantly higher among large containers in both control and treatment groups. This raises the question whether the concentration of pyriproxyfen transferred to the water by the female mosquito was affected by container size/water volume, i.e., larger volumes of water would potentially dilute the effect of pyriproxyfen on larval development. This should be quantified in future studies to understand how much pyriproxyfen is picked up during adult mosquitoes' initial exposure to treatments. This further evaluation is important since we also show differences in emergence rates between small and large containers in control mosquitoes that had not been exposed to pyriproxyfen. This may be expected as mosquitoes could be more attracted to lay eggs in accessible larger containers but would also depend on a variety of factors including nutrient availability and overcrowding by existing larvae.

Exposure to pyriproxyfen reduced fecundity, fertility, and subsequent adult emergence. However, not all mosquitoes exposed to pyriproxyfen experienced the same degree of reduction in immature development, fecundity, and fertility measured here. Comparisons should also be done to evaluate efficacy of pyriproxyfen for other mosquito species (e.g., *Cx. pipiens/quinquefasciatus*, *Ae. aegypti* [L.], *Ae. triseriatus* [Say, Thomas]). We expect variation in these relationships between species, populations, and under different environmental conditions.

These findings strengthen the assumption that temperature, rainfall, and abundance of containers in the landscape and other unknown factors, in part, drive *Ae. albopictus* abundance and could influence the efficacy of barrier adulticide treatments due to degradation of AI with environmental pressure and ubiquitous oviposition sources (Richards et al. 2008, VanDusen et al. 2016). There is likely variation in abundance of water-holding containers, influence of neighboring properties, and other unknown factors that were not assessed in the current laboratory study.

Based on the results of the current pilot study and other studies, pyriproxyfen may be a useful control method for some populations of *Ae. albopictus* or other mosquito species (e.g., *Ae. triseriatus*), especially where resistance to other AIs or cryptic oviposition sources are present (Chism and Apperson 2003, Harris et al. 2013, Faraji and Unlu 2016). Comparisons should be done to evaluate the efficacy of autodissemination stations, barrier treatments, and/or other methods of application for this AI. In addition, the size, level of organic content, occurrence/abundance of water-holding containers in the landscape could be assessed over the mosquito season to test the efficacy of pyriproxyfen at controlling mosquitoes in a variety of container types. The data gained from these studies can inform mosquito control operators about the efficacy of barrier treatments against *Ae. albopictus*. However, field assessments under a variety of environmental conditions must be carried out to test these laboratory-based results.

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REFERENCES CITED

Chandel K, Suman D, Wang U, Unlu I, Williges E, Williams G, Gaugler R. 2016. Targeting a hidden enemy: pyriproxyfen autodissemination strategy for the control of the container mosquito *Aedes albopictus* in cryptic habitats. *PLoS Negl Trop Dis* 10:1–15.

Chism BD, Apperson CS. 2003. Horizontal transfer of the insect growth regulator pyriproxyfen to larval microcosms by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory. *Med Vet Entomol* 17:211–220.

Faraji A, Unlu I. 2016. The eye of the tiger, the thrill of the fight: effective larval and adult control measures against the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae), in North America. *J Med Entomol* 53:1029–1047.

Gaugler R, Suman D, Wang Y. 2012. An autodissemination station for the transfer of an insect growth regulator to mosquito oviposition sites. *Med Vet Entomol* 26:37–45.

Hallman A, Bond C, Buhl K, Stone D. 2015. Pyriproxyfen general fact sheet. National Pesticide Information Center, Oregon State University Extension Services [accessed September 17, 2019]. Available from: http://npic.orst.edu/factsheets/pyriproxifen.html.

Harris C, Lwetoiiera DW, Dongus S, Matowo NS, Lorenz LM, Devine GJ, Majambere S. 2013. Sterilizing effects

of pyriproxyfen on *Anopheles arabiensis* and its potential use in malaria control. *Paras Vect* 6:144.

Hawley WA. 1988. The biology of *Aedes albopictus*. *J Am Mosq Control Assoc* 4:1–39.

Mian LS, Dhillon MS, Dodson L. 2017. Field evaluation of pyriproxyfen against mosquitoes in catch basins in southern California. *J Am Mosq Control Assoc* 33:145–147.

Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D. 2009. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. *Emerg Microbes Infect* 11:1177–1185.

Qualls QA, Mullen GR. 2006. Larval survey of tire-breeding mosquitoes in Alabama. *J Am Mosq Control Assoc* 22:601–608.

Richards SL, Ghosh SK, Zeichner BC, Apperson CS. 2008. Impact of source reduction on the spatial distribution of larvae and pupae of *Aedes albopictus* (Diptera: Culicidae) in suburban neighborhoods of a piedmont community in North Carolina. *J Med Entomol* 45:617–628.

Soin T, Swevers L, Kotzia G, Latrou K, Janssen C, Rouge P, Harada T, Nakagawa Y, Smagge G. 2010. Comparison of the activity of non-steroidal ecdysone agonists between dipteran and lepidopteran insects, using cell-based EcR reporter assays. *Pest Manag Sci* 66:1215–1229.

Unlu I, Farajollahi A, Strickman D, Fonseca DM. 2013. Crouching tiger, hidden trouble: urban sources of *Aedes albopictus* (Diptera: Culicidae) refractory to source-reduction. *PLoS ONE* 8:e77999.

VanDusen AE, Richards SL, Balanay JG. 2016. Evaluation of bifenthrin barrier sprays on foliage in a suburban neighborhood in eastern North Carolina. *Pest Manag Sci* 72:1004–1012.

Yee DA. 2008. Tires as habitats for mosquitoes: a review of studies within the eastern United States. *J Med Entomol* 45:581–593S.

Yee DA, Allgood D, Kneitel JM, Kuehn KA. 2012. Constitutive differences between natural and artificial container mosquito habitats: microorganisms, resources, and habitat parameters. *J Med Entomol* 49:482–491.