RESIDUAL ACTIVITY OF PYRIPROXYFEN AGAINST MOSQUITOES IN CATCH BASINS IN NORTHWESTERN RIVERSIDE COUNTY, SOUTHERN CALIFORNIA

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INTRODUCTION

For decades insect growth regulators (IGRs) have shown great efficacy against a variety of insect pests and disease vectors, coupled with low mammalian toxicity and reduced risk to aquatic nontarget species. As growth modifying substances, IGRs could be mimicking insect juvenile hormone (juvenoids, e.g., methoprene) or interfering with chitin synthesis in insects (substituted benzoylphenyl urea compounds, e.g., dichlobenil derivative, diflubenzuron, or triazine derivative, cyromazine), to name a few (Mian and Hendrick 2007) or technical methoprene against store-product pests (Mian and Mulla 1982b). Formulated products containing methoprene have been effectively used against various pests and disease vectors such as Altosid® against mosquitoes, Apex® against sciarid flies, Extinguish® in fire ant bait, Kabat® against the cigarette beetle, Minex® against the chrysanthemum leaf miner, Precor® against fleas, Strike® against chironomid midges, and Diacon® II (Hendrick 2007) or technical methoprene against stored-product pests (Mian and Mulla 1982a, 1982c, 1983, 1990). Like methoprene, the junevoid pyriproxyfen has been used for decades against a variety of pests of agricultural (Almond moth, San Jose scale), household (ants, cockroaches, and carpet beetles), and medical and veterinary (mosquitoes, flies, fleas, and ticks) importance (NPIC 1995). In earlier studies, pyriproxyfen (0.5G) resulted in a mediocre inhibition of adult emergence (IAE) of Culex peus (Speicer) (now Cx. stigmatosoma Dyar) and Cx. quinquefasciatus Say in dairy wastewater lagoons in southern California (Mulla et al. 1986). However, when it was applied at 0.1 kg to dairy wastewater lagoons in central California, pyriproxyfen provided complete control of Culex spp. for 7–68 days, and its residual activity lasted longer due to residue adsorption onto organic debris in wastewater lagoons (Mulligan and Schaefer 1990). Pyriproxyfen has also been reported to cause complete IAE of Cx. pipiens pallens (L.) and Cx. tritaeniorhynchus Giles for >3 wk in open containers and irrigation ditches at 0.01 ppm, cesspools at 0.05 ppm, and in sewers with wastewater at 0.1 ppm in Japan (Kamumura and Akutagawa 1991); it has also been reported to cause complete IAE of Aedes togoi (Theobald) in brackish water at 0.05 ppm in Korea (Lee 2001). In a field study, Webb et al. (2012) reported a complete control of the Australian salt-marsh mosquito, Ae. vigilax (Skuse), by a granular formulation of pyriproxyfen (Sumilav 0.5G) for 2 wk posttreatment with a high (28%) IAE in control, which they attributed to abnormally cool weather during the test. In autodissemination studies, Gaugler et al. (2012) reported 100% IAE of Ae. albopictus (Skuse) when exposed to pyriproxyfen-charged stations in cage trials and 81% IAE in small room trials. They also reported venereal transfer of pyriproxyfen from contaminated males to virgin female Ae. albopictus. More recently, Mian et al. (2017) found pyriproxyfen to provide 100% IAE of Cx. quinquefasciatus in...
catch basins in southern California for 3 wk. Beyond that time mortality in the treatments was overshadowed by high mortality in control catch basins, possibly due to autodissemination.

The objective of the present study was to evaluate the effectiveness of formulated pyriproxyfen for a yearlong control of immature mosquitoes in catch basins in northwestern Riverside County, southern California.

**MATERIALS AND METHODS**

**Insect growth regulators:** Pyriproxyfen (Nylar® 0.5G) provided by McLaughlin Gormley King Company (MGK), Minneapolis, MN, was tested at rates of 75 g, 100 g, 125 g, 150 g, and 175 g per catch basin and compared with S-methoprene (Altosid 30-day, 3.5% pellets, provided by MGK) at 3.5 g per catch basin for residual activity from July 2017 to June 2018.

**Field study:** The methodology used in this study was based on the MGK protocol for testing and evaluation of Nylar 0.5G versus Altosid pellets in catch basins by T. Janousek and D. A. Dame (unpublished). Pyriproxyfen was tested in catch basins within a 7-mile radius in the contiguous cites of Eastvale and Riverside, county of Riverside, CA. At the Riverside amusement park (33°54'10.52"N, 117°27'55.92"W) and nearby there were 52 catch basins, 10 of which each were treated with 75 g, 125 g, and 175 g of pyriproxyfen and 10 with S-methoprene pellets (Altosid 30-day formulation), and 12 served as controls. The catch basins varied in size from 18 in. (45 cm) in diam, 18 in. (45 cm) deep, with a 12 × 12 in. (30 × 30 cm) removable grate to 34 in. (85 cm) diam, 24 in. (60 cm) deep, with a 2424 (60 × 60 cm) grate with 0.75 in. (1.9 cm) openings between bars. The catch basins at the Eastvale shopping center parking lot (33°9'6.70"N, –117°55'43.46"W) ranged from 12 in. (30 cm) in diam and 18 in. (45 cm) deep to a 36 × 48 in. (90 × 120 cm) heavy grate and up to 6 ft deep (180 cm) with 1.5 in. (3.8 cm) openings between bars. The Eastvale site had 10 catch basins each treated with 100 g and 150 g of pyriproxyfen and 8 kept as controls, for a total of 28. While pyriproxyfen treatments were performed only once at the onset of the study, Altosid applications were repeated monthly or as needed per label directions for use.

Catch basins with observational history of the presence of mosquito developmental stages were selected with each catch basin examined for width, depth, and water level. All catch basins were sampled with a common dipper or a modified sampler and mosquito developmental stages (eggs, larvae, and pupae), and resting adults, if any, were recorded as pretreatment counts. Pyriproxyfen was tested at 5 application rates of 75 g, 100 g, 125 g, 150 g, and 175 g per catch basin. Each treatment was made to 10 catch basins by pouring the formulation onto the water surface inside the catch basin. S-methoprene pellets were applied to 10 catch basins at the rate of 3.5 g per catch basin. Twenty untreated control catch basins were set up at the beginning of the treatments and handled in the same way as the treated catch basins throughout the study period.

At weekly posttreatment intervals, each catch basin was sampled with 5 dips, starting with control catch basins first, followed by low to high treatments. Larvae and pupae were counted to determine dip averages. Separate dippers were used for treatments and control catch basins. The dipper was rinsed with water between treatments. At each sampling interval, pupae were collected from control and treated catch basins with the help of a pipette; they were placed on a filter paper in a Petri dish with a small quantity of water from the catch basin to keep the pupae alive. All pupae collected in the field were brought to the laboratory and reared to adults under laboratory conditions of 80°F (26.6°C), 40–60% RH, and 12:12 light and dark photoperiod. Adults emerging from pupae were identified to species, using the identification keys by Meyer and Durso (1998).

The efficacy of the IGRs was determined each week through 50 wk posttreatment, using the following formula:

\[ \text{IAE} = 100 - 100 \left( \frac{T}{C} \right), \]

where \( T \) is emergence in treatment and \( C \) is the emergence in control catch basins. A value of 100% means no adults emerged and 0% means successful emergence from all pupae. A given catch basin was to be eliminated from consideration if the IAE dropped to 70% or less, meaning that more than 30% of the pupae emerged as viable adults.

During the entire study period pertinent weather data (air temperature, water temperature, sky cover, rain, and wind) and other specific conditions or events, if any, were recorded.

At the end of the field study we also carried out a couple of tests to bioassay water samples from both control and treated catch basins against 4th-stage larvae of a laboratory-maintained strain of *Culex quinquefasciatus*. In the first test, water samples (100 ml each) were collected in Ziploc plastic bags, using a dipper/modified sampler; they were brought to the laboratory and transferred to 150 ml plastic cups. In the second test a week later, water samples were collected in the same manner after stirring the water with the dipper for 30 sec to allow for mixing up any pyriproxyfen residue. Along with the water from control catch basin, a distilled water control was also run. Each treatment or control cup received 25 early 4th-stage larvae of the test mosquito species. The larvae were provided food (rabbit chow with Brewer’s yeast [3:1]) every 3 days up to 10 days. All treatments and controls were replicated 4 times. The tests were maintained at room temperature (75°F [23.8°C]) in the laboratory. Mortality data were recorded to adult emergence up to 10 days.
Table 1. Mosquito species composition in catch basins in northwestern Riverside County, southern California from July 7, 2017, to June 29, 2018.

| Mosquito species     | Males | Females | Total | Percentage |
|----------------------|-------|---------|-------|------------|
| Culex erythrothorax   | 9     | 26      | 35    | 5.5        |
| Cx. quinquefasciatus  | 233   | 360     | 593   | 92.8       |
| Cx. stigmatosoma      | 1     | 2       | 3     | 0.3        |
| Cx. tarsalis          | 2     | 6       | 8     | 1.2        |
| Cx. thriambus         | 0     | 1       | 1     | 0.2        |
| Total                 | 245   | 394     | 693   | 100        |
| Percentage            | 38.8  | 61.2    | 100   |            |

Data analyses: Data obtained in the field study over 50 wk were arranged and statistically analyzed, using the appropriate test of significance (chi-square test; Steel and Torrie 1960). The data generated in laboratory bioassays were analyzed using the analysis of variance, and means were compared, using Duncan’s multiple range test (Duncan 1955).

RESULTS

Mosquito pupal collection from catch basins exclusively yielded Culex spp. Of the 693 adults emerging from pupal samples (Table 1), the predominant species was Cx. quinquefasciatus (92.8%), followed by Cx. erythrothorax (5.5%), Cx. tarsalis (1.2%), Cx. stigmatosoma (0.3%), and Cx. thriambus (0.2%). The female to male sex ratio was 61:39. As shown in Table 2, pyriproxyfen resulted in 83–100% IAE through 48 wk posttreatment beyond which its activity went down to zero at the Eastvale catch basins. However, like S-methoprene, pyriproxyfen activity at the Riverside treated catch basins (75, 125, and 175 g) still remained high (100% IAE) through the 50-wk-long study.

The data on water bioassay against mosquito larvae presented in Table 3 showed that treatment 100 g and 125 g, in the first test, exhibited identical activity, 48% and 47% IAE, respectively as compared to 8% in S-methoprene-treated samples. In the second test, samples from prestirred water in catch basins showed that the 2 highest treatments, 125 g and 175 g of pyriproxyfen, at the Riverside park still yielded mediocre activity of pyriproxyfen (0.5G) against Cx. quinquefasciatus and Cx. quinquefasciatus in dairy wastewater lagoons in southern California (Mulla et al. 1986). In a similar study, using 0.1 g of pyriproxyfen, Mulligan and Schafer (1990) reported complete control of Culex spp. in dairy wastewater lagoons in central California for up to 68 days. Elsewhere, in Japan, Kamimura and Arakawa (1991) found complete control of Cx. pipiens pallens and Cx. tritaeniorynchus in open irrigated ditches treated with 0.01 ppm of pyriproxyfen. In Korea, Lee et al. (2001) reported complete control of Ae. togoi in brackish water treated with pyriproxyfen (0.05 ppm). In Australia, a granular formulation of pyriproxyfen (0.5G) showed complete control of Ae. vigilax for 2 wk posttreatment (Webb et al. 2012). In a later study by Mian et al. (2017), pyriproxyfen (0.5G) at 10 and 50 g per catch basin resulted in 100% control of Cx. quinquefasciatus and other Culex spp. for 3 wk. In the present study, the control catch basins used were at least 500 ft away from a treated catch basin to avoid any chances of autodissemination. In the earlier study by Mian et al. (2017), the only mosquito species reported from catch basin samples was Cx. quinquefasciatus. The present data confirm Cx. quinquefasciatus as the leading species (92.8%), followed by Cx. erythrothorax (5.5%), Cx. tarsalis (1.2%), Cx. stigmatosoma (0.3%), and Cx. thriambus (0.2%).

The present study has shown that, depending on the application rate, pyriproxyfen could be effective for 50 wk against mosquitoes in catch basins treated with 75, 100, and 175 g per catch basin at the Riverside park; its activity lasted for 48 wk at 100 and 150 g per catch basin at the Eastvale site. This appears to be the first yearlong study on the longevity of pyriproxyfen in catch basins under actual field conditions. However, long-term studies of this type also face some challenges that may have played a role in reduced activity of pyriproxyfen at the Eastvale catch basins. The first of these challenges is the availability of sufficient numbers of active

DISCUSSION

Pyriproxyfen has reportedly shown efficacy against different mosquito species applied at variable rates to a variety of habitats. These results include a mediocre activity of pyriproxyfen (0.5G) against Cx. stigmatosoma and Cx. quinquefasciatus in dairy wastewater lagoons in southern California (Mulla et al. 1986). In a similar study, using 0.1 g of pyriproxyfen, Mulligan and Schafer (1990) reported complete control of Culex spp. in dairy wastewater lagoons in central California for up to 68 days. Elsewhere, in Japan, Kamimura and Arakawa (1991) found complete control of Cx. pipiens pallens and Cx. tritaeniorynchus in open irrigated ditches treated with 0.01 ppm of pyriproxyfen. In Korea, Lee et al. (2001) reported complete control of Ae. togoi in brackish water treated with pyriproxyfen (0.05 ppm). In Australia, a granular formulation of pyriproxyfen (0.5G) showed complete control of Ae. vigilax for 2 wk posttreatment (Webb et al. 2012). In a later study by Mian et al. (2017), pyriproxyfen (0.5G) at 10 and 50 g per catch basin resulted in 100% control of Cx. quinquefasciatus and other Culex spp. for 3 wk. In the present study, the control catch basins used were at least 500 ft away from a treated catch basin to avoid any chances of autodissemination. In the earlier study by Mian et al. (2017), the only mosquito species reported from catch basin samples was Cx. quinquefasciatus. The present data confirm Cx. quinquefasciatus as the leading species (92.8%), followed by Cx. erythrothorax (5.5%), Cx. tarsalis (1.2%), Cx. stigmatosoma (0.3%), and Cx. thriambus (0.2%).

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Table 2. Inhibition of adult emergence (% IAE)\(^1\) of *Culex quinquefasciatus* pupae exposed to various rates of pyriproxyfen along with 1 S-methoprene treatment in catch basins in Riverside County, southern California from July 7, 2017, to June 29, 2018.

| Week posttreatment | Pyriproxyfen | S-methoprene | \(\chi^2 (P = 0.05); \text{degree of freedom}\)\(^5\) |
|---------------------|-------------|--------------|-----------------------------------------------|
|                     | 75 g        | 100 g\(^3\)  | 125 g                                       |
| 1                   | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
|                     | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 2\(^6\)             | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 3                   | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 4                   | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 5                   | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 6                   | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 7                   | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 8                   | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 9                   | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 10                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 11                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 12                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 13                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 14                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 15                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 16                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 17                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 18                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 19                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 20                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 21                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 22\(^6\)            | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 23                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 24                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 25                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 26                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 27                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 28\(^6\)            | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 29\(^6\)            | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 30                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 31                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 32                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 33\(^6\)            | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 34\(^6\)            | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 35\(^6\)            | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 36                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 37\(^6\)            | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 38                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 39                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 40                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 41                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 42                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 43                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 44                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 45                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 46                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 47                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 48                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 49                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |
| 50                  | 100 (0/0)   | 100 (0/0)   | 100 (0/0)                                   |

\(^1\) Mean of ≥10 catch basins.
\(^2\) Each % IAE value is followed in parentheses by number of adults emerged in the treatment divided by number adults emerging in control.
\(^3\) The 75 and 150 g data belong to the Eastvale catch basins; all the other data (75 g, 125 g, 175 g, and methoprene) were generated at the Riverside catch basins.
\(^4\) *Significant difference in values \(\chi^2; P = 0.05\); *ns nonsignificant difference; *na analysis was done due to identical data values.
\(^5\) Not available—started a week later.
\(^6\) Some pupae in both control and treatment samples died due to heat during transportation to the lab.
\(^7\) Treated with 30-day Altosid pellets.
\(^8\) Inhibition of adult emergence could not be measured due to zero pupae in control due to probably cold and very dry conditions.
\(^9\) Immatures present in control.
catch basins of similar dimensions in 1 place. In this study, we were able to find catch basins sufficient to test 3 of the 5 pyriproxyfen rates (75 g, 125 g, 175 g) and 1 methoprene rate (3.5 g) plus the control (≈50 catch basins) at the Riverside park. The other 2 rates (100 g and 150 g per catch basin) plus control were tested in catch basins at the Eastvale shopping center within a 7 mi (11.2 km) radius. The catch basins at the latter site were scattered over the parking lot of the shopping center; they were bigger and deeper than those at the former site. The control catch basins that were much smaller were ~500 ft (1.5 km) distant around an artificial, recreational, and residential lake, Swan Lake. Variability in catch basins at the sites certainly had some impact on working around these structures. Moreover, the 2 sites were sampled on 2 different days of the week due merely to the high number of catch basins to be covered all in 1 day.

Sampling challenges in catch basins with removable grates at the Riverside site as well as those at Swan Lake (Eastvale) could easily allow sampling with the common mosquito dipper used by vector control personnel. On the other hand, the bigger catch basins at the Eastvale site with heavy and fixed grates had to be sampled with a modified sampler narrow enough to pass through the grate openings. The use of the modified sampler was certainly done out of necessity due to the size and structure of these catch basins and may have added to some difference in results.

Depending on the sources of runoff and weather conditions, a catch basin may not always carry sufficient water long enough to sustain mosquito developmental stages. All catch basins in the present study were carefully selected based on their previous history of having mosquito breeding according to the mosquito records maintained by the Northwest Mosquito and Vector Control District (NWMVCD), Corona, California.

| Test | Control | Pyriproxyfen | Methoprene |
|------|---------|--------------|------------|
|      | Distilled water | Catch basin | 75 g | 100 g | 125 g | 150 g | 175 g | 3.5 g |
| 1    | 72 b     | 77 b         | 77 b | 40 a | 41 a | 76 b | 74 b | 71 b |
|      | 6        | 0            | 0    | 48   | 47   | 1    | 6    | 8    |
| 2    | 40 b     | 40 b         | 67 c | 54 b | 13 a | 73 c | 21 a | 64 c |
|      | 0        | 0            | 0    | 67   | 0    | 47   | 0    | 0    |

1 Mean of 4 replicates (n = 25/treatment).
2 Means followed by the same letter in a row are not significantly different from one another (Duncan’s Multiple Range Test, P = 0.05; Duncan 1955).
3 51 wk posttreatment.
4 52 wk posttreatment.

Fig. 1. Weather data collected at each sampling week during the 50-wk-long study. Data include air temperature (°F), catch basin water temperature (°F), sky condition (0–4, clear to overcast), rain (in.), and wind (mi/h).
The other very important and most unpredictable challenge could be the act of nature such as inclement weather conditions. This study year appeared to be totally unpredictable, having weather patterns not experienced before such as sustained cold and dry conditions and erratic rainfall—a pattern not conducive to mosquito breeding. One monsoonal storm did wash way the contents of some of the catch basins at the Eastvale site. Our rainfall in southern California was about one-half of what we had the previous year (18 in. [4,890 mm]). Overall, mosquito numbers throughout southern California were reported low during the 2017–18 season.

In conclusion, this study shows that pyriproxyfen can be effective against mosquitoes inhabiting cryptic structures, e.g., catch basins for up to a year, depending on the application rate and prevailing ambient weather conditions.

It is recommended that future studies of this type should include bioassays of treated and control waters against lab-reared mosquitoes to corroborate the outcome of pupal sampling as stipulated in the present protocol.

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