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EFFECTIVENESS AND RESIDUAL ACTIVITY COMPARISON OF GRANULAR FORMULATIONS OF INSECT GROWTH REGULATORS PYRIPROXYFEN AND *s*-METHOPRENE AGAINST FLORIDA MOSQUITOES IN LABORATORY AND OUTDOOR CONDITIONS

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ABSTRACT. Effectiveness and residual activity tests of granular formulations of 2 insect growth regulators (IGRs), *s*-methoprene and pyriproxyfen, against laboratory-reared larvae of 5 colonized mosquitoes, *Aedes aegypti, Aedes albopictus, Aedes taeniorhynchus, Anopheles quadrimaculatus,* and *Culex nigripalpus,* were conducted in the laboratory and outdoors in plastic tubs. *Culex quinquefasciatus* was exposed to these two IGRs in the laboratory only. Each IGR formulation was applied at 0.02 and 0.05 ppm active ingredient (AI) against 5 of the 6 mosquito species both in the laboratory and the outdoor evaluations, whereas *Cx. quinquefasciatus* was exposed to 0.2 and 0.4 ppm AI of *s*-methoprene, and 0.1 and 0.2 ppm AI of pyriproxyfen in the laboratory. *s*-Methoprene at 0.02 and 0.05 ppm AI resulted in variable levels (<39–100%) of inhibition of adult emergence in the 5 species monitored for 6 weeks after treatment under both test conditions. *Aedes taeniorhynchus* was the most susceptible to *s*-methoprene in terms of initial and residual activity. *Culex quinquefasciatus* and *Ae. albopictus* were the most tolerant to *s*-methopene, with maximum emergence inhibitions amounting to 84% in *Cx. quinquefasciatus* at 0.4 ppm and 44.3% in *Ae. albopictus* at 0.05 ppm during the 1st week in the laboratory. Pyriproxyfen at comparable treatment rates to *s*-methoprene caused very high levels (>80–100% in most cases) of initial and residual emergence inhibitions of the tested species in the laboratory as well as outdoors. In several species, pyriproxyfen induced complete inhibition of adult emergence for several weeks after treatment, even at the lower rate of 0.02 ppm. The World Health Organization has recently recommended the use of pyriproxyfen for the control of some mosquito species at specified rates in certain habitats.

KEY WORDS Mosquitoes, *Aedes* spp., *Anopheles quadrimaculatus, Culex* spp., bioassay, pyriproxyfen, *s*-methoprene, granular formulations

INTRODUCTION

The insect growth regulator (IGR) pyriproxyfen is a juvenile hormone mimic that is highly active against a wide variety of insects of public health importance, including fleas, tsetse flies, houseflies, cockroaches, imported fire ants, chironomid midges, and mosquitoes (Hirano et al. 1998). Because of the reported excellent activity of the earlier formulations of pyriproxyfen against mosquitoes in numerous laboratory and field studies worldwide (Hirano et al. 1998), a granular formulation of this IGR containing 0.5% active ingredient (AI) was submitted by Sumitomo Chemical Company, Japan, to the World Health Organization Pesticide Evaluation Scheme (WHOPES) for testing and evaluation against mosquitoes. This paper contains the results of this evaluation conducted in Florida and simultaneously compares the results with the activity of a granular formulation of the IGR *s*-methoprene containing 1.5% AI. The IGRs were tested at 2 equivalent concentrations against laboratory-reared larvae of colonized mosquito species in the laboratory as well as in plastic tubs placed outdoors for their immediate effectiveness and long-term residual activity.

MATERIALS AND METHODS

Mosquito species: Laboratory-reared late 3rd- and early 4th-stage larvae of *Aedes aegypti* (Linnaeus) (colonized in 1982, Florida Medical Entomology Laboratory [FMEL], Vero Beach, FL), *Aedes albopictus* (Skuse) (colonized in 1992, Gainesville, FL), *Aedes taeniorhynchus* (Wiedemann) (colonized in 1965, FMEL, Vero Beach, FL), *Anopheles quadrimaculatus* (Say) (colonized in 1950, Malaria Research Center, Tallahassee, FL), *Culex nigripalpus* Theobald (colonized in 1997, FMEL, Vero Beach, FL), and *Culex quinquefasciatus* Say (colonized in 1997, FMEL, Vero Beach, FL) were used.

Test IGRs: Pyriproxyfen (Sumilarv 0.5% G, lot 5099X92, Sumitomo Chemical Co., Ltd., Osaka, Japan), supplied through WHOPES, and *s*-methoprene (Altosid® XR-G, 1.5% AI, supplied by Wellmark International, Dallas, TX) were tested.

Laboratory evaluations: These evaluations were conducted in white polyethylene trays (46 × 38 × 8 cm), lined with 2-mil-thick polyethylene sheets, containing 6 liters of well water and maintained at 25 ± 1°C in a rearing room (Nayar et al. 1998). One hundred late 3rd- and early 4th-stage larvae of a mosquito species were introduced into each tray along with 200 mg of larval food (6:1 yeast and beef liver powder). After 2–3 h of larval acclima-

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tion, the trays were randomly treated. Each IGR formulation was applied at 0.02 and 0.05 ppm AI against all mosquito species except for *Cx. quinquefasciatus,* which was exposed to 0.2 and 0.4 ppm AI of *s*-methoprene, and 0.1 and 0.2 ppm AI of pyriproxyfen. Because of the percent AI difference in the 2 test formulations, different amounts (by weight) of the 2 formulations were needed to achieve the required ppm AI concentrations of each IGR. Three replicates of each treatment and 3 untreated trays to serve as controls were maintained for each species. All trays were examined daily to score posttreatment larval and pupal mortality or survivorship, and adult emergence. A large pipette was used to collect live pupae and dead larvae and pupae from each tray on a daily basis. The live pupae were maintained in cups placed in mosquito cages to check for emergence. Survivorship in the treated and control trays was determined by counting the number of pupal skins remaining in the corresponding cups. After 1 wk, when all larvae and pupae had either died or survived as adults in the control trays, a fresh batch of 100 laboratory-reared late 3rd- and early 4th-stage larvae of the same mosquito species was introduced to each treated and control trays (if necessary, dead and live larvae and pupae were collected from the treated trays and discarded before the introduction of a 2nd batch of larvae) and routine daily posttreatment mortality and survivorship observations were continued. In this manner, 6 batches (weekly) of mosquito larvae of a species were introduced to the trays to determine residual activity of each formulation. Two hundred milligrams of mosquito larval food was added to each tray at the time of introduction of each new batch of mosquito larvae and on alternate days thereafter.

Outdoor evaluations: These evaluations were conducted in 1-m-diameter polyethylene tubs, lined with 2-mil-thick polyethylene sheets, containing 100 liters of well water (Ali et al. 1994). The tubs were maintained outdoors under a canopy (Sun canopy, Sunshield II, Powell & Powell Supply Company, Fuquay Varina, NC) to protect from rain and direct sunlight. The test procedures, treatment rates, and daily observations for the 2 IGR formulations against each mosquito species in the tubs were the same as described above for the laboratory trays, except that 1 g of the larval food was added to the tubs at the introduction of each batch of 100 mosquito larvae and on alternate days thereafter. Each tub was kept covered with fine-mesh plastic screen to protect from air-borne debris, wild insects, and any oviposition by wild mosquitoes.

Water temperature in the tubs was recorded throughout an evaluation. Four temperature-recording Onset® computers (32 K Waterproof Stowaway TidBit, Onset Computer Corporation, Pocasset, MA) were randomly submerged in 4 separate tubs. Minimum and maximum water temperatures in the tubs recorded daily ranged from 13.4 to 30.2°C during the span of these evaluations conducted between September 1999 and June 2000.

The efficacy of a formulation against a mosquito species in the trays and tubs was assessed as percent inhibition of adult emergence of the species in treatments, and adjusted for any larval or pupal mortalities in corresponding controls with the formula of Mulla et al. (1974):

$$\% \text{ inhibition of emergence} = 100 - 100(T/C),$$

where T is percent emergence in treated containers and C is percent emergence in control containers.

Mean percent reductions of adult emergence in each batch of mosquito species caused by the 2 formulations in trays and tubs were analyzed by 1-way analysis of variance with Tukey–Kramer multiple comparison posttests by using the computer software Instat V. 3.00 for Windows (Graphpad Software, San Diego, CA).

RESULTS AND DISCUSSION

Effectiveness and residual activity of the 2 IGRs against the test mosquitoes in the laboratory trays and in tubs outdoors are shown in Figs. 1 and 2, respectively. Pyriproxyfen at 0.02 and 0.05 ppm rates monitored for 6 wk after treatment induced almost 100% emergence inhibition of *Ae. aegypti* in the laboratory as well as in the tubs, whereas *s*-methoprene was less effective, reducing emergence of this species 22.3–93.7% in the laboratory and 10.3–100% in tubs, even at the higher rate of 0.05 ppm (Figs. 1a, 1b). The activity profile of *s*-methoprene for the 1st 2 weeks after treatment in the laboratory and 1 wk after treatment in the tubs at the high rate of 0.05 ppm was similar to that of pyriproxyfen, but thereafter, pyriproxyfen showed much higher levels of sustained residual activity against *Ae. aegypti* in both test systems. Itoh (1993) reported that a synthetic slow-release formulation of pyriproxyfen (0.05% AI) exhibited prolonged activity against larvae of *Ae. aegypti* even when the treatments were diluted by using and replenishing water in the treated jars.

The high rate of 0.05 ppm of *s*-methoprene produced a maximum of 44.3% (in laboratory) and 32% (in tubs) emergence inhibition in *Ae. albopictus* at 1 wk after treatment (Figs. 1b and 2b). In comparison, pyriproxyfen induced 52.7–100% (at 0.02 ppm) and 93–100% (at 0.05 ppm) emergence inhibition in the laboratory, and sustained 100% emergence inhibition in the tubs for 6 wk after treatment at both treatment rates. Pyriproxyfen against *Ae. albopictus* was distinctly superior over *s*-methoprene in terms of magnitude and duration of activity at equal concentrations of active ingredients of the 2 IGRs. These observations concur with those of the laboratory bioassay study of Ali et al. (1995), which study showed 21.5 times higher toxicity of pyriproxyfen against *Ae. albopictus* than

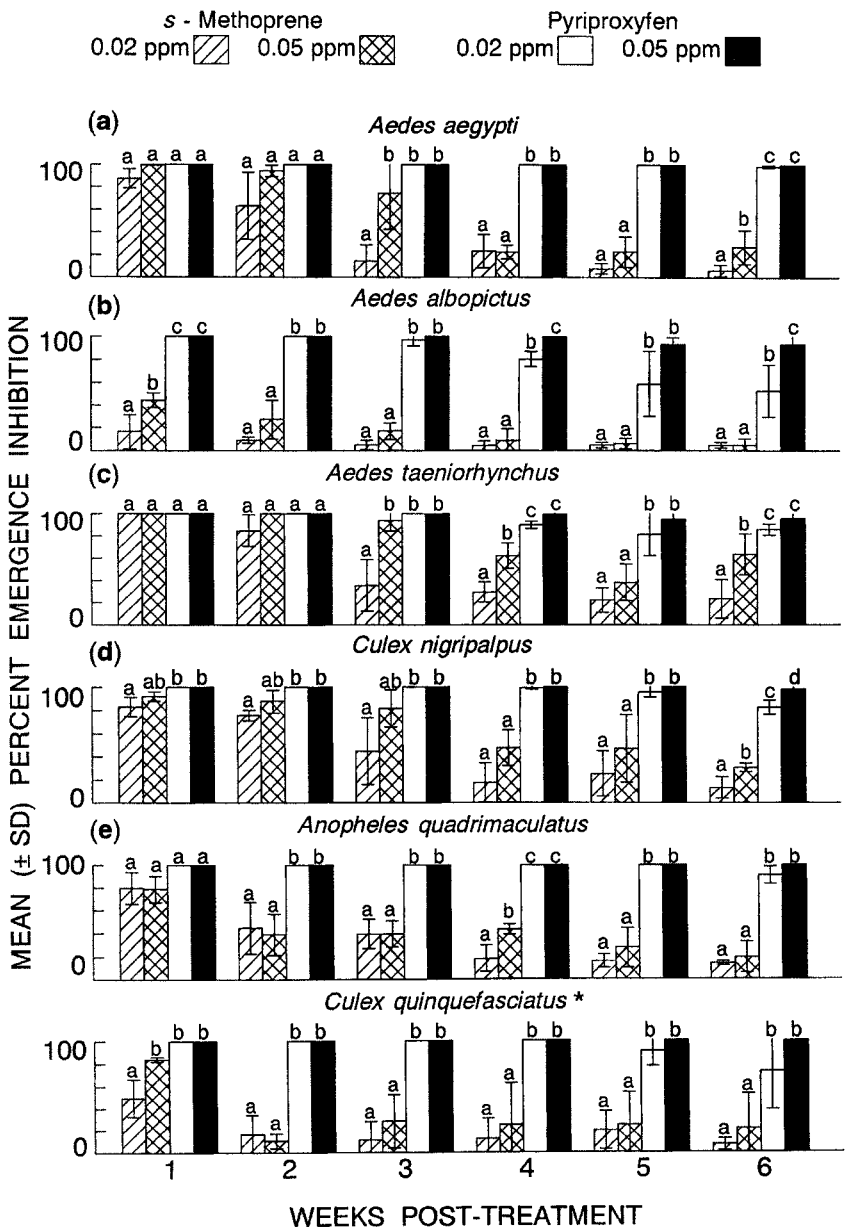

Fig. 1. Posttreatment mean ± SD percent inhibition of adult emergence of laboratory-reared larvae exposed to *s*-methoprene and pyriproxyfen in trays. The larvae of the 1st 5 mosquito species were exposed at 0.02 and 0.05 ppm active ingredient (AI) of each insect growth regulator (IGR). *Larvae of *Culex quinquefasciatus* were exposed to 0.2 and 0.4 ppm AI of each IGR. Means of percent adult emergence inhibition followed by the same letter are not statistically significant ($P > 0.05$).

of *s*-methoprene, when using the technical grade of each IGR.

Residual activity of *s*-methoprene against *Ae. taeniorhynchus* was generally of higher magnitude and duration in outdoor tubs than in the laboratory trays (Figs. 1c and 2c). The higher rates of *s*-methoprene did not consistently produce significantly higher levels of emergence inhibitions in the weekly observations, particularly in the tubs. Pyriproxyfen was superior over *s*-methoprene, producing higher levels of emergence inhibitions of *Ae. taeniorhynchus* at 0.02 ppm as well as at 0.05 ppm in the laboratory and outdoors, specifically during the 3rd to 6th weeks after treatment. No significant difference ($P > 0.05$) was found in emergence inhibition of *Ae. taeniorhynchus* between the low and the high rates of pryriproxyfen in both test systems. The previous laboratory study of Schaefer et al.

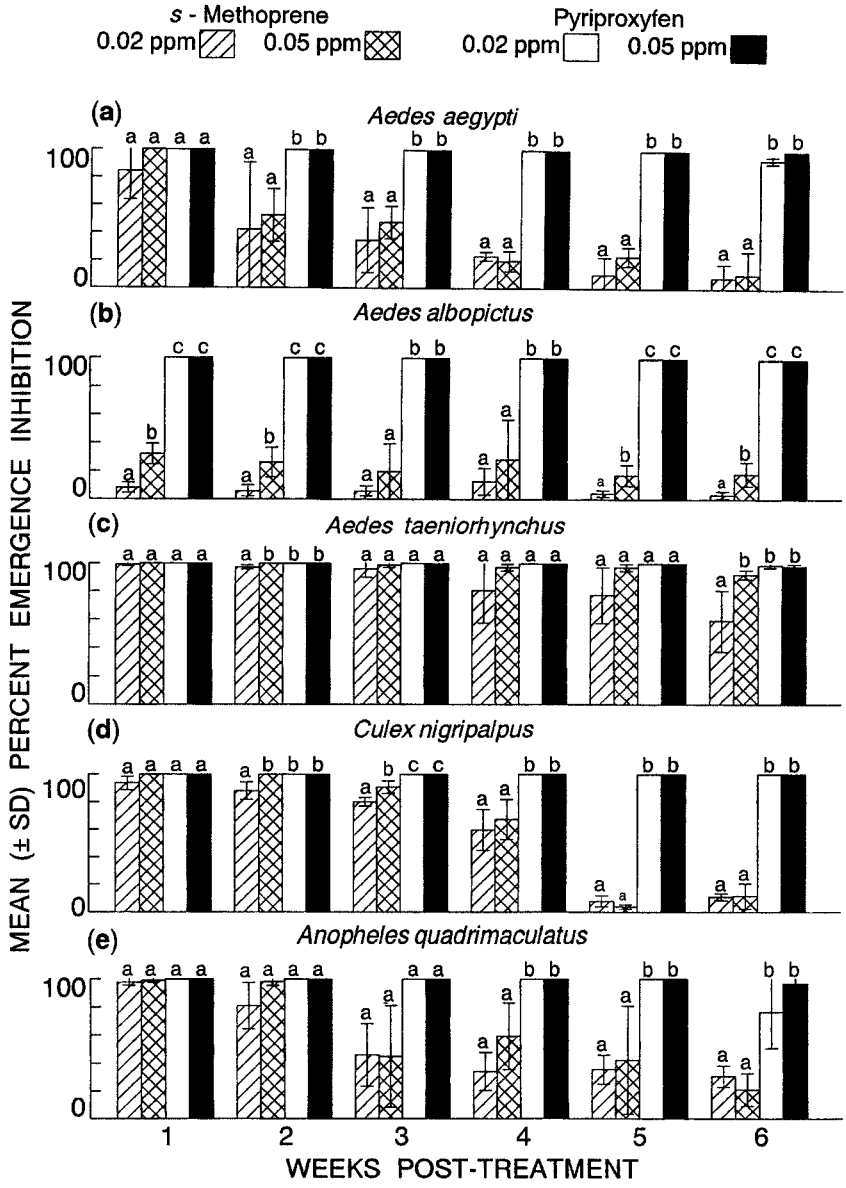

Fig. 2. Posttreatment mean ± SD percent inhibition of adult emergence of laboratory-reared larvae of 5 mosquito species exposed to *s*-methoprene and pyriproxyfen in tubs outdoors at 0.02 and 0.05 ppm active ingredient (AI) of each insect growth regulator. Means of percent adult emergence inhibition of a mosquito species followed by the same letter are not statistically significant ($P > 0.05$).

(1988), which showed pyriproxyfen median lethal concentration and 90% lethal concentration values of 0.01 and 0.052 ppb, respectively, against larvae of *Ae. taeniorhynchus,* is indicative of the excellent activity of this IGR against this mosquito species.

The activity of *s*-methoprene against *Cx. nigripalpus* lasted for 2–3 wk in the laboratory and for 3–4 wk in the tubs at both rates of treatment (Figs. 1d and 2d). In comparison, pyriproxyfen gave consistently superior results; even the lower rate of this IGR completely suppressed emergence of *Cx. nigripalpus* from the tubs during the 6 wk of observation.

Against *An. quadrimaculatus, s*-methoprene at 0.02 and 0.05 ppm in the laboratory as well as in the outdoor tubs caused notably lower levels of emergence inhibition than did pyriproxyfen used at same concentrations, although these differences were not statistically significant for the 1st week after treatment in the laboratory and up to the 3rd week after treatment in the tubs (Figs. 1e and 2e). The former IGR in the laboratory resulted in 14–

79.7% (at 0.02 ppm) and 19–78.8% (at 0.05 ppm) emergence inhibition of *An. quadrimaculatus* during the 1–6 wk after treatment, whereas pyriproxyfen at 0.02 ppm caused 91.3–100% emergence suppression (100% for the 1st 5 wk), and complete emergence suppression for 6 wk after treatment at the higher rate of 0.05 ppm. In the outdoor tubs, *s*-methoprene was highly effective against *An. quadrimaculatus* for only 2 wk after treatment, whereas pyriproxyfen at either rate of treatment induced a complete inhibition of emergence of this mosquito for at least 5 wk after treatment. The superior activity of S-31183 (pyriproxyfen) over *s*-methoprene against *An. quadrimaculatus* was reported previously by Estrada and Mulla (1986).

Culex quinquefasciatus was relatively more tolerant to *s*-methoprene compared to all other mosquito species tested. Even the high rate of 0.4 ppm of this IGR resulted in 84% emergence inhibition of *Cx. quinquefasciatus* at 1 wk after treatment, with activity rapidly declining in subsequent weeks of observation (Fig. 1). Pyriproxyfen at 0.1 ppm and 0.2 ppm (rates lower than *s*-methoprene) completely inhibited adult emergence of *Cx. quinquefasciatus* in the laboratory for at least 4–5 wk. Such activity of pyriproxyfen is compatible with reports of Adames and Rovira (1993) and Chavasse et al. (1995) showing good field control of *Cx. quinquefasciatus* with 0.5% granular and 10% emulsifiable concentrate formulations of pyriproxyfen used at rates of 0.025–0.1 pm AI of the IGR.

s-Methoprene and pyriproxyfen also were tested for effectiveness and residual activity against laboratory-reared larvae of *Ae. albopictus* and *Cx. nigripalpus* of the field strains in the laboratory trays only (data are not included here). The results showed a trend very similar to that observed for the larvae from the laboratory colonies of these species at the same treatment rates. Because methoprene ((*rs*)-methoprene and *s*-methoprene) has been used for mosquito control in Florida for the past 2 decades, the similarities of susceptibility of laboratory colonies to the field strains indicated a lack of any tolerance to *s*-methoprene developed by field populations of *Ae. albopictus* and *Cx. nigripalpus* in the Vero Beach area of Florida.

This study clearly demonstrated the superior activity of pyriproxyfen over *s*-methoprene, on basis of equal concentrations of the active ingredient, against a wide variety of mosquitoes in the laboratory and in experimental tubs placed outdoors. In a majority of cases, pyriproxyfen at the lower rate of 0.02 ppm induced complete inhibition of adult emergence of the tested species for several weeks after treatment. These results suggested that complete inhibition of adult emergence of these mosquitoes may be achieved with rates even lower than the 0.02 ppm AI of pyriproxyfen. The results of this study concerning the initial and residual activity of pyriproxyfen against mosquitoes are in complete agreement with those of several previous investigations employing pyriproxyfen (0.5% G) against mosquitoes in the genera *Aedes, Anopheles,* and *Culex* at rates ranging from 0.01 to 1 ppm AI in a variety of field situations (Kerdpibule 1989, Kamimura and Arakawa 1991, Okazawa et al. 1991, Thongrungiat and Kanda 1991, Adames and Rovira 1993, Kawada et al. 1994). Based on the present results as well as those of other WHOPES-sponsored pyriproxyfen studies and a thorough literature review of laboratory and field activity of pyriproxyfen against mosquitoes and aquatic nontarget organisms (Hirano et al. 1998, WHO 2001), WHOPES recently recommended the use of pyriproxyfen for the control of some mosquito species at specified rates in certain habitats (WHO 2001). Also recently, the Joint FAO/WHO Meeting on Pesticide Residues has considered that, because of extremely low toxicity of pyriproxyfen to mammals, pyriproxyfen GR can be safely added to drinking water at a rate of 0.01 mg AI/liter, for mosquito control (FAO 2001).

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