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Evaluation of effective period of a juvenile hormone mimic, pyriproxyfen, against *Aedes albopictus*: Preliminary experiments in the laboratory and the field.

Masahiro TAKAGI, Yoshio TSUDA and Yoshito WADA

Department of Medical Entomology, Institute of Tropical Medicine, Nagasaki University, 1−12−4 Sakamoto, Nagasaki 852, Japan

Abstract: Effective period of pyriproxyfen against *Aedes albopictus* was evaluated in the laboratory and the field. Experimental containers were treated with 3 different concentrations of pyriproxyfen and placed in the laboratory. In the field, the experimental containers were placed for 3 weeks, and then treated with the same concentrations as the laboratory experiment. Pupae were collected and the emergence of adults was examined every 2 weeks for 8 weeks. Pyriproxyfen was more effective in the laboratory than in the field. The change of water amount was one of the important factors determining the efficiency of pyriproxyfen in the field. No significant differences were observed in the number of collected pupae among concentrations in the laboratory, while smaller number of pupae were collected from the higher concentrations in the field. The duration for the complete inhibition of adult emergence at the highest concentration (0.1 ppm) was 4 and 6 weeks in the field and in the laboratory, respectively.

Key words: juvenile hormone mimic, pyriproxyfen, *Aedes albopictus*, Nagasaki

INTRODUCTION

Insect growth regulators have become an important tool for the control of mosquitoes, because of its high activity, less persistence in the environment and safety to a large number of nontarget organisms.

Efficacy of pyriproxyfen, a juvenile hormone mimic, has been evaluated against a variety of mosquitoes in the laboratory, and the concentration of 50% emergence inhibition (EI_{50}) was estimated for *Anopheles quadrimaculatus*, *Aedes aegypti*, *Culex quinquefasciatus*, and *Cx. tarsalis* (Estrada and Mulla, 1986; Mulla et al., 1986; Mulligan and Schaefer, 1990; Schaefer and Mulligan, 1991). The low values of EI_{50} showed the high activity of pyriproxyfen against these mosquito larvae.

To evaluate the efficacy of pyriproxyfen as a tool for vector control, not only the effective dosage but also the length of effective period should be examined. The duration of complete inhibition was evaluated at a dosage of 0.1 ppm as 5 wk and 2 months against *An.*

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farauti and *An. punctulatus*, respectively, in Solomon Island (Suzuki et al., 1989; Okazawa et al., 1991).

In this study the effective period of pyriproxyfen against *Ae. albopictus* was examined in the laboratory and in the field.

Materials and Methods

Laboratory Experiment: A blue plastic container (14cm in diameter and 17.5cm in depth) containing 1000ml of water was treated with pyriproxyfen and placed in the laboratory. Three different concentrations (0.01, 0.05, and 0.1 ppm) were examined and 5 replications were made for each concentration and the control. Fifteen or 20 mature larvae of *Ae. albopictus* from a laboratory colony were introduced into each container at 0, 2, 4, 6, and 8 weeks after the treatment. Pupae were collected every day for 1 wk and kept in a small vial with clean water. The number of emerging adults and dead pupae were counted. Before the introduction of larvae, the water depth was recorded and water was added to adjust the water amount. The maximum and minimum room temperature were recorded every day during the examination of adult emergence.

Field Experiment: The same plastic containers as in the laboratory experiment containing 1000ml of water were placed at the campus of Nagasaki University School of Medicine on 4, August, 1992. After 3 wk the numbers of larvae and pupae in each container were counted, then the containers were treated with pyriproxyfen at 3 different concentrations (0.01, 0.05, and 0.1 ppm). Five replications were made for each concentration and the control. Fifteen or 20 mature larvae were added and adult emergence was examined by the same way as in the laboratory experiment, except that water was added only when the water depth became <3cm to avoid the complete drying.

Statistical analysis was performed using the Systat statistical software package.

Result and Discussion

Average temperature and water depth were shown in Table 1. Temperature and water

Table 1. Average air temperature (s.d.) and water depth (s.d.) of the experimental container during the experiments.

Week	Temperature				Water depth (cm)	
	Laboratory		Field			
	Max	Min	Max	Min	Laboratory	Field
0	32.6 (0.8)	27.6 (1.4)	32.6 (2.0)	23.6 (1.5)	8.4 (0.3)	8.0 (0.2)
2	31.8 (1.3)	26.1 (1.9)	30.3 (2.2)	23.0 (2.1)	8.4 (0.4)	3.1 (0.6)
4	27.4 (1.7)	25.2 (0.7)	28.1 (1.7)	20.9 (2.7)	8.2 (0.2)	4.6 (1.5)
6	28.3 (1.6)	25.5 (0.9)	22.4 (1.6)	13.8 (1.2)	8.2 (0.2)	9.1 (4.3)
8	30.0 (3.0)	22.0 (0.6)	22.8 (1.8)	11.6 (1.7)	8.3 (0.3)	6.7 (2.8)

depth in the laboratory were kept rather constant. In the field, temperature was low at 6 and 8 wk after treatment and water depth changed greatly during the experiment.

Density of *Ae. albopictus* before the treatment of pyriproxyfen in the field experiment was calculated in Table 2. Variation of the number of larvae and pupae among experimental containers were analyzed by ANOVA and no significant differences in mean densities of larvae and pupae were observed.

The total number of pupae per container was calculated in Table 3. In the laboratory experiment, nearly all introduced mature larvae pupated and no significant differences in the

Table 2. Average numbers (s.d.) of immature *Aedes albopictus* counted in experimental containers before the treatment of pyriproxyfen.

	Pupae	4th	3rd	2nd	1st
0.1	7.0 (5.6)	22.2 (14.6)	3.8 (2.8)	2.6 (2.9)	5.8 (5.5)
0.05	3.2 (1.5)	18.4 (4.4)	8.0 (7.1)	1.2 (1.2)	1.2 (1.9)
0.01	1.6 (1.0)	20.0 (6.1)	5.0 (2.9)	2.2 (0.7)	0 (0)
Control	5.0 (3.4)	12.2 (3.9)	5.4 (5.4)	4.4 (5.5)	1.4 (2.0)
Total	4.2 (3.9)	18.2 (9.2)	5.6 (5.1)	2.6 (3.4)	2.1 (3.8)

Differences between means in the same column are not significantly different ($p<0.05$).

Table 3. Average numbers (s.d.) of pupae collected from experimental containers.

Laboratory Experiment

	Week				
ppm	0	2	4	6	8
0.1	15.0 (0)	14.8 (0.4)	19.8 (0.7)	20.0 (0)	16.6 (1.4)
0.05	14.8 (0.4)	14.8 (0.4)	19.4 (0.8)	20.0 (0)	18.2 (1.5)
0.01	15.0 (0)	13.4 (2.1)	20.0 (0)	20.0 (0)	18.6 (1.5)
Control	15.0 (0)	14.6 (1.2)	20.2 (0.4)	20.0 (0.9)	19.2 (0.7)

Field Experiment

	Week				
ppm	0	2	4	6	8
0.1	20.2 (3.7)	14.0 b (3.8)	13.0 (4.8)	12.4 b (1.4)	3.0 b (1.9)
0.05	18.4 (2.9)	26.2 ab (9.5)	26.0 (10.2)	17.6 ab (5.2)	10.0 ab (2.5)
0.01	22.4 (9.5)	28.2 ab (6.9)	25.6 (7.3)	18.8 ab (3.2)	14.2 a (4.4)
Cont.	14.8 (4.7)	28.8 a (7.8)	26.4 (7.1)	19.8 a (2.6)	19.0 a (7.3)

Means in the same column followed by the same letter or without letter are not significantly different ($p<0.05$).

mean number of pupae were observed among different concentrations. Therefore, mortality of larvae was negligible in the laboratory. On the other hand, the total number of pupae per container decreased with increasing concentration in the field experiment. The results of multiple comparison of means (Tukey HSD test) showed significant differences in the total number of pupae between control and the highest concentration at 2, 6, and 8 wk after the treatment. These results suggest that pyriproxyfen have a lethal effect on *Ae. albopictus* larvae in the field.

Estrada and Mulla (1986) observed that the 2nd-instar larvae of *Cx. tarsalis* and *An. quadrimaculatus* had higher susceptibility to pyriproxyfen than the 4th-instar larvae. As shown in Table 2, *Ae. albopictus* inhabited in the containers in the field, therefore the number of pupae in each container depended not only on the number of introduced mature larvae but also on the density of *Ae. albopictus* larvae naturally breeding in the container. The density of developing larvae at higher concentrations might be low because of the higher mortality of larvae, and this might be one of the reasons for the small number of pupae at the higher concentrations in the field.

The lower mean temperature around 17−18℃ at 6 and 8 wk in the field was another factor relating to the positive correlation between larval mortality and concentration in the

Table 4. Temporal changes in average emergence rates (s.d.) in 3 different concentrations of pyriproxyfen.

Laboratory Experiment

	Concentration (ppm)			
Week	0.1	0.05	0.01	Control
0	0.0 b (0)	0.01 b (0.03)	0.01 b (0.03)	0.99 a (0.03)
2	0.0 b (0)	0.0 b (0)	0.0 b (0)	0.90 a (0.08)
4	0.0 b (0)	0.02 b (0.04)	0.15 b (0.18)	1.0 a (0)
6	0.0 c (0)	0.0 c (0)	0.48 b (0.19)	0.95 a (0.03)
8	0.12 c (0.10)	0.21 bc (0.12)	0.48 b (0.12)	0.89 a (0.09)

Field Experiment

	Concentration (ppm)			
Week	0.1	0.05	0.01	Control
0	0.0 b (0)	0.0 b (0)	0.0 b (0)	0.91 a (0.14)
2	0.0 b (0)	0.0 b (0)	0.12 b (0.11)	0.98 a (0.03)
4	0.0 c (0)	0.0 c (0)	0.49 b (0.29)	0.99 a (0.03)
6	0.02 c (0.04)	0.25 bc (0.31)	0.55 b (0.24)	0.94 a (0.05)
8	0.10 b (0.13)	0.34 b (0.31)	0.82 a (0.13)	0.80 a (0.15)

Means in the same row followed by the same letter are not significantly different (<0.05)

field experiment. The mean developmental time from egg hatch to pupation is as long as 3 weeks at temperatures from 14 to 18℃, whereas at higher favorable temperatures, it takes around 10 days (Hawley, 1988). If larvae are exposed to pyriproxyfen for long period under lower temperature conditions, larval mortality is expected to be higher.

The complete inhibition of adult emergence was observed continuously for 6 and 4 weeks at 0.1 ppm in the laboratory and in the field, respectively (Table 4). The effective period of pyriproxyfen was longer in the laboratory than in the field in all concentrations. The greater changes in water amount in the field (Table 1) was considered to be the main reason for the shorter effective period in the field condition.

The breeding season of *Ae. albopictus* in Nagasaki, Japan is May−September (Mori and Wada, 1978). To inhibit adult emergence of this species by pyriproxyfen, the effective period of concentration at 0.1 ppm is not long enough. Therefore, field evaluations of the effective period of higher concentrations should be made throughout the breeding season in the next study.

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