Trop. Med., 39 (3/4), 89–93, December, 1997

A Field Experiment of the Emergence Inhibition of a Juvenile Hormone Mimic, Pyriproxyfen, Against *Aedes albopictus* in Nagasaki, Japan

Ronald E. MORALES*, Yoshio TSUDA and Masahiro TAKAGI

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

Abstract: A field experiment was conducted to examine the duration of complete inhibition of adult emergence of *Aedes albopictus* at 3 different concentrations of pyriproxyfen (0.1, 1, and 10ppm). Forty plastic containers with 1,000ml of water were placed as ovi-traps at the campus of Nagasaki University School of Medicine on 1 June 1993. Twenty-five ovi-traps were treated with 0.1, 1, and 10ppm of pyriproxyfen and 15 ovi-traps were left without treatment as the control. Pupae of naturally breeding *Ae. albopictus* were collected from the ovi-traps 3 times a week from June to October and the percentage of adult emergence was examined in the laboratory. The emergence of adults was inhibited completely during the whole study period at the highest concentration of 10ppm, whereas the complete inhibition of emergence was never observed at the lowest concentration of 0.1ppm. The negative correlation between the concentration of pyriproxyfen and the density of puape was observed in the whole experimental period, except the late October. The minimum concentration of pyriproxyfen that results in the complete inhibition of adult emergence of *Ae. albopictus* during the whole breeding season was estimated around 1ppm in the field conditions in Nagasaki.

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

INTRODUCTION

Efficacy of pyriproxyfen, a juvenile hormone mimic, has been evaluated against a variety of mosquitoes both in the laboratory and the field (Estrada and Mulla, 1986; Mulla *et al.,* 1986; Mulligan and Schaefer, 1990; Okazawa *et al.,* 1991; Schaefer and Mulligan, 1991; Suzuki *et al.,* 1989; Takagi *et al.,* 1995). Due to the high activity against mosquito larvae and safety to a large number of nontarget organisms, pyriproxyfen and other insect growth

Received for publication, January 12, 1998
Contribution No. 3495 from the Institute of Tropical Medicine, Nagasaki University
*Present address: Faculty of Science, Kagoshima University, 1–21–35 Koorimoto, Kagoshima 890, Japan
Correspondence: Dr. Yoshio Tsuda, Department of Medical Entomology, Institute of Tropical Medicine, Nagasaki University, 1–12–4 Sakamoto, Nagasaki 852, Japan

regulators have become an important tool for the vector control.

Although the length of effective period is an important character of insecticide as well as the effective dosage, only a few studies have been made for pyriproxyfen. The duration of complete inhibition was evaluated at a dosage of 0.1ppm as 5 weeks and 2 months against *Anopheles farauti* and *An. punctulatus,* respectively, in Solomon Island (Suzuki *et al.,* 1989; Okazawa, *et al.,* 1991). For *Ae. albopictus* the complete inhibition of adult emergence at a dosage of 0.1ppm was observed for 4 and 6 weeks in the field and in the laboratory, respectively, in Nagasaki, Japan (Takagi *et al.,* 1995).

The length of breeding season of *Aedes albopictus* differs among localities (Howley, 1988) and they breed from May through October in Nagasaki (Mori and Wada, 1978). There should be a minimum concentration of pyriproxyfen that inhibits the adult emergence completely during the whole breeding season. The present study was conducted to find out the minimum concentration of pyriproxyfen against *Ae. albopictus* in the field conditions in Nagasaki.

Materials and Methods

Forty plastic containers (blue color, 14㎝ in diameter and 17.5㎝ in depth) with 1,000ml of water were placed as ovi-traps at the campus of Nagasaki University School of Medicine on 1 June 1993. Three different concentrations of pyriproxyfen (0.1, 1, and 10ppm) were examined in this study. Fifteen ovi-traps were treated with 0.1ppm of pyriproxyfen on 1 June, and 5 ovi-traps each were treated with 1 and 10ppm on 17 June. Remaining 15 ovi-traps were left without treatment and used as control. Ovi-traps were examined 3 times a week to collect all pupae of *Ae. albopictus* naturally breeding in the ovi-traps. The number of pupae and the depth of water were recorded for each ovi-trap. The maximum and minimum air temperatures of the day were also recorded during the study period. The pupae from the same ovi-trap were collected into a plastic vial (3㎝ in diameter and 6㎝ in depth) with clean water and kept in the laboratory under the conditions of 27℃ and 70% R.H. to examine the percentage of emergence. The data from a half-month were pooled and analyzed. The percentage inhibition of emergence was corrected by Abott's formula. Statistical analysis was performed using the Systat statistical software package.

Results and Discussion

Temporal changes in the average water depth showed that the amount of water in ovi-traps was always large and no ovi-traps were dried up during the study period (Table 1). The annual rainfall in 1993 was exceptionally large (2,842㎜) and 1.44 times larger than the 12 years average (1981−1992) in Nagasaki city. Because of the excessive rainfall, an overflow of the water in ovi-trap was often observed in many ovi-traps in June, July and August. Therefore, the concentration of pyriproxyfen in treated ovi-traps might be affected largely.

Pupae were collected only July, August and early September in the highest concentration of 10ppm, and the emergence of adults was inhibited completely during the study period

Table 1. Average air temperatures (maximum, minimum and mean) and depth of water in ovi-traps placed at the campus of Nagasaki University School of Medicine from June to October 1993.

Period		Air temperature (℃)			Water depth (cm)	
		Max	Min	Mean	Mean	Range
June	early	28.3	19.5	23.9	7.4	6.1- 9.7
	late	27.6	19.3	23.5	12.1	5.6-15.2
July	early	28.4	20.6	24.5	13.7	7.4-15.5
	late	30.2	20.3	25.5	12.7	6.4-14.5
August	early	31.9	23.3	27.6	13.8	11.5-15.1
	late	31.6	21.7	26.7	13.7	11.1-14.8
September	early	30.5	18.9	24.7	13.0	11.8-13.9
	late	28.7	16.5	22.6	12.9	10.8-14.8
October	early	27.0	17.4	22.2	12.0	8.0-14.5
	late	21.7	10.2	16.0	10.3	6.0-13.1

Table 2. Temporal changes in the total numbers of pupae, emerging adults, and the percentages of emerging adults and inhibition of emergence* for *Ae. albopictus* observed in control and treated ovi-traps with 3 different concentrations of pyriproxyfen in Nagasaki 1993.

	June		July		August		September		October		Total
	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	
10ppm **(n = 5)**											
Pupae		0	1	7	11	14	1	0	0	0	34
Emerging adults		-	0	0	0	0	0	-	-	-	0
% emerging adults		-	0	0	0	0	0	-	-	-	0
% inhibition of emergence		-	100	100	100	100	100	-	-	-	100
1ppm **(n = 5)**											
Pupae		0	16	10	16	34	43	24	19	20	182
Emerging adults		-	0	0	0	0	1	0	1	5	7
% emerging adults		-	0	0	0	0	2	0	5	25	3.8
% inhibition of emergence		-	100	100	100	100	98	100	94	66	95.2
0.1ppm **(n = 15)**											
Pupae	0	41	134	88	190	279	182	90	92	40	1136
Emerging adults	-	3	3	6	51	136	136	53	62	29	479
% emerging adults	-	7	2	7	27	49	75	59	67	73	42.2
% inhibition of emergence	-	90	96	91	65	40	22	36	19	1	47.4
Control **(n = 15)**											
Pupae	0	64	118	87	189	193	133	82	89	26	981
Emerging adults	-	48	69	67	147	159	127	76	74	19	786
% emerging adults	-	75	58	77	78	82	95	93	83	73	80.1

*The percentage inhibition of emergence was corrected by Abott's formula.

(Table 2). On the other hand, the complete inhibition of emergence was never observed at the lowest concentration of 0.1ppm and pupae were collected nearly the whole experimental period. Even within 1 month after the treatment, 3 out of 41 larvae emerged as adults in late June. The overall percentage inhibition was calculated as 47.4.

At the intermediate concentration of 1ppm, pupae were collected from early June until late October. The complete inhibition of adult emergence was observed in June, July and late September. Although 1 adult emerged in early September, we concluded that the concentration of 1ppm resulted in the complete inhibition until late September and the inhibition rate started to decrease in October. Mori *et al.* (1981) showed that all eggs laid after October are in diapause and would not hatch until the diapause is broken in February of the next year. If the eggs laid after October will hatch in the next May and breeding places of *Ae. albopictus* will be treated with pyriproxyfen at the beginning of the next breeding season, we can expect the complete inhibition of adult emergence from the overwintering population. Therefore, the decrease in the inhibition rate of adult emergence in October may be negligible to control *Ae. albopictus* population in Nagasaki. Taking account of the heavy rainfall in 1993 and the egg diapause of *Ae. albopictus* in winter, the minimum concentration of pyriproxyfen required to inhibit the adult emergence completely during the whole breeding season was estimated around 1ppm in the field conditions in Nagasaki.

The peak density of pupae was observed in late August, except the concentration of 1ppm where the density of pupae was the highest in early September (Table 3). The negative correlations between the concentration of pyriproxyfen and the density of puape were observed in the whole experimental period, except the late October. The result of analysis of variance of average density of pupae per ovi-trap showed that the difference among concentrations was significant ($F=8.80$, $p<0.001$). The average density of pupae at 10ppm was significantly lower than that of 0.1ppm and the control (Tukey HSD test). The lethal effect of pyriproxyfen on *Ae. albopictus* larvae suggested by Takagi *et al.* (1995) may be the main reason for the observed difference of the density of pupae among different concentrations.

Table 3. Temporal changes in the average number of pupae of *Ae. albopictus* per ovi-trap from June to October 1993 in Nagasaki.

ppm	n	June		July		August		September		October		Mean*	SD
		Early	Late	Early	Late	Early	Late	Early	Late	Early	Late		
10	5		0	0.2	1.4	2.2	2.8	0.2	0	0	0	0.75 a	1.09
1	5		0	3.2	2.0	3.2	6.8	8.6	4.8	3.8	4.0	4.04 ab	2.52
0.1	15	0	2.7	8.9	5.9	12.7	18.6	12.1	6.0	6.1	2.7	8.41 b	5.23
Control	15	0	4.3	7.9	5.8	12.6	12.9	8.9	5.5	5.9	1.7	7.28 b	3.71

*Means with the same letter are not significantly different ($p>0.05$).

Acknowledgement

We thank Dr. Yoshito Wada, the former Professor of Department of Medical Entomology, Institute of Tropical Medicine, Nagasaki University, for valuable suggestions and comments during the course of the study. We are grateful to the JS Research Society, Japan Environmental Sanitation Center for supplying the test compound, pyriproxyfen.

References

1) Estrada, J. G. & Mulla, M. S. (1986): Evaluation of two new insect growth regulators against mosquitoes in the laboratory. J. Am. Mosq. Control Assoc., 2, 57−60.
2) Hawley, W. A. (1988): The biology of *Aedes albopictus.* J. Am. Mosq. Control Assoc. (Suppl.) 1, 1−40.
3) Mori, A., Oda, T. & Wada, Y. (1981): Studies on the egg diapause and overwintering of *Aedes albopictus* in Nagasaki. Trop. Med., 23, 79−90.
4) Mori, A. & Wada, Y. (1978): The seasonal abundance of *Aedes albopictus* in Nagasaki. Trop. Med., 20, 29−37.
5) Mulla, M. S., Darwazeh, H. A., Kennedy, B. & Dawson, D. M. (1986): Evaluation of new insect growth regulators against mosquitoes with notes on nontarget organisms. J. Am. Mosq. Control Assoc., 2, 314−320.
6) Mulligan Ⅲ, F. S. & Schaefer, C. H. (1990): Efficacy of a juvenile hormone mimic, pyriproxyfen (S-31183), for mosquito control in dairy wastewater lagoons. J. Am. Mosq. Control Assoc., 6, 89−92.
7) Okazawa, T., Bakote'e, B., Suzuki, H., Kawada, H. & Kere, N. (1991): Field evaluation of an insect growth regulator, pyriproxyfen, against *Anopheles punctulatus* on north Guadalcanal, Solomon Islands. J. Am. Mosq. Control Assoc., 7, 604−607.
8) Schaefer, C. H. & Mulligan Ⅲ, F. S. (1991): Potential for resistance to pyriproxyfen: a promising new mosquito larvicide. J. Am. Mosq. Control Assoc., 7, 409−411.
9) Suzuki, H., Okazawa, T., Kere, N. & Kawada, H. (1989): Field evaluation of a new growth regulator, pyriproxyfen, against *Anopheles farauti,* the main vector of malaria in the Solomon Islands. Jap. J. Sanit. Zool., 40, 253−257.
10) Takagi, M., Tsuda, Y. & Wada Y. (1995): Evaluation of effective period of a juvenile hormone mimic, pyriproxyfen, against *Aedes albopictus:* Preliminary experiments in the laboratory and the field. Trop. Med., 37, 87−91.