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Field evaluation of an insect growth regulator, pyriproxyfen, against *Culex pipiens pallens* and *Culex tritaeniorhynchus*

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Abstract: A synthetic chemical, 0.5% granules of pyriproxyfen (S-31183), which acts as a juvenile hormone, was tested against *Culex pipiens pallens* and *Cx. tritaeniorhynchus* under field conditions. This compound was extremely effective against larvae of both species which showed high resistance to organophosphorus insecticides. Complete inhibition of adult emergence continued for 3 weeks or more in open polyethylene containers and irrigation ditches at a dosage of 0.01 ppm, in cesspools at 0.05 ppm and in sewers with inflow of house wastewater at 0.1 ppm (AI). Activity of the compound was retained after a drying of the test site for several days. For evaluation of the effect of IGRs in the field, pupal isolates are preferable to larval isolates.

INTRODUCTION

By repeated applications of organophosphorus insecticides *Culex pipiens pallens* Coquillett and *Cx. tritaeniorhynchus* Giles, vectors of filariasis and Japanese encephalitis, respectively, became resistant to these insecticides and so their control is difficult now (*e.g.*, Kamimura and Maruyama, 1983; Maruyama *et al.*, 1984; Watanabe *et al.*, 1990). Recently some insect growth regulators (IGRs) have been synthesized and they are expected to control these resistant mosquitoes (Mian and Mulla, 1982). Pyriproxyfen, one of these IGRs, shows an activity like a juvenile hormone (Syafruddin *et al.*, 1990). This IGR exhibited a high level of activity against *Culex, Aedes, Psorophora* and *Anopheles* mosquitoes (Mulla *et al.*, 1986, 1989; Kawada *et al.*, 1988; Schaefer *et al.*, 1988; Suzuki *et al.*, 1989; Kerdpibule, 1989; Mulligan *et al.*, 1990; Ishii *et al.*, 1990). However, none of those experiments emphasized the efficacy of pyriproxyfen against organophosphorus-resistant mosquitoes.

In the present study we evaluated pyriproxyfen against highly organophosphorus-resistant mosquitoes, *Cx. pipiens pallens* and *Cx. tritaeniorhynchus* in the field.

MATERIALS AND METHODS

The compound evaluated was granular formulation of 0.5% pyriproxyfen (S-31183) which was provided by Sumitomo Chemical Co., Ltd., Osaka, Japan.

The following experiments were carried out in the suburbs of Toyama City (Kamimura, 1991).

A) Polyethylene containers with Cx. pi-

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piens pallens. We placed 15 polyethylene containers (40×60 cm by 15 cm deep) at an open site in the campus of Toyama Medical and Pharmaceutical University to allow *Cx. pipiens pallens* to breed naturally (Fig. 1). After the appearance of mosquito larvae and pupae, 3–4 containers each were treated with pyriproxyfen at dosages of 0.1, 0.01, 0.001 or 0.0001 ppm (AI) on July 23, 1986. Two containers for control remained untreated.

B) Cesspools with Cx. pipiens pallens. Two polluted waste-water cesspools (8×6 m by 0.1 m deep and 6×7 m by 2.51 m deep) in an open site beside a pig shed where many *Cx. pipiens pallens* inhabited were treated with pyriproxyfen at a dosage of 0.05 ppm (AI) on July 28, 1986.

C) Sewers with Cx. pipiens pallens. Four open sewers (10–30 m long, 0.3–0.5 m wide by 0.05–0.2 m deep) where many *Cx. pipiens pallens* inhabited were treated with pyriproxyfen at a dosage of 0.1 ppm (AI) on July 28 or August 4, 1986. In these sewers water was stagnant during the day, but inflow of house wastewater was observed every morning and evening.

D) Ditches with Cx. tritaeniorhynchus. A ditch (300 m long, 4.7 m wide by 0.4 m deep) surrounding a farmhouse and 2 irrigation ditches (2×5 m by 0.12 m deep and 2×10 m by 0.2 m deep) in agricultural land where many *Cx. tritaeniorhynchus* inhabited were treated at a dosage of 0.01 ppm (AI) on July 28 or August 4, 1986.

For evaluation of the compound, the pupal and larval isolate methods were utilized. Pupae were collected from the test sites one day before treatment and at intervals of 1–7 days after treatment till September 16, 1986 (Tables 1–3, Fig. 2). Duplicate samples of 25 pupae each were placed in a plastic cup filled with 100 ml deionized water, and maintained in the laboratory at 25–27°C until all pupae died or emerged.

When pupae were not collected in sufficient number, 3rd and 4th instar larvae were collected with field water. At site 5, 8 and 9 of Tables 2 and 3 few pupae were collected throughout the survey, so that only the larval isolate method was utilized. Twenty-five larvae each were placed in a cup with 100 ml of field water, from where the larvae were collected. They were fed powdered baby food (dried liver and vegetables from Yukijirushi Co., Ltd.). The observation method after pupation was the same as that in the pupal isolates. Mortality readings were taken daily, and dead larvae, pupae and adults were counted and removed. Adults which underwent incomplete emergence and larvae which died before pupation were counted as inhibited.

Fig. 1 Treatment of polyethylene containers in the campus with 0.5% pyriproxyfen granules.

Results

Water temperature in the test sites was 23–30°C and average air temperature was 21.0–29.9°C (mean 25.5°C) during the test period. It rained on August 4, 5, 6, 7, 22, 23 and 24 and September 3, 9 and 10 (Fig. 2). Total precipitation was 185 mm. In pupae from the control site and pretreatment samples from the test sites, mortality in the laboratory was observed at 0–4.5% (mean 1.5%; Tables 1–3).

A) Polyethylene containers with Cx. pipiens pallens

Adult emergence was completely inhibited for 40 days after treatment at a high dosage, 0.1 ppm. All larvae died before pupation in the containers treated at 0.1 ppm for 25 days after treatment. Sufficient number of pupae for evaluation first appeared 27 days after treatment. Due to depression of pupal population, further observation was not continued after then (Table 1, Fig. 2).

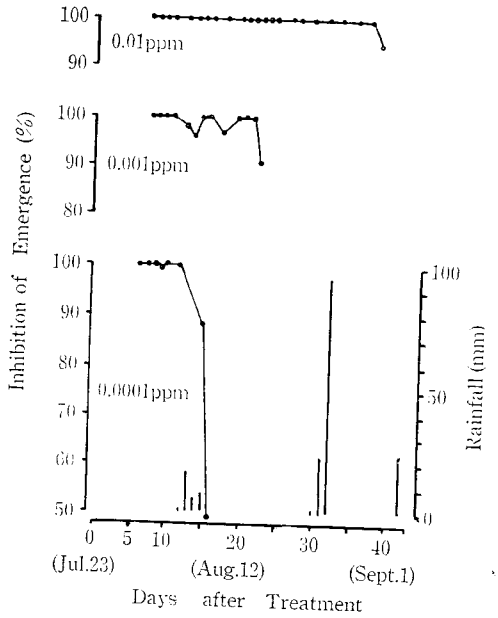

Fig. 2 Residual activities of 0.5% pyriproxyfen granules against *Culex pipiens pallens* in polyethylene containers and rainfall in Toyama.

Emergence was almost inhibited for 38 days and 22 days after treatment at 0.01 and 0.001 ppm, respectively, in spite of several days' rainfall.

Inhibition was almost complete for 12 days after treatment at a low dosage of 0.0001 ppm, but the rate decreased rapidly after rainfall during 13th–19th day after treatment.

B) Cesspools with Cx. pipiens pallens

Complete inhibition of adult emergence was produced during 4–43 days after treatment in the pupal isolates (Table 2).

Nearly half of inhibition for the first 2 weeks in larval isolates was larval death. Nevertheless, the inhibition rate was lower in the larval isolates than in pupal isolates. Some adults emerged normally 1 week after treatment in the larval isolates. After that, the rate of normal emergence was reduced to 0–2%, but the rate gradually increased within 36–50 days after treatment.

One cesspool (Site No. 2 of Table 2) dried up for 4 days from 22nd to 25th day after treatment, and then the water level recovered with rainfall. Third and 4th instar larvae appeared again 36 days after treatment, but the inhibition rate stayed at the 74–85% level during the period from 36 to 56 days after treatment.

Table 1 Residual activity of 0.5% pyriproxyfen granules as measured by inhibition of adult emergence against *Cx. pipiens pallens* in polyethylene containers.

Dose (ppm)	Days after treatment	Inhibition rate of emergence (No. samples)
0.1	27–40	100 (190)
0.01	7–37	100 (2,024)
	38	94.9 (39)
0.001	7–10	100 (1,758)
	12	97.7 (173)
	13	96.1 (51)
	14–15	100 (9)
	17	97.3 (73)
	19–21	100 (175)
	22	91.0 (100)
0.0001	6–8	100 (641)
	9	99.5 (201)
	10–12	100 (160)
	13	87.5 (8)
	17	11.0 (127)
	19	31.5 (124)
0 (control)	6–17	0 (163)
	19	0.5 (218)
	20–31	0 (253)
	33–38	0.6 (172)

C) Sewers with Cx. pipiens pallens

Complete inhibition was produced during 4–22 days after treatment in the pupal isolates. The inhibition rate in the larval isolates was over 80% within 22 days after treatment and then it decreased gradually (Table 2).

D) Irrigation ditches with Cx. tritaeniorhynchus

The inhibition rate in the pupal isolates was more than 98% during the period 4 to 22 days after treatment. The inhibition rate in the pupal isolates was 43–59% for the first 2 days after treatment. The same tendency was observed in the larval isolates. The rate rapidly dropped after rainfall on the

Table 2 Residual activity of 0.5% pyriproxyfen granules as measured by adult emergence against *Cx. pipiens pallens* in the 2 cesspools and the 4 sewers.

Dose (ppm)	Site	Isolation	Days after treatment													
			Pre-	0	1	2	4	7	8	11	15	22	29	36	43	50	56
Cesspool																	
0.05	1	Pupae	4.0	3.7	69.4	98.0	100	100	100	—	—	100	97.2	—	100	—	—
			(101)	(54)	(196)	(100)	(108)	(137)	(98)			(89)	(36)		(55)		
	2	Pupae	—	—	—	—	100	100	100	100	100	100	*	—	—	—	—
							(50)	(37)	(47)	(34)	(232)	(198)					
	1	Larvae	—	—	—	—	—	—	—	—	100	—	—	79.4	—	46.8	—
											(34)			(97)		(94)	
	2	Larvae	—	—	—	—	—	87.2	97.9	—	—	—	*	85.3	78.8	80.0	73.7
								(39)	(85)					(75)	(113)	(25)	(38)
Sewer																	
0.1	3,4	Pupae	—	—	100	—	—	100	—	—	100	100	—	—	—	—	—
					(5)			(64)			(159)	(172)					
	6	Pupae	3.1	9.3	33.3	97.7	99.0	97.5	86.7	—	—	100	—	—	25.4	20.4	—
			(98)	(54)	(6)	(85)	(101)	(40)	(15)			(227)			(67)	(54)	
	3	Larvae	4.5	95.2	—	93.5	95.7	—	90.9	—	90.6	88.9	71.1	75.3	—	—	—
			(94)	(21)		(46)	(46)		(22)		(85)	(45)	(76)	(81)			
	4	Larvae	0	—	85.4	97.2	93.7	80.0	—	—	—	—	79.3	91.9	58.1	55.2	—
			(36)		(41)	(36)	(63)	(40)					(58)	(99)	(105)	(29)	
	5	Larvae	3.4	100	100	100	100	—	94.5	—	95.0	90.5	64.5	—	—	—	—
			(29)	(28)	(36)	(52)	(69)		(55)		(121)	(63)	(76)				
	6	Larvae	—	—	61.5	—	—	95.7	87.5	—	86.6	—	67.9	31.1	—	—	—
					(39)			(46)	(16)		(142)		(78)	(132)			

( ): No. of samples. *: Dried up.

Table 3 Residual activity of 0.5% pyriproxyfen granules as measured by adult emergence against *Cx. tritaeniorhynchus* in the 3 ditches.

Dose (ppm)	Site	Inhibition	Days after treatment										
			Pre-	0	1	2	4	7	15	22	29	36	43
0.01	7	Pupae	0	42.9	59.0	86.7	100	100	98.2	100	—	—	—
			(86)	(21)	(78)	(150)	(91)	(157)	(109)	(5)	—	—	—
	7	Larvae	1.8	100	—	—	—	—	—	—	74.4	52.0	32.0
			(55)	(23)							(82)	(75)	(25)
	8	Larvae	2.1	2.0	95.7	97.5	97.2	97.3	73.9	92.9	83.3	37.8	40.7
			(48)	(50)	(47)	(40)	(36)	(37)	(92)	(85)	(60)	(111)	(113)
	9	Larvae	—	100	100	100	100	97.8	*	92.0	21.1	16.2	—
				(25)	(29)	(26)	(49)	(45)		(25)	(104)	(74)	

( ): No. samples. * Dried up.

29th day after treatment, both in pupal and larval isolates (Table 3).

One irrigation ditch (Site No. 9 of Table 3) dried up for 4 days, August 15–18, and then the water level recovered with rainfall. The inhibition rate in larval isolates after the dry-up was still 92% 22 days after treatment, but the rate decreased rapidly after rainfall during 29–36 days after treatment.

Discussion

Pyriproxyfen effectively inhibited adult emergence of *Cx. pipiens pallens* and *Cx. tritaeniorhynchus* for more than 3 weeks in ditches with wastewater inflow at a dosage of 0.1 ppm and in those with stagnant wastewater of 0.01 ppm. This IGR produced complete inhibition of emergence of *Cx. pipiens pallens* for rainless 12 days in the polyethylene containers without mud on the bottom at a very low dosage of 0.0001 ppm, but the effect decreased rapidly when the concentration was diluted by rain water (Fig. 2).

The high effect of granular formulation was retained in the cesspools and irrigating ditches after drying up for several days in the course of the experiments.

However, the effective component did not remain in the bottom mud for a long period when an emulsion was sprayed (Schaefer *et al.*, 1988). We thought that granules of this IGR sank to the bottom, and the effective component was slowly released; thus, the effective component remained in the substratum for a long time.

It is already confirmed that emergence inhibition rates were higher in pupal isolates than in larval isolates (Mulligan and Schaefer, 1990). The same results were obtained in the present study. Concentration was expected to be higher in the bottom than the upper water for a long time when granular formulation was applied. This IGR is active against the larval stage, and inactive against pupae (Hatakoshi *et al.*, 1987). The age sensitivity of larvae differs in instars (Estrada and Mulla, 1986). Through eating bottom mud and/or floating fine particles containing the active component, larval midguts and Malpighian tubes are destroyed (Syafruddin *et al.*, 1990). Therefore, field-collected pupae might contact higher concentration on the bottom through touch and/or eating mud at their larval stage. In the larval isolate only the field-collected water was used, so larvae could not contact the bottom mud at the stage they were sensitive to the chemical. To determine the effect of IGRs in the field, pupal isolates are stable and are preferable to larval isolates. In the plots treated at high dosage of 0.05–0.1 ppm, many larvae died and pupal populations were depressed, and so the actual mortality in the test sites at high dosages is expected to be higher than that obtained in the pupal isolates.

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摘　要

アカイエカ，コガタイエカに対する昆虫成長制御剤ピリプロキシフェンの野外効力試験

幼若ホルモン様活性を示すピリプロキシフェン（S-31183）0.5%粒剤の，有機燐剤抵抗性を呈すアカイエカ，コガタイエカに対する野外効力試験を行った．その結果，開放的なポリ容器と排水溝では，0.01 ppm，停滞した汚水槽では 0.05 ppm，流れのある下水溝では 0.1 ppm の散布で，3 週間以上ほぼ完全に羽化を阻害した．発生源が一時的に干上がっても，その後も効力が持続した．採集蛹での羽化阻害成績は，実際の死亡率を過少評価しやすいが，安定していて，幼虫と発生源の水を用いた成績よりもすぐれている．