Laboratory and Field Evaluation of Methoprene and Its Slow-Release Formulation, Altosid® 10F, against the Sprinkler Sewage Filter Fly, *Psychoda alternata* SAY (Diptera: Psychodidae)

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(Received 16 March, 1992; Accepted 21 October, 1992)

The effects of methoprene (isopropyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate) and its slow-release formulation, Altosid® 10F containing 10% methoprene, were evaluated against *Psychoda alternata* both in laboratory and field conditions. Methoprene was effective on *P. alternata*, causing larval death, pupal death, failure of emergence, and abnormal adults. The IC₅₀ values of methoprene and its slow-release formulation were 0.0014 µg/ml and 0.0023 µg/ml, respectively. Three seconds submersion of the 4th instar larvae in this slow-release formulation (3.9 µg/ml methoprene) produced a marked emergence inhibition effect. A field test was conducted against a natural population of *P. alternata* in a 10-person septic tank in Naruto City. After the introduction of 2.5 g of this slow-release formulation into the tank, the adults of *P. alternata* disappeared 1 week after the treatment for a period of over two months.

Key words: *Psychoda alternata*, Altosid® 10F, methoprene, JHM, control

INTRODUCTION

The sprinkler sewage filter fly, *Psychoda alternata* SAY and the moth fly, *Clogmia albipunctata* (WILLISTON), are nuisance insects that enter dwellings when they occur in considerable numbers in septic tanks, sinks, and basement drains (METCALF, 1962; YABE et al., 1969). Moreover, these insects are known to cause myiasis in man (TOKUNAGA, 1953; KAMIMURA and ARAKAWA, 1986).

Methods used to control *P. alternata* have included submersion in water, to drown the larvae, and insecticide application using mainly organophosphorus insecticides (ISHII and HARADA, 1961). Since the early 1980s, Altosid® 10F, an insecticide containing methoprene, a juvenile hormone mimic, has gained acceptance for controlling insects of medical and environmental importance (KAMEI et al., 1982; ISHII et al., 1987; KAMEI et al., 1991). It has been established that Altosid® 10F is effective in the control of *C. albipunctata* (KAMEI et al., 1992).

This paper describes the results of a laboratory test using methoprene and its slow-release formulation, Altosid® 10F, on laboratory-reared colonies of *P. alternata*, and also the effects of the slow-release formulation on this insect occurring in a septic tank.

MATERIALS AND METHODS

Insects. A *P. alternata* colony was collected from the drainage of a poultry farm in

Tokushima City in May, 1980 and maintained in a glass container by feeding on dried beer yeast at 26–27°C and on a 13L–11D regime in the laboratory. The 4th instar larvae of 5th generations of the strain mentioned above were used in the laboratory test. Larval period and adult longevity of the *P. alternata* were 9–15 and 5–6 d, respectively (YASUTOMI and UMEYA, 1983).

Chemicals. Methoprene (isopropyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate) of 96.6% purity and its slow-release formulation, Altosid® 10F containing 10% methoprene were used. Both chemicals were obtained from Zoecon Corporation, USA.

Laboratory assay. One ml of ethyl alcohol solution of methoprene, or its slow-release formulation, was diluted 1,000 times with distilled water, and 50 ml of this solution was added to a plastic container (9.0 cm dia., 7.5 cm ht.) containing 50 ml of stock water which had been prepared using distilled water and dried beer yeast ad-lib.

To prevent the drowning of test larvae, a piece of filter paper (15 cm dia.) was folded into a triangular pyramid and set in the plastic container. The 4th instar larvae of *P. alternata* were then introduced into each container and reared under laboratory conditions by feeding on dried beer yeast. The effect of this slow-release formulation was also evaluated by immersing the 4th instar larvae for only 3 s in solutions of this formulation. After the excess solution on the larval body surface was absorbed by the filter paper, the larvae were released into methoprene-free stock water.

Evaluation of insecticidal effects. Each laboratory test comprised at least 2 replications per concentration and more than 5 concentrations per insecticide. Observations were continued until pupae died or adults emerged. There were 5 possible consequences for treated larvae: larval death, pupal death, death when partially emerged, abnormal emergence, and normal emergence. The emergence inhibition rate was calculated by the following formula:

$$\text{Emergence inhibition rate} = \left(1 - \frac{\text{number of normal emergence}}{\text{number of larvae used}}\right) \times 100.$$

Fig. 1. Schematic diagram of tested septic tank. Pi: inflow pipe, S: precipitation separation compartment, A: aeration compartment, Pr: precipitation compartment, Dir: disinfection compartment, Po: outflow pipe, Ca: cap of the tank (adult population on this cap was collected).

Table 1. Effects of methoprene on the 4th instar larvae of *Psychoda alternata*

Concentrations ($\mu\text{g/ml}$)	No. of larvae tested	Mortality (%)				Emergence inhibition ^a (%)
		Larvae	Pupae	Adults		
				failure	deformed	
5	119	12.6	87.4	0	0	100
0.5	105	18.1	18.1	1.0	0	100
0.05	88	6.8	92.1	1.0	0	100
0.005	112	12.5	58.9	19.6	0.9	92.0 (90.3) ^b
0.0005	107	15.0	2.8	0	5.6	23.3 (7.6)
0.00005	78	7.7	10.3	0	3.9	21.8 (5.7)
0.000005	97	3.1	11.3	2.1	7.2	21.3 (5.1)
Control	70	7.1	5.7	1.4	2.9	17.1 (0)

^a Before ABBOTT's correction.^b Corrected emergence inhibition rate.Concentration-inhibition regression equation: $Y=1.8214X+10.2084$; $IC_{50}=0.0014 \mu\text{g/ml}$.

This emergence inhibition rate was further corrected by taking account of the rate of natural emergence failure (ABBOTT's formula) thus obtaining a corrected emergence inhibition rate. The resultant survival data were subjected to probit analysis.

Field trial. A field test was conducted against a natural population of *P. alternata* present in a 10-person septic tank of a kindergarten in Naruto City, Tokushima (Fig. 1). A 400-fold dilution of this slow-release formulation (2.5 g) was sprayed into the septic tank with a hand sprayer. The application was performed on June 10, 1981. An efficacy evaluation of this slow-release formulation was carried out by collecting the the adults resting on the cap of the septic tank.

RESULTS

Laboratory evaluations

Table 1 shows the effects of methoprene on the 4th instar larvae of *P. alternata* in laboratory assays. Percentages of larval and pupal death on controls were 7.1 and 5.7% respectively, and 82.9% of larvae developed to normal adults. However, methoprene treatment at concentrations of 0.005 $\mu\text{g/ml}$ and over showed 58.9–92.1% pupal death rates, and high larval mortality. Moreover, the 0.005 $\mu\text{g/ml}$ methoprene resulted in 19.6% partially emerged pupal deaths. The concentration-inhibition regression equation for the effects of methoprene on the 4th instar larvae of *P. alternata* was $Y=1.8214X+10.2084$, where Y was the probit of emergence inhibition and X was the log concentration ($\mu\text{g/ml}$), at an IC_{50} value of 0.0014 $\mu\text{g/ml}$.

Table 2 shows the effects of this slow-release formulation on the 4th instar larvae of *P. alternata* in laboratory assays. A 100% inhibition was observed for this slow-release formulation at concentrations of 0.05 $\mu\text{g/ml}$ and over (methoprene concentrations), similar to the results with methoprene itself (Table 1). In these concentrations, almost all larvae died at the pupal stage. The concentration-inhibition regression equation and IC_{50} value of this slow-release formulation against the 4th instar larvae of *P. alternata* were $Y=0.4889X+6.2942$ and 0.0023 $\mu\text{g/ml}$, respectively.

Table 2. Effects of Altosid® 10F on the 4th instar larvae of *Psychoda alternata*

Concentrations ^a ($\mu\text{g/ml}$)	No. of larvae tested	Mortality (%)				Emergence inhibition ^b (%)
		Larvae	Pupae	Adults		
				failure	deformed	
5	62	17.7	82.3	0	0	100
0.5	72	18.1	80.6	0	1.4	100
0.05	64	14.1	76.6	7.8	1.6	100
0.005	58	10.4	44.8	0	12.1	67.2 (61.3) ^c
0.0005	67	22.4	11.9	0	13.4	47.8 (38.5)
0.00005	71	5.6	8.5	1.4	7.0	22.5 (8.7)
0.000005	61	19.7	3.3	0	0	28.9 (16.3)
Control	33	3.0	3.0	3.0	6.1	15.1 (0)

^a Methoprene concentrations.^b Before ABBOTT's correction.^c Corrected emergence inhibition rate.Concentration-inhibition regression equation: $Y=0.4889X+6.2942$; $IC_{50}=0.0023 \mu\text{g/ml}$.Table 3. Effects of short time exposure of Altosid® 10F on the 4th instar larvae of *Psychoda alternata*^a

Concentrations ^b ($\mu\text{g/ml}$)	No. of larvae tested	Mortality (%)				Emergence inhibition ^c (%)
		Larvae	Pupae	Adults		
				failure	deformed	
250	35	22.9	77.1	0	0	100
125	40	35.0	62.5	0	2.5	100
62.5	56	32.1	67.9	0	0	100
31.3	34	20.6	76.5	2.9	0	100
15.7	34	29.4	35.3	2.9	2.9	100
7.8	34	23.5	70.6	2.9	2.9	100
3.9	26	7.7	84.6	3.9	0	96.1 (94.5) ^d
2.0	37	8.1	35.1	5.4	10.8	59.5 (43.1)
Control	52	28.9	0	0	0	28.8 (0)

^a Larvae were dipped in the test solutions for 3 s.^b Methoprene concentrations.^c Before ABBOTT's correction.^d Corrected emergence inhibition rate.

Short-time submersion evaluation

It is possible that *P. alternata* larvae may encounter high concentrations of methoprene when this slow-release formulation is applied in a field, because recommended spraying dilution rate is 200–2,000 times of this slow-release formulation (resulting in 50–500 $\mu\text{g/ml}$ methoprene concentration). Therefore, we evaluated the effects of 2.0 to 250 $\mu\text{g/ml}$ of this slow-release formulation (methoprene concentrations) applied to the 4th instar larvae for 3 s. As shown in Table 3, a 100% emergence inhibition was observed above 3.9 $\mu\text{g/ml}$ of this formulation, and 35.1–84.6% pupal death was noted

Fig. 2. Number of adult *Psychoda alternata* collected from the 10-person septic tank before and after treatment with 2.5 g Altosid® 10F (400-fold dilution).

in this experiment. Treatment with 2.0 $\mu\text{g}/\text{ml}$ of this formulation resulted in normal emergence of 40.5%.

Field test

Figure 2 shows the adult emergence of *P. alternata* from the septic tank treated with 2.5 g of this formulation. A range of 113–272 adults was observed before the treatment and 224 adults were observed the day after the treatment. However, no adults were observed 6 d after the treatment. This effect continued for up to 72 d after the treatment, the last day of the investigation.

DISCUSSION

Methoprene and its slow-release formulation have potent emergence inhibition effects on the 4th instar larvae of *P. alternata* with IC_{50} values of 0.0014 $\mu\text{g}/\text{ml}$ and 0.0023 $\mu\text{g}/\text{ml}$, respectively (Table 1 and 2). These IC_{50} values against *P. alternata* are 3–7 times and 3.8–6.2 times higher than those against *C. albipunctata* and *Culex pipiens pallens* in the same larval stage, respectively (KAMEI et al., 1992; BUEI et al., 1975).

C. albipunctata and *P. alternata* live in a similar habitat, and body length of *P. alternata* larva is smaller than that of *C. albipunctata*. However, susceptibility of *P. alternata* to methoprene was lower than that of *C. albipunctata*, whereas the pupal death rates of *C. albipunctata* and *P. alternata* at 0.0005–5 $\mu\text{g}/\text{ml}$ of methoprene were similar: 12.5–88.0% (KAMEI et al., 1992) and 2.8–92.1%, respectively.

Methoprene exhibited no significant activity to larvae of *P. alternata*, except at the 4th instar, but showed a strong inhibitory effect on the emergence of *Cx. pipiens pallens*.

when they were treated with more than 2 $\mu\text{g}/\text{ml}$ Altosid® 10F at 1st instar (KAMEI et al., 1981).

When the solution of this slow-release formulation is applied to the surface of sewage, *P. alternata* larvae may encounter a high concentration of methoprene, since this is where these larvae live in the "fluid column." Although the results of the short-submersion tests indicated a moderately lethal effect on the larvae, this slow-release formulation showed extremely strong emergence inhibition at concentrations of 3.9 $\mu\text{g}/\text{ml}$ and above. Thus, application of the insecticide at high concentration and low volume on sewage containing live larvae would be most effective.

The seasonal prevalence of moth flies breeding in a rural area of Yokohama was surveyed from June to October in 1967 and throughout the year in 1968 by YABE et al. (1969). Their data indicated that adults of *P. alternata* were commonly collected by black-light traps (Nozawa type) throughout the year in 1968 and 3 peaks were seen in May, June to July, and September to October, the highest being in June to July. In the present study, field application was effective over a period of 2 months, and adults did not emerge on sewage treated with 2.5 g of this slow-release formulation (Fig. 2). This effect must have been the result of the application of this formulation, and not of a natural fluctuation of population judging from the seasonal prevalence of *P. alternata* noted above.

In general, active sludge microorganisms live in the aeration compartment of the septic tank (Fig. 1). Altosid® 10F below 4 $\mu\text{g}/\text{ml}$ (methoprene) has no effect on the growth of active sludge microorganisms (KAMEI et al., 1992). The larvae of *P. alternata* live mainly on the scum surface of the precipitation separation compartment (Fig. 1). Even if this slow-release formulation were applied at high concentration to the scum surface of the precipitation compartment, it would not affect the active sludge microorganisms because the concentration of this slow-release formulation (methoprene) would be diluted below 4 $\mu\text{g}/\text{ml}$ by the large volume of water in the aeration compartment.

Considering all these characteristics and its effectiveness, Altosid® 10F is a reasonable agent for the control of moth flies.

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