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# EVALUATION OF NEW PYRETHROIDS AGAINST IMMATURE MOSQUITOES AND THEIR EFFECTS ON NONTARGET ORGANISMS

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ABSTRACT. In the laboratory, 5 new pyrethroids were screened against larvae and pupae of *Culex quinquefasciatus*. Two of the materials proved highly active, yielding 90% mortality in larvae at 0.07–0.46 ppb. Three of the materials were highly active against pupae, yielding 90% mortality at 1–4 ppb.

Two of the most active pyrethroids (cypermethrin and S–3206 or fenpropathrin: α-cyano-3-phenoxybenzyl 2, 2, 3, 3-tetramethyl cyclopropanecarboxylate) were evaluated under field conditions against larvae and pupae of *Cx. tarsalis* and *Psorophora columbiae*. Cypermethrin produced 90–100% mortality in larvae of the former species at 3–5 g AI/ha while S–3206 yielded 90–100% control at 27–55 g AI/ha. Both materials were equally effective against the second species, yielding high level to complete control of larvae at the low rates of about 6–11 g/ha. These rates also produced high level to complete control of pupae. The activity of these materials against pupae provides a decided advantage for use of these materials in mosquito control programs.

Under field conditions, both cypermethrin and S-3206 showed no adverse effects on diving beetle adults. Cypermethrin was also relatively innocuous to dragonfly naiads and ostracods at effective larvicidal rates. However, this material reduced mayfly naiads to a very low level and recovery was not noticeable 2 weeks later. S-3206 adversely affected both mayfly and dragonfly naiads, but had no effects on ostracods except for a short period. The affected organisms recovered in 2 weeks after treatment.

## INTRODUCTION

During the past few years a number of photostable pyrethroids have become available for the control of a variety of insect pests. Some of these materials have proven to be more active than some of the most highly active organophosphorous compounds such as temephos, chlorpyriphos, malathion, fenthion, and naled against several groups of insects of public health importance, such as mosquitoes and chironomid and biting midges (Ali and Mulla 1980, Darwazeh et al. 1978, Kline et al. 1981, and Mulla et al. 1980). Decamethrin and permethrin, for example, produced excellent reduction in the number of endophilic mosquitoes, *Anopheles gambiae* s.l. and *An. funestus* Giles for 24 weeks when applied as emulsifiable or suspension concentrates to interior surfaces of houses in Kenya, and the reduction in the number of resting adults on treated surfaces is attributed partly to repellent effects; the residual sprays also reduced biting activities of

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*Mansonia* spp. in houses (Taylor et al. 1981).

Most synthetic pyrethroids evaluated as larvicides showed some adverse effects on some nontarget aquatic macroinvertebrates such as mayfly and dragonfly naiads at mosquito larvicidal rates of 1.1–27.5 g AI/ha (0.001–0.025 lb AI/A); these organisms, however, were affected for a short period, and these populations began to recover within 7–14 days after treatment (Darwazeh and Mulla 1981, Mulla et al. 1980). At mosquito larvicidal rates, a number of these pyrethroids were reported (Mulla et al. 1978) to be harmless to selected species of freshwater fishes, including rainbow trout *Salmo gairdneri* Richardson, mosquito fish *Gambusia affinis* (Baird and Girard), desert pupfish *Cyprinodon macularius* (Baird and Girard), and *Tilapia mossambica* (Peters). Numerous (5–6) successive weekly applications of the pyrethroids, permethrin, decamethrin and pyrenone® tossits induced no reduction in mosquito fish and desert pupfish populations at larvicidal and 5X these rates (Darwazeh and Mulla 1981, Mulla et al. 1981).

These findings indicate that some synthetic pyrethroids in general are highly active against insects of medical importance, but have temporary impact on some other components of the environment. The following studies, therefore, were conducted to evaluate some newly discovered and developed pyrethroids against mosquito larvae and pupae in the laboratory and under field conditions, and also to study their short-term effects on some nontarget aquatic invertebrates.

## METHODS AND MATERIALS

LABORATORY. One percent stock solutions of technical materials were prepared in acetone (w/v) and serial dilutions in acetone were made as needed. To achieve proper concentration, the required amount of a toxicant solution (0.1–1.0 ml) was added to 4 oz disposable cups (Sweetheart Cup Div., Baltimore, MD) containing 20 4th stage larvae or pupae in 100 ml of tap water. Each material was tested on 2–3 occasions and at 3–4 different concentrations by utilizing 3 replicates per concentration, as well as checks receiving 1 ml acetone only. After 24 hr of exposure in a holding room at 25 ± 1°C, mortality was noted and corrected mortality values were subjected to log probit regression analysis by using a Compucorp 145 E desk model computer to obtain the $LC_{50}$ and $LC_{90}$ in ppb, slope of the dosage response lines, and the correlation coefficients. Materials tested included cypermethrin (Ammo®) (FMC Corp., Ag. Chemical Div., Middleport, NY), S–2703, S–2852, S–3206 (fenpropathrin) and S–4068 (Sumitomo Chemical Co. Ltd., Osaka, Japan). Chemical descriptions of these pyrethroids are as follows:

S–2703: αCyano-3-phenoxybenzyl d-*cis, trans* chrysanthemate.

S-2852: (E)-1-ethynyl-2-methylpenta-2-enc-yl-(+)-*trans, cis* chrysanthemate.

cypermethrin (Ammo): (±)α-Cyano-(3-phenoxyphenyl)methyl (±) *cis, trans* 3-(2,2-dichloroethenyl)-2,2-diethyl cyclopropane-carboxylate.

S-3206 (fenpropathrin): α-Cyano-3-phenoxybenzyl 2,2,3,3-tetra-methyl cyclopropanecarboxylate.

S-4068: 2-methyl-3-(prop-2-ynyl)-4-oxo-cyclopent-2-enyl-d-*cis, trans* chrysanthemate.

FIELD. Two materials were available in emulsifiable concentrate formulations for field evaluation. S-3206 (30%) EC and Ammo® (30%) EC were evaluated against larvae of the mosquito *Culex tarsalis* Coquillett in experimental ponds, and *Psorophora columbiae* (Dyar and Knab) in irrigated pastures in Palo Verde Valley of southern California.

In the experimental ponds, the studies were conducted at the University of California Aquatic and Vector Control Research Facility in the Coachella Valley; this facility was described by Mulla et al. (1982). In brief, the ponds measure 18 × 18 ft (30 $m^2$), fully vegetated along the sides, and water depth was maintained constant (30 cm), by the use of float valves. The mean water pH was 9.4 and temperatures are given in the tables. During these studies, mosquito populations consisted mainly of larvae and pupae of *Cx. tarsalis*. Each material was tested at 3–4 different rates, utilizing 3 replicates per rate. In each test, 3 ponds were left untreated as a check. The required amount of a toxicant was mixed with 120 ml of water, and applied with a one-liter all-purpose household sprayer. Prior to treatment and 2, 7, and 14 days after treatment, 5 dips per pond were taken; they were concentrated into one composite sample after removing excess water through 150 mesh stainless steel strainer cloth. The composite sample was preserved with 95% alcohol, and all organisms present were counted and identified under a dissecting microscope in the laboratory.

Pyrethroids (S-3206 and Ammo) were evaluated against *Ps. columbiae* larvae in 1/18 acre plots (224 m$^2$) in El Rancho Del Rio, 8 km south of Palo Verde, California, east of State Highway 78 in Imperial County. Each material was applied at 3 different rates, utilizing 3 plots per rate in the same irrigation check. Three plots were left untreated as checks in each test. The required amount of each material was mixed with 4 liters of water, and applied with a 4-liter stainless steel sprayer. Prior to treatment and 12- and 24-hr post-treatment, 10 dips per plot were taken, and percent reduction of mosquito larval population was determined. At the time of treatment, 3rd and 4th stage larvae of *Ps. columbiae* were present in 20–30 cm deep water, covering the entire plots. Bermuda grass in the field, 10–15 cm in height, was submerged in the irrigation water. Water pH in plots was approximately 7.9, and air and water temperatures during these studies are included in the tables. Percent reduction in field plots with *Ps. columbiae* larvae was calculated on the basis of larval counts obtained in post-treatment vs. pretreatment. In the experimental ponds where *Culex* occurred, due to the gradual decline of larvae in the check ponds, percent reduction was calculated (using 3rd and 4th) according to the formula of Mulla et al. (1971) giving:

$(\%R) = 100 - (\frac{C_1 \times T_2}{T_1 \times C_2})100$, where $C_1$ = mean no. larvae pre-treatment in check ponds, $T_1$ = mean no. larvae pre-treatment in treated ponds, $C_2$ = mean no. larvae post-treatment in check ponds, and $T_2$ = mean no. larvae post-treatment in treated ponds.

This calculation provides for the natural declines of populations in treated and untreated plots.

Results obtained from all field evaluations were subjected to statistical analysis by using a Compucorp 145 E Computer.

## RESULTS AND DISCUSSION

LABORATORY. All pyrethroids evaluated in the laboratory exhibited excellent biological activity against both larvae and pupae of *Cx. quinquefaciatus* Say. The most effective material, cypermethrin, produced 90% mortality in 4th stage larvae at 0.07 ppb. S-3206 also displayed high levels of activity against 4th with an LC$_{90}$ of 0.4 ppb, but was one-sixth as effective as cypermethrin. S-2703, S-4068, and S-2852 also showed excellent activity against the larvae, causing 90% mortality at 3, 4 and 9 ppb, respectively (Table 1).

Cypermethrin was the most active material tested against pupae, causing 90% mortality at 1.0 ppb. S-2703 showed essentially similar levels of activity against pupae and the larvae, causing 90% mortality in pupae at 3.9 ppb. S-4068, S-3206, and S-2852 were less active against pupae with an LC$_{90}$ of 13.8, 21.6 and 26.9 ppb, respectively. S-3206 showed excellent activity against the larvae, but was far less active (50-folds) against pupae.

FIELD. In the field, Ammo® 2.5 EC (cypermethrin) produced 94% and complete control of *Cx. tarsalis* larvae in experimental ponds at the rates of 2.75–5.5 g AI/ha (0.0025–0.005 lb AI/A) within 2 days of treatment. One week after treat-

Table 1. Evaluation of new pyrethroids against 4th stage larvae and pupae of *Culex quinquefasciatus* in the laboratory.

| Material | $LC_{50}$–$LC_{90}$ (ppb)[a] | Slope | Correlation coefficient |
|---|---|---|---|
| | *Larvae* | | |
| Cypermethrin (Ammo®) | 0.05–0.07 | 6.66 | 0.98 |
| S-3206 (fenpropathrin) | 0.27–0.46 | 5.37 | 0.94 |
| S-2703 | 0.80–3.00 | 2.30 | 0.86 |
| S-4068 | 1.60–4.00 | 3.15 | 0.94 |
| S-2852 | 5.10–9.00 | 5.19 | 0.90 |
| | *Pupae* | | |
| Cypermethrin (Ammo®) | 0.40–1.00 | 3.20 | 0.93 |
| S-2703 (fenpropathrin) | 1.40–3.90 | 2.95 | 0.93 |
| S-4068 | 3.50–13.80 | 2.14 | 0.83 |
| S-3206 | 6.20–21.60 | 2.36 | 0.94 |
| S-2852 | 12.10–26.90 | 3.71 | 0.98 |

[a] Values obtained through log probit regression analysis by using Compucorp 145 E Computer.

ment, however, reduction at the 2 lower rates was negligible (30–46%) compared to 82 and 99% at the higher rates. At all 4 rates of application there was negligible reduction 2 weeks after treatment. It is interesting that the 3 high rates suppressed pupal populations completely. Even after 14 days of treatment, no pupae occurred, though the larval population prevailed at high level. S-3206 EC (30%) was one-tenth as effective as Ammo against the same mosquito species in the experimental ponds, causing 94 and 100% control 2 days after treatment at the rates of 27.5 and 55 g AI/ha (0.025–0.05 lb AI/A). At both rates, excellent reduction (91 and 97%) was obtained 7 days after treatment, but declined to a negligible level 2 weeks after treatment (Table 2). It is noteworthy that pupal populations were drastically or completely suppressed for up to 14 days post-treatment, indicating that the efficacy of this material probably lasts for more than 2 weeks.

Against the floodwater mosquito, *Ps. columbiae,* both Ammo and S-3206 initially produced similar results, causing 100 and 98% reduction 12 hr after treatment at the rate of 5.5 g AI/ha (0.005 lb AI/A), while 81 and 95% reduction was obtained 24 hr after treatment. At the higher rate of 11 g AI/ha (0.01 lb/A), S-3206 eliminated larval populations completely 12 (data not included) and 24 hr after treatment (Table 3). Slightly better results obtained 12 hr after treatment were due to the large amount of water in the plots covering the entire plot, where surviving mosquito larvae were thinly dispersed. Twenty-four hr after treatment, water was found in low spots and in trenches along the borders, where surviving larvae became concentrated. Larval counts in check plots also were 2–3 times higher 24 hr post-treatment than the pre-treatment counts (see Table 3). This aspect should be noted in studies on floodwater mosquitoes where standing water rapidly dries up.

As shown in Tables 2–3, Ammo was equally effective against larvae of both stagnant and floodwater mosquitoes at the rate of 5.5 g/ha (0.005 lb/A). S-3206, however, was just as effective as Ammo against a floodwater mosquito, *Ps. columbiae,* but was far less effective against *Cx. tarsalis,* requiring 27.5 g/ha (0.025 lb/A) for satisfactory control.

Both materials (Ammo and S-3206), at all larvicidal rates applied, did not exhibit noticeable effects on adult diving beetles (*Berosus metallliceps:* Dytiscidae). Mayfly naiads (*Callibaetis pacificus* Seeman) and dragonfly naiads (*Erythemis*

Table 2. Evaluation of new pyrethroids against *Culex tarsalis* in experimental ponds (Coachella Valley Aquatic Vector Control Research Facility, southern California).

| | | | Mean no. of larvae and pupae/5 dips pre-, and post-treatment (days) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Post-treatment | | | | | | | | | | | | |
| Material and | Rate | | Pre-treatment | | | 2 | | | | 7 | | | | 14 | | | | |
| formulation | lb/A | (g/ha) | 1–2 | 3–4 | P | 1–2 | 3–4 | P | (%R)[d] | 1–2 | 3–4 | P | (%R)[d] | 1–2 | 3–4 | P | (%R)[d] |
| | | | | | | *October 6, 1981*[ac] | | | | | | | | | | | | |
| Cypermethrin | | | | | | | | | | | | | | | | | | |
| (Ammo®) | 0.0010 | (1.10) | 16 | 21 | 6 | 5 | 11 | 3 | 58b | 12 | 8 | 1 | 46bc | 58 | 10 | 5 | ob |
| EC 2.5 | 0.0025 | (2.75) | 28 | 35 | 18 | 0 | 4 | 1 | 94a | 43 | 1 | 0 | 30bc | 112 | 36 | 0 | ob |
| (30%) | 0.005 | (5.50) | 17 | 28 | 18 | 0 | 0 | 1 | 100a | 8 | 0 | 0 | 82ab | 30 | 4 | 0 | 24ab |
| | 0.010 | (11.00) | 50 | 26 | 7 | 0 | 0 | 1 | 100a | 1 | 0 | 0 | 99a | 13 | 1 | 0 | 70a |
| Check | — | — | 43 | 37 | 7 | 34 | 46 | 7 | 0c | 32 | 44 | 5 | 5c | 45 | 32 | 6 | 4b |
| | | | | | | *October 13, 1981*[bc] | | | | | | | | | | | | |
| Fenpropathrin | | | | | | | | | | | | | | | | | | |
| (S-3206) | 0.010 | (11.00) | 9 | 21 | 2 | 4 | 13 | 5 | 43bc | 19 | 4 | 0 | 23a | 41 | 23 | 1 | 0a |
| (30%) | 0.025 | (27.50) | 56 | 38 | 7 | 1 | 5 | 11 | 94ab | 7 | 1 | 2 | 91a | 65 | 14 | 0 | 16a |
| | 0.050 | (55.00) | 45 | 32 | 20 | 0 | 0 | 2 | 100a | 2 | 0 | 0 | 97a | 42 | 19 | 0 | 21a |
| Check | — | — | 32 | 44 | 5 | 33 | 52 | 7 | 0c | 45 | 31 | 6 | 0b | 30 | 12 | 1 | 45a |

[a] Water temperature mean minimum 17.2°C; mean maximum 27.7°C.

[b] Water temperature mean minimum 15°C; mean maximum 22.2°C.

[c] Means followed by same letters in columns in each test are not significantly different from each other at the 5% probability level.

[d] Percent reduction.

Table 3. Evaluation of new pyrethroids against *Psorophora columbiae* larvae in irrigated fields of Palo Verde Valley (Imperial County, southern California).

| Material and formulation | Rate lb AI/A | Rate (g AI/ha) | Mean no. of larvae/10 dips Pre-treatment | Mean no. of larvae/10 dips Post-treatment (24h) | Mean[c] % reduction |
|---|---|---|---|---|---|
| | | *August 21, 1981*[a] | | | |
| Ammo | 0.0010 | (1.10) | 79 | 22 | 72[a] |
| EC 2.5 | 0.0025 | (2.75) | 21 | 7 | 67a |
| | 0.0050 | (5.50) | 26 | 5 | 81a |
| Check | — | | 27 | 77 | 0b |
| | | *August 18, 1982*[b] | | | |
| S-3206 | 0.0010 | (1.10) | 790 | 505 | 36a |
| (30%) EC | 0.0025 | (2.75) | 550 | 175 | 68a |
| | 0.0050 | (5.50) | 370 | 120 | 68a |
| Check | — | | 400 | 675 | 0a |
| | | *August 21, 1981*[a] | | | |
| S-3206 | 0.005 | (5.50) | 44 | 2 | 95a |
| (30%) EC | 0.010 | (11.00) | 39 | 0 | 100a |
| | 0.025 | (27.50) | 55 | 0 | 100a |
| Check | — | | 27 | 77 | 0b |

[a] Water temperature mean minimum 26.6°; mean maximum 36.6°C; air temperature 43°C.

[b] Water temperature mean minimum 26.1°C; mean maximum 36.1°C; air temperature 42.7°C.

[c] Means followed by the same letters in each test are not significantly different from each other at the 5% probability level.

*simplicicollis* Say) were completely or markedly eliminated for a short period. Ostracods were not markedly affected by Ammo, but were affected for about a week in S-3206 treatments. The ostracods began to recover within one week, while the mayfly and dragonfly naiads were affected for more than 2 weeks (Table 4).

From these studies it is apparent that Ammo could be used effectively for the control of stagnant and floodwater mosquito larvae at the low rates of 5.5–11.0 g/ha (0.005 - 0.01 lb/A), depending on water depth, larval density and vegetative growth. At the same rate, S-3206 will produce excellent results against floodwater mosquitoes, but higher rates (27.5 g/ha) are required for the control of *Cx. tarsalis*. Both materials appear to be excellent candidates for mosquito control due to their high activity against larvae as well as pupae of stagnant and floodwater mosquitoes, without having long-lasting effects on nontarget organisms. These materials appear to be nonresidual, and break down rapidly in water, as the target mosquito and most nontarget invertebrate populations recovered within 2–3 weeks after a given treatment.

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Table 4. Effect of new pyrethroids on nontarget invertebrates in experimental ponds (Coachella Valley Aquatic and Vector Control Research Facility, southern California).

| | | | Mean no. of nontarget invertebrates/5 dips pre- and post-treatment (days) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Material and | Rate | | Mayfly naiads | | | | Dragonfly naiads | | | | Diving beetle adults | | | | Ostracods | | | |
| formulation | lb AI/A | (g AI/ha) | Pre- | 2 | 7 | 14 | Pre- | 2 | 7 | 14 | Pre- | 2 | 7 | 14 | Pre- | 2 | 7 | 14 |
| | | | | | | | *October 6, 1981*[a] | | | | | | | | | | | |
| Ammo | 0.001 | (1.10) | 20 | 9 | 1 | 1 | 0 | 0 | 1 | 5 | 9 | 9 | 4 | 12 | 1 | 8 | 42 | 75 |
| EC 2.5 | 0.0025 | (2.75) | 33 | 12 | 1 | 1 | 0 | 0 | 0 | 3 | 7 | 8 | 9 | 9 | 0 | 1 | 4 | 14 |
| | 0.0050 | (5.50) | 29 | 0 | 0 | 0 | 1 | 0 | 0 | 6 | 18 | 23 | 10 | 23 | 0 | 1 | 4 | 19 |
| | 0.0100 | (11.00) | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 16 | 10 | 8 | 12 | 7 | 6 | 43 |
| Check | | — | 17 | 52 | 20 | 21 | 0 | 2 | 2 | 8 | 10 | 7 | 9 | 5 | 1 | 3 | 12 | 47 |
| | | | | | | | *October 13, 1981*[b] | | | | | | | | | | | |
| S-3206 | 0.010 | (11.00) | 25 | 0 | 0 | 1 | 2 | 1 | 1 | 8 | 2 | 28 | 11 | 2 | 5 | 0 | 6 | 56 |
| (30%) | 0.025 | (27.50) | 22 | 0 | 0 | 1 | 7 | 0 | 0 | 3 | 10 | 30 | 21 | 4 | 37 | 2 | 67 | 112 |
| | 0.050 | (55.00) | 2 | 0 | 0 | 1 | 5 | 0 | 1 | 4 | 11 | 45 | 30 | 8 | 24 | 0 | 1 | 13 |
| Check | | — | 20 | 21 | 21 | 18 | 2 | 2 | 8 | 10 | 9 | 4 | 5 | 1 | 12 | 5 | 47 | 50 |

[a] Water temperature mean minimum 17.2°; mean maximum 27.7°C.

[b] Water temperature mean minimum 15°; mean maximum 22.2°C.

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# RED EYE AND VERMILLION EYE, RECESSIVE MUTANTS ON THE RIGHT ARM OF CHROMOSOME 2 IN *ANOPHELES ALBIMANUS*[1]

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ABSTRACT. Two new eye color mutants, *red eye (re)* and *vermillion eye (ve)* arose spontaneously in laboratory stocks of *Anopheles albimanus.* Both mutants are recessive and expressed during the larval, pupal and adult stages. In individuals homozygous for *re*, the eyes darken with age in the adult stage, but in *ve* homozygotes there is no change in the bright red color. The loci for both mutants are on the right arm of chromosome 2, but the mutants are not allelic. *Vermillion eye* homozygotes are weak and a pure stock has been established and maintained with difficulty.

## INTRODUCTION

For the past several years we have been involved in basic studies on the genetics of *Anopheles albimanus* Wiedemann. The linkage groups and mode of inheritance of several mutants have been reported for this species of neotropical mosquito which is an important vector of human malaria. A current list of genetic markers in *An. albimanus* was given in a recent report by Narang et al. (1981).

In this present report, we describe two new non-allelic, recessive, eye-color mutants, *red eye (re)* and *vermillion eye (ve).*

## METHODS AND MATERIALS

Established procedures were used for the rearing and maintenance of the mosquitoes (Rabbani and Seawright 1976, Benedict et al. 1979). Appropriate crosses (Tables 1–4) were used to determine the mode of inheritance and linkage group for the two mutants. Other mutant markers used during the linkage study

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