# Laboratory and Field Evaluation of the Efficacy of Four Insecticides for *Aedes vigilax* (Diptera: Culicidae) and Toxicity to the Nontarget Shrimp *Leander tenuicornis* (Decapoda: Palaemonidae)

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**ABSTRACT** Laboratory and field evaluations were conducted in southeastern Queensland, Australia, to determine the toxicities of 2 organophosphate compounds (temephos and pirimiphos-methyl), an insect growth regulator (*s*-methoprene), and *Bacillus thuringiensis* variety *israelensis* de Barjac (*Bti*) to *Aedes vigilax* (Skuse), an Australian saltmarsh mosquito vector of Ross River virus. The toxicity of these compounds to *Leander tenuicornis* Say, a cohabiting nontarget shrimp species, was also assessed. *s*-Methoprene and *Bti* were found to be the most selective for *Ae. vigilax*, with selectivity ratios ($LC_{95}$ nontarget/$LC_{95}$ target) of 255,000 and 38,000, respectively. In contrast, selectivity ratios of 13 and 0.01 were calculated for temephos and pirimiphos-methyl, respectively. As predicted by the laboratory studies, the field applications of *s*-methoprene and *Bti* were highly effective against *Ae. vigilax*, while not affecting *L. tenuicornis* survival. In contrast, although temephos and pirimiphos-methyl were both effective against *Ae. vigilax*, these products also killed 100% of caged *L. tenuicornis*. *s*-Methoprene and *Bti* did not affect water quality, whereas temephos and pirimiphos-methyl significantly influenced pH and turbidity. Accordingly, based on the high selectivity ratios, excellent field efficacy, and lack of influence on abiotic water characteristics, *s*-methoprene and *Bti* were ideal for insecticide control of *Ae. vigilax* in Australian saltmarsh pools.

**KEY WORDS** *Aedes vigilax*, *Leander tenuicornis*, insecticides, water quality, nontarget shrimp, mosquito control

*Aedes vigilax* (Skuse) is the major saltmarsh mosquito vector of Ross River virus in Queensland and the major mosquito pest along the Australian coast from New South Wales northward to Perth and near Adelaide (Kay 1982). Local government bodies regularly spray mangrove and saltmarsh habitats with a range of chemical insecticides in attempts to control this mosquito. These same habitats are important nursery environments for a wide diversity of nontarget species and their food organisms, including crustaceans of ecological and commercial importance (Morton et al. 1988, Sumpton and Greenwood 1990).

There are growing concerns in Australia over the use of organophosphorous compounds (Gehrke 1988, Mortimer and Hughes 1991, AEPA 1994, Mortimer and Chapman 1995, Brown et al. 1997). Indications are that organophosphorous applications often can have hazardous or unknown effects on associated nontarget species. In contrast, *s*-methoprene (Sawby et al. 1992; Ross et al. 1994a, b), an insect growth regulator, and *Bacillus thuringiensis* variety *israelensis* (*Bti*) (Hershey et al. 1995; Brown et al. 1997, 1998b), an entomopathogenic bacterium, can control mosquitoes with minimal environmental impact. Accordingly, they are being applied with increasing frequency to saltmarsh and mangrove habitats in Australia.

Brown et al. (1997) evaluated the acute toxicity of selected insecticides to nontarget fish, copepod, and shrimp species collected from local government treatment areas in southeastern Queensland. The palaemonid shrimp, *Leander tenuicornis* Say, was identified as an appropriate indicator species for toxicity studies. In addition to being sensitive to toxic effects in saltwater systems, the shrimp is abundant in shallow estuarine and wetland habitats of southeastern Queensland, and is of known importance in estuarine food webs (Wadley 1978). There also is a coincidence between its maximum breeding season and that of mosquitoes, and hence mosquito control programs.

This study was designed to assess the efficacy of 4 commonly used insecticides for *Ae. vigilax* and their effects on cohabiting *L. tenuicornis*. We first assessed the current 24-h concentration–response relationship of these insecticides for both the target and nontarget species. A selectivity ratio ($LC_{95}$ nontarget/$LC_{95}$ target) (Holck and Meek 1987) was then calculated for each insecticide. These laboratory studies were then followed by field evaluations, because both environmental and biological factors can affect concentrations of treatment compounds. Studies have shown

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that tidal flushing and dispersal (Pierce et al. 1989), adsorption to organic matter (Cooney 1995), the size and life-history stages of nontarget species (Mian and Mulla 1992), and synergistic effects with other pollutants (Rand 1995, Klein 1995) all influence the impact of insecticide applications. Accordingly, this study evaluated the efficacy with which these insecticides control *Ae. vigilax* in saltmarsh pools, evaluated possible toxicity to the cohabiting shrimp *L. tenuicornis*, and monitored abiotic water characteristics for possible changes as a consequence of treatment with the insecticides.

## Materials and Methods

**Collection, Maintenance, and Identification of Test Species.** Late-juvenile/adult shrimp and late 2nd-/early 3rd-instar *Ae. vigilax* were collected from saltmarsh pools near Coomera Marina (27° 54′ S, 153° 17′ E) in southeastern Queensland. The shrimp were collected with 2-mm mesh bait fish traps (25 by 25 by 45 cm, Mossop's Tackle, Brisbane, Australia), and long-handled 280-μm mesh hand-held nets (10 by 20 cm aperture) facilitated collection of the mosquitoes. Collected specimens were placed in aerated habitat water for transport to the laboratory in Brisbane 80 km away. Additional habitat water also was collected for subsequent maintenance and experimental purposes. All water for experimentation and maintenance of test animals was passed through a 100-μm mesh net before use to remove detritus. In the laboratory, shrimp were transferred into aerated aquaria (24 by 22 by 46 cm) for a 3- to 4-d acclimation period. The mosquitoes were maintained for 24 h in 20-liter drums. Only individuals active after the acclimation period were used in trials. *L. tenuicornis* was identified according to the description in Wadley (1978), and *Ae. vigilax* according to Marks (1982).

**Insecticides Evaluated.** To evaluate the effects of insecticides used in field applications, we tested the following 4 insecticides: (1) Abate 100 E ([AI] 10% temephos applied at 0.1 kg/ha, Cyanamid Australia, Baulkham Hills, NSW, Australia); (2) Actellic ([AI] 90% pirimiphos-methyl applied at 0.56 kg/ha, ICI Crop Care, Melbourne, VIC, Australia); (3) VectoBac12AS ([AI] 1.279 by $10^9$ International Toxic Units (ITU) *Bti*/liter, applied at 1.0 liter/ha, Hoechst Schering AgrEvo, Pennant Hills, NSW, Australia); and (4) Altosid Liquid Larvicide ([AI] 20% *s*-methoprene applied at 0.06 kg/ha, Sandoz, Dallas, TX). Based on the application rate and the percentage of active ingredients, the estimated field concentration in a 15 cm deep pool was calculated for each insecticide.

**Laboratory Shrimp Bioassays.** Static exposure assays were designed and implemented according to criteria specified by American Society for Testing and Materials (ASTM 1980) for acute toxicity testing of macroinvertebrates. The test animals were exposed to serial dilutions of an insecticide in filtered habitat water, with no change of water for the duration of the assays. Three replicates each of 20 late-juvenile/adult specimens were introduced into 12-liter glass aquaria (20 by 20 by 30 cm) containing 5 liters of test concentration. Three control containers holding 20 test specimens each in habitat water without insecticide were used for each bioassay.

**Laboratory Mosquito Bioassays.** Laboratory bioassays, based on standard methods for testing of larval susceptibility (WHO 1981), were used to determine the concentration–response relationship between the selected insecticides and *Ae. vigilax*. Recently, Ritchie et al. (1997), using this method, developed $LC_{95}$ data on *s*-methoprene and *Ae. vigilax* from southeastern Queensland. Because these data were developed at the Queensland Institute of Medical Research, we used their results as a means of avoiding replication of effort.

Larvae were exposed to serial dilutions of insecticide in filtered habitat water. Five replicates each of 20 late 3rd/early 4th instars were introduced into 250-ml glass beakers containing 200 ml of test concentration. Five control beakers holding 20 test larvae each in habitat water without insecticide were used for each bioassay.

Initially, for both the mosquito and shrimp bioassays, a number of range finding tests with widely spread exposure concentrations were conducted. Based on these tests, a narrow range of concentrations that straddled the effective range were conducted. The numbers surviving were counted at 24 h. Death, or the lack of reaction to gentle prodding with a glass pipette, was the measured deleterious response. The assays were conducted at 25°C and a photoperiod of 12:12 (L:D) h. The test specimens were not fed for 24 h before, or during, testing to minimize variability caused by nutritional and metabolic condition. Test specimens were removed individually from the holding aquaria and distributed randomly among the test containers.

**Field Bioassays.** The field trials were conducted by exposing caged field-collected adult *L. tenuicornis* and late 3rd-/early 4th-instar *Ae. vigilax* to the selected insecticides in small (≈3–5 $m^2$, 5–18 cm deep) saltmarsh pools near Coomera Marina. The insecticides were applied by hand at the rates detailed above in *Insecticides Evaluated*. A tape-meter was used to measure the dimensions of each pool. Based on these measurements, the volume was calculated and the dosage adjusted to give the estimated field concentration, as detailed in Table 1. The temephos and pirimiphos-methyl evaluations were conducted concurrently. The *s*-methoprene and *Bti* evaluations were conducted on separate occasions.

Toxicity was assessed in open-topped, 280-μm mesh cylinders (20 cm deep by 100 cm diameter). For each insecticide, 3 treatment and 3 control pools were selected for both species, with 1 cylinder placed in each pool. As the organophosphate evaluations occurred concurrently, 1 set of untreated control pools was used for the evaluation of both products. For assessment of *Ae. vigilax* control efficacy, 100 larvae were placed in each cylinder ≈2 h before treatment. Toxicity to *L. tenuicornis* was measured by placing 20 shrimp per cylinder in each of the selected pools. The numbers

Table 1. Selectivity ratios ($LC_{95}$ nontarget/$LC_{95}$ target) for *L. tenuicornis* and *Ae. vigilax*, respectively, based on concentrations of insecticides causing 95% mortality after 24 h laboratory exposure

| Active ingredient | EFC | *L. tenuicornis* | | | | | *Ae. vigilax* | | | | | SR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $LC_{95}$[a] | *n* | Slope (SE) | $\chi^2$ | *P* | $LC_{95}$[a] | *n* | Slope (SE) | $\chi^2$ | *P* | |
| Temephos, ppm | 0.06 | 0.05 (0.04, 0.06) | 300 | 3.9 (0.6) | 28.7 | <0.01 | 0.004 (0.003, 0.005) | 500 | 2.7 (0.2) | 6.2 | 0.9 | 13 (10, 15) |
| Pirimiphosmethyl, ppm | 0.30 | <0.00004[b] | 300 | | | | 0.003 (0.003, 0.003) | 500 | 9.5 (1.1) | 12.1 | 0.5 | <0.01[b] |
| *Bti* (ITU) | $1.3 \times 10^9$ | $1.500 \times 10^9$ ($800$ to $2,200 \times 10^9$) | 300 | 2.8 (0.8) | 30.4 | <0.01 | $0.04 \times 10^9$ ($0.03$ to $0.05 \times 10^9$) | 500 | 3.3 (0.2) | 7.4 | 0.7 | 38,000 (30 to 50 × $10^3$) |
| *s*-Methoprene, ppm | 0.008 | 51 (35, 75) | 300 | 2.3 (0.3) | 12.8 | <0.01 | 0.0002[c] (0.0001, 0.0003) | | | | | 255,000 (17 to 51 × $10^4$) |

EFC, estimated field concentration of active ingredient for 15 cm deep pools. *P* refers to the probability corresponding to maximum likelihood chi-square statistic for goodness-of-fit of the model. SR, selectivity ratio. ITU, international toxic units/mg.

[a] Values in parentheses are the 95% lower and upper confidence limits.

[b] Conservative estimate as we were unable to determine the full concentration-response curve.

[c] *s*-Methoprene $LC_{95}$ data for *Ae. vigilax* derived from Ritchie et al. (1997).

surviving at 24 h after application were counted and percentage mortality calculated. Additionally, because *s*-methoprene is a sustained release formulation, mosquito pupal mortality and shrimp survival were assessed every 24 h for 120 h. Because abiotic factors can affect the toxicity of a substance (Cooney 1995), salinity (g/liter), pH, water temperature (°C), dissolved oxygen (mg/liter), and turbidity (nepholometric turbidity units were recorded before treatment and at 24 h using a portable field laboratory (Horiba, Kyoto, Japan).

**Analysis of Laboratory Bioassay Data.** Probit models were used to model mortality as a function of insecticide dose. Zero concentrations were analyzed as concentrations of 0.000001 ppm to avoid infinite logarithmically transformed values. Approximately linear plots of the probit values by log (dose) indicated that the assumptions associated with fitting these probit models were met. The SPSS-PC+ version 4.0 PROBIT procedure (Norusis 1990) was used for these analyses.

**Analysis of Field Bioassay Data.** To homogenize variances, arcsine square-root transformations were performed on the proportions of control and treatment mortalities at 24 h. Paired *t*-tests were then used to determine if significant differences occurred between control and treatment mortalities for each insecticide evaluation. Paired *t*-tests were then used to analyze the significance of differences in control versus treatment changes in each of the abiotic water variables.

## Results

**Laboratory Shrimp Bioassays.** Pirimiphos-methyl was the most toxic compound tested against *L. tenuicornis*, with an $LC_{95}$ of <0.00004 ppm (Table 1; Fig. 1). The estimated field concentration for pirimiphos-methyl was ≈7,500 times the $LC_{95}$ value for *L. tenuicornis*. Of the 2 organophosphorous compounds evaluated, temephos was the least toxic to *L. tenuicornis*, with an $LC_{95}$ value of 0.05 ppm, which was 83% of the estimated field concentration. *s*-Methoprene was least toxic to *L. tenuicornis* with an $LC_{95}$ value that represented 6,375 times the estimated field concentration. *Bti* also exhibited low acute toxicity to *L. tenuicornis*, with an $LC_{95}$ value that was 1,154 times the estimated field concentration.

**Laboratory Mosquito Bioassays.** $LC_{95}$ values calculated for 3rd-instar *Ae. vigilax* exposed to pirimphosmethyl and temephos were similar (Table 1; Fig. 1). However, the estimated field concentration for pirimphos-methyl was 5 times greater than that for temephos. The estimated field concentrations for *Bti* and *s*-methoprene were 33 and 40 times the $LC_{95}$ values for *Ae. vigilax*, respectively. The $LC_{95}$ values for temephos and pirimiphos-methyl were 20 (95% CL 10–50) and 15 (95% CL 10–30) times greater, respectively, than the $LC_{95}$ value for *s*-methoprene.

**Selectivity Ratios.** *s*-Methoprene had the greatest selectivity for *Ae. vigilax* compared with *L. tenuicornis*, followed by *Bti*, temephos, and pirimiphos-methyl (Table 1).

**Field Bioassays.** The 4 insecticides killed 100% of the caged *Ae. vigilax* at the estimated field concentration (Table 2), as predicted by the laboratory $LC_{95}$ values (Table 1). The laboratory bioassays were also good predictors of nontarget effects in the field. Shrimp mortality was significantly greater with temephos and pirimiphos-methyl than with *Bti* or *s*-methoprene. Treatment with the organophosphorous compounds killed 100% of caged shrimp within 24 h. The field application of *s*-methoprene and *Bti* did not affect shrimp survival.

There was no evidence of statistically significant changes in the measured abiotic water characteristics as a consequence of treatment with *s*-methoprene and *Bti* (Table 3). In contrast, the temephos and pirimiphos-methyl treatments decreased pH and increased turbidity.

## Discussion

Pirimiphos-methyl (trade name Actellic) was described as being "only moderately toxic to fish," with an $LC_{50}$ value of 0.34 ppm in a 24-h static bioassay for the rainbow trout (*Salmo gairdneri* Richardson) (Actellic 1990). In contrast, we have found that at the recommended application rate, this compound is toxic

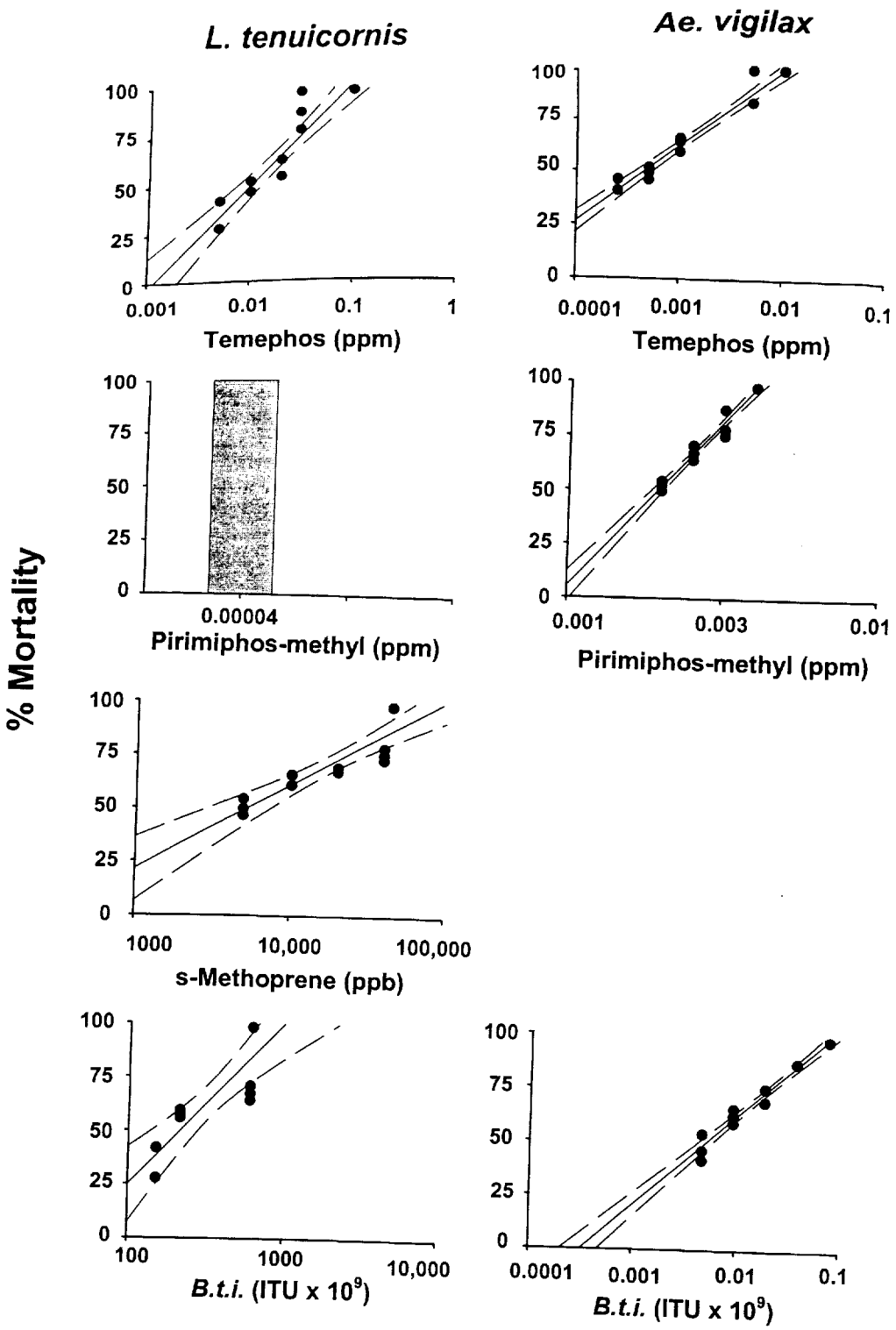


Fig. 1. Observed (circles) and predicted (solid line fitted using probit regression equations in Table 1; dashed lines indicate 95% CI) mortality of *Ae. vigilax* and *L. tenuicornis* treated with 4 insecticides. It was not practical to determine a full regression equation for the effects of pirimiphos-methyl on *L. tenuicornis*, because 100% mortality was recorded at 0.00004 ppm, which is 0.01% of the estimated field concentrations.

**Table 2. Field evaluation of the toxicity of 4 insecticides to a nontarget shrimp (*L. tenuicornis*) and target mosquito (*Ae. vigilax*) species in saltmarsh pools**

| Active ingredient | % *L. tenuicornis* mortality ± SD after 24 h | | | | % *Ae. vigilax* mortality ± SD after 24 h | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Treatment | *t* value | *P* | Control | Treatment | *t* value | *P* |
| Temephos, ppm | 8 ± 5 | 100 ± 0 | −1.28 | <0.01 | 9 ± 4 | 100 ± 0 | −1.27 | <0.01 |
| Pirimiphos-methyl, ppm | 8 ± 5 | 100 ± 0 | −1.28 | <0.01 | 9 ± 4 | 100 ± 0 | −1.27 | <0.01 |
| *Bti* (ITU) | 5 ± 9 | 8 ± 8 | −0.35 | 0.75 | 6 ± 5 | 100 ± 0 | −1.31 | <0.01 |
| *s*-Methoprene, ppm[a] | 8 ± 6 | 7 ± 3 | 0.39 | 0.72 | 4 ± 5 | 100 ± 0 | −15.1 | <0.01 |

Degrees of freedom = 4 for all treatments. ITU, international toxic units/mg.
[a] Because *s*-methoprene is an insect growth regulator, the final mortality counts were recorded at 120 h after treatment.

to the fish *Pseudomugil signifer* Kner (Pacific Blue-eye), with an $LC_{50}$ value of 0.09 ppm (Brown et al. 1998a). Field trials showed that the compound was moderately toxic to *Daphnia pulex* (Leydig), with a static 24-h bioassay $LC_{50}$ value of 0.36 ppm (Actellic 1990). In our study, pirimiphos-methyl was highly toxic to the nontarget shrimp, with an $LC_{95}$ value that was ≈0.01% of that cited for *S. gairdneri*. Accordingly, because this compound is also 100 times more toxic to *L. tenuicornis* than the cohabiting target mosquito *Ae. vigilax*, the use of this product in habitats that support shrimp species is strongly discouraged.

**Table 3. Mean change ± SE in abiotic water characteristics of saltmarsh pools 24 h after treatment with insecticides**

| Abiotic parameter | Treatment | Change | 95% CI for difference of means | *t* value | *P* |
|---|---|---|---|---|---|
| pH | Control[a] | 8.9 ± 0.2 | | | |
| | Temephos | 7.1 ± 0.2 | 1.42–2.23 | 12.6 | 0.02 |
| | Pirimiphos-methyl | 6.8 ± 0.1 | 1.80–2.43 | 18.8 | <0.01 |
| | Control | 8.5 ± 0.2 | | | |
| | *Bti* | 8.4 ± 0.2 | −0.44–0.63 | 0.5 | 0.64 |
| | Control | 6.7 ± 0.3 | | | |
| | *s*-Methoprene | 6.3 ± 0.1 | −0.189–0.949 | 1.86 | 0.14 |
| Turbidity (NTUs) | Control[a] | 69.7 ± 8.6 | | | |
| | Temephos | 127.7 ± 23.4 | −98.0–18.0 | −4.02 | 0.02 |
| | Pirimiphos-methyl | 117.0 ± 18.5 | −80.1–14.6 | −4.01 | 0.02 |
| | Control | 37.7 ± 5.1 | | | |
| | *Bti* | 30.7 ± 4.0 | −3.47–17.5 | 1.86 | 0.14 |
| | Control | 10.3 ± 1.5 | | | |
| | *s*-Methoprene | 15.0 ± 3.5 | −10.7–1.4 | −2.13 | 0.10 |
| Dissolved oxygen (mg/liter) | Control[a] | 5.18 ± 0.24 | | | |
| | Temephos | 6.02 ± 0.27 | −1.42–0.27 | −4.06 | 0.02 |
| | Pirimiphos-methyl | 4.66 ± 0.48 | −0.35–1.37 | 1.67 | 0.17 |
| | Control | 6.99 ± 0.51 | | | |
| | *Bti* | 7.21 ± 1.05 | −2.10–1.65 | −0.34 | 0.75 |
| | Control | 3.87 ± 0.69 | | | |
| | *s*-Methoprene | 4.32 ± 0.83 | −2.19–1.28 | 1.28 | 0.51 |
| Temp, °C | Control[a] | 36.6 ± 0.1 | | | |
| | Temephos | 35.1 ± 0.8 | −2.85–0.218 | −3.24 | 0.03 |
| | Pirimiphos-methyl | 36.6 ± 0.6 | −1.41–0.546 | −1.23 | 0.29 |
| | Control | 31.6 ± 0.4 | | | |
| | *Bti* | 31.6 ± 0.4 | −0.86–0.86 | 0.0 | 1.00 |
| | Control | 23.3 ± 0.5 | | | |
| | *s*-Methoprene | 23.3 ± 0.2 | −0.465–1.19 | 0.89 | 0.29 |
| Salinity (g/liter) | Control[a] | 7.03 ± 1.40 | | | |
| | Temephos | 6.67 ± 1.25 | −2.64–3.38 | 0.34 | 0.75 |
| | Pirimiphos-methyl | 6.97 ± 1.46 | −3.18–3.32 | 0.06 | 0.96 |
| | Control | 6.37 ± 0.95 | | | |
| | *Bti* | 7.07 ± 0.15 | −2.23–0.84 | −1.27 | 0.27 |
| | Control | 23.7 ± 5.12 | | | |
| | *S*-Methoprene | 20.1 ± 4.92 | −7.72–15.0 | 0.89 | 0.42 |

Paired *t*-tests (df = 4) were used to analyze the significance of differences in changes of the abiotic water variable before and after insecticide treatment. Nepholometric turbidity units.
[a] Temephos and pirimiphos-methyl evaluations were conducted at the same time. Hence, one set of untreated control pools were used for the evaluation of both products.

Our results also indicate that temephos should be used cautiously. Although this product exhibited greater selectivity for *Ae. vigilax* than pirimiphos-methyl, field applications in closed, shallow, saltmarsh pools will result in high mortality of *L. tenuicornis*. This is consistent with previous findings on the toxicity of temephos to nontarget species of crustaceans, including shrimp, mysids, cladocerans, and crabs (Ward and Busch 1976, Mulla et al. 1978, Mortimer and Chapman 1995, Pierce et al. 1996). Furthermore, although organisms using saltmarsh habitats often experience broadly fluctuating environmental conditions, the fact that the organophosphorous compounds influenced pH and turbidity in the field also gives some cause for concern. We can only postulate that the increased turbidity was the result of stressed organisms disturbing sediment.

The high selectivity ratios, excellent field efficacy, and lack of influence on abiotic characteristics show that *s*-methoprene and *Bti* are ideal for the control of *Ae. vigilax* in Australian saltmarsh habitats.

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