

FIELD EFFICACY AND NONTARGET EFFECTS OF THE MOSQUITO LARVICIDES TEMEPHOS, METHOPRENE, AND *BACILLUS THURINGIENSIS* VAR. *ISRAELENIS* IN FLORIDA MANGROVE SWAMPS

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ABSTRACT. We compared the efficacy and nontarget effects of temephos, *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*), and methoprene applied by helicopter to control mosquito larvae in mangrove swamps on Sanibel Island, FL, in May 1997. Three sites per treatment and 3 untreated sites were used. Temephos (Abate[®]) was applied at 37 ml/ha (43% active ingredient [AI]), *B.t.i.* granules (Vectobac G[®]) were applied at 5.606 kg/ha (200 International Toxic Units/mg), and methoprene (Altosid[®] ALL) was applied at 213 ml/ha (5% AI). Efficacy was quantified by monitoring the survival of caged and uncaged larval *Aedes taeniorhynchus*. We quantified mortality of sentinel nontarget amphipods (Talitridae) at all sites, monitored the effect of temephos on flying arthropods using light traps, and collected dead insects in tarps suspended under mangroves in areas treated with either temephos or methoprene. Each pesticide showed good overall efficacy but occasional failures occurred. No detectable mortality of amphipods or flying insects attributable to pesticides was found. The inconsistent field efficacies of the pesticides indicate a need for reinspection of treated sites in this habitat.

KEY WORDS *B.t.i.*, Abate[®], Altosid[®], mosquito control, *Aedes taeniorhynchus*

INTRODUCTION

Coastal marshes and mangrove swamps are often managed for mosquito control because salt-marsh mosquitoes can emerge in numbers that threaten the health of humans and livestock. The salt-marsh mosquito *Aedes taeniorhynchus* (Wiedemann) can make our study area, Sanibel Island, FL, nearly uninhabitable, and it can harm livestock (Addison and Ritchie 1993). *Aedes taeniorhynchus* can fly long distances to obtain blood meals (Ritchie and Montague 1995), so one of the best methods of controlling this species is to kill the larvae in local breeding sites before they disperse as adults. *Aedes taeniorhynchus* larvae develop in temporary pools that form in depressions above the upper intertidal zone in mangrove swamps and salt marshes. They often occur in such large numbers that emergence of even a small percentage of the population can create problems for residents. Because salt marshes and mangrove swamps are productive habitats that sustain a variety of wildlife (review, Mitsch and Gosselink 1993), environmentally sound mosquito management is a conservation priority. We conducted a large-scale field study on 3 larvicides, temephos, *Bacillus thuringiensis* var. *israelensis* (de Barjac) (*B.t.i.*), and methoprene, to assess whether these larvicides could control mosquitoes in mangrove areas without causing substantial mortality of nontarget amphipods and canopy insects.

The 3 pesticides have different modes of action and expected nontarget effects. All are relatively

safe for vertebrates at levels used in mosquito control, but vary in risk to invertebrates. Temephos is an organophosphate pesticide that acts by inhibiting cholinesterase, and it is toxic to insects and some other nontarget invertebrates (Smith 1987, Brown et al. 1996). The bacterium *B.t.i.* is a microbial insecticide. It controls mosquitoes with toxins whose action is specific to nematoceran dipterans (e.g., mosquitoes and black flies), and is expected to have little effect on other macroinvertebrates (Back et al. 1985, Federici 1995). Methoprene is similar in structure to insect juvenile hormone, and it causes mortality in mosquitoes by interfering with metamorphosis. Although the action of methoprene is not specific to mosquitoes, many insects are insensitive to the levels of methoprene used in mosquito control (Hershey et al. 1995, but see Gelbic et al. 1994). Methoprene is not expected to affect adult insects. Methoprene and *B.t.i.* were recently reported to have negative indirect effects on predatory insects (Hershey et al. 1998). However, declines of predators occurred only after 2-3 years of frequent application of the materials at maximum label rates, and no such effects have been documented during normal use of these materials.

This study offers a side-by-side comparison of the field efficacy of the pesticides when applied by aircraft to swamps composed of mangroves and grasses. To compare the nontarget effects of the pesticides, we monitored the survival of a common amphipod (Talitridae) in treated and control sites. We also tested whether temephos would kill invertebrates inhabiting the mangrove canopy. Nontarget insects in the mangrove canopy could be affected by aerial applications of temephos because it is a contact poison.

MATERIALS AND METHODS

Study sites: The study was conducted in May 1997, during the 1st larviciding operations of that

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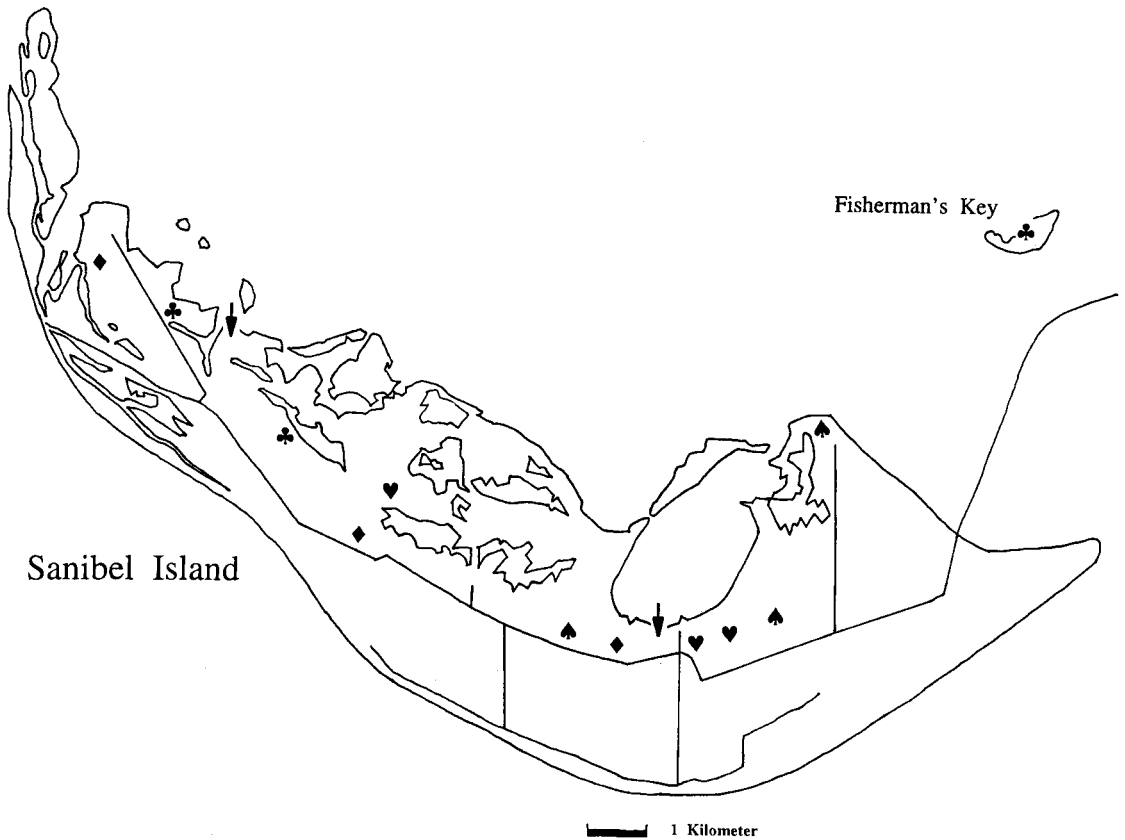


Fig. 1. Map of a study area, Sanibel Island, FL, showing the location of mangrove areas treated with mosquito control pesticides or used as controls. Sites ranged in size from 3.2 to 27.6 ha. Treatments correspond to symbols as follows: ♣ temephos, ◆ *Bacillus thuringiensis* var. *israelensis*, ♠ methoprene, ♥ control.

year. We established 11 study sites on Sanibel Island plus one on nearby Fisherman's Key (Fig. 1). Sites ranged in size from 3.2 to 27.6 ha. The dominant species in all areas were red mangrove (*Rhizophora mangle* (L.)) and black mangrove (*Avicennia germinans* (L.)), with a few areas of grasses or white mangroves (*Laguncularia racemosa* (Gaertn)) in some sites. These sites typically flood during high-tide cycles that coincide with onshore winds, and the water usually disappears within several days (G. Wichterman, personal communication). All sites flooded with rain and tide waters during a storm on May 12, 1997, and 1st-stage *Ae. taeniorhynchus* appeared the next day. Each larvicide was applied to 3 sites and 3 control sites were not treated. Control sites flooded simultaneously with treated sites but produced few mosquitoes. We were unable to assign control sites at random with respect to the presence of mosquitoes, because even small untreated areas can produce enormous numbers of mosquitoes that attack nearby residents.

We sampled study sites on the day before pesticide application and on the subsequent 6 days. At all sites, we monitored survival of caged mosquito larvae and amphipods, and sampled uncaged mos-

quitoes in treated sites using dippers. We evaluated whether temephos would affect terrestrial insects by comparing insect collections from sites treated with temephos to sites that were either untreated or treated with methoprene. Details are given below.

Pesticide application: Lee County Mosquito Control District personnel applied all pesticides by helicopter, and cleaned application gear between applications of different materials. The *B.t.i.* granules (Vectobac G[®], Abbott Laboratories, North Chicago, IL) were applied on May 14, 1997, at a rate of 5.606 kg/ha (200 International Toxic Units per mg; 5 lb/acre). Temephos and methoprene were applied on May 15, 1997, except for 1 application of temephos to Fisherman's Key on May 16, 1997. Temephos (Abate[®], Clarke Mosquito Control Products, Roselle, IL) was applied at 37 ml/ha (43% active ingredient [AI]; 0.5 fl oz/acre). Methoprene (Altosid[®] ALL, Wellmark International, Bensenville, IL) was applied at 213 ml/ha (5% AI, 3 oz/acre). One *B.t.i.* site was retreated with temephos after 2 days to prevent emergence of mosquitoes that survived the *B.t.i.* application.

Sentinels: We used field-collected *Ae. taeniorhynchus* larvae and amphipods in the family Tali-

tridae as sentinels to monitor insecticide activity. *Aedes taeniorhynchus* and the amphipods were the only aquatic macroinvertebrates consistently present in study sites after flooding, probably because the habitat is ephemeral. Other aquatic macroinvertebrates occasionally observed during field work were fiddler crabs, water boatmen (Corixidae), and dytiscid beetles. Sentinels were held in floating predator-exclusion cages. Cages were 1.9-liter plastic buckets that were suspended in the water by a ring of styrofoam and attached to a stake by string. Cages had 2 screened windows measuring 5×10.5 cm, and had screened lids that were removed during insecticide applications. Before pesticide application on May 14, 25 2nd-stage mosquito larvae were placed in each of 2 cages per site, and 25 amphipods were placed in each of 2 cages per site. We recorded sentinel organism survival each day.

Dip samples: We monitored pesticide efficacy using 3 transects of 15 mosquito-dipper samples per site (45 dips of 350 ml each). We used a random dipping pattern at all sites except for 1 site treated with methoprene. This site contained large numbers of mosquitoes that formed dense aggregations, and random sampling underestimated their abundance. Beginning on May 18, we targeted aggregations in our dip transects to check for mosquitoes dying as mature 4th instars or pupae, as is typical of methoprene. On this date we also put 10 4th-stage larvae into each of 3 additional sentinel cages, to see what proportion of previously uncaged mosquitoes would metamorphose. Aggregations did not pose sampling problems at other sites because mosquitoes either were dead by the time aggregations formed or were too sparse to form aggregations.

We used analysis of variance (ANOVA) to compare the abundance of mosquitoes before vs. after treatment in treated sites. Abundances were transformed as $\ln(n + 1)$ to normalize their distribution. Data were the mean of the number per dip averaged over 2 pretreatment dates for temephos (May 14, 15), compared to the mean abundance of 2 post-treatment dates (May 18, 19). Only 1 pretreatment date was available for the *B.t.i.* sites (May 14), and we used May 15 as the posttreatment date because 1 site had to be retreated with temephos after May 15. Natural mortality cannot be factored out of the data set, but the data allow a comparison of the relative proportion of mosquitoes emerging from each treatment. Analysis of the sentinel data from controls showed whether mortality could be attributed to pesticides. Unfortunately, we could not perform a statistical test for uncaged mosquitoes exposed to methoprene because we had suitable data from only 1 site. The 2nd site dried, and larval aggregation at the 3rd caused an apparent rise in sample numbers after treatment. However, sentinel data were available for statistical tests of the effect of methoprene.

Canopy insects: Preliminary sampling showed

that insects were too sparse in the mangrove canopy for effective sampling by sweep net (15 canopy sweeps typically yielded fewer than 4 insects). Therefore, we tested whether temephos affected flying insect abundance by collecting insects with Centers for Disease Control light traps (CDC traps) and ultraviolet light traps (UV traps) in 2 sites treated with temephos (Wulfert's Point and West Impoundment Swale), and in 2 sites that are not larvicided (Pole Line and Tarpon Bay). We placed 2 CDC traps and 1 UV trap in each site, at least 20 m apart. The vegetation was thick in these areas and so it is unlikely that the traps attracted insects from outside the designated sites. We could not see from 1 trap to the next and had to follow flagged trails to find them. However, insects were free to fly in and out of sites during the study. We collected insects on the night before temephos was applied, and on the subsequent 4 nights. We used ANOVA to determine whether treated areas showed greater differences in abundance than controls after the pesticide application date. Data points were transformed as $[\ln(\text{pretreatment abundance}) - \ln(\text{posttreatment abundance})]$, where the pretreatment data were collected May 14 p.m. to May 15 a.m., and the posttreatment data were collected May 16 p.m. to May 17 a.m.. The posttreatment collection occurred on the night after the highest mortality was seen in larval mosquitoes.

Not all insects are attracted to light traps and some cannot fly, so we used a 2nd method to test whether temephos caused mortality of canopy insects. We suspended 3 3.8-m² tarps under the mangrove canopy in all sites treated with methoprene and temephos to collect any insects killed by insecticide application. We used methoprene sites as controls for the effects of temephos, because both insecticides are applied as spray from helicopters. Methoprene should not kill canopy insects immediately because few of the insects would be likely to metamorphose during the brief study. The center of each tarp was weighted to form a funneled shape so that insects would not be swept out of the tarps by breezes. We collected all dead or moribund insects from these tarps on the 2 days following insecticide applications.

RESULTS

Sentinel organisms

All 3 materials killed sentinel mosquitoes. Heavy mortality occurred in treated sites but survival was high in controls (Fig. 2A). Ninety-five percent of sentinel mosquitoes died in 2 of 3 sites treated with temephos, but all survived in the 3rd site, Fisherman's Key. This site was treated 1 day later, and contained older 4th-stage larvae that may have been less susceptible to temephos (G. Wichterman, personal communication). The effect of temephos was significant when we eliminated this site from sta-

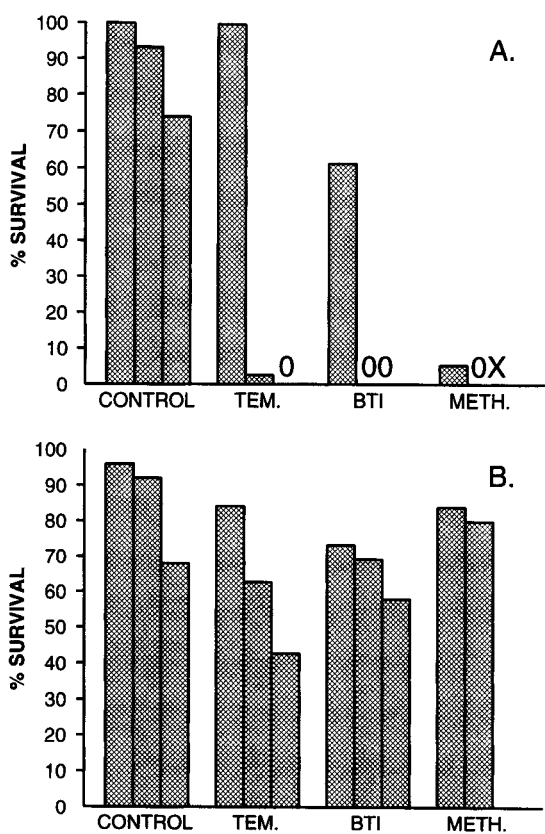


Fig. 2. Survival of *Aedes taeniorhynchus* larvae (A) and Talitridae amphipods (B) held in cages in Florida mangrove areas that were treated with temephos, *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*), or methoprene, or untreated. Bars represent the proportion of 50 sentinel organisms surviving in 1 site until 4 days posttreatment, except for the 1st *B.t.i.* site, where data are from day 2 posttreatment; this site was later retreated with temephos. Two cages of 25 of each type of organism were used per site, with 3 sites per treatment. CONTROL = untreated, TEM. = temephos, BTI = *B.t.i.*, METH. = methoprene. 0 = no survival, × = loss of site through drying.

tistical analysis (df 1,3, $F = 34.5$, $P = 0.01$). The *B.t.i.* also killed sentinel mosquitoes (ANOVA, df 1,4, $F = 8.8$, $P = 0.04$). All sentinel mosquitoes died in 2 treated sites but 60% survived in the 3rd site until the 2nd day posttreatment, when they were killed with temephos. Survival in the cages was higher than in the uncaged population (see *Mosquito dip transects* below). This could result from poor distribution of *B.t.i.* granules into the cages. Methoprene prevented the maturation of all sentinel mosquitoes at 1 site, but approximately 5% matured at a 2nd site where larvae were abundant and formed aggregations. We had also caged 30 additional larvae at this site that had been free-swimming for 5 days postapplication, and 10% of these emerged. The 3rd site dried shortly after pesticides were applied, and was eliminated from the

analysis. Methoprene significantly decreased sentinel mosquito survival (ANOVA, df 1,3, $F = 29.9$, $P = 0.01$).

No effects were detected of any of the 3 insecticides on amphipod survival (Fig. 2B; ANOVAs on: temephos vs. control, df 1,3, $F = 1.4$, $P = 0.32$; *B.t.i.* vs. control, df 1,4, $F = 3.6$, $P = 0.13$; methoprene vs. control, df 1,3, $F = 0.22$, $P = 0.67$). We used data from day 4 for all but 1 site because all materials should cause mortality within a few days of treatment, and later data would contain more noise from natural mortality. For 1 of the *B.t.i.* sites we used survival data from the 2nd day posttreatment because the site was subsequently retreated with temephos.

Mosquito dip transects: Dip transects for mosquito larvae showed similar mortality to that obtained from the sentinel data (Fig. 3), but no effects were statistically significant at the $P = 0.05$ level. For temephos, numbers of uncaged mosquito larvae dropped dramatically at the 2 sites where sentinel mosquitoes showed poor survival. However, at 1 of these sites approximately 30% of mosquitoes survived to emergence even though the sentinel data indicated survival of less than 5%. The temephos application completely failed to control mosquitoes at Fisherman's Key, where the larvae were treated as late 4th instars.

All sites treated with *B.t.i.* showed decreases in mosquito populations (Fig. 3B), although 1 site could not exhibit much of a decrease because very few larvae were present initially. An ANOVA indicated a strong trend toward an effect of *B.t.i.* on mosquito abundance whether or not this site was included (all sites, df 1,4, $F = 4.9$, $P < 0.09$; 1 site excluded, df 1,3, $F = 13.0$, $P < 0.07$).

Methoprene killed nearly all mosquitoes at metamorphosis in 1 of the 2 sites that remained wet until mosquitoes emerged (Fig. 3C). At the 2nd site, the final dip sample consisted of 35% shed pupal skins and 65% dead pupae or larvae and many biting adults were present, showing that methoprene did not yield adequate control. This was the same site that showed emergence of 5% of the original caged sentinels and 10% of field-exposed mosquitoes that were caged on day 5. Dip samples at both sites contained many dead pupae, 4th-stage larvae, and partially emerged adults, as is typical of the action of methoprene.

Canopy insects: Analysis of light trap data did not indicate any consistent loss of insects from treated sites (Fig. 4). No significant decreases occurred in the abundance of flying insects captured in CDC traps in sites treated with temephos (ANOVA, df 1,2, $F = 4.3$, $P = 0.17$). Results from UV light traps were similar. Unfortunately, 2 of the UV traps failed during the pretreatment night, 1 from a treated site and the other in a control. Therefore, instead of analyzing pre- vs. posttreatment differences for UV traps, we compared the posttreatment abundance of insects in controls vs. treated areas

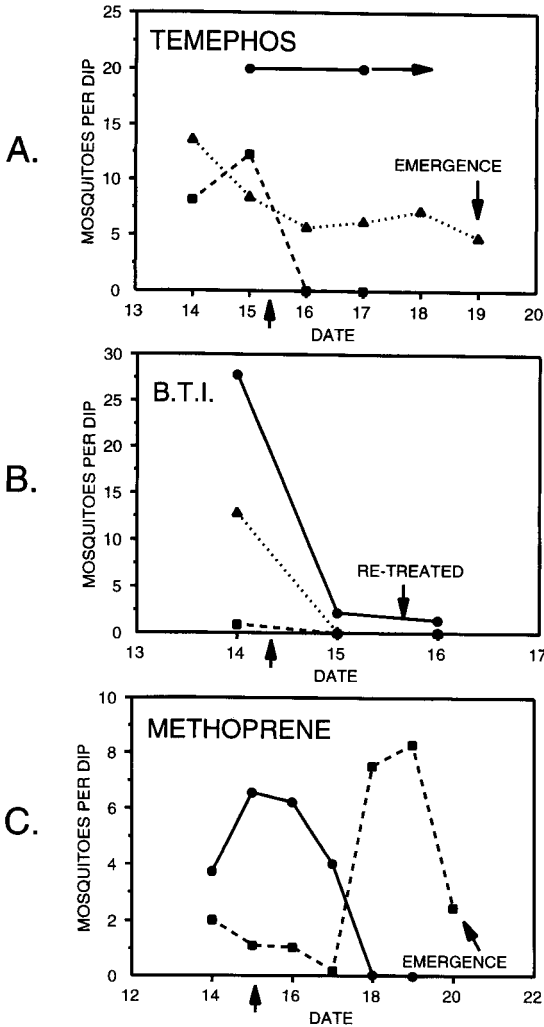


Fig. 3. Numbers of *Aedes taeniorhynchus* mosquito larvae per dip in 3 transects of 15 mosquito-dipper samples per site, per date, in Florida mangrove areas treated with (A) temephos, (B) *Bacillus thuringiensis* var. *israelensis*, or (C) methoprene. Each line represents a separate site. The arrow on the x axis indicates the date of pesticide application.

on May 17, the 1st night when all traps ran properly. No effect of temephos (ANOVA, df 1,2, F = 2.1, P = 0.28) was detected. Most of the insects captured in light traps were small flies in the families Psychodidae and Ceratopogonidae. Other common taxa included mosquitoes, crane flies, moths, beetles, and Hymenoptera.

Table 1. Numbers of insects caught in 3.8-m² tarps hung below mangrove canopy sprayed with either methoprene or temephos, during 2 days after pesticide application.

	Ants	Wasps	Beetles	Moths	Spiders	Flies	Bugs	Other	Total
Temephos	91	2	3	0	3	6	0	3	108
Methoprene	17	3	19	1	6	2	9	4	61

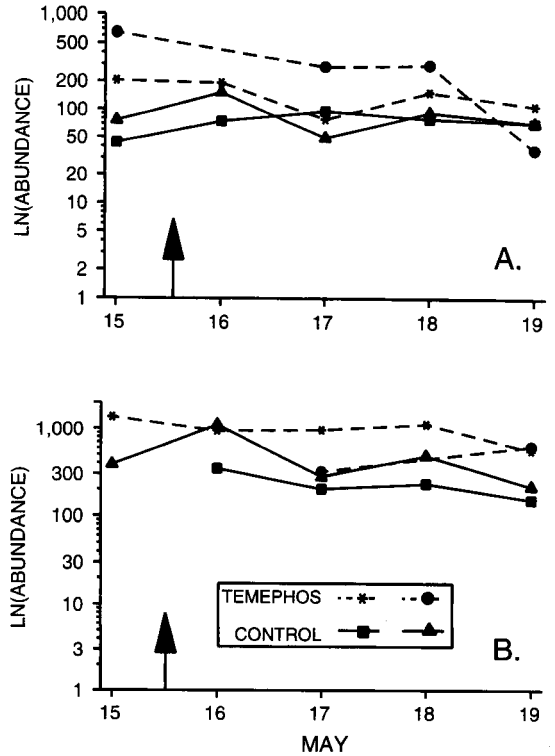


Fig. 4. Light trap catches of flying insects in Florida mangrove areas that were treated with temephos for mosquito control (dashed lines), or left untreated (solid lines). Two Centers for Disease Control light traps (A) and 1 ultraviolet light trap (B) were placed at each site. The arrow on the x axis indicates the date of pesticide application.

Tarps strung under mangroves treated with temephos and methoprene collected similar numbers of dead arthropods in each treatment over the 48 h after pesticide application (ANOVA, df 1,4, F = 0.206, P = 0.67; Table 1).

DISCUSSION

All 3 pesticides caused significant mortality of sentinel mosquito larvae in treated sites compared to controls, demonstrating that the pesticides (rather than predation or other natural sources of mortality) were responsible for reducing mosquito populations. The mortality of sentinel mosquitoes paralleled decreases in natural populations in treated sites. However, sentinel mosquitoes sometimes showed greater or lesser declines after pesticide ap-

plication than natural populations. These differences could result from uneven distribution of pesticides over the sites or different levels of exposure due to the cages.

Larvicides were often effective in killing uncaged *Ae. taeniorhynchus* larvae underneath the mangrove canopy, yielding average control levels of 80–90%. This is a good level of control for this habitat, which is difficult to treat because the canopy can interfere with delivery of the materials and the water is often hidden from view. However, in at least 1 site per material, the larvicides failed to yield the high level of control necessary for salt-marsh mosquitoes.

Temephos failed to control mosquitoes adequately at 2 sites, including 1 complete failure. This may have been due to a 1-day delay in treating the site, because older *Ae. taeniorhynchus* larvae that are approaching pupation are less susceptible to temephos (G. Wichterman, personal communication).

The *B.t.i.* reduced dip-counts of mosquitoes to 0 at 2 sites but a 3rd site had to be retreated with temephos to further reduce emergence from 14% to nearly 0. Mosquitoes were more abundant at this site than at any of the others (Fig. 3), and *B.t.i.* sometimes fails to control dense populations of mosquitoes if they deplete the toxin from the water before all obtain a lethal dose (e.g., Mulla et al. 1990, Becker et al. 1992).

Finally, methoprene yielded 1 success and 1 partial failure. Dame et al. (1998) recently demonstrated resistance to methoprene for a population of *Ae. taeniorhynchus* on Captiva Island that had been exposed to extended-release, 150-day Altosid briquets over a period of 6 years. Captiva and Sanibel islands are part of the same land mass, and it is possible that the population of *Ae. taeniorhynchus* is also the same. This could explain the emergence of mosquitoes from our methoprene-treated sites, although uneven application of materials is also a possibility.

The occasional failures of the 3 larvicides demonstrate that reinspection and retreatment of breeding sites are very important in ensuring an effective mosquito control program. However, a 2nd application of larvicide is only feasible for sites treated with *B.t.i.* early in larval development, or before the late 4th stage with temephos. Methoprene does not afford this opportunity because it kills mosquitoes during pupation and emergence, when it is too late to re-treat with a larvicide.

Pesticides did not cause detectable mortality of amphipods. The amphipods had been collected in aquatic samples from the sites and were sometimes seen swimming in the water; however, they often climbed up the screens of the sentinel buckets and rested above the waterline. This could have reduced their exposure to pesticides. Most Talitridae are semiaquatic species, which explains their abundance in the intermittently flooded high marsh.

Comparatively few other studies have been pub-

lished of the nontarget effects of these pesticides on salt marshes or mangrove swamp fauna with which to compare our results, but we review those available below. We found few aquatic nontarget organisms in our ephemeral sites; however, we report studies of macroinvertebrates or fish that may enter the high marsh during more extended periods of flooding. As a caveat in interpreting these studies, all but the 1st 2 presented were conducted in laboratories. Laboratory studies often result in greater exposure of organisms to toxins because of clean water conditions and the inability of organisms to behaviorally avoid contaminants.

In a field study, the granular formulation of temephos was reported to cause behavioral changes in fiddler crabs. The fiddler crabs actively collected and ingested the granules, which were applied at 63 g AI/ha (Ward and Busch 1976, Ward et al. 1976). Crabs may have been attracted by the celatom in the granules. This effect is not expected with the liquid temephos used in our study area because the liquid is applied at a lower rate and the crabs cannot collect it. The 2nd field study was performed by Pierce et al. (1989), who quantified the effects of liquid temephos on 2 species of shrimp, juvenile snook, and sheepshead minnows. Temephos was applied at twice the application rate used in our study. Although temephos was safe for the fish, results for the shrimp were unclear because 32% mortality occurred at 1 of their 3 treated sites. However, this mortality was not correlated with the amount of temephos in the water.

Roberts (1995) found that *B.t.i.* had no effect on either a salt-marsh gammaroid amphipod or a shrimp. McKenney and Celestial (1996) found that methoprene killed a marine mysid shrimp exposed to 125 µg/liter over 4 days, with sublethal effects occurring at levels as low as 2 µg/liter. In a comparative study, Lee and Scott (1989) used the mummichog fish (*Fundulus heteroclitus*) to compare the acute toxicities of *B.t.i.*, methoprene, and temephos. Temephos was more toxic than the other larvicides, but all 3 were safe for the fish at the expected water concentration for mosquito control. Brown et al. (1996) compared the toxicity of *B.t.i.*, methoprene, and temephos to an Australian estuarine shrimp (*Leander tenuicornis*). Methoprene and *B.t.i.* were not toxic at levels used in mosquito control. The median lethal concentration of temephos was 0.01 ppm, and concentrations of temephos this high may occur under operational conditions in Florida mangroves. The expected concentration of temephos applied at 14 g (0.5 oz) of 43% AI temephos/acre (as in our study) is 0.026 ppm, with realized concentrations of 0.12–0.0045 ppm temephos found in surface and midwater samples, respectively (Pierce et al. 1996). However, whether laboratory studies on an Australian shrimp can predict toxicity for southeastern North America intertidal fauna under field conditions is not known. In conclusion, although temephos had some documented toxic ef-

fects, little field evidence exists that it causes mortality of nontarget macroinvertebrates or fish when applied at rates used in mosquito control. Relatively low potential exists for mortality of aquatic nontargets in our study area because few species utilize the high marsh during the brief period that it is flooded, and the more species-rich low marsh is rarely treated because regular tidal flooding reduces or prevents mosquito breeding.

This is the 1st study of whether temephos affects mangrove canopy arthropods. We did not find significant decreases in nocturnal flying insects in sites treated with temephos compared with controls. We also compared the number of insects collected in tarps under canopy treated with temephos vs. methoprene, and found a similarly low number of dead insects (an average of 10/tarp/day). In conclusion, temephos, *B.t.i.*, and methoprene effectively controlled *Ae. taeniorynchus* without observable mortality of the nontarget amphipods, and temephos did not cause detectable changes in the abundance of flying insects.

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