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INFLUENCE OF TEMPERATURE AND CONCENTRATION OF VECTOBAC® ON CONTROL OF THE SALT-MARSH MOSQUITO, *OCHLEROTATUS SQUAMIGER*, IN MONTEREY COUNTY, CALIFORNIA

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ABSTRACT. Laboratory susceptibility bioassays were conducted to determine the efficacy of VectoBac® TP (*Bacillus thuringiensis* var. *israelensis* [*Bti*]) at different concentrations and temperatures against the salt-marsh mosquito *Ochlerotatus squamiger*. Bioassays on late 3rd- and early 4th-stage larvae, read at 72 h and 14°C produced an LD_{90} of 0.223 mg/liter, whereas more than double this dose was required to produce similar mortality at 6°C. A field trial in the winter of 2001–02 of an aerially applied VectoBac TP formulation in Salinas, CA, corroborated laboratory bioassay observations by producing 97–100% control of *Oc. squamiger* at 72 h postapplication. Inconsistencies in mortality with field applications of VectoBac TP previously observed by North Salinas Valley Mosquito Abatement personnel were most likely caused by uneven application rates and varying temperatures and water volumes.

KEY WORDS *Ochlerotatus squamiger, Bacillus thuringiensis israelensis,* field control, laboratory susceptibility assays

INTRODUCTION

Ochlerotatus squamiger Coquillet (formerly *Aedes squamiger*) is a univoltine nuisance mosquito that breeds in the brackish intertidal pools associated with pickleweed (various species of *Salicornia*) and cattails (*Typha*) along the California coastline from Sonoma County to Baja Mexico (Bohart and Washino 1978). Morphological similarities and isoenzyme allele profiles place *Oc. squamiger* in the *Ochlerotatus stimulans* group, within which it is most closely related to *Ochlerotatus washinoi* Lanzaro and Eldridge (formerly coastal *Aedes increpitus* Dyar). Three to 6 egg hatch cycles occur from December to March that coincide with tidal pool flooding at high tides and rainfall. Larvae are found in waters ranging from 4 to 14°C and take approximately 48 days from hatching to adult emergence. If left untreated, multiple egg hatchings result in large numbers of *Oc. squamiger,* creating a major nuisance for people living in surrounding areas and outlying foothills. In addition, periodic isolations of California encephalitis–like virus have been made in Morro Bay populations (Eldridge et al. 1991, Fulhorst et al. 1996). Consequently, *Oc. squamiger* is a target species for control. Most districts currently use granular, aqueous suspension, water dispersible granular, and technical powder formulations of *Bacillus thuringiensis* var. *israelensis* (*Bti*) for control of mosquito larvae on more than 1 million acres in the USA (DeChant, unpublished data). These formulations are used extensively in floodwaters, salt marshes, rice fields, pastures, and ditches. The use of *Bti* is increasing for several reasons, including development of resistance by mosquitoes to other insecticides, *Bti*'s safety to nontarget organisms, and advancements in formulation such as water-dispersible granules (Su and Mulla 1999).

In the winter of 2000, concerns expressed by North Salinas Valley (California) Mosquito Abatement District (MAD) personnel about the efficacy of VectoBac® TP against salt-marsh *Oc. squamiger* populations in Salinas were brought to our attention. They found that in areas treated with VectoBac TP, control was not as effective when compared with the results obtained with Baytex (Peter Ghormley, personal communication) or as would be expected by published reports of *Bti* control against *Oc. squamiger* (Garcia et al. 1982, Webb and Dhillon 1984). In field trials by Webb and Dhillon (1984) and Garcia et al. (1982), previously available commercial *Bti* formulations, such as Bactimos® WP and VectoBac WP, were used. Webb and Dhillon (1984) used the Bactimos WP formulation in laboratory susceptibility bioassays against late 3rd and early 4th instars, and they gave no indication of whether assays were conducted in fresh or brackish water or at what temperature. All laboratory susceptibility assays were conducted on early 4th instars so that comparisons could be made to previous work by Webb and Dhillon (1984).

The goal of this study was to ascertain the susceptibility of Monterey County *Oc. squamiger* populations against currently available *Bti* formulations at various temperatures in the laboratory. For comparative purposes, larval probit dose susceptibility profiles to 1 other *Bti* formulation (VectoBac WDG) and *Bacillus sphaericus* (*Bs,* VectoLex®

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WDG) were conducted at 10°C in brackish water, which is more typical of *Oc. squamiger* habitat conditions. Aerial applications of *Bti* against *Oc. squamiger* in Salinas were then conducted to corroborate laboratory observations.

MATERIALS AND METHODS

Laboratory Bti *susceptibility assays: Ochlerotatus squamiger* were collected from brackish tidal pools in Salinas as 2nd and 3rd instars and raised in enameled pans at 10°C in the tidal pool water that they were collected in. A few branches of pickleweed were added to each pan because the larvae grew healthier and more synchronized in its presence. Ground LabDiet® 5001 rodent diet (PMI® Nutrition International, Inc., Brentwood, MO) was used as an additional food source. Larvae were allowed to mature to the early 4th stage, at which time they were used for the bioassay.

The *Bti* susceptibility bioassays were conducted in waxed paper cups (Sweetheart, #S-304, Sweetheart Cup Company, Owings Mill, MD) on 4 replicates of 15 larvae in each replicate per concentration of formulation. The formulations used were VectoBac TP and VectoBac WDG. Each cup contained 99 ml of field-collected tidal pool water, 5 mg of ground rodent food, and varying concentrations of VectoBac WDG (37.4% *Bti,* 3,000 International Toxic Units [ITU]/mg) or VectoBac Technical Powder (100% *Bti,* 5,000 ITU/mg) diluted and delivered in 1 ml of tap water per cup. Controls contained only tidal pool water, 1 ml of tap water, and ground rodent diet. After the addition of the bacterial solution, the test solution was stirred briefly to ensure uniform mixture. The larval cups were then held at 10°C for the duration of the assay, and percent larval mortality was recorded from each cup every 24 h up to 72 h after addition of larvicide. For a comparison, an assay with VectoLex WDG (51% *Bs,* 650 *Bs* ITU/mg) was also conducted in the same manner as described above. Larval mortality counts were subjected to probit analysis (POLO PC LeOra Software, Berkeley, CA) to determine LD_{50} and LD_{90} levels from data after 72 h exposure (Finney 1972).

During the winter and spring months, tidal pool temperatures can vary from 4 to 14°C. Hence, VectoBac TP susceptibility assays were also conducted at 6 and 14°C to ascertain whether VectoBac TP LD_{50} and LD_{90} levels varied between temperatures.

Field trial: Three tidal pools near Moss Landing in Monterey County, subject to influence by Elkhorn Slough, were selected to carry out the field evaluation of VectoBac TP and Baytex against *Oc. squamiger.* Baytex was applied to another field for comparative purposes because the North Salinas Valley MAD was, at the time of this experiment, still applying Baytex for control of *Oc. squamiger* in a limited fashion, as allowed by California law until their supplies are depleted. Water depth ranged from 0 to greater than 1 m, with an estimated mean of about 30 cm. Larvae resting on the surface were sampled with a standard mosquito dipper (BioQuip Products Inc., Gardena, CA) where water depths ranged from 10 to 40 cm. The control tidal pool was 24.3 ha, whereas the VectoBac TP and Baytex pools were each 14.2 ha. The *Bti* was applied at the maximum label rate as a mixture of 909.01 g VectoBac Technical Powder (Valent Biosciences, Libertyville, IL) with 386.4 ml Golden Bear® GB-1111 oil (Golden Bear Oil Specialties, Inc., Oildale, CA) and 21.140 kg sand/ha as per label instructions. This application is equivalent to 4.5 billion ITU/ha and, at the given mean water depth, is equivalent to 1,473 ITU/liter of water. The VectoBac TP mixture was dispersed at approximately 10:00 a.m. from an Isolair (model 2600-20H, Troutdale, OR) hydraulic-powered broadcaster bucket at the 5-mm setting. The spray system was attached to a Bell OH-58 helicopter that flew 6 m above ground at 64.3 km/h with a swath width of 10 m. Baytex concentrate (Bayer Corp., Kansas City, MO) was applied at a rate of 59.14 ml active ingredient per hectare, which was prepared by mixing 59.14 ml of Baytex concentrate with 1,833 ml of water. The Baytex mixture was applied from an Isolair brand hydraulic-powered spray system (model 3900) with 6 CP® nozzles evenly spaced with 90° deflectors across an 8-m boom, each set at 2.07×10^2 kPa and openings of 1.55 mm. The spray system was attached to a Bell OH-58 helicopter that flew 2.5 m above ground at 80 km/h with a swath width of 20 m.

Within each tidal pool site, 5, 10-m-radius sampling stations were set up at areas where *Oc. squamiger* larval densities were highest as determined by prior dipping. Spacing between the center of stations varied, but was never less than 30 m. Each station consisted of 20 flagged dipping locations positioned within 10 m of the center. A sentinel cage was placed in the center of each station. The sentinel cages were made from rectangular Rubbermaid® 4630 plastic boxes (40.6 × 28.5 × 17.5 cm, Rubbermaid Home Products, Wooster, OH) stabilized in the water by blocks of styrofoam wired to their sides and tethered to a stake. Additionally, each sampling station in the VectoBac TP field included, next to each sentinel cage, a 10.41-liter Rubbermaid 2963 catch-bucket that served to capture VectoBac TP sand granules dispersed by the helicopter to examine evenness of application. Catch-buckets were filled with tap water and rested on the tidal pool substrate with their rims above the marsh water level. Each sentinel cage was half-filled with tidal pool water. One hundred field-collected *Oc. squamiger* larvae (mixed 1st-, 2nd-, and a few 3rd-stage larvae) were then added to each sentinel cage in order to observe mortality over the test period.

Immediately after aerial application, the

Table 1. Summary of probit analysis of the effect of *Bacillus thuringiensis* var. *israelensis* (*Bti*) on larval mortality of Monterey County, CA, *Ochlerotatus squamiger* exposed as 4th-stage larvae.

Bti larvicide agent	LD_{50} (mg/liter, 95% confidence interval)	LD_{90} (mg/liter, 95% confidence interval)	Slope	Index[1]
VectoBac WDG (assay run at 10°C)	0.14723 (0.12105–0.17894)	0.3958 (0.30887–0.5583)	2.984 ± 0.147	0.047
VectoBac TP[2] (assay run at 6°C)	0.22615 (0.20106–0.2551)	0.55226 (0.46339–0.69659)	3.305 ± 0.206	0.029
VectoBac TP[2] (assay run at 10°C)	0.12244 (0.10878–0.13634)	0.26627 (0.23326–0.31479)	3.798 ± 0.246	0.029
VectoBac TP[3] (assay run at 14°C)	0.09028 (0.07818–0.10292)	0.22288 (0.18856–0.27758)	3.265 ± 0.233	0.035

[1] Index of significance for potency estimation: for good data sets, value should be less than 0.4 (Finney 1972).
[2] Correlation coefficient of dose/mortality as determined by Microsoft Excel linear regression (Microsoft Corp. 1997), $r > 0.93$.
[3] $r > 0.75$.

VectoBac TP sand granules were removed from the buckets, left to dry 48 h, and weighed. In the Baytex and VectoBac TP fields, mosquito larvae were sampled with 1 dip at each flagged location 24 h before treatment and every 24 h after application for a period of 72 h posttreatment. During the field trial, temperature was measured, and rainfall data was retrieved from the National Weather Service Web site for the town of Salinas (21.5 km from the test site). Sampling in the control field was set up in the same manner, except sampling started at 0 h (i.e., the day the other fields were treated). Each time dipping was performed at a sampling station, sentinel cage mortality was assessed. Analysis of variance with Microsoft® Excel (Microsoft Corp., Redmond, WA) was performed on larva counts from each station to test whether decline in larva numbers was significant.

RESULTS

Laboratory susceptibility assays: Ochlerotatus squamiger were more susceptible to *Bti* (VectoBac TP and VectoBac WDG) than *Bs* (VectoLex WDG). Concentrations of VectoLex WDG of up to 10 mg/liter (above the maximum label rates) failed to produce even 50% mortality; therefore, we decided to discontinue this test for meaningful probit analysis. VectoBac WDG produced 50% mortality at 0.1472 mg/liter and an LD_{90} of 0.3958 mg/liter (Table 1) at 10°C. VectoBac TP formulation yielded an LD_{50} of 0.1224 mg/liter and an LD_{90} of 0.2663 mg/liter at 10°C (Table 1), whereas at 6°C, the LD_{50} and LD_{90} were 0.2262 and 0.5523 mg/liter, respectively. At 14°C, VectoBac TP was more effective at killing the larvae than at 6 or 10°C, as indicated by lower LD_{50} (0.0903 mg/liter) and LD_{90} (0.2229 mg/liter) levels. The data of Webb and Dhillon (1984), which terminate after 48 h, indicate 95% mortality at 0.1 mg/liter and also at 0.5 mg/liter among 3rd- and 4th-stage larvae at unspecified temperatures.

In our laboratory assays with *Bti* TP at 10°C, significant ($P \leq 0.05$) mortality occurred between 48 and 72 h (data not shown). In addition, field trial data showed a significant increase in mortality from 48 to 72 h (Fig. 1). Because of this significant increase in death, all of the data from laboratory assays and the field trial are based on a 72-h, rather than 48-h, assessment, as was done in the previous study by Webb and Dhillon (1984). We think that this longer evaluation time gives a more accurate representation of what occurs during typical control treatments in the field.

The VectoBac TP sand mixture uses Golden Bear GB-1111 oil to adhere the technical powder to the sand granules. The Golden Bear GB-1111 oil is itself a registered larvicide; however, the amount of oil applied in the VectoBac TP sand mixture compared with the recommended label rate for the oil as a larvicide is small: less than 2%. In order to rule out the possibility of the oil acting as a larvicide in the field, we conducted assays in the laboratory with the amount of GB-1111 per surface area applied in our field trial. The amount of Golden Bear GB-1111 used as part of the VectoBac TP sand mix produced no mortality when used alone in Rubbermaid boxes with *Oc. squamiger* in the laboratory (data not shown).

Field study: VectoBac TP was chosen for field evaluation because of its high active ingredient content and because the label allowed for it to be adhered with oil to sand granules, which would effectively carry it to the bottom, where *Oc. squamiger* typically feed. During the period of the field trial, ambient temperatures in the tidal pool ranged from a minimum of 9°C to a maximum of 14°C, and it rained intermittently (total, 29 mm) for 48 h after application of VectoBac TP and Baytex. Considerable amounts of rainwater accumulated at the control site within 24 h, connecting small pools and allowing larvae, which had been dense in small pools, to disperse widely. This event might explain the variation in numbers of larvae recorded at all the stations in the untreated control site.

Greater than 90% control of 1st-, 2nd-, and 3rd-stage *Oc. squamiger* larvae was achieved in the

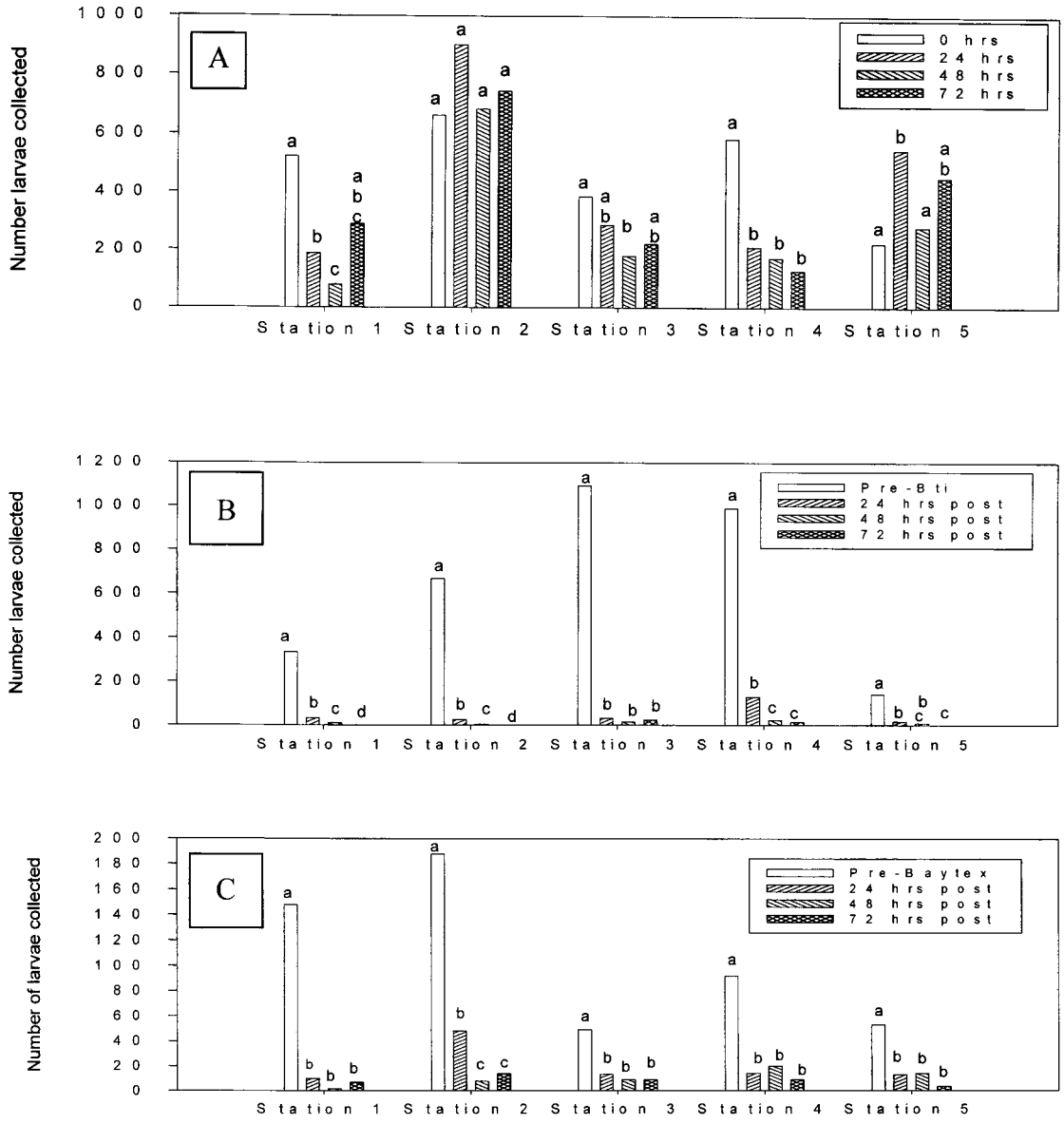

Fig. 1. Numbers of larvae of *Oc. squamiger* sampled in A) untreated, B) VectoBac TP–treated, and C) Baytex-treated tidal marshes. Matched letters indicate no significant difference ($P \leq 0.05$) within a given station over time.

Table 2. Mean percent mortality observed in the 5 sentinel cages per field.

Time (h)	% mortality		
	Control	*Bti*	Baytex
0 (pretreatment)	0.0	0.0	0.0
24	1.2	99.4	98.4
48	2.0	99.8	99.0
72	2.0	100	99.4

sentinel cages and in the tidal pools after applications of Baytex and VectoBac TP sand mixture (Fig. 1 and Table 2). Although each station differed in numbers of larvae before treatment, the overall reduction in larval populations proved to be statistically equivalent ($P \leq 0.05$) by 72 h postapplication of larvicide. In the tidal pools treated with VectoBac TP, some variation in percent mortality occurred between stations in the first 48 h, probably because of unevenness of *Bti* distribution, but by 72 h after treatment, larval reduction had evened out to equivalent levels ($P \leq 0.05$) at all stations. Baytex caused higher mortality within 24 h than

VectoBac TP, which was generally slower, taking up to 72 h posttreatment to reach maximum effect in the tidal marshes (Fig. 1). However, higher mortality was recorded after 72 h in the tidal marshes treated with VectoBac TP (99.3%) than in those treated with Baytex (90.4%). More rapid mortality of larvae was observed in the sentinel cages treated with VectoBac TP than in the tidal marsh itself (Table 2).

At an application rate of 22.39 kg/ha VectoBac TP sand mixture, we expected to capture 0.114 g dry weight sand granule mixture in each bucket. The weights of sand mixture actually captured in each of the 5 buckets were 0.14, 0.12, 0.101, 0.053, and 0.081 g (mean, 0.0989 ± 0.0344 g). Application rates hence varied from slightly more than (station 1) to slightly less than half (station 4) of the expected 22.39 kg/ha. Despite this variation in application rates, 100% control was attained with VectoBac TP in 3 of the 5 stations; station 3 attained 97.8% mortality, and station 4 attained 98.6%. All stations except number 3 continued to show a significant decline in larvae numbers from 24 to 72 h.

DISCUSSION

Low efficacy of *B. sphaericus* against *Oc. squamiger* was not unexpected, as susceptibility within *Ochlerotatus* and *Aedes* seems to be quite variable. Mulligan et al. (1978) demonstrated that *Bs* strain 1593 was much less effective against *Aedes nigromaculis, Ae. sierrensis,* and *Ae. melanimon* than *Culex quinquefasciatus* or *Cx. tarsalis.* However, later studies have shown increased effectiveness of *Bs* strain 2362 against *Ae. nigromaculis* (Mulla et al. 1988) and *Ae. melanimon* (Mulla et al. 1985) in warm water.

VectoBac WDG formulation proved a little less effective than the technical powder formulation, but this result was expected because the technical powder contained higher concentrations of bacteria than WDG. Because we decided to conduct field trials with technical powder, we conducted comparisons of susceptibility to *Bti* at different temperatures with the technical powder formulations so that we could justifiably draw conclusions between laboratory and field observations. The effects of temperature on the ability of *B. thuringiensis* to cause mortality have been documented in *Aedes stimulans* (Walker) (Wraight et al. 1981) and blackfly larvae (Lacey et al. 1978, Molloy et al. 1981), with all showing a decrease in efficacy at lower temperatures. There is currently no consistent consensus as to the cause of lowered mortality at lower temperatures. A common thought is that lowered metabolism and lowered rates, or even cessation, of feeding at lower temperatures cause ingestion of less toxin (Mulla 1990). On the other hand, Lacey et al. (1978) did not notice a significant decrease in the feeding rate of the blackfly larvae from 10 to 4°C, despite a 2 times drop in larval mortality. They accounted for this difference in larval mortality to increased gut pH, with increased temperature (Dadd 1976) favoring effectiveness of *Bti* (Faust et al. 1967). Our assays indicated that lowering the temperature by 8°C from 14 to 6°C required 2.5 times more *Bti* to achieve similar levels of mortality (50 and 90%) of *Oc. squamiger* larvae. We did not attempt to compare growth and feeding rates at different temperatures; thus, we are unable to provide an explanation for lowered efficacy at lower temperature. However, we were able to demonstrate that high levels of mortality are still achievable within the manufacturer's suggested label rate at 6°C, which is at the low end of a typical tidal pool's water temperature.

The larvae at station 4 in the VectoBac TP–treated tidal pool were effectively controlled despite receiving half of the expected VectoBac TP sand mixture (684.8 ITU/liter), a dose that compares to a little higher than the laboratory LD_{50} level (610 ITU/liter at 10°C). This higher mortality observed in the field at this lower dose was most likely a result of the higher susceptibility of 1st and 2nd instars, which predominated when the field study was conducted. This indicates that although the concentration of *Bti* was still high enough at station 4 to produce effective larval control on 1st and 2nd instars, control might have been compromised had they been at the late 4th stage. Care should be taken to apply the VectoBac TP sand mixture evenly at a rate of 22.39 kg/ha in the field. Water volume will also vary throughout the treatment area, leading to lower concentrations of bacteria. Had the VectoBac TP sand mixture been applied unevenly at half the recommended label rate (11.19 kg/ha) for nonpolluted waters such as tidal pools, some areas might not have received a lethal dose. The VectoBac TP field, though averaging about 30 cm in depth over most of the field, did have a much deeper channel running through it. Some of the few live larvae dipped at the 72-h sampling were from a flagged spot in this channel, suggesting a sublethal dose was achieved because of the deeper water. The more rapid mortality of larvae in sentinel cages (shallower water) than in the tidal pool supports this suggestion.

During this field trial, the VectoBac TP mixture effectively controlled mosquitoes in the tidal pools. Approximately 1 month after the above field trial was conducted, however, a similar application of VectoBac TP was made in the same type of marsh environment near Monterey, and effective control was not achieved. In this case, rain increased the water depth from about 30 cm to approximately 45 cm, and the water temperature was approximately 5°C. Estimates from larval dipping by MAD personnel during this 2nd trial indicated approximately 50% control at 72 h (Peter Ghormley, personal communication). Lab data show that at 6°C, the LD_{50} of VectoBac TP was 1.85 times higher than

at 10°C, and the LD_{90} was more than twice as high as at 10°C. This alone could explain the ineffectiveness of VectoBac TP in the 2nd field trial, because the effect of temperature can reduce the amount of kill to unacceptable levels.

Inconsistencies in larval control in the salt marsh are probably caused by a combination of factors, including temperature variation, uneven application rates, and water volume in treatment areas, because our laboratory bioassays demonstrate that *Bti* in both formulations is a competent killer of *Oc. squamiger.*

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