Journal of the American Mosquito Control Association, 12(1):33–38, 1996

EFFECT OF TADPOLE SHRIMP, *TRIOPS LONGICAUDATUS,* (NOTOSTRACA: TRIOPSIDAE), ON THE EFFICACY OF THE MICROBIAL CONTROL AGENT *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* IN EXPERIMENTAL MICROCOSMS

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ABSTRACT. Laboratory bioassays using *Culex quinquefasciatus* larvae evaluated the effect of tadpole shrimp on the persistence of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) in water collected from the surface of field microcosms. Time elapsed since *B.t.i.* treatment, as well as presence or absence of soil and tadpole shrimp, affected *B.t.i.* persistence at the water surface in 15- and 30-cm total depths. The presence of tadpole shrimp slowed the natural decline in *B.t.i.* effectiveness over time, but this effect was depressed when soil was present. Tadpole shrimp foraged throughout the water column and stirred up the substrate, keeping more particles in suspension at the surface than in microcosms with no shrimp, in microcosms with water depths of 15 and 30 cm.

INTRODUCTION

Bacillus thuringiensis var. *israelensis* de Barjac (*B.t.i.*) is an effective mosquito larvicide. Its high specificity and negligible toxicity to nontarget organisms make it an attractive alternative to chemical mosquito control compounds, and it is widely used throughout the world in black fly and mosquito control programs. However, the duration of adequate toxicity for mosquito control is quite short, lasting a few days to 1 wk (Mulligan et al. 1980, Mulla 1985), thus necessitating repetitive treatments when controlling larvae in semipermanent or permanent aquatic habitats. This decline in efficacy can be attributed to a variety of biotic and abiotic factors, including mosquito physiology and feeding behavior (Aly et al. 1988, Rashad and Mulla 1989), larval density (Mulla et al. 1990, Becker et al. 1992), temperature (Mulla et al. 1990, Becker et al. 1992), sunlight (Morris 1983, Becker et al. 1992), presence of suspended particulates and spore settling rates (Sheeran and Fisher 1992, Yousten et al. 1992), and pollution (Mulla et al. 1982, Ali et al. 1989), as well as effects of coexistent fauna (Blaustein and Margalit 1991).

One factor that may limit the effectiveness of *B.t.i.* in the field is lack of suspension of the toxic parasporal bodies in the feeding zone of mosquito larvae in the water column. Yousten et al. (1992) examined settling rates in the laboratory with water collected from a pond. Although spores settled only slightly in 24 h, toxicity declined by one order of magnitude within 10 min and nearly as much again by 120 min, indicating faster settling of the insecticidal parasporal body. In the field, Ignoffo et al. (1981) showed that water samples taken from the surface of *B.t.i.*-treated waters were less toxic to mosquito larvae than were water samples taken from the bottom of the water column.

The tadpole shrimp (TPS), *Triops longicaudatus* (LeConte) (Notostraca: Triopsidae), is a freshwater branchiopod predator of mosquito larvae developing in temporary habitats such as flood-irrigated agricultural fields, dry lakes, depressions, and temporary ponds. Its effectiveness in mosquito control has been demonstrated in the laboratory and in replicated field trials (Tietze and Mulla 1990, 1991; Fry and Mulla 1994). Tadpole shrimp are not adversely affected by *B.t.i.* (Fry-O'Brien, unpublished data), but the effect of TPS on *B.t.i.* efficacy is unknown.

Tadpole shrimp burrow into pond sediments and forage throughout the water column. The presence of TPS is often indicated by high water turbidity due to stirring up of the substrate. This activity may extend the suspension of *B.t.i.*, thus increasing its availability to mosquito larvae. The high turbidity in TPS-inhabited waters may also slow photodegradation of bacterial toxins by reducing penetration of ultraviolet light.

However, the presence of some crustaceans, such as the fairy shrimp, *Branchipus schaefferi* Fischer, and the ostracod, *Cypridopsis vidua* Muller, has been shown to decrease the effectiveness of *B.t.i.* under some conditions (Blaustein and Margalit 1991). The mechanism is unknown, but, in the case of *B. schaefferi,* could not be attributed to removal of *B.t.i.* by crustacean feeding.

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This study was initiated to determine the effect of TPS on the efficacy of *B.t.i.* against mosquito larvae. Laboratory bioassays were used to compare the effects of TPS on *B.t.i.* efficacy in the presence or absence of a soil substrate. The effect of TPS on particle suspension was also examined by weighing solids present in surface water.

MATERIALS AND METHODS

Tadpole shrimp used in these experiments were procured from natural populations in ponds at the Aquatic and Vector Control facility at the University of California, Riverside, CA.

Experiments 1 and 2 in the field consisted of 4 treatments with 5 replicates (in 1-m^2 fiberglass tubs): no soil and no TPS (−−), soil and no TPS (+−), no soil and with TPS (−+), and both soil and TPS (++). The soil used in these experiments was a sandy loam collected at the Agricultural Experiment Station at the University of California, Riverside. This soil was devoid of TPS eggs. Tubs were randomly assigned to treatment groups. In experiment 1 (September 1993), approximately 4 cm of soil was placed in the bottom of each of the 10 tubs used for the 2 treatments with soil. All 20 tubs were filled with 15 cm of water. Seventy-five mature TPS were placed in each of 10 tubs (5 with soil, 5 without soil). Each tub was treated with Vectobac® 12AS (Abbott Laboratories, North Chicago, IL) at a concentration of 0.25 ppm (3.75 ml of formulation diluted in approximately 50 ml water) using a hand-held, 1-liter spray bottle.

For laboratory bioassays against mosquito larvae, approximately 800 ml of water was collected from the surface of each tub with a 500-ml beaker by dipping from the top 2.54 cm. Collections were made at 24, 48, 72, and 96 h after treatment. This water was transported to the laboratory and tested for toxicity against mosquito larvae.

In experiment 2 (October 1993), the methods of experiment 1 were repeated except that the water depth was doubled to 30 cm. The same treatment rate (3.75 ml of formulation) of Vectobac 12AS was used, and surface water collections were made in the same manner 12, 24, 48, 72, 96, and 120 h after treatment. The water was transported to the laboratory for bioassay.

Bioassays using water collected from the experimental tubs were conducted using 4th-instar *Culex quinquefasciatus* Say from an established laboratory colony. Eighty larvae per replicate were distributed in 4 Dixie™ cups (20 larvae per cup), each containing 200 ml of water from the microcosms. Larvae were not starved before assay, and no supplemental food was added. The cups were placed in a holding room at approximately 26°C. Mosquito mortality was recorded after 24 h.

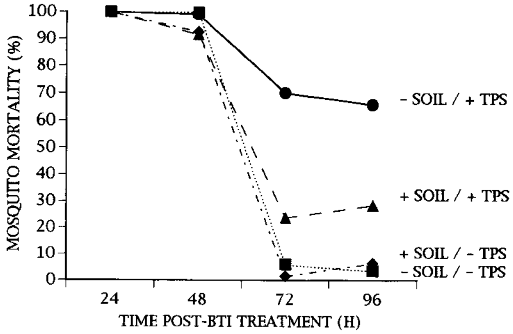

Fig. 1. Effect of tadpole shrimp and soil on *Bacillus thuringiensis* var. *israelensis* efficacy over time. Bioassays using larval mosquitoes were conducted in water collected from treatments in field microcosms. Water depth = 15 cm.

The amounts of suspended solids in the treatment groups were compared at each sampling time for both experiments. Only treatments containing soil were used (++ and +−). Whatman #2 11-cm filter circles were labeled, completely wetted in distilled water, and oven-dried for 24 h at 37.8 ± 2.8°C. The weight of each dried filter paper was recorded. Water samples from the field were shaken vigorously, and 200 ml was poured onto filter paper in a Buchner funnel. Water was removed by vacuum, leaving suspended solids on the filter. The filters were then oven-dried for 24 h at the same temperature as before and weighed with 0.001-g precision on an analytical balance. The differences in weight between the dried filter papers alone and the filter papers with sediment treatments were determined.

The results of mosquito bioassays among treatments were compared using multiple regression on arcsine-transformed data (Feldman et al. 1988). The soil × TPS interaction was not significant and was not included in the final regression model. Included variables were hours post-*B.t.i.* treatment, soil, and TPS. The effect of TPS on suspended particulates was compared using multiple regression on ln(g/L + 1)-transformed data (Feldman et al. 1988). The final regression model included the variables hours post-*B.t.i.* treatment and TPS.

RESULTS

In laboratory bioassays of *B.t.i.*-treated water from field microcosms, 3 variables, hours post-treatment, soil, and TPS, contributed to mosquito mortality in the field experiment conducted in September ($F = 79.04$, $P < 0.0001$; Fig. 1). Re-

Table 1. Regression coefficients (arcsine-transformed data), standard errors (SE), and probability-values (*P*) for factors affecting efficacy of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) in experimental microcosms.

Factor[1]	September[2] ($R^2 = 0.76$) Coefficient	SE	*P*	October[3] ($R^2 = 0.62$) Coefficient	SE	*P*
Hours post-*B.t.i.*	−0.02	0.001	0.0001	−0.01	0.001	0.0001
Soil	−0.33	0.07	0.05	−0.38	0.07	0.0001
Tadpole shrimp	0.22	0.07	0.001	0.15	0.07	0.05

[1] Soil × tadpole shrimp interaction not included in the final regression model ($P > 0.1$).

[2] Fifteen-centimeter water depth; 16.7–28.9°C.

[3] Thirty-centimeter water depth; 10.6–24.4°C.

gression coefficients are shown in Table 1. In this experiment, the water depth was 15 cm and the water temperature ranged from 16.7 to 28.9°C. As expected, the *B.t.i.* efficacy, as measured by mosquito mortality, declined in all treatments over the 96-h study period. Mosquito mortality was highest in water from the −+ treatment after 96 h in September (66.0 ± 5.0%). There was a reduction in effectiveness in the ++ treatment (28.5 ± 13.0% larval mortality), although larval mortality was higher in both treatments where TPS were present as compared with treatments without TPS. The mean larval mortality was 6.5 ± 3.0% in the +− treatment and 3.5 ± 1.0% in the −− treatment. In September, then, both the length of time after *B.t.i.* treatment and the presence of soil reduced efficacy of *B.t.i.*, whereas the presence of TPS increased efficacy of *B.t.i.*

In test 2, the water was adjusted to 30 cm deep and water temperature ranged from 10.6 to 24.4°C. The same 3 variables affected *B.t.i.* efficacy (F = 51.91, $P < 0.0001$; Fig. 2). The regression coefficients are shown in Table 1. Again, the efficacy of *B.t.i.* decreased over time, but after 120 h, water samples from the −+ treatment still resulted in 94.8 ± 2.0% mosquito mortality in laboratory bioassay. There was a reduction in *B.t.i.* efficacy when soil was present (47.6 ± 14.0% larval mortality). There was little difference between the ++ treatment and the −− treatment (40.0 ± 14.0% larval mortality), but again there was a reduction in *B.t.i.* efficacy (5.0 ± 1.0% larval mortality) in the +− treatment as compared with the −− treatment. In October, even though the water was twice as deep, the results were comparable with those in September. The greatest *B.t.i.* efficacy at the end of the test period was seen in the treatments with TPS present.

Because soil reduced efficacy of *B.t.i.* in both the presence and the absence of TPS, the amounts of suspended particulates at the water surface were compared. In September, the amount of particulates did not change over time but was higher at the surface when TPS were present (F = 46.63, $P < 0.0001$; Fig. 3). Regression coefficients are shown in Table 2. In October, similar results were seen (F = 17.08, $P < 0.0001$; Fig. 4 and Table 2). There was less

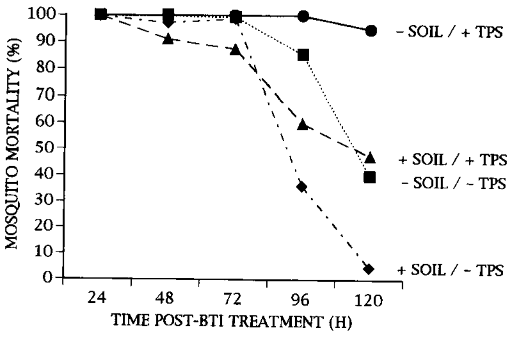

Fig. 2. Effect of tadpole shrimp and soil on *Bacillus thuringiensis* var. *israelensis* efficacy over time. Bioassays using larval mosquitoes were conducted in water collected from treatments in field microcosms. Water depth = 30 cm.

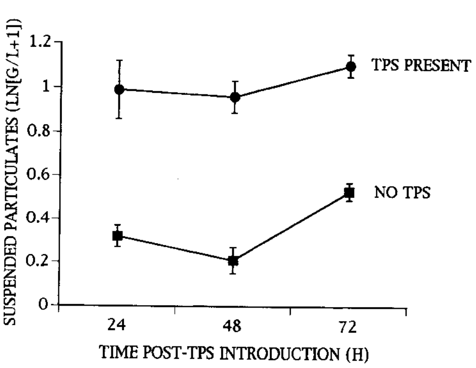

Fig. 3. Relationship between tadpole shrimp and particulate materials at the water surface in field microcosms. Water depth = 15 cm, temperature = 16.7–28.9°C.

Table 2. Regression coefficients (ln[x + 1]-transformed data), standard errors (SE), and probability-values (P) for factors affecting suspended particulates in experimental microcosms.

	September[2] ($R^2 = 0.78$)			October[3] ($R^2 = 0.42$)		
Factor[1]	Coefficient	SE	P	Coefficient	SE	P
Hours post-*B.t.i.*	-5.8×10^{-5}	3.6×10^{-5}	0.1	-5.1×10^{-5}	2.8×10^{-5}	0.1
Tadpole shrimp	0.01	7.1×10^{-4}	0.0001	0.01	9.6×10^{-4}	0.0001

[1] Hours × tadpole shrimp interaction not included in the final regression model ($P > 0.1$).
[2] Fifteen centimeter water depth; 16.7–28.9°C.
[3] Thirty centimeter water depth; 10.6–24.4°C.

particulate matter in suspension at the surface in October than in September (Figs. 3 and 4), which was probably caused by the greater water depth in the October experiment.

DISCUSSION

Our results are consistent with the findings of Sheeran and Fisher (1992) in that agitation, by TPS activity in this study, was the most important factor in maintaining the persistence of *B.t.i.* in the mosquito larvae feeding zones in the experimental microcosms. They also found that sedimentation increased settling of *B.t.i.*, which in turn reduced its efficacy by making it unavailable to filtering or browsing mosquito larvae. This was supported by our findings because the presence of soil depressed the effectiveness of *B.t.i.* over time.

In addition, the presence of TPS could facilitate adherence of *B.t.i.* to suspended organic and inorganic solids by increasing physical contact. Adherence of *B.t.i.* to suspended solids could reduce its availability to mosquito larvae (Margalit and Bobroglo 1984, Ohana et al. 1987, Yousten et al. 1992, Tousignant et al. 1993), thus resulting in a reduction of *B.t.i.* effectiveness. In our study, no reduction in efficacy could be attributed to this cause. In contrast, higher mosquito mortality was found in the soil treatments that contained TPS than in treatments with soil alone. This suggests either that adherence of *B.t.i.* to soil particles is not increased in the presence of TPS (and may even be inhibited by water movement) or that the solids and adherent *B.t.i.* were small enough and remained in suspension long enough for ingestion by feeding mosquitoes.

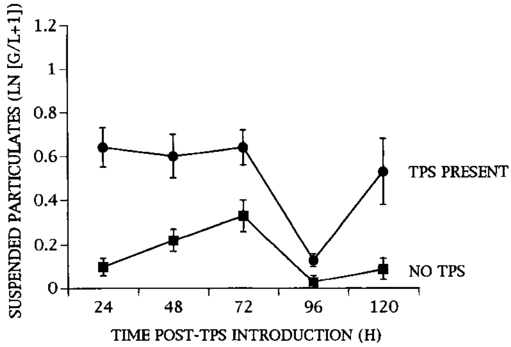

Fig. 4. Relationship between tadpole shrimp and particulate materials at the water surface in field microcosms. Water depth = 30 cm, temperature = 10.6–24.4°C.

Hypothetically, sedimentation of solids with *B.t.i.* attached increases duration of *B.t.i.* toxicity. Toxin particles settled on the substrate are less exposed to light and photodegradation. If *B.t.i.* can then become dislodged and resuspended by the action of TPS, it may retain overall toxicity longer. Further research is needed to verify whether or not this phenomenon occurs.

Water temperature is another important factor influencing the effect of TPS on *B.t.i.* efficacy. Slow feeding rates of mosquito larvae at cool temperatures will reduce efficacy of *B.t.i.*, but cool temperatures may also reduce TPS activity. Reduction in TPS activity may allow *B.t.i.* to settle more rapidly out of the range of the feeding zone of mosquito larvae. The effect of temperature on TPS activity and *B.t.i.* suspension should be examined.

Unlike Blaustein and Margalit (1991), we found an increase in effectiveness of *B.t.i.* in the presence of a crustacean. In addition, TPS are already under consideration as biological control agents against mosquitoes developing in temporary habitats (Tietze 1990[2]), so there is potential for integration of *B.t.i.* and TPS. Some floodwater mosquitoes, such as *Psorophora columbiae* (Dyar and Knab), still develop faster than TPS and escape predation (Tietze 1991). Under these circumstances in particular, a combination of *B.t.i.* with TPS may prove to be an effective control measure. Mulla (1990) discussed integrated mosquito control with *B.t.i.* and natural

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[2] Tietze, N. S. 1990. The tadpole shrimp, *Triops longicaudatus* (Notostraca: Triopsidae), for biological control of mosquitoes. Ph.D. thesis. University of California, Riverside.

invertebrate predators, and in field studies, Walton and Mulla (1991) demonstrated a synergistic action of *B.t.i.* with the mosquitofish, *Gambusia affinis* (Baird and Gerard). The enhanced control was seen because of the immediate effect of *B.t.i.* (0–14 days), followed by increasing predation by *Gambusia* as its abundance increased. Similar results may be expected with the use of *B.t.i.* in conjunction with TPS. *Bacillus thuringiensis* var. *israelensis* can control mosquitoes developing within the first 4 days, after which TPS will be large enough to prey upon subsequent species and cohorts of mosquitoes (Tietze and Mulla 1989, 1991). In *B.t.i.*-treated waters, mosquito larvae may appear within 3–4 days after treatment (Mulla 1990), so the fact that TPS can be effective within this short period of time is very important. Further control may also be achieved by the activity of TPS, which will keep the remaining *B.t.i.* in suspension and available to mosquito larvae.

Service (1983) suggested that integrated control using larvicides such as *B.t.i.* and predators such as TPS may ultimately fail because of food shortages caused by the larvicide. Because TPS are omnivorous, there should be no larvicide-induced food shortage in this system.

This study demonstrates that *B.t.i.* and TPS may be used as complementary mosquito control agents in temporary aquatic habitats. *Bacillus thuringiensis* var. *israelensis* can effectively control mosquitoes for a short period of time. As the its effects of *B.t.i.* begin to decline, TPS will become large enough to consume mosquito larvae. It may be possible to achieve continuous control of mosquitoes under these circumstances. Enhancement of *B.t.i.* efficacy in the presence of TPS is an important added bonus in this scenario. The apparent mechanism is increased *B.t.i.* suspension, which keeps toxins available to feeding mosquito larvae.

ACKNOWLEDGMENTS

We thank Jeff Beehler and John Chaney for rearing mosquitoes and Doug Kuney for invaluable field assistance. Brad Mullens and Robert Luck reviewed and improved the manuscript. This research was supported by the University of California, Special Funds for Mosquito Research.

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