EFFICACY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST LARVAE OF THE SOUTHERN BUFFALO GNAT, *CNEPHIA PECUARUM* (DIPTERA: SIMULIIDAE), AND THE INFLUENCE OF WATER TEMPERATURE[1]

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ABSTRACT. Susceptibility of southern buffalo gnat larvae to *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) was studied during 1986–88. Tests were conducted using Vectobac 12AS® in a mini-gutter aquatic bioassay system. Mini-gutter tests using a 4.5 ppm *B.t.i.* concentration and a 10 min exposure period confirmed a positive correlation between water temperature and *B.t.i.* effectiveness. Significantly lower larval mortality occurred as water temperature decreased below 9°C, indicating that proper timing of *B.t.i.* application is essential to maximize larval control. Even with this temperature limitation, larval control using *B.t.i.* should provide an economically effective means of preventing outbreaks of the southern buffalo gnat.

INTRODUCTION

The southern buffalo gnat, *Cnephia pecuarum* Riley, was once considered the great scourge of man and livestock in the Mississippi River Valley (James and Harwood 1969). After the late 1930s, the occurrence and importance of this black fly had diminished to the point where Laird (1981) reported it was no longer a significant pest. Crosskey (1990) indicated that *C. pecuarum* was a pest of the past. However, in 1979 the southern buffalo gnat began to reclaim its role as a major economic pest of livestock production in both Arkansas and Texas.

Cnephia pecuarum is a unique pest relative to the aquatic development of its immature stages, seasonal occurrence and control strategies. Bradley (1935a, 1935b) recorded the last available information concerning the biology and behavior of this species. Egg hatch of this univoltine pest in the Sulphur River drainage system, area of first resurgence, generally occurs from October to December. Historically, larval development coincides with water temperatures ranging from 2 to 20°C. Adult emergence occurs from November through March with peak activity in late February.

Recent emphasis has been placed on the use of the biological insecticide *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) for control of immature mosquitoes and black flies. Efficacy has been reported for a wide range of black fly species (Lacey and Mulla 1977, Undeen and Berl 1979, Gaugler and Finney 1982) with negligible impact on other non-target aquatic invertebrates (Colbo and Undeen 1980, Lacey et al. 1982, Back et al. 1985, Merritt et al. 1989). However, a positive correlation has been reported between water temperature and *B.t.i.* induced larval mortality for several black fly species (Lacey et al. 1978). This indicates that water temperature may be an important consideration in development of an effective control program. The purpose of these investigations were: 1) determine the susceptibility of *C. pecuarum* larvae to *B.t.i.*, 2) determine the relationship between water temperature and *B.t.i.* efficacy, and 3) determine the optimal timing of larvicide application to *C. pecuarum* populations in the Sulphur River drainage system in Arkansas and Texas.

MATERIALS AND METHODS

During the fall and spring of 1986–87 and 1987–88, tests to determine *B.t.i.* effectiveness were conducted using an artificial river system, the "mini-gutter." The mini-gutter system used was comparable to that described by Wilton and Travis (1965) and Lacey et al. (1982). The mini-gutter system was composed of a watertight wooden holding tank to which were attached 8 polyvinyl chloride (PVC) rain gutters which served as individual simulated river channels (Fig. 1). The holding tank (1.2 × 0.3 × 2.4 m) held a water volume of 680 liters. To facilitate larval attachment, each 3.04 m gutter was banded with fine sand at 0.3 m intervals using PVC glue. Water depth and velocity in each mini-gutter was regulated by capping the point of water entry into the gutter and adjusting the gutter height at the point of water discharge.

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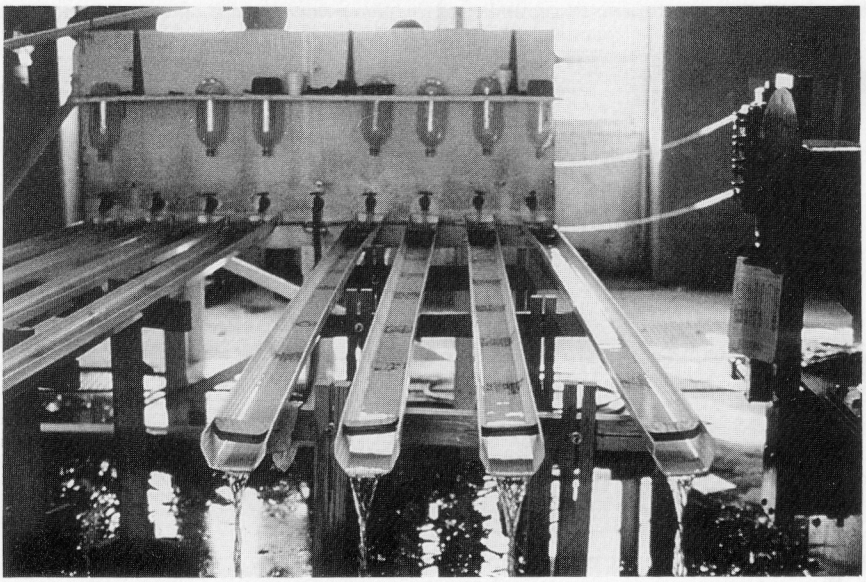

Fig. 1. Mini-gutter system for simulated river tests.

Water flow to each gutter was controlled with 2.54 cm water faucets.

The mini-gutter system was set up in the flood control release structure of Wright Patman Dam, thereby allowing water to be pumped directly from a discharge gate of the Sulfur River into the holding tank. The submergible water pump provided sufficient water volume (95 liters/min) to maintain a constant head pressure at maximum gutter flow with excess water discharged through a side drain in the holding tank. Water leaving the gutter system was drained back into the exit side of the discharge gate with no recycling of water through the gutter system.

Larvae for each mini-gutter test were collected by boat from submerged tree limbs and leaf packs in the Sulphur River and transported to the laboratory in insulated containers. For each test, in excess of 100 *C. pecuarum* larvae were placed in each gutter at a reduced rate of water discharge and allowed to attach for approximately 30 minutes. After larval attachment, water discharge to each gutter was increased to approximately 7 liters/min. Larvae were then allowed to adjust to the increased water velocity for 1 hour.

Immediately prior to insecticide treatment, larval density in each gutter was adjusted to ca. 100 larvae by removing excess larvae with a modified eye dropper. After an additional 30 min for stabilization, exact larval density was recorded for each gutter. Since exact numbers of larvae were recorded for each gutter, testing was conducted across the complete spectrum of larval instars with no attempt to target a single developmental stage. Small minnow nets (10 × 10 cm) lined with nylon organdy were then placed at the end of each gutter to collect drifting larvae. Water temperature was then recorded and insecticide treatments begun.

Treatment was accomplished by dripping *B.t.i.* into 6 randomly chosen gutters using modified 2 liter bottles suspended above each gutter (Fig. 1). The cap of each bottle was drilled with a 0.79 mm hole to serve as point of application. To maintain constant discharge from each bottle, a 6.35 mm air intake hole was provided in the bottom of each bottle. Larvicide dose for each gutter was determined according to rate of discharge in each individual gutter. Treatment bottles were calibrated by determining the amount of solution necessary to deliver the desired insecticide concentration over the desired period of larval exposure. Two wooden baffle blocks mounted in each gutter, immediately downstream from the point of insecticide application and water inflow, enhanced *B.t.i.* mixing in each gutter. Two gutters not exposed to insecticide served as experimental controls.

Testing was conducted primarily at a 4.5 ppm *B.t.i.* concentration on the basis of preliminary cup test investigations and the suggestions of company representatives and L. Lacey. It was decided to concentrate testing over a 10 min period of larval exposure due to the high turbidity and slow downstream movement of the Sulphur River. One additional test was conducted at a reduced exposure period (5 min) to verify the most effective yet economical rate of larvicide application.

Table 1. Effect of water temperature on susceptibility of *Cnephia pecuarum* to *B.t.i.* at a concentration of 4.5 ppm and 10 min exposure period.

Water temp. (°C)	No. tests	Total no. exposed	Mean kill* (%)	
13.0	6	676	100.0	A
11.8	3	258	83.2	ABC
10.8	3	345	90.4	AB
10.0	6	1,174	92.9	AB
9.8	5	511	95.1	A
9.0	13	2,614	90.4	AB
8.5	9	1,989	62.5	DE
7.8	9	1,411	68.2	CDE
7.5	3	593	76.6	BCD
7.3	3	460	52.1	E
6.5	9	1,423	66.9	CDE
6.0	8	1,083	72.6	CD
5.8	7	1,405	65.2	DE
5.5	6	928	61.7	DE
5.3	5	404	52.3	E

* Means followed by the same letter are not significantly different ($P > 0.05$).

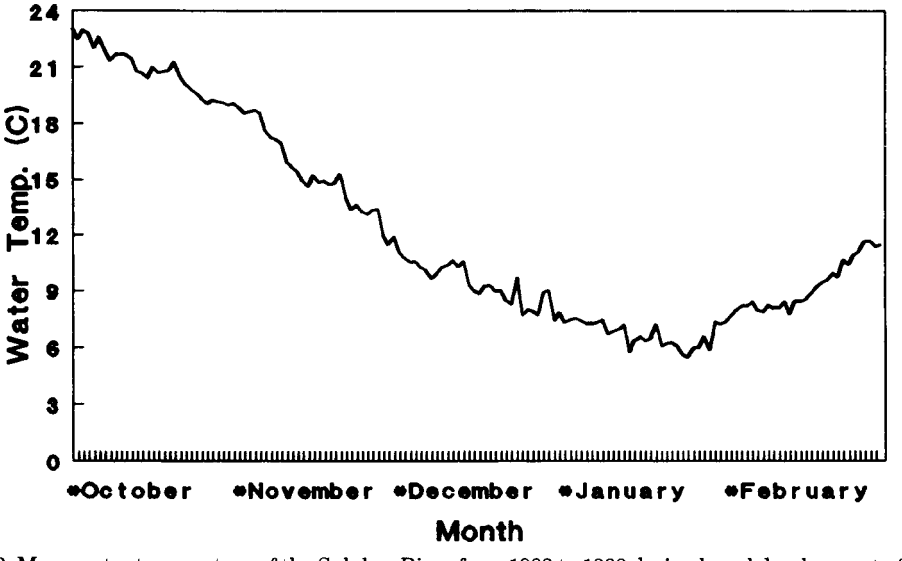

Fig. 2. Mean water temperature of the Sulphur River from 1983 to 1988 during larval development of *Cnephia pecuarum*.

Larval mortality in each cup was determined 24 h posttreatment by touching each larva with a fine camel hair brush. Absence of reflex curling motion was evaluated as dead or dying. Larval mortality for each gutter was determined by examining larvae remaining in the gutter as well as those collected in drift nets. Collected data for each gutter was corrected for control mortality according to Abbott (1925). Data were then statistically analyzed ($P >= 0.05$) using the General Linear Model (GLM). Where significant differences were determined during GLM analysis, means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

Investigation of the effectiveness of *B.t.i.* against *C. pecuarum* larvae in a mini-gutter system during 1986 and 1987 indicated larval susceptibility to this product. The effectiveness of a 4.5 ppm concentration of *B.t.i.* against *C. pecuarum* larvae is illustrated in Table 1. Statistical analysis of these data indicated a significant decrease in larval mortality for *C. pecuarum* as water temperature decreased below 9.0°C. Control mortality never exceeded 1.9% across the range of experimental temperatures tested. These data indicate that a *B.t.i.* concentration

of 4.5 ppm is an effective, economical rate for *B.t.i.* application against *C. pecuarum* larvae.

Larval mortality showed a positive correlation to water temperature (r=0.79) at a 4.5 ppm *B.t.i.* concentration. Lacey et al. (1978) noted a comparable correlation of larval mortality frequency and water temperature for *S. vittatum* Zetterstedt. In addition, a decrease in period of larval exposure from 10 to 5 min at a water temperature of 7.25°C resulted in a significant decrease in larval mortality frequency, 52.1 and 14.7%, respectively. Therefore, these data confirmed that optimum control could best be achieved using a 10 min period of larval exposure.

The variability in larval mortality frequency observed in these tests resulted from seasonal variation in water temperature. To this extent, some variability was observed in relation to tests conducted during early and late season and between years conducted at the same water temperature. In general, early season tests provided slightly more effective larval control than did late season. Yearly variation in larval mortality control frequency was limited to only a few experimental water temperatures and had only slight impact on overall results.

These results may indicate a change in larval physiology of *C. pecuarum* or an inactivation of the δ-endotoxin as a result of low water temperature. Lacey et al. (1978) determined a significant decrease in feeding rate for *S. vittatum* as water temperature decreased below 15°C. Dadd (1976) found that gut pH in mosquitoes is lowered in relation to chilling. However, Lacey et al. (1978) determined that midgut pH of *S. vittatum* did not change sufficiently to prevent endotoxin activity. Additional tests would be necessary to determine water temperature influenced changes in feeding rate and gut pH for *C. pecuarum* larvae before ruling out these factors. It is also impossible to dismiss water quality effects, as temperature directly influences both the physical and chemical properties of water.

While instar composition was not determined, observations indicated no difference in mortality relative to larval developmental stage of *C. pecuarum* at the tested water temperatures. Similar findings were reported by Lacey et al. (1978) for *S. vittatum*. In contrast, decreased penultimate larval instar susceptibility was reported by Guillet and Escaffre (1979) for *Simulium damnosum* Theobald and by Molloy et al. (1981) for *S. vittatum*. While mortality in penultimate *C. pecuarum* larvae was observed, limited pupation occurred during late season *B.t.i.* tests. Susceptibility of the penultimate instar appears dependent upon timing of insecticide exposure, as all exposed pupae were noted to be active 24 h post-treatment. As shown for mosquitoes, lower susceptibility may occur in the final instar as a result of a reduction and/or cessation of feeding prior to pupation. However, as *B.t.i.*-exposed pupae were not retained to determine long term survival, additional studies would be necessary to quantify a change in penultimate instar susceptibility.

Historical water temperature data for the Sulphur River from 1983 to 1988 are illustrated in Fig. 2. As noted previously, the seasonal occurance of larval *C. pecuarum* typically extends from October to February, corresponding to water temperatures of 2° to 20°C. Therefore, results of these investigations indicate that mid-December is the cutoff date to obtain maximum larval control of *C. pecuarum* in the Sulphur River drainage system and avert emergence of extensive adult populations. Additional investigations are warranted to determine the feasibility of regulating early season river levels to ensure a synchronous egg hatch, thereby maximizing control with one *B.t.i.* application. Nevertheless, results indicate that a successful control strategy can be developed to prevent outbreaks of *C. pecuarum* larvae relying on the use of *B.t.i.* at a 4.5 ppm concentration.

Overall, these tests indicate: 1) *C. pecuarum* larvae are susceptible to *B.t.i.*, 2) degree of susceptibility is directly related to water temperature, 3) control measures should be implemented before water temperature decreases below 9°C, and 4) larval control can be achieved using a *B.t.i.* concentration (4.5 ppm) and exposure period (10 min) which would be both effective and economical for large-scale river application.

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