EFFICACY OF *BACILLUS THURINGIENSIS* (H-14) FOR LARVAL *AEDES* MOSQUITO CONTROL IN INTERMOUNTAIN MEADOWS IN WYOMING

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ABSTRACT. One square meter field enclosures made of steel flashing, and natural ponds were used to determine the efficacy of Bactimos® formulations of *Bacillus thuringiensis* (H-14) for control of late instar *Aedes dorsalis, Ae. fitchii* and *Ae. melanimon* mosquito larvae in intermountain meadows. Low temperatures caused decreased efficacy of the formulations tested in the laboratory, and spring temperature extremes lowered efficacy in field tests. Adequate control of fourth instar larvae was obtained in field studies in a 24 hour period when water temperatures were ≥ 12°C at a treatment rate of 0.1 mg/liter.

INTRODUCTION

Bacillus thuringiensis serotype H-14, is an excellent candidate for biological control of larval mosquitoes (de Barjac 1978, Goldberg and Margalit 1977) and has been evaluated in trials under a variety of climatic conditions in North America (Garcia and Des Rochers 1979, Sebastien and Brust 1981, Dame et al. 1981, Wraight et al. 1982, Hembree et al. 1980, Eldridge and Callicrate 1982). Generally, these tests have been at low altitudes, where daily temperature and light intensity fluctuations are not as extreme as those in mountainous areas of Wyoming.

Aedes dorsalis (Meigen) and *Aedes melanimon* Dyar immature development may take as long as 57 days at variable water temperatures existing in river oxbow depressions along the Big and Little Laramie rivers and in irrigation pasture pools (Johnson 1978).[3] During late May and early June, water temperature in these areas varies as much as 25°C in a 24 hr period. Larviciding for mosquito control in this region extends through the period when pupae are found in many pastures (Lloyd and Kumar 1979). The experiment described below was designed to determine the efficacy of Bactimos® formulations of *B. thuringiensis* (H-14) on late instar indigenous *Aedes* mosquitoes in an intermountain meadow environment.

MATERIALS AND METHODS

Formulations of *B. thuringiensis* (H-14) were supplied by Biochem Products, P. O. Box 264, Monchanin, DE 19710. The wettable powder (WP) formulation had a potency of 3500 AA units per miligram of product, and based on the label, up to 0.1 mg/liter were recommended for late instar larval control. Bactimos flowable concentrate (FC), contained 1000 AA units per milligram of product and the label recommendation was for up to 1.42 liters/0.4047 ha for water averaging 50.8 cm in depth.

The field research was performed at the Paradise Farm Research Unit, University of Wyoming. In May 1981, third and fourth instar *Aedes* larvae were collected from naturally flooded depressions along the Little Laramie River and held in the laboratory at 12.8±2°C for at least 12 hours. Groups of 500 larvae were placed in field enclosures, where they were held for an additional 28 hours prior to treatment. Enclosures were made of 35.5 cm aluminum standard flashing, riveted to make a 1 m^2 circle. Each circle was pressed ca. 10 cm into the sod in a 0.5 ha depression in an irrigated meadow. Enclosures were banked with sod on the outside to prevent mosquito larva escape. In 1981, enclosures were emptied of water several times, to remove indigenous mosquitoes, prior to the addition of test mosquitoes. Indigenous larvae retained in each experimental enclosure were used as target mosquitoes, in 1982, as soon as fourth instar larvae were present.

Population estimates were obtained by taking 30 dips with a 0.47 liter enameled dipper from each enclosure. Twenty dips were from the edge of the enclosure, 9 halfway between the margin and the center, and one from the center. In 1981, this procedure recovered 10–11% of the larvae that were placed in untreated enclosures 48 hr earlier. Larger ponds were sampled at 50 randomly selected marked sites. Larvae were placed in alcohol and identified using the key of Harmston and Lawson (1968).

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[3] Johnson, G. 1978. Pre-adult development and survival of *Aedes* mosquitoes indigenous to Albany County, Wyoming and other selected mosquitoes in temperature controlled chambers and semi-natural habitats. Unpublished Ph.D. Dissertation. University of Wyoming, Laramie, Wyoming.

Water and air temperatures were monitored continously with a Weather Measure Co. T601 2 point thermograph (±1°C accuracy). The water probe was covered with a 71 × 61 cm solar shield and immersed in an area between 2 enclosures. The air probe was in a Stevenson shelter at 2 m.

The volume of water in each 1 m² enclosure was estimated by measuring water depth at 12 sites within the enclosure. The appropriate dose of Bactimos WP or FC was applied in 2 liters of distilled water from a sprinkling can between 0600–0800 hr.

In a preliminary laboratory test against fourth instar *Ae. dorsalis* and *Ae. melanimon* held at temperatures between 4 (16 hr) and 22°C (8 hr) within a 24 hr period, the LC_{50} and LC_{95} were ca. 0.1 and 0.3 mg WP per liter, respectively. Three replications of these 2 concentrations plus one higher, 0.9 mg/liter, were evaluated in field enclosure trials in 1981. The 0.9 mg/liter WP concentration in 14 liters of water was later applied to a 208 m² natural pond (15,000 liters) that had been filled with captured third and fourth instar *Ae. dorsalis* and *Ae. melanimon*. Application was made to this pond by a CO_2 powered backpack sprayer (Grow et al. 1973) at 283 KPa.

On June 29, 1982, natural ponds of 0.50 and 0.18 ha were treated, respectively, with 0.1 mg/liter wettable powder and 0.1 mg/liter flowable concentrate delivered by CO_2 powered backpack sprayer at 283 KPa.

Laboratory tests of temperature dependence of Bactimos FC efficacy at 24 hr were modeled after World Health Organization guidelines (World Health Organization 1975). The tests used 5 repetitions of 20 larvae each of 100 ml distilled water per concentration. Field collected, unfed, 4th instar *Aedes fitchii* (Felt and Young) larvae were held in environmental chambers with a 15 hr photophase, 9 hr scotophase for LC_{50} and LC_{95} determinations at constant temperatures of 10, 13, 19, 22 and 25°C.

RESULTS

In 1981, percent control was determined by comparison between the number of living larvae collected from the check enclosures, and those collected from treatment enclosures. Temperatures of 11–22°C with means of 15.8 and 15.9°C respectively for the two 24 hr intervals, existed in the enclosures during the period (June 10–12, 1981). As shown in Table 1, 0.9 mg/liter treatments were 100% effective at 24 hr. In enclosures treated with 0.3 and 0.1 mg/liter Bactimos WP, 88 and 55% control was observed 24 hrs after treatment. Decreased numbers of larvae captured 48 hr after treatment were a result of previous sampling during the two repetitions at the 24 hr sample as well as increased mortality. Based on identification of surviving larvae at 24 hr, treatment enclosures contained larvae in percentages very similar to those of check enclosures. Species composition of populations surviving treatment was not significantly different from control populations, which were 43±4% *Ae. fitchii*, 39±9% *Ae. dorsalis*, and 16±4% *Ae. melanimon* ($p>0.10$ Duncan's New Multiple Range Test).

Table 1. Mean percent control of mosquito larvae in enclosures 24 and 48 hr after treatment with Bactimos® in 1981. Temperature variation 11–22°C. Three replicates per treatment.

Treatment	24 hr Larvae/ 30 dips	24 hr % control	48 hr Larvae/ sample	48 hr % control
check	49.8	0 a	31.4	0 a
0.1 mg/liter	22.2	55 b	7.7	75 b
0.3 mg/liter	6.0	88 c	1.4	96 c
0.9 mg/liter	0	100 d	0	100 c

Means, in the same column, followed by the same letter are not significantly different from each other (Waller-Duncan test k ratio = 100≅to an α level of 0.05).

Treatment of 208 m² pond with 0.9 mg/liter Bactimos WP resulted in 100% 24 hr mortality, as larval sampling changed from 61.5 per 50 dip repetition to 0. Water temperatures ranged from 10 to 29°C during the 24 hr period. The larval population treated was 94% *Ae. dorsalis* and 6% *Ae. melanimon*.

Comparisons between the efficacy of Bactimos FC and WP formulations in field enclosures in 1982 are given in Table 2. There were

Table 2. Percent reduction in third and fourth instar indigenous larval *Aedes* 24 and 48 hr following treatment with two formulations of Bactimos®. 1982. (Three enclosures per mean).

Hours post treatment	Active ingredient in m/g liter	Wettable powder	Flowable concentrate
24	Control	– a	– a
	0.1	52.1 b	49.7 b
	0.3	87.7 c	71.3 bc
	0.5	93.3 c	87.6 c
48	Control	– a	– a
	0.1	62.2 b	53.3 b
	0.3	98.0 c	85.8 c
	0.5	99.3 c	95.9 c

Means, in the same column, followed by the same letter are not significantly different from each other (Waller-Duncan test k ratio = 100≅ to an α level of .05).

no significant differences in efficacy between the two formulations at any of the treatment levels. Water temperatures ranged from 5.5 to 16°C (mean 9.9) with 9 hr of this time below 10°C on the first 24 hr and from 5.5 to 19°C (mean 11.6) with 7 hr under 10°C in the second 24 hr. Because of below 0°C air temperature, ice was removed from the water surface prior to larva sampling at 48 hr. The percent reduction of larval numbers in treated enclosures was calculated by the following formula:

$$\% \text{ reduction} = 100 - \frac{\text{posttreatment mean of treated}}{\text{pretreatment mean of treated}}$$

divided by

$$\frac{\text{posttreatment mean in untreated}}{\text{pretreatment mean in untreated}} \times 100$$

Based on subsequent identification of larvae surviving 24 hr after treatment, none of the three primary species (63± 12% *Ae. fitchii,* 18± 5% *Ae. melanimon,* and 7± 6% *Ae. dorsalis*) found at this site was statistically more susceptible ($p>0.10$) to Bactimos than the other two. There was significantly higher mortality in the 0.3 and 0.5 mg/liter treatment than in the 0.1 mg/liter treatments 48 hr after application regardless of formulation. The 0.3 mg/liter flowable concentrate treatment was not significantly different from the 0.1 or 0.5 mg/liter treatments 24 hr after application.

In the ponds treated in late June, temperatures ranged from 12.2 to 24°C during the first 24 hr and from 13.9 to 18.9°C from 24 to 48 hrs. Ninety-seven percent control was achieved with the FC formulation, and 91% control with wettable powder 24 hrs after treatment. Forty-eight hrs after treatment, no surviving larvae were found in either pond. *Ae. dorsalis* (87%), *Aedes campestris* Dyar and Knab (10%) and *Aedes cataphylla* Dyar (3%) constituted the primary species in the treated ponds.

Laboratory toxicity studies on third stage *Ae. fitchii* in 1982, using the Bactimos FC formulation at constant temperatures (Table 3) demonstrated a close correlation between susceptibility to Bactimos and temperature (Fig. 1). LC_{50} 95% fiducial limits at 10, 13 and 19°C did not overlap. Over 19°C LC_{50} concentrations of one temperature regime fell within the 95% fiducial limits of the next temperature. A direct and positive relationship between increasing temperature and lower dosage for LC_{50} ($R^2 = 0.86$, $F = 18.99$; $PR>F = 0.023$) and LC_{95} ($R^2 = 0.96$, $F = 64.84$; $PR> F = 0.004$) was apparent by linear regression analysis.

Table 3. Twenty-four hour LC_{50} and LC_{95} values for fourth instar *Aedes fitchii* exposed to Bactimos® flowable concentrate at 10, 13, 19, 22 and 25°C.

Temperature (°C)	Concentration and 95% fiducial limits in mg/liter			
	LC_{50}	range	LC_{95}	range
10°	0.255	0.221–0.289	0.510	0.423–0.711
13°	0.139	0.110–0.172	0.447	0.320–0.727
19°	0.076	0.061–0.097	0.253	0.176–0.476
22°	0.058	0.044–0.075	0.237	0.160–0.471
25°	0.045	0.034–0.058	0.198	0.137–0.372

DISCUSSION

The positive correlation between increasing temperature and *B. thuringiensis* (H-14) efficacy shown in our experiments has been observed with *Aedes* mosquitoes (Wraight et al. 1981), as well as with black flies (Lacey et al. 1978, Molloy et al. 1981). At least two mechanisms may account for an increase in mortality at higher temperatures; increased rates of toxin activation, increasing physical degradation of ingested particles by the host with accompanying toxin release, and/or increased feeding rates by mosquito larvae increasing the uptake of *B. thuringiensis* (H-14).

Activities, including ingestion, by *Ae. dorsalis* are decreased markedly at temperatures near 10°C (Rees and Nielsen 1947). Other target species in our field trials may be conjectured to have activity thresholds near the same temperature, increasing the probability that they were not active when Bactimos formulations were first applied. Field temperatures were near this activity threshold for substantial portions of the experimental period, decreasing the probability of Bactimos ingestion by larvae. The mortality of indigenous larvae in laboratory trials increased markedly, within the range of temperatures tested, as temperatures increased up to 25°C. During field enclosure trials in 1982, low temperatures reached 5.5°C, which is well below the activity threshold of *Ae. dorsalis,* and rose only to 16 and 19°C within the 24 and 48 hr periods respectively. During the period of inactivity by the mosquitoes for 7–9 hr per day, following treatment, Bactimos loss to environmental factors such as adsorption to soil particles (Ignoffo et al. 1981; Van Essen and Hembree 1982) or inactivation by ultraviolet light radiation (Ignoffo et al. 1981) particularly in shallow grassy portions of the enclosure (depth ≅ 5 cm or less) where large numbers of larvae were often found, may have further decreased the effectiveness of the applied doses.

In order to effectively control *Aedes* mosquitoes with *B. thuringiensis* (H-14) under conditions similar to those at which our experiments took place, it would seem necessary to

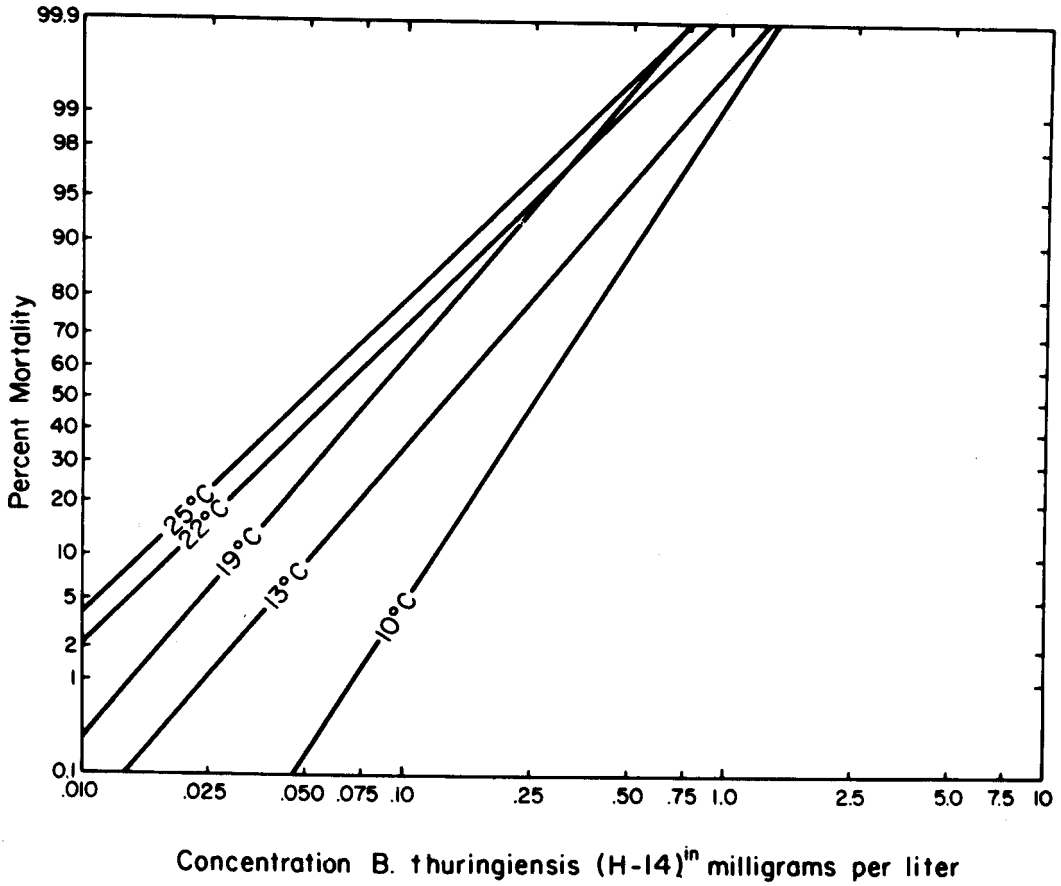

Fig. 1. Twenty-four hr efficacy of Bactimos® against 3rd and 4th instar *Aedes* larvae at 10, 13, 19, 22 and 25°C.

delay treatment until water temperatures were well into the activity range for any species under consideration. Unfortunately, this conflicts with current established patterns for aerial larval control which dictate that applications cease as winds increase to over 8 kph, causing excessive insecticide drift. Ground application therefore will be necessary for satisfactory control.

In later portions of the larval development season, when nighttime temperatures do not drop to a point close to the activity threshold, Bactimos is efficacious and *B. thuringiensis* (H-14) would seem to be a welcome, seasonal, addition to the larval mosquito control arsenal in intermountain meadows.

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