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EFFECT OF LOW TEMPERATURE ON FEEDING RATE OF *AEDES STIMULANS* LARVAE AND EFFICACY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* (H-14)

EDWARD D. WALKER

Department of Entomology, Michigan State University, East Lansing, MI 48824

ABSTRACT. Experiments were conducted to determine the effects of low temperature (0 and 4°C), vs. a high temperature (22°C), on the feeding rate of *Aedes stimulans* larvae, and their susceptibility to *Bacillus thuringiensis* var. *israelensis* (H-14) (*B.t.i.*). Third-instar *Ae. stimulans* slowed but did not halt feeding at 0 and 4°C compared to 22°C. Susceptibility of larvae, as measured by LC_{50} values, to *B.t.i.* was highest at 22°C (LC_{50} = 0.1 ppm), and lower at 4°C (LC_{50} = 0.2 ppm) and 0°C (LC_{50} = 0.9 ppm). The data from the feeding and susceptibility experiments suggest that decreased efficacy of *B.t.i.* at low temperatures may occur because the rate of larval feeding decreases. Low water temperature should be a consideration during operational applications of *B.t.i.* for control of larvae in cold-water habitats, such as the spring *Aedes* species.

INTRODUCTION

A range of environmental and biological factors affect the activity of *Bacillus thuringiensis* var. *israelensis* serotype H-14 (*B.t.i.*) against mosquito larvae, including water temperature, sunlight, density of larvae, larval instar, concentration of larval food, and certain water chemistry parameters such as concentration of dissolved organic matter (Becker et al. 1992). Generally, toxic activity of *B.t.i.* decreases with decreasing water temperature for both mosquito and black fly larvae, so correspondingly the median lethal concentrations (LC_{50}s) increase for a given formulation or experimental preparation as temperature decreases (Wraight et al. 1981, 1987; Ignoffo et al. 1983; Lacey 1985; Mulla et al. 1990).

At least 2 gaps exist in our understanding of the relationship between temperature and activity of *B.t.i.* against mosquitoes. First, most studies have been done with temperatures ranging to as low as 5°C (see studies cited above), yet water temperatures often range to as low as 0°C in spring *Aedes* larval habitats, particularly in March and April when other conditions are optimal for applications of *B.t.i.* (Knepper and Walker 1989; Knepper et al. 1991, 1994). Data are needed on activity of *B.t.i.* in the critical temperature range between 0 and 5°C. Second, whether larvae discontinue or retard feeding at low temperatures is unknown, but if they do, then they may not ingest sufficient *B.t.i.* to receive a lethal dose. Thus, the apparent effect of low temperature on decreased activity of *B.t.i.* may be indirectly mediated through decreased larval feeding activity. The objectives of this study were: 1) to measure feeding rate of larvae of a representative spring *Aedes* species at low water temperatures, and 2) to determine whether activity of *B.t.i.* is decreased or delayed at temperatures lower than those previously examined in other studies.

MATERIALS AND METHODS

Aedes stimulans (Walker) larvae were collected from a pool in Ingham Co., MI, in April 1991. Larvae were held at 4°C in plastic buckets filled with water and supplied with leaf detritus taken from the habitat, until experiments were performed beginning the day after collection.

To study the effect of temperature on feeding of larvae, 10 3rd-instars were placed in glass tubes with 10 ml of tap water, allowed no access to food for 24 h, and held at 4°C. A suspension of 5 g of yellow brewer's yeast in 1 liter of water was prepared, divided into 3 portions, and allowed to adjust to temperatures of 0°C (ice water bath kept in a refrigerator), 4°C (refrigerator), or 22°C (incubator). Tubes containing larvae were transferred to the ice water bath, the refrigerator, or the incubator, and larvae allowed to acclimate for 2 h. Then water from the tubes was drawn down with a pipette, the yeast suspension (10 ml) added, and larvae allowed to feed at the experimental temperature. At various intervals in minutes after the experiment began, tubes were emptied and larval gut filling recorded by inspection under a dissecting microscope and grading by an index. The gut-filling index ranged from 0 to 9, where 0 indicated neither the thorax nor any of the abdominal segments contained yellow color (indicating the presence of yeast in the gut); 1 indicated that the thorax only had yellow color; 2 indicated that the thorax and first abdominal segment had yellow color, and so on; a score of 9 was rated when the larval gut was completely

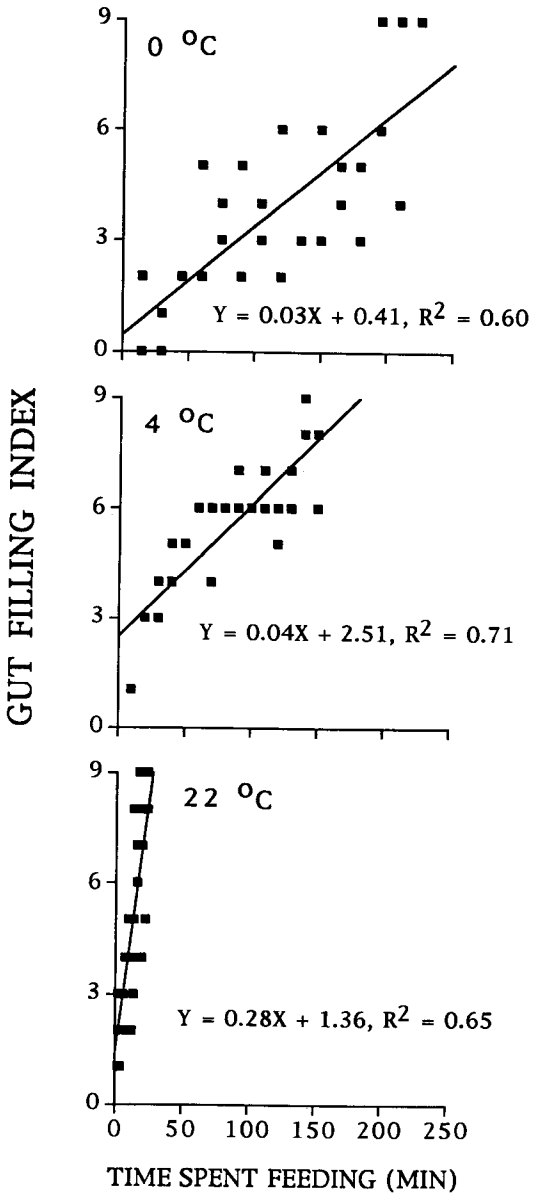

Fig. 1. Rate of filling of guts (gut-filling index) of 3rd-instar *Aedes stimulans* held at 3 constant temperatures.

filled (i.e., thorax and all 8 abdominal segments had yellow color). The intervals of time at which tubes were removed depended upon the temperature at which they were held. Twenty-seven tubes of 10 larvae each were used for each temperature, and the gut-filling index was averaged for all 10 larvae for each time interval of the experiment. The experiment lasted 250 min. Least-squares regression was performed of gut-filling index on time spent feeding, and slopes of regression lines compared with analysis of covariance (ANCOVA) (Steele and Torrie 1980).

The effect of temperature on activity of *B.t.i.* was determined as follows: Stock solutions of *B.t.i.* (Teknar HP-D, lot #9602579, Zoecon Corp., Dallas, TX; ~1,500 AAU/mg) were prepared with distilled water in dilution series starting with 10 ppm, 1 ppm, and then serial 2-fold dilutions to 0.03 ppm. Larvae held in tubes of distilled water were used as controls. Groups of 10 3rd-instar *Ae. stimulans* were placed in tubes as before, acclimated to temperatures of 0, 4, or 22°C as previously described, the water removed, and then dilutions of the *B.t.i.* solution (held at the 3 different temperatures for 2 h as described above) placed in the tubes with larvae. Three tubes served as replicates for each dilution at each temperature. Tubes were held at one of the 3 experimental temperatures, and larval mortality was checked after 12, 24, and 48 h. Mortality of larvae at 12, 24, and 48 h after exposure to *B.t.i.* was compared among temperatures with repeated-measures analysis of variance (ANOVA), given that each tube was inspected successively 3 times to count dead larvae. The LC_{50} values for each temperature were estimated with probit analysis using mortality data from the 48-h time interval (Finney 1971). Slopes of the linear regression of probit transformations of mortality on $\log_{10}$(dose of *B.t.i.* in ppm) were tested for homogeneity with ANCOVA.

RESULTS AND DISCUSSION

The feeding experiments with 3rd-instar *Ae. stimulans* larvae showed that these mosquitoes slowed but continued feeding at 0 and 4°C (Fig. 1). Larvae filled their guts with yeast (i.e., reached a gut-filling index of 9) in 30 min at 22°C, 150 min at 4°C, and 200 min at 0°C. Pairwise comparisons of slopes of lines from regression of gut-filling index on time spent feeding (see regression equations in Fig. 1) showed that there was no difference in gut-filling rates of larvae at 0 and 4°C (ANCOVA, $F = 0.03$, $df = 1$, $P > 0.05$), but that there were significant differences in gut-filling rates between larvae held at 0 or 22°C (ANCOVA, $F = 36.9$, $df = 1$, $P < 0.001$), and between larvae held at 4 or 22°C (ANCOVA, $F = 66.0$, $df = 1$, $P < 0.001$).

In dose-response experiments where temperature was varied and test animals checked at 12, 24, and 48 h after initiation, larval mortality was apparent at all dosages, except 0 ppm controls, at 12 and 24 h among larvae held at 22°C (Fig. 2). In contrast, mortality was scant among larvae held at 0 and 4°C until 48 h after initiation of the experiment (Fig. 2). Mortality of 100% was not observed at any dose of *B.t.i.* for larvae held

at 0°C; moreover, larval mortality in control tubes at this temperature was negligible. Repeated-measures ANOVA showed a highly significant effect of temperature on mortality ($F = 328.1$, $df = 2$, $P < 0.001$), a highly significant effect of dose on mortality ($F = 112.2$, $df = 7$, $P < 0.001$), and a significant interaction between temperature and dose ($F = 30.6$, $df = 14$, $P < 0.001$). Probit analyses provided estimates of LC_{50} values at 48 h after initiation of the experiment, as follows: 0°C, 0.9 ppm (0.4–3.3 ppm, 95% CI); 4°C, 0.2 ppm (0.08–0.4 ppm, 95% CI); and 22°C, 0.1 ppm (0.07–0.15 ppm, 95% CI). Pairwise comparisons of slopes of lines from regression of percent mortality of larvae, expressed as probits, on $\log_{10}$ (dose of *B.t.i.*) showed that there were highly significant differences in dose–response between larvae held at 0 or 22°C (ANCOVA, $F = 247.8$, $df = 1$, $P < 0.001$), between larvae held at 0 and 4°C (ANCOVA, $F = 9.36$, $df = 1$, $P < 0.001$), and between larvae held at 4 or 22°C (ANCOVA, $F = 245.8$, $df = 1$, $P < 0.001$).

This study documents that 3rd-instar *Ae. stimulans* larvae slowed, but did not discontinue, their feeding activity at temperatures of 0 and 4°C. Rate of gut filling was similar for larvae held at 0 and 4°C, but much more rapid for larvae held at 22°C. The fact that larvae fed at the melting point of water (i.e., 0°C) shows that *Ae. stimulans* are capable of ingesting food (and *B.t.i.*) at very low temperatures, but it does not demonstrate that they assimilate nutrients or grow at these temperatures. Whether *Ae. stimulans* larvae would develop at the low temperatures in experiments here is not known, but larvae of some other northern *Aedes* species develop at temperatures as low as 1–2°C (Clements 1992:153). The experimental results strongly suggest that complete discontinuation of larval feeding is unlikely to be the explanation for the decreased efficacy of *B.t.i.* at low temperatures observed here.

An analysis of dose–mortality responses of *Ae. stimulans* when they were provided *B.t.i.* at different temperatures shows that acute mortality is lessened, and delayed, in larvae held at low temperatures compared to 22°C. Because of this delay, LC_{50} estimates could not be made with probit analyses until 48 h after initial exposure to the concentrations of *B.t.i.* In contrast, high mortality at the higher concentrations of *B.t.i.* occurred within 12 h. The median lethal concentration of *B.t.i.* at 0°C (0.9 ppm) was 9-fold higher than that at 22°C (0.1 ppm), and 4-fold higher than that at only 4°C (0.2 ppm). The negligible mortality observed at the 2 low temperatures at 12 and 24 h postexposure contrasts with the readily observed mortality at these temperatures after 48 h. However, larvae at lower temperatures will feed more slowly and may have

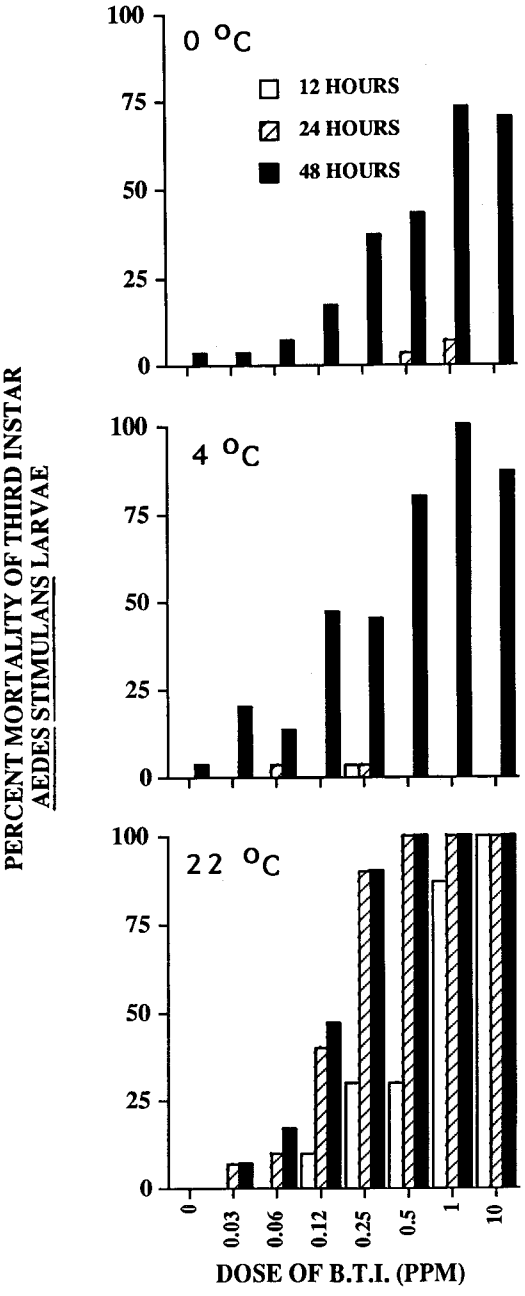

Fig. 2. Mean percent mortality of 3rd-instar *Aedes stimulans* at 12, 24, and 48 h of exposure to a range of concentrations of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) with groups of larvae held at 3 different constant temperatures.

accumulated a lethal dose of *B.t.i.* much slower than larvae held at the high temperature. Thus, slower feeding rate could explain the differences in timing of mortality observed in the dose-response experiment (Fig. 2). Another explanation

is that activity of proteolytic enzymes in the gut and binding of the *B.t.i.* toxins to midgut epithelial cells, both physiological processes comprising part of the mode of action of *B.t.i.* (Porter et al. 1993), were reduced at 0 to 4°C as well. However, these processes were not studied specifically here. From an operational standpoint, applications of *B.t.i.* into woodland pool habitats of spring *Aedes* when the water temperature is in the range of 0–4°C may not achieve the desired level of larval mortality. Water temperatures of woodland pools with *Ae. stimulans* larvae commonly occur within this range (Westwood et al. 1983; Knepper et al. 1991, 1994), thus low water temperature is an important consideration for planning *B.t.i.* applications to spring *Aedes* habitats.

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REFERENCES CITED

Becker, N., M. Zgomba, M. Ludwig, D. Petric and F. Rettich. 1992. Factors influencing the activity of *Bacillus thuringiensis* var. *israelensis* treatments. J. Am. Mosq. Control Assoc. 8:285–289.

Clements, A. R. 1992. The biology of mosquitoes, Volume 1. Development, nutrition, and reproduction. Chapman & Hall, New York.

Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge Univ. Press, London.

Ignoffo, C. M., C. Garcia, M. Kroha and T. L. Couch. 1983. The effects of temperature and water on the insecticidal activity and spore viability of wettable powder formulations of *Bacillus thuringiensis* var. *israelensis*. J. Kans. Entomol. Soc. 56:88–92.

Knepper, R. G. and E. D. Walker. 1989. Effect of *Bacillus thuringiensis israelensis* (H-14) on the isopod *Asellus forbesi* and spring *Aedes* mosquitoes in Michigan. J. Am. Mosq. Control Assoc. 5:596–598.

Knepper, R. G., S. A. Wagner and E. D. Walker. 1991. Aerially-applied, liquid *Bacillus thuringiensis* var. *israelensis* (H-14) for control of spring *Aedes* mosquitoes in Michigan. J. Am. Mosq. Control Assoc. 7:307–309.

Knepper, R. G., S. A. Wagner, E. Abel and E. D. Walker. 1994. Fixed-wing, aerial application of liquid *Bacillus thuringiensis* (H-14) (Acrobe) for control of spring *Aedes* mosquitoes in Michigan. J. Am. Mosq. Control Assoc. 10:42–44.

Lacey, L. A. 1985. *Bacillus thuringiensis* serotype H-14 (Bacteria), pp. 132–158. *In:* H. C. Chapman (ed.). Biological control of mosquitoes. Bull. Am. Mosq. Control Assoc. 6.

Mulla, M. S., B. A. Frederici, H. A. Darwazeh and L. Ede. 1990. Effect of some environmental factors on the efficacy of *Bacillus sphaericus* 2362 and *Bacillus thuringiensis* (H-14) against mosquitoes. Bull. Soc. Vector Ecol. 15:166–176.

Porter, A. G., E. W. Davidson and J.-W. Liu. 1993. Mosquitocidal toxins of bacilli and their genetic manipulation for effective biological control of mosquitoes. Microbiol. Rev. 57:838–861.

Steele, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics, 2nd ed. McGraw-Hill, New York.

Westwood, A. R., G. A. Surgeoner and B. V. Helson. 1983. Survival of spring *Aedes* spp. mosquito (Diptera: Culicidae) larvae in ice-covered pools. Can. Entomol. 115:195–197.

Wraight, S. P., D. P. Molloy and S. Singer. 1987. Studies on the culicine host range of *Bacillus sphaericus* and *Bacillus thuringiensis* var. *israelensis* with notes on the effects of temperature and instar on bacterial efficacy. J. Invertebr. Pathol. 49:291–302.

Wraight, S. P., D. P. Molloy, H. Jamnback and P. McCoy. 1981. Effects of temperature and instar on the efficacy of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* strain 1593 against *Aedes stimulans* larvae. J. Invertebr. Pathol. 38:78–87.