Journal of the American Mosquito Control Association, 14(1):69–71, 1998

LABORATORY BIOASSAY TO COMPARE SUSCEPTIBILITIES OF *AEDES AEGYPTI* AND *ANOPHELES ALBIMANUS* TO *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AS AFFECTED BY THEIR FEEDING RATES

FARIDA MAHMOOD[1]

Entomology and Nematology Department, P. O. Box 110620, University of Florida, Gainesville, FL 32611-0620

ABSTRACT. This study presents the effect of differences in the feeding rates of *Aedes aegypti* and *Anopheles albimanus* on their susceptibilities to *Bacillus thuringiensis israelensis*. *Aedes aegypti* was more susceptible than *An. albimanus* because of its faster rate of feeding. *Aedes aegypti* ingested 11.5 times more spores than did *An. albimanus*, resulting in lower LT_{50} values. *Anopheles albimanus* larvae fed at a slower rate and required fewer spores than *Ae. aegypti* to induce 50% mortality. These findings support earlier reports of much higher concentrations of *B. thuringiensis* required to kill various anopheline species.

KEY WORDS *Aedes aegypti*, *Anopheles albimanus*, *Bacillus thuringiensis*, feeding rate, susceptibilities

INTRODUCTION

The rapid increase in mosquito resistance to various chemical insecticides and growing public concern over environmental pollution has resulted in alternatives for mosquito control such as the use of biological agents and insect growth regulators. Goldberg and Margalit (1977) isolated a bacterial mosquito pathogen that was designated by de Barjac (1978a) as *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*). Laboratory bioassays and field applications of this entomopathogen have shown biological control of several mosquito species and black flies (Ignoffo et al. 1981, Ali et al. 1984, de Barjac and Sutherland 1990). Most anopheline species, with the exception of *Anopheles franciscanus* McCracken, are less susceptible to *B.t.i.* than are mosquito larvae of other genera (Garcia et al. 1980, Sun et al. 1980, Washino and Garcia 1980, Dame et al. 1981, Ali et al. 1984, Mulla 1985). Nugud and White (1982) suggested that either anophelines are less susceptible, i.e., they require higher concentrations of *B.t.i.*, or their surface feeding habit does not allow them to ingest sufficient amounts of bacterial spores, since most formulations tend to sink rather quickly. Earlier, Sun et al. (1980) showed no effect of water depths between 1 and 5 cm on susceptibilities. These authors suggested that a difference in feeding behavior might account for differences in susceptibilities.

Anopheles larvae filter-feed on food particles present at the surface of water or a few centimeters below it, whereas *Culex* and *Aedes* larvae not only feed faster but are capable of filter-feeding at much deeper water depths (Aly et al. 1988). Since anophelines are important vectors of malarial parasites and have exhibited resistance to various insecticides from different geographical areas, biological insecticides are a basic necessity. Much higher concentrations of *B.t.i.* are required to induce mortality in anopheline larvae than in *Aedes aegypti* larvae (Goldberg and Margalit 1977, Tyrell et al. 1979, Mulla et al. 1982). The present study was conducted to compare spore intake in relation to LT_{50} (time taken to kill 50% of exposed larvae) of a very susceptible mosquito species, *Ae. aegypti*, and a relatively tolerant species, *An. albimanus* (Ali et al. 1984).

MATERIALS AND METHODS

Strains: Anopheles albimanus (El Salvador) and *Ae. aegypti* (Orlando) strains were used in this study. Mosquitoes were reared at 26 ± 2°C, 16:8 (L:D) photoperiod, and 85% relative humidity. Larvae were provided Baby E tetramine fish food, and early 4th-instar larvae (1 day after molting) of both species were tested.

Determination of LT_{50}: In this study, we used larvae from 2 different mosquito genera with different feeding rates, *Ae. aegypti* and *An. albimanus*. Technical grade *B.t.i.* powder (ABG 6164) was suspended in deionized water at a concentration of 5 µg/ml. The spores were homogeneously dispersed by sonicating the above suspension for 30 min. Three replicates of 20 larvae of each species were exposed to 100 ml of the *B.t.i.* suspension in 300-ml disposable waxed paper cups. Three replicates containing 20 larvae of each species were simultaneously exposed to 100 ml of deionized water as above and served as controls. The larvae were observed at 15-min intervals under a dissecting microscope at 20× magnification until all larvae died. The larval response was categorized as active (feeding actively), paralyzed (not active but mouth brushes still moving), and dead (inactive, no movement). The above experiment was repeated 3 times, resulting in 9 replicates per species.

Determination of ingestion rate: The larvae of both species were exposed to the above *B.t.i.* suspension by 3 different types of treatments. In the 1st treatment, 2 separate groups of 20 larvae each of

[1] Present address: Mosquito Research and Control, Headlee Research Laboratories, Department of Entomology, P. O. Box 231, Cook College, Rutgers the State University of New Jersey, New Brunswick, NJ 08901.

Table 1. A comparison of *Bacillus thuringiensis* H-14 spore ingestion rates of *Aedes aegypti* and *Anopheles albimanus* after being exposed to 5 µg/ml *B.t.i.* aqueous suspensions.

Exposure time	*An. albimanus* CFUs/larva ± SD (*n*)[1]	*Ae. aegypti* CFUs/larva ± SD (*n*)[1]
30 sec	1,636 ± 1,098 (10)	545 ± 365 (10)
½LT_{50}	48,300 ± 36,150 (10)	536,250 ± 160,093 (9)
Corrected ½LT_{50}[2]	46,664	535,705
LT_{50}	6,461 ± 5,025 (13)	56,727 ± 29,509 (9)
Corrected LT_{50}[3]	4,825	56,182

[1] Colony forming units/larva ± standard deviation; *n* = sample size.
[2] CFUs/larva at ½LT_{50} − CFUs/larva after 30 sec.
[3] CFUs/larva at LT_{50} − CFUs/larva after 30 sec.

both species were exposed for only 30 sec to *B.t.i.* suspension and later surface-sterilized with 95% ethanol to determine the number of spores on the larval surface. In the 2nd treatment, 2 groups of 20 larvae of *Ae. aegypti* and *An. albimanus* were exposed to the above *B.t.i.* suspension for 120 and 200 min, respectively. The above times were calculated as their respective LT_{50} values from the first experiment. Since δ-endotoxin causes paralysis and affects the feeding rate, in the 3rd treatment, 2 groups of 20 larvae each of *Ae. aegypti* and *An. albimanus* were exposed to the above *B.t.i.* suspension for their respective ½LT_{50} values, i.e., 60 and 100 min. Two groups of 20 larvae of each species were exposed only to deionized water for each treatment as controls.

After exposure to the above conditions, 10–15 randomly picked larvae of each species were surface-sterilized in 95% ethanol for 30 sec and rinsed in 500 ml of deionized sterile water. Larvae were homogenized individually in separate, all-glass homogenizers and transferred to separate tubes containing 3 ml of water. All larval homogenates were pasteurized for 12 min at 80°C in a water bath. From each larval homogenate, 0.1 ml of 10^{-2} dilutions were plated on culture plates containing tryptose blood agar base. The plates were kept in an incubator at 26°C, and colony-forming units (CFUs) were counted for each plate after 24 h. All plates were incubated for an additional 24 h to allow sporulation. Mean CFUs of 2nd and 3rd treatments were corrected by deleting the number of CFUs of the 1st treatment. The *B.t.i.* colonies were identified using colony morphology and presence of parasporal crystals (Lacey and Kline 1983).

Statistical methods: Percent mortality was corrected by using Abbott's (1925) formula. The LT_{50} values for each species were calculated by using regression analysis of log time probit percent mortality (Finney 1971). Half LT_{50} value was determined from the LT_{50} value. The corrected mean CFUs/larva at ½LT_{50} and LT_{50} values of both species were compared to each other by chi-square at ($P < 0.05$).

RESULTS AND DISCUSSION

Determination of LT_{50}: A comparison of LT_{50} values of both species showed that *Ae. aegypti* larvae were more susceptible and their LT_{50} was 121 min ($y = -14.02 + 9.13x$). The effect of δ-endotoxin became apparent on *An. albimanus* larvae later, and their LT_{50} was 203 min ($y = -17.49 + 9.74x$). Present observations agreed well with earlier observations for different anopheline species (Sun et al. 1980, Nugud and White 1982, Ali et al. 1984).

The difference in the LT_{50} values of both species was also related to the time required for the appearance of δ-endotoxin intoxication. Cessation of feeding activity, accompanied by paralysis, was observed in *Ae. aegypti* larvae 30–45 min after exposure to *B.t.i.* In comparison, these symptoms appeared after 2 h in *An. albimanus.* The δ-endotoxin intoxication occurs as a result of the release of parasporal crystal and its further degradation in the alkaline pH of the midgut.

Significantly more *B.t.i.* spores were ingested by *Ae. aegypti* after exposure for ½LT_{50} and LT_{50} times ($P < 0.05$). Table 1 depicts a comparison of the number of CFUs/larva of *B.t.i.* ingested by *Ae. aegypti* and *An. albimanus* at different times after exposure to 5 µg/ml of bacterial suspension. *Aedes aegypti* larvae ingested 11.5 and 11.6 times more spores after their ½LT_{50} and LT_{50} times, respectively, than did *An. albimanus* larvae. The results demonstrate that *Ae. aegypti* larvae ingested almost 11 times more *B.t.i.* spores than did *An. albimanus.* Both *Ae. aegypti* and *An. albimanus* had 9.7 and 9.5 times more CFUs/larva, respectively, after being exposed for their ½LT_{50} times than after being exposed for their LT_{50} times. Although we do not have experimental evidence, the detection of smaller numbers of spores at the LT_{50} times might be a result of degradation of spores and release of parasporal crystal at the alkaline midgut pH accompanied by paralysis, resulting in lower spore intake and increased spore defecation with time.

In general, earlier reports have depicted *Anopheles* larvae as much more tolerant to the same concentration of *B.t.i.* than are *Aedes* and *Culex* mosquitoes

(de Barjac 1978a, Mulla et al. 1982). Under similar conditions, *An. stephensi* and *An. quadrimaculatus* required 3 times more concentrated suspensions to inflict 50% mortality than did *Ae. aegypti* (de Barjac 1978b, Mulla et al. 1982).

Anopheles albimanus fed at a slower rate, supporting earlier studies in which much higher concentrations of *B.t.i.* were required to induce similar mortality in anopheline than in *Aedes* or *Culex* larvae. Aly et al. (1988) showed that the LC_{50} (lethal concentration at 50% mortality) values of *An. albimanus* and *Ae. aegypti* were affected by increasing larval densities. Increasing concentrations of toxin were necessary to induce the same levels of mortality. The average amount of toxin/larva inducing 50% mortality decreased 43–64% in *An. albimanus* and 34–44% in *Ae. aegypti* when larval densities increased from 0.15 to 1.5 individuals/ml of water. Aly et al. (1988) suggested that the relative tolerance of anopheline larvae was due to their slower filtration rate. The present results seem to agree with their conclusions, since it was found that a much smaller number of spores were ingested by *An. albimanus* at their $\frac{1}{2}LT_{50}$ and LT_{50} times than by *Ae. aegypti* (Table 1).

In mosquitoes, the rate of ingestion of *B.t.i.* plays a role in the activity and effectiveness of δ-endotoxin by affecting the response of an individual in various manners. The effect of a given dosage of toxin produces different results depending on whether the lethal dose is administered all at once or in small doses over a longer period (Aly et al. 1988). Present results are also supported by the study of Manasherob et al. (1996) in which they encapsulated high concentrations of *B.t.i.* spores in a motile protozoan, *Tetrahymena pyriformis*. The activity of *B.t.i.* was enhanced by 8 times when *An. stephensi* larvae were exposed to the same concentrations of *B.t.i.* with and without encapsulation, suggesting that more encapsulated spores were ingested within a shorter period. Further studies are required to determine the quantitative rate of spore intake and the actual concentration of δ-endotoxin required for killing *An. albimanus* and *Ae. aegypti* larvae.

ACKNOWLEDGMENTS

This research was conducted at the Department of Entomology and Nematology, University of Florida, Gainesville, FL. I appreciate the help of D. J. Boucias of the Entomology and Nematology Department and J. A. Seawright of the USDA-ARS Center for Medical, Agricultural and Veterinary Entomology. This is University of Florida Journal Series No. R-06029.

REFERENCES CITED

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265–267.

Ali, A., D. M. Sauerman and J. K. Nayar. 1984. Pathogenicity of an industrial formulation of *Bacillus thuringiensis* serovar *israelensis* to larvae of some culicine mosquitoes in the laboratory. Fla. Entomol. 67:193–197.

Aly, C., M. S. Mulla, B. Xu and W. Schnetter. 1988. Rate of ingestion by mosquito larvae (Diptera: Culicidae) as a factor in the effectiveness of a bacterial stomach toxin. J. Med. Entomol. 25:191–196.

Dame, D. A., K. E. Savage, M. V. Meisch and S. L. Oldacre. 1981. Assessment of industrial formulation of *Bacillus thuringiensis* var. *israelensis*. Mosq. News 41: 540–546.

de Barjac, H. 1978a. Une nouvelle variete de *Bacillus thuringiensis* tres toxique pou les mostiques: *B. thuringiensis* var *israelensis* serotype 14. C. R. Acad. Sci. Paris D 286:797–800.

de Barjac, H. 1978b. Toxcite de *Bacillus thuringiensis* var. *israelensis* pour les larves d' *Aedes aegypti* et d' *Anopheles stephensi*. C. R. Acad. Sci. Paris D 286:797–800.

de Barjac, H. and D. J. Sutherland (editors). 1990. Part 1, pp. 3–217. *In:* Bacterial control of mosquitoes and blackflies. Rutgers Univ. Press, New Brunswick, NJ.

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

Garcia, R., B. DesRoches and W. Tozer. 1980. Studies on the toxicity of *Bacillus thuringiensis* var. *israelensis* against mosquito larvae. Univ. Calif. Mosq. Control Res. Annu. Rep. 54–57.

Goldberg, L. J. and J. Margalit. 1977. A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univitatus, Aedes aegypti,* and *Culex pipiens*. Mosq. News 37:355–358.

Ignoffo, C. M., C. Garacia, M. J. Kroha, T. Fukuda and T. L. Couch. 1981. Laboratory tests to evaluate the potential efficacy of *Bacillus thuringiensis* var *israelensis* for use against mosquitoes. Mosq. News 42:85–93.

Lacey, L. A. and D. L. Kline. 1983. Laboratory bioassay of *Bacillus thuringiensis* (H-14) against *Culicoides* spp. and *Leptoconops* spp. (Ceratopogonidae). Mosq. News 43:502–503.

Manasherob, R., E. Ben-Dov, J. Margalit, A. Zaritsky and Z. Barak. 1996. Raising activity of *Bacillus thuringiensis* var *israelensis* against *Anopheles stephensi* larvae by encapsulation in *Tetrahymena pyriformis* (Hymenostomatida: Tetrahymenidae). J. Am. Mosq. Control Assoc. 12:627–631.

Mulla, M. S. 1985. Field evaluation and efficacy of bacterial agents and their formulations against mosquito larvae, pp. 227–250. *In:* M. Laird and J. W. Miles (eds.). Integrated mosquito control methodologies, Volume 2. Academic Press, New York.

Mulla, M. S., B. A. Federici and H. A. Darwazeh. 1982. Larvicidal efficacy of *Bacillus thuringiensis* serotype H-14 against stagnant-water mosquitoes and its effects on nontarget organisms. Environ. Entomol. 11:788–795.

Nugud, A. D. and G. B. White. 1982. Evaluation of *Bacillus thuringiensis* serotype H-14 formulation of larvicides for *Anopheles arabiensis* (species B of *Anopheles gambiae* complex). Mosq. News 42:36–40.

Sun, C., G. P. Georghiou and K. Weiss. 1980. Toxicity of *Bacillus thuringiensis* var *israelensis* to mosquito larvae variously resistant to conventional insecticides. Mosq. News 40:614–618.

Tyrell, D. J., L. I. Davidson, L. A. Bulla and W. A. Ramoska. 1979. Toxicity of parasporal crystals of *Bacillus thuringiensis* subsp. *israelensis* to mosquitoes. Appl. Environ. Microbiol. 38:656–658.

Washino, R. K. and R. Garcia. 1980. Comparison of three formulations of *Bacillus thuringiensis* var *israelensis* H-14 in a California rice field. Univ. Calif. Mosq. Control Res. Annu. Rep. 62–63.