INTEGRATED USE OF PLANARIA (*DUGESIA DOROTOCEPHALA*) AND *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST *AEDES TAENIORHYNCHUS*: A LABORATORY BIOASSAY[1]

M. J. PERICH, P. M. CLAIR[2] AND L. R. BOOBAR

U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, MD 21702-5010

ABSTRACT. The effectiveness of integrating *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) and the predatory planaria, *Dugesia dorotocephala* against *Aedes taeniorhynchus* was determined under controlled laboratory conditions. There was no significant effect ($P > 0.05$) of *B.t.i.* on *D. dorotocephala* either by direct association or through ingestion of *B.t.i.* dosed larvae. Planaria, alone, and *B.t.i.* combined with planaria, both provided significant ($P < 0.05$) reduction of *Ae. taeniorhynchus* populations through the 12-week evaluation.

INTRODUCTION

Aedes taeniorhynchus (Wied.), a vector of Venezuelan equine encephalitis and Eastern equine encephalitis in the salt marsh and coastal areas of North and South America (Knight and Stone 1977), has become resistant to most organic insecticides (Brown 1986). In addition to resistance, many conventional insecticides used to control *Ae. taeniorhynchus* populations are harmful to nontarget organisms (Galindo 1972) and require frequent reapplication (Giglioli et al. 1979), creating the need for alternative control strategies.

One possible alternative control strategy against *Ae. taeniorhynchus* is the integrated use of the predatory planarian, *Dugesia dorotocephala* (Woodworth), and the microbial larvicide, *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*). *Bacillus thuringiensis* var. *israelensis* has been shown to be efficacious against naturally occurring populations of *Ae. taeniorhynchus* larvae with no significant effect on nontarget organisms in the salt marsh (Purcell 1981). Short-term persistence in mosquito larval habitats is a major disadvantage of *B.t.i.* (Mulla 1985, Garcia and Sweeney 1986) that limits its practical use for the control of *Ae. taeniorhynchus*. *Dugesia dorotocephala* has been reported to be an effective predator of mosquito larvae (Legner 1977, Medved and Legner 1974) and shown to be potentially safe with nontarget aquatic organisms (Perich and Boobar 1990). This planaria species offers potential as a biological control agent against *Ae. taeniorhynchus* due to its tolerance of saline water (Rivera 1989),[3] its ability for mass production (Legner and Tsai 1978) and the ease of application (Darby et al. 1988). Rapid increase in pest populations prior to the establishment of the introduced predator population often limits the use of any predator as a biological control agent (Huffaker and Messenger 1976) and is a major limitation in the use of planaria against *Ae. taeniorhynchus* populations.

The described disadvantages of each component used as a single control agent against *Ae. taeniorhynchus* can potentially be overcome by combining *B.t.i.* with *D. dorotocephala*. The objectives of this study were to evaluate the effectiveness and compatibility of integrating *B.t.i.* and planaria against *Ae. taeniorhynchus* under laboratory conditions.

MATERIALS AND METHODS

All *B.t.i.* treatments were prepared from Teknar®, a commercial *B.t.i.* formulation, as agitated water-based suspensions at an application concentration of 0.59 liters/ha (0.5 pints/acre). The potency of the *B.t.i.* was 3,000 *Aedes aegypti* international toxic units/mg.

Dugesia dorotocephala were from a stock colony maintained at this laboratory for 2 years and originally were collected from a stream in Keedysville, MD. Species identification was confirmed with reference to Kenk (1972). All *D. dorotocephala* used in the tests were 15–20 mm long.

B.t.i. *effects on* D. dorotocephala: The effects of *B.t.i.* on *D. dorotocephala* were determined by direct association between *B.t.i.* and planaria, and planarian consumption of *B.t.i.*-dosed mos-

[1] The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

[2] Present address: U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011.

[3] Rivera, V. R. 1989. Physical and behavioral effects of water quality parameters on various species of planaria and their predation rates of five mosquito species. M.S. thesis. Hood College, Frederick, MD.

Reprinted from Journal of the American Mosquito Control Association
Vol 6 December 1990 Number 4

quito larvae. Direct association was done by placing 10 *D dorotocephala* into five 100 × 50 mm glass crystalizing dishes filled with filtered well water treated with the *B.t.i.* suspension. Five additional dishes containing 10 planaria each and untreated filtered well water received *B.t.i.*-dosed 3rd instar *Ae. taeniorhynchus* larvae, in which larvae were dosed using a microinjection apparatus to place the *B.t.i.* directly into the mosquito larvae intestines through the anal pore (Misch and Anderson 1986) to assure intoxication. Five dishes containing 10 untreated planaria and untreated filtered well water served as controls. The *Ae. taeniorhynchus* larvae were from an insecticide susceptible colony maintained at this laboratory for 10 years. Three times per week for the entire study, 25 treated 3rd instar *Ae. taeniorhynchus* larvae were placed into each *B.t.i.* dosed larvae treatment dish, and 25 untreated larvae were placed into each *B.t.i.* direct association treatment and untreated control designated dish. The mortality and reproduction of the treated planaria were compared with the untreated planaria, with the number of live planaria counted weekly.

The duration of the bioassay was 30 days, with the cumulative number of live *D. dorotocephala* used as the criterion to determine any *B.t.i.* effect. Data were subjected to analysis of variance procedure (ANOVA [SAS/STAT 1985]) for testing the null hypothesis that *B.t.i.* had no effect.

Efficacy of B.t.i. *and* D. dorotocephala *against* Ae. taeniorhynchus: The efficacy of *B.t.i.* and *D. dorotocephala* individually, and combined, against laboratory populations of *Ae. taeniorhynchus* was determined by a comparison bioassay in which each treatment (*B.t.i.*, planaria, *B.t.i.* + planaria) and untreated controls were replicated 3 times. Initially, 300 first instar larvae from a laboratory colony of *Ae. taeniorhynchus* were placed into 37 × 20 × 7 cm enamel-lined pans containing 2 liters of filtered well water and 3.8 g of sea salt (0.19% salinity). Fresh water was replenished, as needed, to compensate for evaporation. The water in each pan was continuously oxygenated with an air stone and air pump to maintain the dissolved oxygen content between 4 and 6 ppm. The pH of the water was maintained using 1 M phosphoric acid or sodium hydroxide between 7.5 and 8.0. The bioassay was done in an environmentally controlled room maintained at 26 ± 1°C, 80 ± 5% RH with a 12 h light/dark photoperiod.

Pans designated either *B.t.i.* only, or *B.t.i.* combined with planaria, were dosed at the start of the study with the agitated water-based *B.t.i.* suspension, for a final *B.t.i.* concentration of 0.59 liters/ha (0.5 pints/acre). Pans assigned to have either the planaria only, or *B.t.i.* combined with planaria treatment received 10 *D. dorotocephala* per pan at the start of the bioassay. The number of planaria was recorded weekly.

Mosquito larvae were fed 0.5 g of a 1:1:1 liver powder, yeast and ground hog chow mixture 3 times per week. The diet for the planaria was supplemented each week by placing 1 g of fresh beef liver in each pan for 24 h. Every week, 300 additional 1st instar *Ae. taeniorhynchus* larvae were added to each pan to simulate natural population renewal due to hatching of eggs present in the immediate area.

As larvae pupated, they were counted and placed in a 250 ml beaker containing 200 ml of filtered well water. Pupae were then placed in 30 × 30 × 30 cm aluminum cages and allowed to emerge. This did not affect treatment results due to the mode of action of *B.t.i.* as a gastric toxin (Margalit and Dean 1985) and prior evaluations which determined that *D. dorotocephala* do not significantly prey on *Ae. taeniorhynchus* pupae. This finding is opposite of that reported against *Culex stigmatosoma* Dyar (as *Cx. peus* Speiser) pupae (Yu and Legner 1976). Each cage contained a cotton wick saturated with a 10% sucrose solution and a beaker of moist sphagnum moss for oviposition. Rabbits (*Oryctolagus cunniculus*) were used to bloodfeed the adults for 15 min 3 times a week. Moss was collected weekly and replaced with fresh moss. After one week of room drying, eggs were rinsed off the moss, hatched, larvae counted, and the first instar larvae were placed back in the corresponding larval tray, in addition to the 300 first instar larvae added weekly.

The cumulative mean number of pupae per week for each treatment was used as the criterion in evaluating *B.t.i.* and planaria efficacy against *Ae. taeniorhynchus*. The bioassay was conducted until the mean number of pupae in each treatment was not significantly different from the number of the control, or until 12 weeks. Data were subjected to an analysis of variance procedure (ANOVA [SAS/STAT 1985]) for testing the null hypotheses that treatment had no effect on *Ae. taeniorhynchus* populations. Means calculated for each treatment regime and control were separated by use of Duncan's (1955) multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

No significant effect of *B.t.i.* on *D. dorotocephala* either by direct association or through ingestion of *B.t.i.*-dosed larvae was determined ($P > 0.05$; df = 14; F = 2.79; ANOVA). Both *D. dorotocephala* dosed directly with *B.t.i.*, and those fed *B.t.i.*-dosed mosquito larvae, were found to reproduce asexually at a higher level than the untreated control group (Fig. 1), a

finding similar to that reported by Levy and Miller (1978) who exposed planaria to synthetic organic pesticides and insect growth regulators.

The single *B.t.i.* treatment, as expected, provided 100% control of the *Ae. taeniorhynchus* population through 2 weeks (Table 1). The mean number of pupae from the *B.t.i.* treated pans at 3 weeks was not significantly ($P > 0.05$) different from the mean number of pupae from the untreated control pans. Further evaluation of the *B.t.i.* treatment was stopped after 3 weeks. These results indicated that either all applied *B.t.i.* toxin crystals were consumed by the larvae within 2 weeks or that some undetermined factor caused this treatment to be effective for only 2 weeks. Due to the laboratory conditions of limited organic materials in the water and the shallowness of the test pans (7 cm), neither *B.t.i.* adsorption to organic particles nor settling to a level below the larval feeding zone is a likely factor in the limited persistence of this treatment.

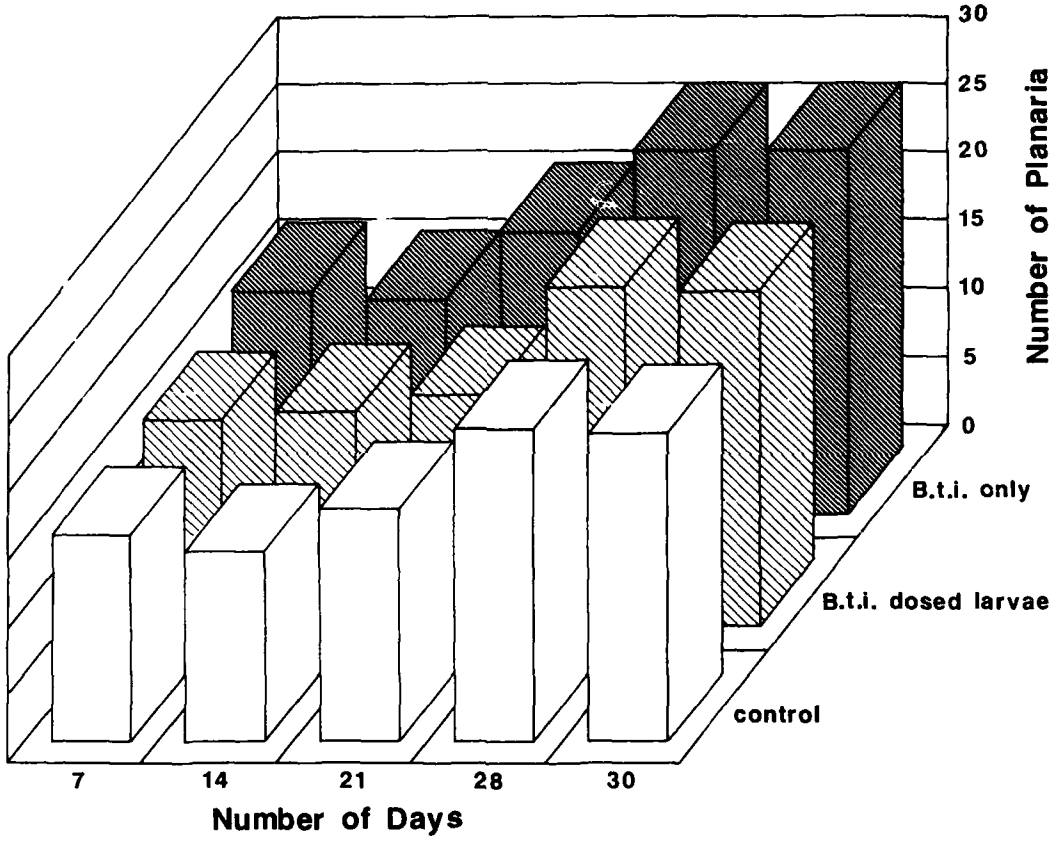

Fig. 1. Effects of *Dugesia dorotocephala* exposure to *B.t.i.* as measured by the mean number of live planaria over time.

Table 1. Weekly mean number of *Aedes taeniorhynchus* pupae removed from pans inoculated with *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) and *Dugesia dorotocephala* individually and combined.

	Mean number of pupae collected from the indicated treatment pans[1]											
	Weeks											
Treatment	1	2	3	4[2]	5	6	7	8	9	10	11	12
Control	73A	95A	108A	143A	162A	165A	114A	114A	152A	138A	137A	178A
B.t.i.	0B	0B	80A	—	—	—	—	—	—	—	—	—
Planaria	24C	1B	1B	41B	36B	32B	28B	1B	1B	2B	1B	1B
B.t.i. + planaria	0B	0B	0B	12B	12B	1C	1BC	1B	1B	1B	1B	1B

[1] Means in the same column followed by the same letter not significantly different ($P < 0.05$; N = 5; Duncan's multiple range test [SAS/STAT 1985]).

[2] Evaluation of *B.t.i.* inoculated pans terminated.

Table 2. Weekly mean number of *Dugesia dorotocephala* in planaria and *B.t.i.* plus planaria treatment pans.

Treatment	x̄ no. *D. dorotocephala* per week											
	1	2	3	4	5	6	7	8	9	10	11	12
Planaria	10	18	26	30	33	34	43	52	56	59	63	65
Planaria + *B.t.i.*	10	15	24	27	31	32	34	37	45	51	58	62

The planaria-only treatment provided significant reduction ($P < 0.05$) of the mosquito test population throughout the 12 week bioassay as compared with the untreated control group (Table 1). The *D. dorotocephala* provided 67% reduction of the *Ae. taeniorhynchus* population for the first week and reduction was increased to 99% for the following 2 weeks. On week 4, introduction of progeny from surviving mosquitoes of week 1, in addition to the weekly 300 first instar larvae, increased the prey to planaria ratio, which caused a subsequent increase in the cumulative mean percent of surviving pupae from 1 to 35%. The increase in prey to planaria ratio also stimulated asexual multiplication of the planaria which doubled within 5 weeks (Table 2), similar to that reported by Tsai and Legner (1977). There was a subsequent decrease in the cumulative mean percent of surviving pupae back to 1% by the 8th week. Planaria were observed to kill more mosquito larvae than were consumed, with larvae becoming attached to each other by the mucus secreted by the *D. dorotocephala* and settling to the bottom and drowning. The prolonged and significant ($P < 0.05$) reduction of *Ae. taeniorhynchus* by *D. dorotocephala* in this study agrees with the reported control of *Culex tarsalis* Coquillett, *Cx. stigmatasoma* and *Cx. quinquefasciatus* Say in earthen test ponds (Legner 1977), and of *Cx. stigmatasoma* in rice fields (Yu and Legner 1976).

The treatment combination of *B.t.i.* and planaria also provided significant ($P < 0.05$) reduction of the *Ae. taeniorhynchus* for the entire 12 week bioassay as compared with the untreated control group (Table 1). The initial 100% control can be attributed to the *B.t.i.* component, after which the *D. dorotocephala* maintained reduction of the test population. The increase in the mean number of *Ae. taeniorhynchus* pupae in weeks 4 and 5 could be due to a decrease in *D. dorotocephala* feeding activity which occurs periodically in nature (Pennak 1978).

Todd and Giglioli (1983) reported failure to implement biocontrol against *Ae. taeniorhynchus* populations using predators, specifically, 3 species of minnows, due to 2 factors: (1) immediate hatching of mosquito eggs before the predator populations could become established, resulting in a high prey to predator ratio; and (2) dilution of the predator population during initial swamp flooding. Results from the present study indicate that the reported limiting factors (Todd and Giglioli 1983) might be resolved by the combination treatment of *B.t.i.* and *D. dorotocephala*. The *B.t.i.* component would provide initial control allowing the planaria population to become established. Once established, the *D. dorotocephala* population should increase rapidly in response to the increase in the available mosquito prey and provide significant suppression of the mosquito population.

In conclusion, *B.t.i.* has no detrimental effect on the predator *D. dorotocephala* and can be integrated with planaria against *Ae. taeniorhynchus*. The *D. dorotocephala*, once established, should provide prolonged significant ($P < 0.05$) reduction of *Ae. taeniorhynchus* population as measured under the laboratory conditions of this study. The treatment combination of *B.t.i.* and *D. dorotocephala* appears from this study to offer potential as an alternative control strategy against *Ae. taeniorhynchus* and warrants field evaluation.

REFERENCES CITED

Brown, A. W. A. 1986. Insecticide resistance in mosquitoes: a pragmatic review. J. Am. Mosq. Control Assoc. 2:123–140.

Darby, W. M., L. R. Boobar and M. R. Sardelis. 1988. A method for dispensing planaria (*Dugesia dorotocephala*) for mosquito control. J. Am. Mosq. Control Assoc. 4:545–546.

Duncan, P. B. 1955. Multiple range test and multiple F tests. Biometrics 11:1–42.

Galindo, P. 1972. Control of VEE vectors. Pan. Am. Sanit. Bur. Sci. Publ. 243:394–395.

Garcia, R. and A. W. Sweeney. 1986. The use of microbial pathogens for the control of mosquitoes. Agric. Ecosystems Environ. 15:201–208.

Giglioli, M. E. C., E. J. Gerberg and R. G. Todd. 1979. Large scale field tests and environmental assessments of Sumithion (fenitrothion) against adult mosquitoes in Grand Cayman, West Indies. Mosq. News 39:781–793.

Huffaker, C. B. and P. S. Messenger. 1976. Theory and practice of biological control. Academic Press Inc., New York.

Kenk, R. 1972. Freshwater planarians [Turbellaria] of North America. Biota of freshwater ecosystems. *Identification Manual* No. 1, Smithsonian Inst., Washington, DC.

Knight, K. L. and A. Stone. 1977. A catalog of the mosquitoes of the world (Diptera: Culicidae). Thomas Say Foundation Vol. 6. College Park, MD.

Legner, E. F. 1977. Response of *Culex* spp. larvae and

their natural insect predators to two inoculation rates with *Dugesia dorotocephala* (Woodworth) in shallow ponds. Mosq. News 37:435–440.

Legner, E. F. and S. C. Tsai. 1978. Increasing fission rate of the planarian mosquito predator *Dugesia dorotocephala* through biological filtration. Entomophaga 23:293–298.

Levy, R. and T. W. Miller, Jr. 1978. Tolerance of the planarian *Dugesia dorotocephala* to high concentrations of pesticides and growth regulators. Entomophaga 23:31–34.

Margalit, J. and D. Dean. 1985. The story of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*). J. Am. Mosq. Control Assoc. 1:1–7.

Medved, R. A. and E. F. Legner. 1974. Feeding and reproduction of the planarian, *Dugesia dorotocephala* (Woodworth), in presence of *Culex peus* Speiser. Environ. Entomol. 3:637–641.

Misch, D. W. and L. M. Anderson. 1986. A technique and apparatus for placement of test substances into the intestines of mosquito larvae. Entomol. Exp. Appl. 41:179–183.

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, Vol. 2. Academic Press, London.

Pennak, R. W. 1978. Freshwater invertebrates of the United States. 2nd ed. Wiley, New York.

Perich, M. J. and L. R. Boobar. 1990. Effects of predator *Dugesia dorotocephala* (Tricladida: Turbellaria) on selected nontarget aquatic organisms: laboratory bioassay. Entomophaga 35:79–83.

Purcell, B. H. 1981. Effects of *Bacillus thuringiensis* var. *israelensis* on *Aedes taeniorhynchus* and some nontarget organisms in the salt marsh. Mosq. News 41:476–483.

SAS/STAT. 1985. Guide for personal computers. 6th ed. SAS Institute Inc., Cary, NC.

Tsai, S. C. and E. F. Legner. 1977. Exponential growth in culture of the planarian mosquito predator *Dugesia dorotocephala* (Woodworth). Mosq. News 37:474–478.

Todd, R. G. and M. E. C. Giglioli. 1983. The failure of *Gambusia puncticulata* and other minnows to control *Aedes taeniorhynchus* in a mangrove swamp on Grand Cayman, West Indies. Mosq. News 43:419–425.

Yu, H. and E. F. Legner. 1976. Regulation of aquatic Diptera by planaria. Entomophaga 21:3–12.