MICROBIAL LARVICIDES FOR THE CONTROL OF NUISANCE AQUATIC MIDGES (DIPTERA: CHIRONOMIDAE) INHABITING MESOCOSMS AND MAN-MADE LAKES IN CALIFORNIA

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ABSTRACT. *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) and *B. sphaericus* were evaluated for chironomid larvicidal activity in freshwater mesocosms. Of two *B.t.i.* formulations, the technical powder ABG-6164 provided excellent control of chironomines (94%) at the rate of 11.2 kg/ha whereas the liquid concentrate, Vectobac 6 AS, achieved only moderate control (57%) at the rate of 22.4 kg/ha. In contrast, similar rates of *B. sphaericus* products, ABG-6184 technical powder and BSP-2 flowable concentrate, produced no significant reduction. Lake studies were initiated to determine which *B.t.i.* formulations were practical and effective in large-scale situations. Two sinking granular corn grit formulations worked with varying degrees of success but were deemed too bulky for extensive applications. *B.t.i.* technical powder preparations with high potency ratings of 5,000 and 12,430 ITU/mg gave excellent control of *Chironomus* spp. (100% and 87%) when used at the rates of 6.7 and 2.8 kg/ha, respectively. None of the treatments reduced larval populations of the tanypodines, *Procladius* and *Tanypus*.

INTRODUCTION

Nonbiting midges of the family Chironomidae are one of the most common and abundant groups of organisms in inland natural and man-made aquatic ecosystems (Ali 1980). They breed in a variety of habitats such as streams, rivers, lagoons, ditches, freshwater ponds, sewage oxidation ponds, flood control channels, reservoirs and lakes. In temperate and subtropical regions, midges complete their development from egg to adult in 2–4 wk and emerge in large numbers especially from shallow eutrophic bodies of water. Some water spreading basins in southern California have been reported to support midge larval densities as high as $20{,}000/m^2$ (Ali and Mulla 1978, Bay and Anderson 1965).

The larvae of chironomids play a beneficial role in aquatic ecosystems. They are involved in recycling organic wastes and they constitute a good source of food for fish, waterfowl (especially shore birds) and other biota (Ali and Mulla 1979), but the larvae that inhabit domestic reservoirs may contaminate portable water and even be preserved in soft drinks that are subsequently condemned for presence of insect parts (M. S. Mulla, personal observations). The adults emerging from rivers, ponds, water spreading basins, lakes, flood control channels and other habitats surrounded by residential, recreational, commercial and industrial establishments can become a nuisance by creating aesthetic as well as economic and medical problems (Mulla 1974). They may cause serious problems for industries located in the proximity of breeding sites such as plastic and paint industries, which suffer economic losses where adult midges fly into and imbed in the final products (Ali 1980). Therefore, for economic and health reasons, control of midges may become necessary in some communities.

In southern California chironomid midges develop in many types of aquatic habitats, such as natural rivers, man-made lakes and ponds, flood control channels, water spreading basins and sewage oxidation ponds. In some of these sources located amid urban and periurban areas, heavy populations of chironomid midges have been encountered that cause nuisance and economic problems. Since the 1970s there have been many residential-recreational lake developments in California. With these lakes, it is an economic advantage to configure the shoreline by constructing inlets or fingers where more homes along the increased waterfront can be built. Such lakes provide ideal breeding sites for midge populations, which in turn cause nuisance and economic problems especially to the residents of the areas.

These studies were carried out to evaluate the efficacy of 2 microbial control agents *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) and *B. sphaericus* strain 2362 against midge larvae in man-made lakes and experimental mesocosms (ponds).

MATERIALS AND METHODS

Study areas: The studies were conducted in mesocosms and lakes in California. Mesocosms are defined here as small discreet experimental plots representative of larger-size ecosystems possessing similar species composition and environmental features. They are identical and can be replicated in experimental designs studying biology, ecology and control of organisms. The mesocosm studies were carried out at the Aquatic and Vector Control Research Facilities in the Coachella Valley and Riverside each having 64 and 40 outdoor man-made mesocosms 30 m^2 × 30 cm deep and 27 m^2 × 30 cm deep, respectively. The mesocosms in the Coachella Valley have low growing submerged vegetation on the bottom and margins, whereas those in Riverside are free of plants. Water temperature in the ponds during the course of the study was 9.5–17°C (Coachella Valley) and 23.5–30°C (Riverside). The lake studies were conducted at 3 different man-made residential-recreational lakes: Woodward Lake in Fresno, Woodbridge North Lake in Irvine, and Lake Calabasas in Calabasas, California. Woodward Lake at the time of the study (1988–89) was 2 years old with surface area of 21.6 ha, had an average depth of 2.1 m and water temperature of 24–26°C during the study. Woodbridge North Lake was 11 years old (in 1988), 12 ha and 2.1 m deep with 12–24°C water temperature. Lake Calabasas was 22 years old (1989), 8.4 ha and approximately 1.8 m deep with a water temperature of 19.5–24°C.

Mesocosm studies: Six bacterial formulations were evaluated: four formulations of *B.t.i.* and 2 formulations of *B. sphaericus* strain 2362. The *B.t.i.* products tested were: Vectobac 6 AS, aqueous suspension (600 ITU/mg), Vectobac ABG-6164, technical powder (5,000 ITU/mg), tested in Coachella Valley; and 2 formulations of corn grit granules ABG 6264 (400 ITU/mg), ABG 6253 (200 ITU/mg), tested in Riverside, all provided by Abbott Laboratories, N. Chicago, IL 60064. The corn grit formulations with a particle size of 1–2 mm were heavy, sinking rapidly to the bottom. The *B. sphaericus* products tested were BSP-2, liquid (2 × 10^7 spores/g) from Solvay and Company, Brussels, Belgium, and ABG-6184, primary powder (7.6 × 10^{10} spores/g) from Abbott Laboratories, both tested in Coachella Valley. Liquid and powder formulations were mixed with water and applied using plastic squeeze bottles. The corn grit granules were applied by hand-broadcasting. The rates applied to the mesocosms (Table 1) were considered to be high (about 5–10 times) in comparison with rates used against mosquitoes (Mulla 1986, Mulla and Darwazeh 1985, Mulla et al. 1988). Vectobac 6 AS was applied at rates of 11.2 and 22.4 kg/ha, Vectobac technical powder ABG-6164 at 5.6 and 11.2 kg/ha, and the *B. sphaericus* formulations, BSP-2 at 22.4 kg/ha and ABG-6184 at 11.2 kg/ha. The 2 corn grit formulations ABG-6253 and ABG-6264 were applied at the rates of 11.2 to 44.8 kg/ha. Each treatment including checks were replicated 3 times in the mesocosms.

Lake studies: Evaluation of *B.t.i.* formulations was carried out in 3 residential-recreational lakes in California. At Woodward Lake, the corn grit formulation ABG-6253 (200 ITU/mg) of *B.t.i.* was evaluated. This formulation was applied from a boat using a backpack granular blower. It was applied at the rates of 13.5, 28 and 56 kg/ha into 3 finger-like inlets, each rate applied to a separate finger, and a fourth untreated finger was left as check. Each finger covered 1.2–2 ha.

At Woodbridge North Lake, *B.t.i.* ABG-6164, technical powder (12,430 ITU/mg) and 2 corn grit formulations: ABG-6253 (200 ITU/mg) and ABG-6264 (400 ITU/mg) were evaluated. The powder formulation was mixed with water and applied from a pressurized spray tank in front of the boat at the rates of 1.4 and 2.8 kg/ha. Each rate was applied to one section of the lake which covered 1.6 ha (including a section for check). In another test the corn grit formulation ABG-6253 was evaluated at the rate of 56 kg/ha. In a final test the more potent corn grit formulation ABG-6264 was tested at the rates of 11.2 and 19.1 kg/ha. Both granular formulations were applied by a backpack blower from the boat.

At Lake Calabasas, *B.t.i.* Vectobac technical

Table 1. Field evaluation of *B.t.i.* and *B. sphaericus* formulations against chironomine[a] larvae in field mesocosms—Coachella Valley.

Formulation[b]	Rate (kg/ha)	Percent control days post-treatment 3	7	14
B.t.i., Vectobac 6 AS	11.2	7	37	13
(600 ITU/mg)	22.4	9	32	57
B.t.i., Vectobac ABG-6164 TP	5.6	49	88	98
(5,000 ITU/mg)	11.2	52	94	100
Bs 2362, BSP-2 FC	22.4	0	28	38
(2 × 10^7 spores/g)				
Bs 2362, ABG-6184 TP	11.2	6	0	26
(7.6 × 10^{10} spores/g)				

[a] The predominant species was *Chironomus decorus*, with a few *C. fulvipilus* and *Paralauterborniella elachista*.
[b] AS = aqueous suspension; TP = technical powder; FC = flowable concentrate.

powder (5,000 ITU/mg) was evaluated at the rates of 2.2, 4.5 and 6.7 kg/ha. The formulation was mixed with water and sprayed in front of the boat from a pressurized spray tank to ensure good mixing with lake water. Several swaths were made to insure uniform coverage. Each rate was applied to one section of the lake that covered 1.6 ha with one section serving as check.

LARVAL SAMPLING

Mesocosm studies: Larvae were sampled by using a 7.5 × 15 cm scoop (0.01 m^2 area) in the Coachella Valley and 15 × 15 cm scoop (0.02 m^2 area) in Riverside. Two samples were taken from each mesocosm before treatment, 3 days post-treatment and then weekly thereafter for 2 wk. Each sample was washed through a 40-mesh screen (pore size 50 × 65 μm) to remove silt and then the residues including larvae transferred to white enameled pans for counting and identification using the procedures detailed in Mulla et al. (1971, 1974).

Lake studies: Larvae were sampled at Woodward Lake with an Ekman dredge (0.02 m^2 area) removing about 5-cm deep samples of mud. Six samples per finger (plot) were taken for pretreatment assessment and subsequently after treatment on a weekly basis for 8 wk. Larvae were counted and segregated into chironomines and tanypodines as described above. These 2 groups respond very differently to *B.t.i.* formulations.

At Woodbridge North Lake, larval sampling was done as in the previous experiment using an Ekman dredge but 8 samples were taken in each section (plot) and counted as chironomines and tanypodines. Samples were taken before treatment and after treatment on a weekly basis for 3–6 wk.

At Lake Calabasas, larvae were sampled as before using the dredge, with 5 samples per section (plot) for pretreatment and post-treatment assessments, which were carried out on a weekly basis for 5 wk, and the larvae were segregated and counted into separate genera.

The population trends in treated and untreated plots are presented as mean number of larvae per unit area. The densities are either presented in tables or in graphs. In some experiments the percent control was determined by using Mulla's formula (Mulla et al. 1971), whereas in others it was not necessary to calculate level of control.

$$\% \text{ control} = 100 - \left(\frac{C_1}{T_1} \times \frac{T_2}{C_2}\right) 100$$

where: C_1 = mean no. larvae pretreatment in check plots, T_1 = mean no. larvae pretreatment in treated plots, C_2 = mean no. larvae post-treatment in check plots, T_2 = mean no. larvae post-treatment in treated plots.

RESULTS AND DISCUSSION

Mesocosm studies: At the Aquatic and Vector Control Research Facility in the Coachella Valley, the chironomid fauna inhabiting the ponds consisted mostly of *Chironomus decorus* Johannsen, *C. fulvipilus* Rempel and a few *Paralauterborniella elachista* (Townes) (all in subfamily Chironominae); the mean numbers of larvae of all species ranging between 5,769–9,644/m^2 before treatment. Vectobac ABG-6164 (TP) applied at the rates of 5.6 and 11.2 kg/ha yielded the highest level of control (98 and 100%, respectively) at 2 wk post-treatment (Table 1). The Vectobac 6 AS formulation at the rates of 11.2 and 22.4 kg/ha yielded 37% control at the lower rate 1 wk post-treatment and 57% at the higher rate 2 wk post-treatment. BSP-2 (FC) applied at the rate of 22.4 kg/ha and ABG-6184 (TP) at the rate of 11.2 kg/ha had no significant effect on larvae providing only 38 and 26% control, respectively, at 2 wk post-treatment. These results suggest that at least the 2 *Chironomus* species were greatly affected by *B.t.i.*, and the effectiveness was not apparent until 2 wk after treatment. The *B. sphaericus* formulations were considered to be ineffective against chironomid larvae even at the very high rates used.

Results with *B.t.i.* corn grit granular formulations (10/20 mesh) applied at 2 rates to mesocosms at the University of California, Riverside, facility were variable depending on the species. The corn grit formulation ABG 6253 (200 ITU/mg) having low potency and applied at 22.4 and 44.8 kg/ha produced a high level of reduction (84%) in *Chironomus* sp. larvae at the high rate of treatment at 1 wk post-treatment (Table 2). The high potency corn grit formulation ABG 6264 (400 ITU/mg) yielded 100% control of *Chironomus* larvae at the higher rate of 22.4 kg/ha. The results with the more potent corn grit formulation (ABG-6264, 400 ITU/mg) were more promising on the basis of equivalent dosages of the formulation with lower potency. As can be seen from the data the mean number of larvae of each species declined in the check plots 1 wk after start of the experiment. This decline is considered in calculating percent control by the formula.

Larvae of *Dicrotendipes* sp. (subfamily Chironominae) were also greatly reduced especially at the higher rates of ABG-6253 (77% reduction) and ABG-6264 (100%) at 1 wk post-treatment. A high level of reduction was also noted at the lower dosages of each formulation (65 and 86%, respectively) (Table 2).

Both corn grit formulations yielded little or

Table 2. Efficacy of *B.t.i.* corn grit granules against chironomid midge larvae in outdoor mesocosms at the University of California, Riverside, CA.

Formulation	Rate (kg/ha)	Mean no. larvae/m^2 Pre-treatment	1 week	% control
	Procladius sp.			
ABG 6264 (400 ITU/mg)	11.2	1,421	861	24
	22.4	732	689	0
ABG 6253 (200 ITU/mg)	22.4	689	689	0
	44.8	1,292	861	17
Check	—	861	689	—
	Paratanytarsus sp.			
ABG 6264 (400 ITU/mg)	11.2	2,153	2,885	0
	22.4	947	388	44
ABG 6253 (200 ITU/mg)	22.4	3,143	1,679	27
	44.8	3,531	4,908	0
Check	—	1,464	1,076	—
	Chironomus sp.			
ABG 6264 (400 ITU/mg)	11.2	775	43	81
	22.4	1,765	0	100
ABG 6253 (200 ITU/mg)	22.4	861	172	32
	44.8	904	43	84
Check	—	732	215	—
	Dicrotendipes sp.			
ABG 6264 (400 ITU/mg)	11.2	1,163	129	86
	22.4	517	0	100
ABG 6253 (200ITU/mg)	22.4	1,851	517	65
	44.8	947	172	77
Check	—	861	689	—

no reduction in the larvae of *Procladius* sp. (subfamily Tanypodinae) and *Paratanytarsus* sp. (subfamily Chironominae). These species seem to be refractory to *B.t.i.* On the other hand *Chironomus* sp. and the closely related *Dicrotendipes* sp. seem to be susceptible to *B.t.i.* preparations. It is thus obvious that various genera or species show different levels of susceptibility to *B.t.i.* formulations.

Lake studies: Woodward Lake was inhabited by *Chironomus decorus* of the subfamily Chironominae and *Procladius bellus* (Loew), and *Tanypus grodhausi* Sublette of the subfamily Tanypodinae. The chironomine and tanypodine larval densities varied considerably from plot to plot before treatment (2,110–8,008/m^2 and 344–689/m^2, respectively). *B.t.i.* ABG-6253 (corn grit granules) was applied at 3 rates of 13.5, 28 and 56 kg/ha. The results of this test as population trends are shown in Fig. 1 with the percent control given here. The 13.5 kg/ha rate showed only a low level of efficacy against chironomines producing 22% control at 2 wk post-treatment whereas the higher rates of 28 and 56 kg/ha produced 83% and 96% control 2 wk after treatment, respectively (Fig. 1A). Control of over 70% lasted for 4 wk at both these high rates. The tanypodine larvae on the other hand were unaffected by ABG-6253 even at the high rate of 56 kg/ha (Fig. 1B). It is apparent that not all chironomid larvae are susceptible to *B.t.i.*, and those that are susceptible require about 5–10× the established mosquito larvicidal rates on a per unit surface area basis (Mulla 1986, Mulla and Darwazeh 1985, Mulla et al. 1988). The lack of efficacy of *B.t.i.* against tanypodines was also documented in the mesocosm studies and in a lake (Mulla et al. 1991).

At Woodbridge North Lake in Irvine, three formulations of *B.t.i.* were evaluated. The chironomid midge fauna was composed of chironomines (mostly *C. decorus*) and tanypodines (mostly *Procladius sublettei* Roback and *Tanypus neopunctipennis* Sublette). The chironomines were found in greater preponderance than the tanypodines. The mean numbers of chironomine larvae in the 3 tests ranged between 2,024–3,444/m^2 and tanypodine larvae between 431–1,119/m^2 before treatment.

In the first experiment in Woodbridge North Lake, ABG 6164, a highly potent technical powder (12,430 ITU/mg), was evaluated at the rates of 1.4 and 2.8 kg/ha. The lower rate yielded good level of control of chironomines (maximum reduction of 73%) 2 wk after treatment (Fig. 2A). This moderate level of control lasted for about

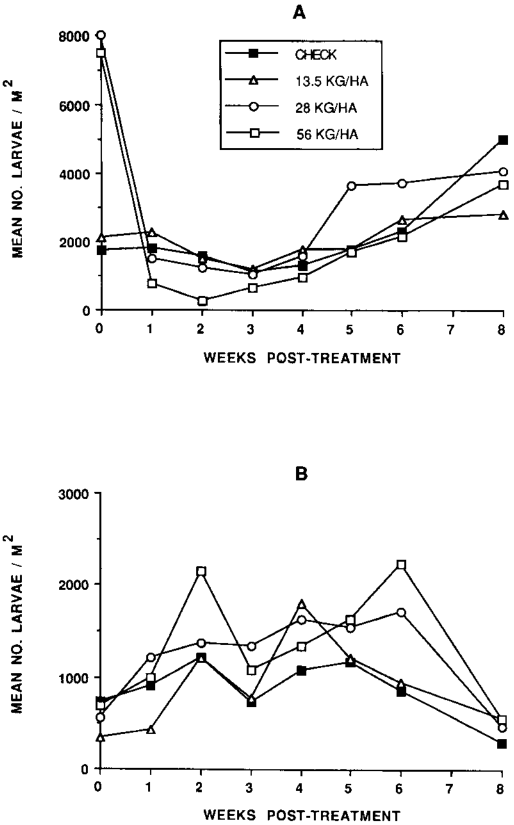

Fig. 1. Efficacy of *B.t.i.* corn grit granules (ABG-6253, 200 ITU/mg) against chironomid midges in Woodward Lake, Fresno, CA. (A) *Chironomus decorus*, (B) tanypodines consisting of mostly *Procladius bellus* and *Tanypus grodhausi* occurring in equal proportion.

4 wk. The high rate of application produced maximum control of 87% three wk after treatment, and control lasted for the duration of the experiment. No effect was noted on tanypodine larvae (only 20–29% reduction), population levels remaining stable and at similar densities in both the check and treated (Fig. 2B).

In the second experiment in Woodbridge North Lake, ABG-6253 corn grit granules (200 ITU/mg) was evaluated at the rate of 56 kg/ha, which produced 42–67% control of chironomines for 3 weeks (Fig. 3A). Thereafter, the population in the treated reached the same level as that in the check section. The formulation did not seem to affect the tanypodine larvae as the population levels remained stable and similar to the check (Fig. 3B).

In the third experiment at Woodbridge North Lake, the potent corn grit formulation ABG-6264 (400 ITU/mg) was employed. At both rates of application (11.2 and 19.1 kg/ha) this formulation yielded mediocre level of reduction (32 and 47%) of *C. decorus* for about 2 wk (Fig. 4). Three weeks after treatment, the population levels were higher than those in the untreated section. Lack of adequate efficacy of this formulation could be due to the low level of dosages applied. Further studies are needed to elucidate the efficacy of corn grit formulations.

In the last experiment of this series Vectobac technical powder (5,000 ITU/mg) was evaluated against *C. decorus* larvae which was the dominant species in Lake Calabasas with densities of 10,333–14,983 larvae/m^2 before treatment. The powder preparation was applied at rates of 2.2, 4.5 and 6.7 kg/ha (Table 3). The lower rate of 2.2 kg/ha yielded a maximum control of 66% at 2 wk after treatment, the level of control then declined. The intermediate rate (4.5 kg/ha) yielded higher level of control whereas the 6.7 kg/ha rate gave 95% control during the first week and reached 100% control at 2 and 3 wk post-treatment, then declined. Only the 2 higher rates yielded over 70% control for up to 4 wk

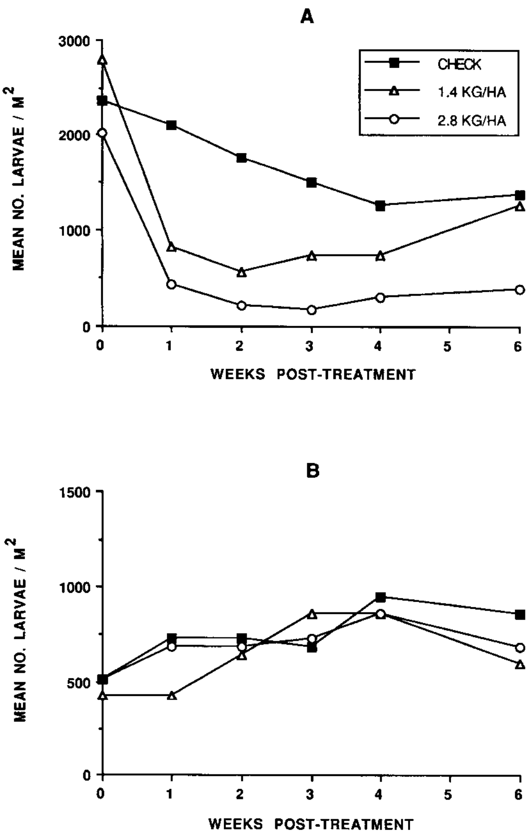

Fig. 2. Efficacy of *B.t.i.* technical powder (ABG-6164, 12,430 ITU/mg) against (A) *Chironomus decorus* and (B) tanypodine midges consisting of *Procladius sublettei* and *Tanypus neopunctipennis* in Woodbridge North Lake, Irvine, CA.

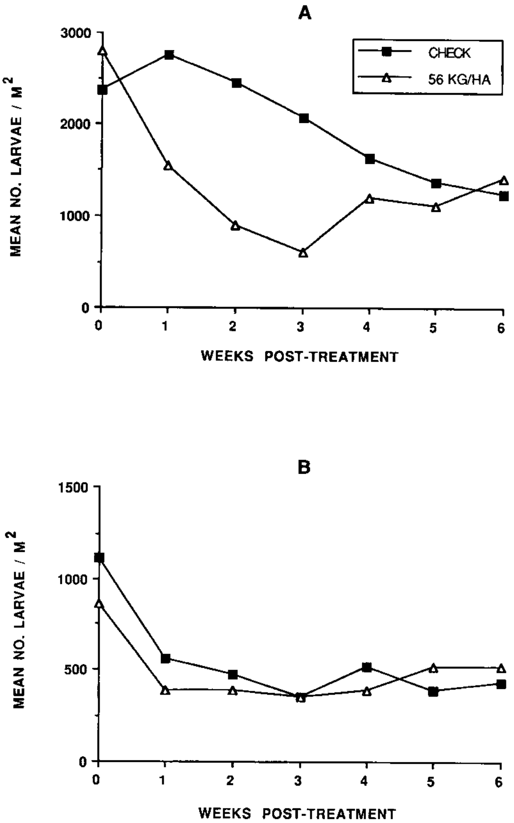

Fig. 3. Efficacy of *B.t.i.* corn grit granules (ABG-6253, 200 ITU/mg) against (A) *Chironomus decorus* and (B) tanypodine midges consisting of *Procladius sublettei* and *Tanypus neopunctipennis* in Woodbridge North Lake, Irvine, CA.

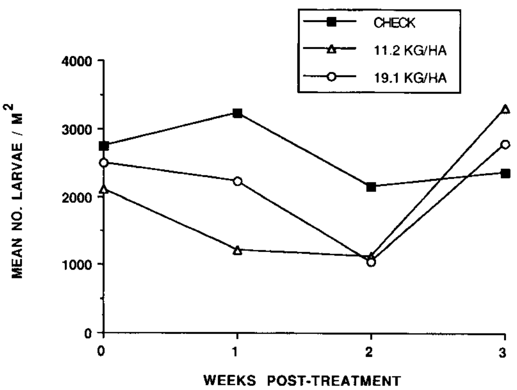

Fig. 4. Efficacy of *B.t.i.* corn grit granules (ABG-6264, 400 ITU/mg) against *Chironomus decorus* in Woodbridge North Lake, Irvine, CA.

after treatment (these rates are again about 10 times the mosquito larvicidal rates).

As a result of these studies in mesocosms and several lakes, it is evident that *B.t.i.* at 5–10 times mosquito larvicidal rates (on surface area basis) can yield practical initial and sustained control (up to 4–6 wk) of chironomine (*Chironomus* spp. and related species) midge larvae. However, the tanypodine larvae were not affected by any of the treatments. The dosage, if expressed as concentration (mg/liter) in the entire volume of the lake, will be equivalent to that for mosquito larvae (breeding in shallow water) as the water depth in lakes is generally over 1.5 m. Thus, the use of *B.t.i.* formulations (powder, liquid or granules) can provide an additional and environmentally safe option for the control of nuisance aquatic midges. But *B. sphaericus* seems to be more specific to mosquitoes; it produced no noticeable reduction in midge larvae. Dosages much greater than 5–10 times mosquito larvicidal rates will be necessary to control larvae of some chironomid midges.

Table 3. Efficacy of Vectobac TP (5,000 ITU/mg) against *Chironomus decorus* larvae in a residential-recreational lake, Lake Calabasas.

Weeks post-treatment	Percent control at rate (kg/ha) 2.2	4.5	6.7
1	34	62	95
2	66	87	100
3	62	90	100
4	57	84	96
5	41	34	28

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