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PROTOCOL FOR THE INTRODUCTION OF NEW *BACILLUS THURINGIENSIS ISRAELENSIS* PRODUCTS INTO THE ROUTINE MOSQUITO CONTROL PROGRAM IN GERMANY

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ABSTRACT. The efficacy of new and frequently used formulations of *Bacillus thuringiensis israelensis* (*B.t.i.*) has been evaluated in the laboratory and in the field conditions under which they will be used in Germany. The principles governing the successful introduction of new formulations of microbial control agents into routine programs have been highlighted. The potency of the formulations in use (Teknar®, Bactimos®, and Vectobac®) and their efficacy against the indigenous mosquito species *Aedes vexans, Aedes cantans,* and *Culex pipiens* have been assessed to determine the minimum effective dosage in the laboratory and the optimum effective dosage in small field trials. These results should ensure the proper selection of the most appropriate formulation and dosage for the particular climatic and ecological conditions.

INTRODUCTION

In many mosquito control programs, *Bacillus thuringiensis israelensis* (*B.t.i.*) has proved to be highly effective against a wide variety of mosquito species in different climatic zones of the world and to be extremely safe environmentally (Margalit and Dean 1985, Becker and Xu 1989, de Barjac and Sutherland 1990, Karch et al. 1991, Becker and Margalit 1993). For instance, since 1981 *B.t.i.* has been widely used in Germany against floodwater and snow-melt mosquitoes such as *Aedes vexans* (Meigen) and *Aedes cantans* (Meigen), as well as against *Culex pipiens* Linn. So far in the course of the program, more than 60 tons of fluid and powdered *B.t.i.* formulations have been applied successfully to more than 70,000 ha in Germany, causing a substantial reduction in the mosquito population without any harmful impact on the environment. In other countries of Europe, such as the Czech Republic, France, Hungary, Italy, Russia, Spain, and Switzerland, the introduction of the *B.t.i.* method into regular mosquito control programs is making good progress (Rettich 1986, Eröss 1988).

The efficacy of a microbial control agent such as *B.t.i.* is influenced by a great variety of biotic and abiotic factors: the susceptibility of the target mosquito species, the stage of development, the feeding behavior of mosquito larvae, the temperature and quality of water, the intensity of sunlight, the density of larval mosquito populations, and the presence of filter-feeding nontarget organisms (Lacey and Oldacre 1983, Mulla et al. 1990, Becker et al. 1992).

The characteristics of the formulations in use, such as potency, settling rate, and shelf life, also can influence the effectiveness of microbial control agents. It is important therefore to understand the impact of these factors on routine treatment, especially in regard to the calculation of the dosage, the selection of the right formulation for each particular environmental situation, and the appropriate timing for the treatment. Before using the different formulations in routine programs, it is desirable to evaluate them thoroughly in the laboratory in order to assess 1) the potency of the formations (especially of fluid formations after storage of several months), 2) their efficacy against indigenous mosquito species in the laboratory to determine the minimum effective dosage (LC_{99} values), and 3) the optimum effective dosage by means of small field tests.

The formulations of microbial control agents are being improved continuously to optimize their use in routine programs. The aim of this study was to evaluate the efficacy of new and frequently used *B.t.i.* formulations in the laboratory and in the field conditions under which they will be used in Germany. Furthermore, the principles governing the successful introduction of new formulations of microbial control agents into routine programs needed to be highlighted. The fundamental factors deciding the efficacy and cost of a campaign are the selection of the correct formulation, e.g., as fluid, powder, granules, pellets, or tablets, and the technique for its application, which must be individually tailored for each different situation.

MATERIALS AND METHODS

The following commercially available *B.t.i.* formulations were used: two Teknar® formulations (technical concentrates [TC]) provided by the Sandoz Crop Protection Corp. (Chicago, IL) designated as Teknar TC® 13,500 AAU/mg (lot:

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58120L) and Teknar TC® 35,000 AAU/mg (lot: 5500640); Bactimos PP® (lot: 0004); and Vectobac TP® (lot: 62027 PG). All 4 were used for laboratory and field experiments. The speed of settling of Teknar TC 13,500 AAU/mg (lot: 58120L) was compared with that of the aqueous suspension formulation of Teknar HP-D (lot: 5046610).

Assessment of the potency and the minimum effective dosage in the laboratory: Before the new formulations were used in the field, their actual potency and their efficacy were evaluated against *Aedes aegypti* (Linn.) and the indigenous mosquito species in the laboratory (minimum effective dosage).

All bioassays were run according to World Health Organization (WHO) guidelines (World Health Organization 1981) at 6 different concentrations with controls in 3 replicates. Fifty milligrams of *B.t.i.* products were added to 10 ml of distilled water and homogenized in a mixing machine (IKA Combimag Reo) at 700 rpm for 20 min, then mixed with a Vortex shaker for 15 min. One milliliter was taken from the homogenized solution and added to 49 ml of distilled water to form the stock solution of 100 mg/liter. Twenty-five early 4th-instar larvae of *Ae. aegypti* in 2 ml of water were added to plastic disposable cups filled with 148 ml of distilled water. Larvae were not fed during the tests. Mortality readings were taken after 24 h. The water temperature during the tests were 23 ± 1°C. The LC_{50}, LC_{90}, and LC_{99} values were calculated by using log/probit analysis (Finney 1971).

The potency of the formulations (international toxic units [ITU]) was determined by the following formula:

$$\text{ITU/mg} = \frac{15{,}000 \text{ ITU} \times LC_{50} \text{ standard (IPS-82)}}{LC_{50} \text{ sample}}$$

Different instars of the indigenous species *Ae. cantans, Ae. vexans* and *Cx. pipiens* collected in the field were used for assessment of the susceptibility of the various species and of the larval instars. For tests with field-collected larvae, the procedure for bioassays was adapted to the specific needs of the study. Water from the larval habitat was used instead of distilled water to avoid any dramatic change in the living conditions of the larvae that might affect the evaluation of the susceptibility of the species in question. Depending on the concentration required, amounts ranging from 1.5 to 300 µl of the stock solution were added to the test vessels. Each formulation was run 4 times at 5–7 concentrations. The LC_{99} values determined for field-collected larvae were defined as the minimum effective dosage and served as guideline for the assessment of the field trials.

Assessment of the optimum effective dosage in the field: Based on the results achieved in the laboratory, the optimum effective dosage for field applications was determined. The series of trials to determine the optimum effective dosage of *B.t.i.* formulations in the campaign against larvae of the snow-melt mosquito *Ae. cantans* were conducted in a boggy wood in the Upper Rhine Valley. The wood consists mainly of *Alnus glutinosa* and is a typical mass larval habitat for *Ae. cantans.* There are numerous uniform hollows in this area, each about 5 m wide and 25 m long. These hollows originated some 50 years ago in the course of sediment extraction. They have now developed into natural larval habitats for *Ae. cantans.* The hollows fill with water during early spring when the level of the water table rises following snow melt and rain. The bottom of the pools generally is covered with a dense layer of leaf litter. The larvae of *Ae. cantans* usually hatch from the egg in February or March, when the water temperature is about 5°C. After a prolonged larval development, the adults emerge mainly at the beginning of May. At the time of the investigation in March and April the hollows were flooded to a depth of about 25 cm. The hollows flooded at more or less the same time in spring, so that the larvae of *Ae. cantans* developed almost synchronously. This gave the advantage that effective dosages could be determined for each individual larval instar. The water was not polluted, and the water temperature ranged from 7 to 13°C and pH from 5.5 to 6.8 during the period of the trials.

The larval population of *Ae. cantans* was associated with isolated specimens of *Aedes rusticus* (Rossi), *Aedes communis* (De Geer), *Aedes punctor* (Kirby), *Aedes cataphylla* Dyar and *Culiseta morsitans* (Theobald). As nontarget organisms, crustaceans such as *Daphnia* sp., *Asellus aquaticus,* copepods, trichopterans such as *Limnephilus flavicornis* and *Phryganea* sp., and *Mochlonyx culicivorax* occurred in low numbers.

Treatment: According to the volume of the water bodies and the results achieved in the bioassays, preweighted amounts of the *B.t.i.* formulations were suspended each in 2 liters of filter-screened tap water and sprayed evenly onto the surface of the ponds using a hand-operated compression sprayer. Four to 6 increasing concentrations of each product were applied (0.005, 0.01, 0.02, 0.3, 0.05, and 0.1 mg/liter). All trials were carried out in triplicate, and the concentrations were chosen to produce at least 3 larval mortalities between 20 and 95% for computerized LC_{50}, LC_{90}, and LC_{99} values. Two ponds per series were left untreated as controls.

Larval densities were monitored by using a 1-liter dipper at the edges of the ponds at equal distances. At each pond before treatment and 1, 3, 5, and 7 days after treatment, 10 dips were taken and mortality rates were calculated. The larval instars were recorded separately. Only the larvae of *Ae. cantans* were taken into consideration. Larval samples were taken to the laboratory for species identification.

Aerial application: The aerial application of tailor-made *B.t.i.* sand granules was carried out at 2 localities in southwestern Germany: against 4th-instar larvae of *Ae. vexans* at Philippsburg in the flood plain of the River Rhine, an area overgrown with *Salix alba* and *Populus canadensis*, and against 3rd–4th-instar larvae of *Ae. cantans* in an alder wood at St. Leon-Rot. In each area, 1 ha of characteristic and densely vegetated habitat was chosen for granular application by helicopter.

The granules were prepared in a cement mixer by using dry quartz sand (grain size 0.6–2 mm), vegetable oil, and Teknar TC. Three mixtures were tested: a) 50 kg sand, 0.8 liter sunflower oil, and 0.9 kg Teknar TC 13,500 AAU/mg; b) 50 kg sand, 1.3 liter sunflower oil, and 1.8 kg Teknar TC 13,500 AAU/mg; c) 50 kg sand, 0.8 liter sunflower oil, and 0.7 kg Teknar TC 35,000 AAU/mg.

The granules were dispensed by a Bell 47 helicopter equipped with a "Simplex Granule Spreader" flying at a speed of 60 km/h and a height of 25 m above the ground. At each test area, 25 kg of sand granules/ha were applied, resulting in dosages of 0.45 and 0.9 kg of Teknar TC 13,500 AAU/mg and 0.35 kg of Teknar TC 35,000 AAU/mg per hectare. Before and after the aerial treatment, the larval density was recorded following the dipping method described above.

The homogenity of the granule distribution was checked by plastic bowls (surface, 0.1 m^2) that were placed in each test area along 2 lines, across the flight direction of the helicopter, separated to 50 m. Twenty-five bowls, 4 m apart, were placed along each line (total length, 100 m). After application, the quantity of granules per bowl was measured. This confirmed the more or less equal distribution of granules.

Settling rate trials: The impact of the settling rate on the efficacy of the powder and of the aqueous suspension formulations of Teknar (Teknar TC 13,500 AAU/mg vs. Teknar HP-D) was assessed by suspending each formulation in a stock concentration of 100 mg/liter. The solution was kept at a room temperature of 22°C. After 5, 25, and 125 min and 24 h of settling, appropriate amounts of this stock suspension were taken carefully with a pipette from the upper 10 mm of the stock suspension and used for preparing bioassays according to the WHO standard method mentioned above, in order to assess the remaining active material in the uppermost layer of the suspension at different time intervals.

RESULTS

Potency of the products: The bioassays with *Ae. aegypti* proved that the activity of the tested products was more or less identical with the potency given on the product labels. The average potency was 13,500 ITU/mg for Teknar TC (13,500 AAU/mg), 34,344 ITU/mg for Teknar (35,000 AAU/mg), 7,866 ITU/mg for Bactimos, and 5,766 ITU/mg for Vectobac TP.

Susceptibility of the indigenous species and instars—assessment of the minimum effective dosage (LC_{99}) in the laboratory: Comparison of the $LC_{50}/LC_{90}/LC_{99}$ values shows that 2nd-instar larvae of *Ae. cantans* are about twice as susceptible to the *B.t.i.* products on trial as 3rd-instar larvae. In turn, 3rd-instar larvae are about twice as susceptible as 4th-instar larvae (Table 1). Under the more or less standardized conditions of the bioassay, the minimum effective dosage is dependent mainly on the activity of the compound being used, the species being tested, and the larval instar. For example, with Teknar TC 13,500 AAU/mg, the minimum effective dosage is 0.023 mg/liter for 2nd-instar larvae of *Ae. cantans* and 0.038 mg/liter for 3rd-instar larvae. With Teknar TC 35,000 AAU/mg, the figure for 3rd-instar larvae of *Ae. cantans* is 0.022 mg/liter. With Bactimos and Vectobac, the minimum dosage for 3rd-instar larvae of *Ae. cantans* is some 2–4 times higher than with the 2 Teknar products (Bactimos: 0.073 mg/liter; Vectobac: 0.08 mg/liter). Although there were no essential differences in susceptibility between the larvae of *Ae. cantans* and *Ae. vexans*, *Culex* larvae were found to be 2–4 times less susceptible than *Aedes* larvae of the same instar (Tables 1 and 2).

Field evaluation of the optimum effective dosage: When 2nd-instar larvae of the snow-melt mosquito *Ae. cantans* were present in the water of the larval habitats, the optimum effective dosage for Teknar 13,500 AAU/mg was 0.03 mg/liter (Table 3) (3 days after treatment LC_{99} = 0.026 mg/liter). When the more than doubly active Teknar 35,000 AAU/mg was used against 3rd-instar larvae of the same species, the optimum effective dosage was 0.02 mg/liter. The optimum dosages for controlling early 3rd-instar larvae of *Ae. cantans* were 0.07 mg/liter for Bactimos PP (Table 3) and 0.1 mg/liter for Vectobac TP. Here too, the differences established for the optimum dosage can be linked with the different activity of the products. Mortality rates were sig-

Table 1. Susceptibility of different larval instars of the snow-melt mosquito *Aedes cantans* against Teknar TC (13,500 and 35,000 AAU/mg), Bactimos PP, and Vectobac TP (LC-values in mg/liter; 95% fiducial limits in parentheses).

Instar	LC_{50}	LC_{90}	LC_{99}
Teknar TC 13,500 AAU/mg			
2nd	0.002 (0.002–0.003)	0.008 (0.007–0.011)	0.023 (0.016–0.041)
3rd	0.005 (0.002–0.011)	0.016 (0.009–0.049)	0.038 (0.034–0.051)
4th	0.013 (0.011–0.014)	0.033 (0.023–0.041)	0.072 (0.055–0.103)
Teknar TC 35,000 AAU/mg			
2nd	0.002 (0.002–0.003)	0.005 (0.005–0.007)	0.010 (0.008–0.015)
3rd	0.003 (0.002–0.005)	0.009 (0.006–0.021)	0.022 (0.012–0.062)
4th	0.008 (0.007–0.008)	0.022 (0.019–0.026)	0.051 (0.041–0.069)
Bactimos PP			
3rd	0.010 (0.008–0.020)	0.032 (0.023–0.039)	0.073 (0.053–0.134)
4th	0.015 (0.012–0.017)	0.054 (0.044–0.071)	0.154 (0.108–0.268)
Vectobac TP			
3rd	0.015 (0.013–0.019)	0.039 (0.032–0.051)	0.080 (0.048–0.223)
4th	0.028 (0.020–0.036)	0.072 (0.052–0.132)	0.158 (0.096–0.445)

nificantly lower after one operational day than they were after 3 or 5 days.

Aerial application: Teknar TC-1989 sand granules applied by helicopter in dosages of 0.45 and 0.9 kg *B.t.i.*/ha against 3rd–4th-instar larvae of *Ae. cantans* caused 99.7–100% mortality after 7 days (Table 4). Teknar TC 35,000 AAU/mg applied by the same method at a rate of 0.35 kg of *B.t.i.* against 4th-instar larvae of *Ae. vexans* caused 99.5–100% mortality after 3 days.

Impact of the settling: When the powder formulation were used, less than 50% of the delta-endotoxin remained in the top 1 cm of the water layer after 25 min, after 2 h less than 20% remained, and after 24 h, less than 2% (Table 5). Twenty-four h after application of the suspen-

Table 2. Susceptibility of 4th-instar larvae of *Aedes vexans* and *Culex pipiens* against Teknar TC 13,500 and 35,000 AAU/mg (LC-values in mg/liter; 95% fiducial limits in parentheses).

Species	LC_{50}	LC_{90}	LC_{99}
Teknar TC 13,500 AAU/mg			
Ae. vexans	0.013 (0.012–0.014)	0.035 (0.030–0.045)	0.080 (0.060–0.120)
Cx. pipiens	0.023 (0.015–0.032)	0.110 (0.068–0.371)	0.387 (0.165–0.871)
Teknar TC 35,000 AAU/mg			
Ae. vexans	0.005 (0.003–0.007)	0.014 (0.011–0.023)	0.031 (0.020–0.086)
Cx. pipiens	0.011 (0.009–0.011)	0.041 (0.035–0.048)	0.122 (0.094–0.171)

Table 3. Field evaluation of Teknar TC (13,500) against 2-instar larvae of *Aedes cantans* and of Teknar TC 35,000 AAU/mg, Bactimos, and Vectobac against 3rd- (and early 4th-) instar larvae of *Ae. cantans* (LC-values in mg/liter; 95% fiducial limits in parentheses).

Product	Days post-treatment	LC_{50}	LC_{90}	LC_{99}
Teknar TC 13,500	1	0.008 (0.006–0.010)	0.025 (0.016–0.044)	0.066 (0.040–0.169)
	3	0.003 (0.002–0.004)	0.010 (0.009–0.013)	0.026 (0.019–0.050)
	7	0.002 (0.001–0.003)	0.008 (0.006–0.010)	0.025 (0.017–0.053)
Teknar TC 35,000	1	0.008 (0.007–0.009)	0.028 (0.023–0.037)	0.076 (0.054–0.127)
	3	0.005 (0.004–0.006)	0.011 (0.009–0.013)	0.021 (0.016–0.032)
	7	0.004 (0.003–0.005)	0.009 (0.008–0.012)	0.019 (0.014–0.032)
Bactimos PP	1	0.025 (0.021–0.028)	0.059 (0.050–0.072)	0.119 (0.092–0.175)
	5	0.018 (0.015–0.021)	0.037 (0.033–0.047)	0.068 (0.053–0.104)
	7	0.018 (0.016–0.021)	0.036 (0.032–0.045)	0.062 (0.049–0.094)
Vectobac TP	1	0.030 (0.021–0.039)	0.076 (0.052–0.134)	0.160 (0.100–0.455)
	5	0.020 (0.016–0.023)	0.048 (0.041–0.060)	0.099 (0.076–0.154)
	7	0.014 (0.010–0.018)	0.039 (0.033–0.050)	0.088 (0.065–0.155)

sion, the LC_{50} value rose to 0.75 mg/liter, compared with 0.01 mg/liter at the beginning of the experiment. On the other hand, when the fluid concentrate (Teknar HP-D) was applied, more than 80% of the toxin still remained in the upper layer of the water after 25 min and more than 60% after 2 h. But even with the fluid solution, more than 90% of the toxin had settled into the deeper water layers after 24 h.

DISCUSSION

The successful use of microbial control agents is based upon thorough preparations for the campaign. The prerequisites are 1) A precise knowledge must be obtained of the larval habitats, which must be carefully mapped, characterized, and also numbered so they can be identified rapidly during routine operations. 2) A precise assessment must be made of the entomological data, such as the composition of and fluctuations in the larval and adult mosquito populations. Adequate information must be obtained on the climatic factors that influence mosquito densities, such as the occurrence of rainy seasons. 3) The potency and efficacy of the control agent have to be assessed in the laboratory and at various larval habitats, the most appropriate formulation has to be selected, and the sequence of follow-up treatments has to be adapted to the local situation. 4) The spray equipment has to be adapted to the specific characteristics of the product. 5) A proper design of the control strategy must be made, based on the results obtained in the preparatory phase.

This study showed that *B.t.i.* formulations have continually been improved. Because of the greater potency of the powder formulations, 20–100 g of the products tested per hectare of water surface are sufficient to kill the larvae of floodwater mosquitoes. This calculation is based on a water body 10 cm in depth. This is the zone in which the larvae of the floodwater mosquitoes tested here generally are found.

When no significant natural mortality can be expected (e.g., due to the absence of numerous

Table 4. Efficacy of Teknar TC sand granules applied by helicopter against *Aedes vexans* and *Aedes cantans* larvae in the Upper Rhine Valley.

Product	Dosage (kg/ha)	Species (instars)	No. larvae/10 dips[1] Pre-treatment	Post-treatment 2 days	Post-treatment 3 days
Teknar TC 35,500 AAU/mg	0.35	*Ae. vexans* (L4)[2]	823	9 (98.9)[3]	4 (99.5)
Teknar TC 35,000 AAU/mg	0.35	*Ae. vexans* (L4)	75	5 (93.3)	0 (100)
Teknar TC 13,500 AAU/mg	0.45	*Ae. cantans* (L3)	805	2 (99.8)	3 (99.6)
Teknar TC 13,500 AAU/mg	0.90	*Ae. cantans* (L4)	711	28 (96.1)	3 (99.6)
Teknar TC 13,500 AAU/mg	0.90	*Ae. cantans* (L4)	153	0 (100)	0 (100)

[1] Volume of one dip = 1 liter.
[2] Figures in parentheses are percentages of reduction.
[3] L3–L4 = larval instars.

predators or no fast decline of the water level), the costs can be reduced by operating against the early instars. Only half the dosage required to kill 3rd-instar larvae is needed for 2nd-instar larvae. If 4th-instar larvae are present, then the dosage must be doubled again.

Table 5. Comparison of the impact of settling on the larvicidal activity of a liquid and a powder (Teknar) formulation (LC-values in mg/liter).

Time of settling	LC_{50}	LC_{90}	LC_{99}
Teknar TC powder (13,500 AAU/mg)			
0 min	0.010 (100)[1]	0.022 (100)	0.042 (100)
5 min	0.022 (45)	0.046 (48)	0.083 (50)
25 min	0.027 (36)	0.053 (42)	0.090 (46)
125 min	0.079 (12)	0.155 (14)	0.268 (15)
24 h	0.075 (<2)	1.5 (<2)	2.3 (<2)
Teknar HP-D FC (3,000 AAU/mg)			
0 min	0.155 (100)	0.313 (100)	0.556 (100)
5 min	0.166 (94)	0.342 (92)	0.617 (91)
25 min	0.183 (85)	0.382 (82)	0.694 (80)
125 min	0.258 (60)	0.441 (71)	0.683 (81)
24 h	1.7 (9)	6.2 (5)	14.0 (4)

[1] Figures in parentheses are percentages of remaining active ingredient.

The precepts laid down here for the trials of new formulations have proved themselves in practice. If we compare the LC_{99} values (minimum effective dosage) obtained in the laboratory with the values obtained in field experiments for the optimum dosage, it is clear that in the campaign against floodwater mosquitoes the minimum dosage is about the same as the optimum dosage. Following the definitions used here, the minimum dosage in the bioassay was established after 24 h. However, the optimum dosage in the field can be determined only after an adequate operating period (about 3 days). If the LC_{99} values are studied in the field after 24 h, then the values in the field are 2–3 times higher (Tables 1 and 3). This difference in efficacy between laboratory and field is balanced by establishing the optimum dosage through the longer operating period (time factor, persistence). A comparison of the LC values also furnishes valuable lessons for the practical application. In general the LC_{90} value is 2–3 times greater than the LC_{50} value (see Tables 1 and 3). A similar difference is found when the LC_{90} and LC_{99} values are compared. The LC_{99} values are 5–12 times greater than the LC_{50} values. To obtain the optimum dosage for a campaign against the floodwater mosquitoes of the Upper Rhine region, we need to begin with a dosage that is some 2–3 times the LC_{90} value and some 10 times the LC_{50} value. In the light of the values obtained during trials for determining the optimum dosage in the field, we recommend choosing test concentrations that are 2, 4, and even 8 times the value of the LC_{90} value obtained in the laboratory for any particular product.

The physical behavior of formulations can influence even the choice of the best formulation for each particular situation. For example, if surface-feeding *Anopheles* larvae are to be controlled, then it is best to use a fluid formulation because the toxins in these concentrates appear to remain longer in the upper layers of the water body (Table 5). This is probably because there is a smaller detergent content in the fluid concentrates compared with the powder formulations, which need this higher detergent content to facilitate a rapid solution in water during the preparation of the suspension. When water temperatures are low, which as a rule reduces the feeding/filter rate of the larvae, we recommend using a fluid concentrate so the toxin will remain in the water for a sufficiently long period for the larvae to take in a lethal amount of the toxin. In general, and in spite of these disadvantages, the powder formulations are more effective than the fluid concentrates because of their greater activity.

When *B.t.i.* sand granules are used, a quantity of *B.t.i.* powder about 10 times that employed in ground applications must be used to achieve a comparable effect (Table 4). This probably is due to losses of the active agent during application. Powder is lost during its dispersal with compressed air because of abrasion of the sand grains, despite the binding agent (vegetable oil), and it is also lost in the vegetation above the water surface.

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REFERENCES CITED

Becker, N. and J. Margalit. 1993. Control of dipteran pests by *Bacillus thuringiensis*, pp. 147–170. *In:* P. Entwistle, M. J. Bailey, J. Cory and S. Higgs (eds.). *Bacillus thuringiensis*: its uses and future as a biological insecticide. John Wiley & Sons, Ltd., Sussex, England.

Becker, N. and B. Xu. 1989. Microbial control of mosquitoes in Germany and the Hubei Province of the People's Republic of China. Israel J. Entomol. 23:39–44.

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.

de Barjac, H. and D. J. Sutherland (editors). 1990. Bacterial control of mosquitoes and black flies. Rutgers Univ. Press, London.

Eröss, J. 1988. Mosquito control by larvicides Abate, Viodat, Teknar. Parasitol. Hungarica 21:99–103. [In Hungarian.]

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

Karch, S., Z. A. Manzambi and J. J. Salaun. 1991. Field trials with Vectolex (*Bacillus sphaericus*) and Vectobac (*Bacillus thuringiensis* H-14) against *Anopheles gambiae* and *Culex quinquefasciatus* breeding in Zaire. J. Am. Mosq. Control Assoc. 7:176–179.

Lacey, L. and S. Oldacre. 1983. The effect of temperature, larval ages and species of mosquito on the activity of an isolate of *Bacillus thuringiensis* var. *darmstadiensis* toxic for mosquito larvae. Mosq. News 43:176–180.

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

Mulla, M. S., H. A. Darwazeh and M. Zgomba. 1990. Effect of some environmental factors on the efficacy of *Bacillus sphaericus* 2362 and *Bacillus thuringiensis* (H-14) against mosquitoes. Bull. Soc. Vector Ecol. 15:166–175.

Rettich, F. 1986. Our experience with the use of *Bacillus thuringiensis* H-14 in mosquito control in Czechoslovakia. Dipterologica Bohemoslovaca 4:129–133.

World Health Organization. 1981. Report of informal consultation on standardization of *Bacillus thuringiensis* H-14. TDR/BCV/BTH-14/811, WHO/VBC/81-828.