

## A SEMIFIELD EVALUATION OF VECTOBAC® DT (ABG-6499), A NEW FORMULATION OF *BACILLUS THURINGIENSIS ISRAELENSIS* FOR CONTROL OF *Aedes albopictus*

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**ABSTRACT.** We evaluated the effectiveness and duration of effectiveness of a new formulation of *Bacillus thuringiensis* var. *israelensis* (*Bti*) for control of larval *Aedes albopictus*. The product tested was Vectobac® DT (ABG-6499), a *Bti* tablet formulation containing 3.4% of active ingredient (3,400 ITU/mg) supplied by SCAE Valent BioSciences Italy S.r.l. The study was conducted at the Botanical Garden of the University of Rome "La Sapienza" between June and September 2002, the most favorable season for the development of *Ae. albopictus* in Italy. Black 20-liter plastic buckets containing water and an organic substrate were used as experimental breeding sites. The number of larvae in these buckets was estimated weekly, and positive buckets were treated with the recommended dose of the larvicide. The results showed that Vectobac DT induced 100% larval mortality after 24 h in all experimental breeding sites during the entire study period. Nonetheless, in most cases, the larvicidal activity only lasted about 48 h; thus, effective mosquito control would require that treatment be performed every 8–10 days in this habitat.

**KEY WORDS** *Aedes albopictus*, *Bacillus thuringiensis israelensis*, mosquito control, insecticide formulations, Italy

### INTRODUCTION

*Bacillus thuringiensis israelensis* (*Bti*) has been shown to be a very effective microbial larvicide in the control of mosquitoes and blackflies, and it is not harmful to nontarget species. Nonetheless, the larvicidal activity of most of the commercially available *Bti* formulations is short-lasting, except in habitats lacking soil or organic substrate; thus, these formulations need to be used frequently, resulting in high costs of management (Regis et al. 2001). Although some formulations have been reported to have a slow release of the active ingredient (Nasci et al. 1994, Su and Mulla 1999, Prabakaran et al. 2001, Maldonado Blanco et al. 2002), a truly long-lasting formulation still needs to be developed (Regis et al. 2001).

The objective of the present study was to evaluate the effectiveness and the residual effect of a new *Bti* formulation, Vectobac® DT, in the control of the larvae of *Aedes albopictus* (Skuse), a mosquito that is native to Southeast Asia and is a daytime, outdoor mosquito that preferentially bites humans (Hawley 1988). The study was conducted in 2002 in Rome, Italy, where *Ae. albopictus* has been present since the late summer of 1997 (Romi et al. 1999).

### MATERIALS AND METHODS

**Study area:** Rome is the 1st large city in Italy to have experienced extensive infestation of *Ae. albopictus* (Romi et al. 1999). In Rome, larvae are found from March through November. Although a few adult females have been found to be active in December, the peak of adult abundance occurs in late August and early September (Di Luca et al. 2001). The specific area selected for this study was

the Botanical Garden of the University of Rome "La Sapienza," which is located in a densely populated central area of the city. This garden is approximately 1 ha and is almost entirely shaded by trees. Numerous containers are present on the grounds for the experimental cultivation of aquatic plants. These containers serve as developmental sites for larval *Ae. albopictus*, making this garden one of the most heavily infested areas in the city.

**Monitoring:** To monitor the abundance of *Ae. albopictus* and to determine the point at which this abundance was sufficient for evaluating the effectiveness of Vectobac DT, we placed 6 ovitraps in shaded areas of the garden at a distance of at least 50 m from each other. The ovitraps consisted of 500-ml black plastic pots filled with water and containing a 15 × 3-cm Masonite® stick as a wet surface for laying eggs. To estimate the number of eggs of *Ae. albopictus* the Masonite sticks were brought weekly to the Laboratory of Parasitology of the Istituto Superiore di Sanità (the National Health Institute of Italy) and analyzed with a stereomicroscope. The Masonite sticks removed from the ovitraps were immediately replaced with new sticks, and the pots were refilled to replace the evaporated water. The ovitraps were placed in the garden from May 9 (7 wk before beginning treatment with Vectobac DT) to October 11 (5 wk after treatment was discontinued).

**Experimental breeding containers:** Once the abundance of *Ae. albopictus* was sufficient for evaluating the effectiveness of Vectobac DT (a mean of at least 50 eggs/ovitraps), we created experimental breeding sites in the garden. These sites consisted of 8 black plastic buckets (30 cm in diameter and 34 cm in height). The buckets were filled with approximately 20 liters of tap water, and a Masonite

stick (45 × 5 cm) was placed in each bucket as a surface for egg laying. Each bucket was placed next to an ovitrap (designated as buckets 1–6); an additional 2 buckets (buckets 7 and 8) were not treated and were used as controls. To estimate the number of larval and pupal *Ae. albopictus*, each bucket was emptied weekly into a plastic container without removing the organic material (e.g., leaves and soil) settled at the bottom, so as to better simulate a natural situation. The number of larvae in these containers was estimated by eye and recorded as a range, by using the following categories: 0, no larvae; A, 1–50; B, 50–200; C, 200–500; and D, >500; the preimaginal stages also were recorded. The water and the Masonite sticks were then placed back in the buckets. To determine whether or not our estimates of the number of larvae and pupae were accurate, each week we counted all of the larvae in 1 treated and 1 nontreated bucket (buckets 1 and 7) by filtering the water through a sieve. After counting, the larvae in bucket 1 were placed back in the bucket, whereas, to avoid additional infestation, the larvae from both bucket 7 and the other untreated bucket (bucket 8) were discarded each week. When positive for larvae, buckets 1–6 were treated with Vectobac DT, as described below. Mortality was recorded 24 h after treatment. After the mortality check, the Masonite sticks from both the treated and nontreated buckets were turned over and put back in the buckets to allow the eggs on the sticks to hatch. This procedure was repeated weekly, beginning on June 27 (when egg abundance was sufficient for evaluating treatment effectiveness); the treatment period lasted for 11 wk, ending on September 5, when the University required that the garden be disinfested with pyrethroids, which drastically reduced the mosquito population. The treatment period corresponded to the most suitable season for reproduction of *Ae. albopictus* in Rome. In only 1 case (bucket 1, week 1) did the 24-h mortality check reveal the presence of live larvae; in this case, the bucket was immediately retreated and another mortality check was performed 24 h later.

**Larvicidal treatment:** Vectobac DT is a *Bti* formulation (ABG-6499) in the form of a tablet containing 3.4% of active ingredient (3,400 ITU/mg) supplied by SCAE Valent BioSciences Italy S.r.l. The dosage recommended by the manufacturer is 1 tablet (0.384 g) per 50 liters of water. Following this recommendation, we placed one half of a tablet (0.192 g) in the buckets that were positive for larvae. To ensure that breaking the tablet in half had no effect on the duration of effectiveness, starting on August 22 (week 9 of the treatment period) an entire tablet was used for the treatment of buckets 1–3.

**Statistical analysis:** The analysis of variance, with grouping factor treatment and repeated measures, was used to evaluate the pre- and posttreatment difference in larval abundance between treat-

ed and nontreated buckets. Multiple comparisons were conducted by using Tukey's test. The effectiveness of the 2 different dosages of Vectobac DT tablets was compared by the Mann-Whitney test. The adopted level of significance was  $\alpha = 0.05$ . All statistical analyses were performed by BMDP (1998) statistical software (Saugus, MA) (Dixon 1988).

All data from monitoring and from the bioassay were entered and managed by Microsoft® Excel software (Redmond, WA). Meteorological data were provided by a meteorological station located in the center of Rome (Mangianti and Perini 2002).

## RESULTS

### Monitoring

The mean number of eggs found in the ovitraps by week and the weekly temperature and rainfall during the monitoring period are shown in Fig. 1. The 6 ovitraps were positive for eggs beginning in the 1st week of monitoring, and the garden remained infested with *Ae. albopictus* during the entire monitoring period. Seven weeks into monitoring, the mean number of eggs reached 50 eggs/ovitrap, which was considered to be sufficient for evaluating the effectiveness of treatment. The weekly abundance of the species, evaluated as the mean number of eggs of the 6 ovitraps, increased almost constantly up to mid-July, reaching a peak of more than 100 eggs/ovitrap (Fig. 1); it then followed an erratic trend until being reduced to zero after the insecticide treatment in early September. As expected, and as previously observed in Rome (Toma et al. 2003), peaks in egg abundance occurred approximately 1 wk after peaks in rainfall (Fig. 1). During the monitoring period, a total of 398.2 mm of rain was recorded, the mean daily temperature ranged from 18 to 28.9°C, the photoperiod ranged from 15.6 to 12 h daylight, and the relative humidity ranged from 49.7 to 77.5% (with a mean of approximately 50%).

### Mosquito species

In addition to *Ae. albopictus*, the larvae of 2 other species of mosquitoes (*Culiseta longiareolata* (Macquart) and *Culex pipiens* (L.)) were found in all of the experimental buckets and, although rarely, also in the ovitraps. At all weekly observations, *Ae. albopictus* represented more than 80% of mosquitoes in the buckets, and *Cs. longiareolata* and *Cx. pipiens* represented less than 15% and 5% of mosquitoes, respectively. Larvae of both *Cs. longiareolata* and *Cx. pipiens* were susceptible to treatment (data not shown).

### Effectiveness of treatment

The results of the evaluation of the effectiveness are shown in Table 1. At 24 h after treatment, larval

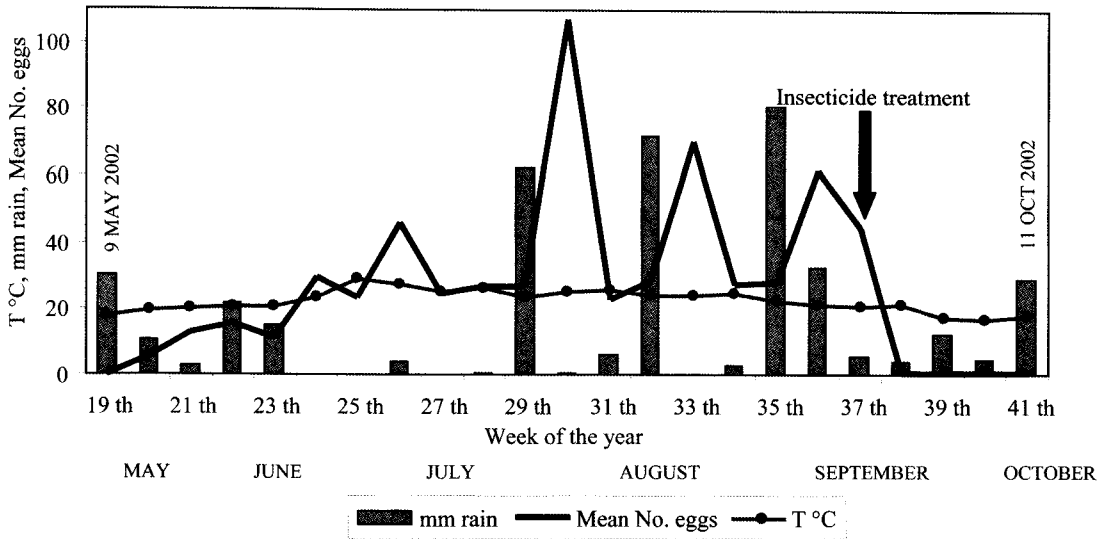


Fig. 1. Monitoring of abundance of *Aedes albopictus* in the study area, Rome, May–October 2002. The black line represents the mean number of eggs by ovitrap, the gray line the mean daily temperature (°C), and the bars the amount of rainfall (mm).

mortality was 100% for all of the treated buckets, with the exception of bucket 1 at week 1. However, at the successive weekly pretreatment observations, in approximately 80% of the cases, the treated buckets were again positive for larval *Ae. albopictus*, with abundance reaching 200–500 (category C) in some buckets. In the remaining 20% of cases, the treated buckets remained negative in the successive 1 or 2 wk; this was observed at least once for all of the buckets except bucket 5.

The difference in larval abundance between the treated buckets (buckets 1–6) and those not treated (buckets 7 and 8) was statistically significant ( $F = 6.14$ ,  $df$  1,20,  $P = 0.00222$ ). With regard to the accuracy of our estimated ranges of larval abundance, the larval counts performed on buckets 1 and 7 were consistently within the ranges estimated by eye. Figure 2 shows the weekly trend in larval abundance in buckets 1 and 7, which were considered as representative of all of the buckets. As shown in Table 1, the number of larval *Ae. albopictus* in buckets 1 and 7 ranged from a minimum of 37 (bucket 1, week 1) to a maximum of 793 (bucket 7, week 4).

Most of the larvae in the treated buckets were 2nd and 3rd instars; some 1st and early 4th instars also were recorded, whereas no late 4th instars or pupae were observed. In the untreated control buckets, which were consistently positive throughout the monitoring period, late preimaginal stages were prevalent.

When comparing the buckets treated with the recommended dose, which in this case was one half of a tablet, to those treated with a double dose (a whole tablet), no significant difference in larval

abundance or instar in the week after treatment was observed.

During the bioassay period, 262.2 mm of rain was recorded, the mean daily temperature ranged from 27.4 to 21.6°C, the photoperiod ranged from 15.6 to 13.5 h daylight, and the relative humidity ranged from 49.7 to 77.5% (with a mean of approximately 50%). The water temperature in the buckets (recorded weekly at 12:00 p.m.) ranged from 22.5 to 26.3°C.

### DISCUSSION

The results of this study showed that Vectobac DT induced 100% larval mortality when used to control *Ae. albopictus* in experimental containers, confirming the previously reported effectiveness and rapidity of *Bti*-based products against this species (Nasci et al. 1994, Ali et al. 1995, Sulaiman et al. 1997). However, in most cases, the treated buckets were again positive for larval *Ae. albopictus* at the successive weekly observations. This finding, together with the observation that the early development stages were prevalent in the treated buckets and the late stages were prevalent in the untreated buckets, and considering that temperatures consistently above 25°C allow eggs to develop into pupae in about 6–8 days (Hawley 1988), suggests that the larvicidal activity only lasted for approximately 48 h in most cases.

Although many data are available in the literature on the effectiveness and duration of different *Bti*-based formulations in the control of *Aedes* spp., relatively few laboratory and field studies have assessed formulations with a presumably slow release

Table 1. Efficacy of Vectobac® DT on larval *Aedes albopictus* in a semifield assay. Experimental breeding containers (buckets 1–6) were treated weekly, and larval mortality was evaluated 24 h after treatment. Containers 7 and 8 were untreated controls. Larval abundance is reported as a range (0, no larvae or pupae; A, 1–50; B, 50–200; C, 200–500; D, >500), with the exceptions of buckets 1 and 7, for which the larvae were counted individually. Stage of larvae is also reported (I, 1st; II, 2nd; III, 3rd; IV, 4th; P, pupae).

Week <sup>1</sup>	Bucket							
	1	2	3	4	5	6	7	8
1 + 24 h	698 (II, III)	D (II, III)	D (II, III)	D (II, III)	D (II, III)	D (II–III)	676 (II, III)	D (II, III)
2 + 24 h	37 (II, III)	0	0	0	0	0	694 (III, IV)	D (II–IV)
3 + 24 h	0	A (II, III)	B (I–III)	A (II, III)	A (II, III)	0	711 (II, III)	D (III, IV)
4 + 24 h	615 (I–III)	0	0	0	0	0	720 (III, IV)	D (III, IV, P)
5 + 24 h	172 (II, III)	0	0	0	0	0	701 (II–IV)	D (II–IV)
6 + 24 h	0	B (II, III)	0	0	0	0	731 (III, IV, P)	D (III, IV)
7 + 24 h	0	0	0	0	0	B (II–III)	702 (II, III)	D (II–IV)
8 + 24 h	0	A (II, III)	B (II–IV)	A (I, II)	C (II, III)	0	793 (III, IV)	D (III, IV, P)
9 + 24 h	43 (II, III)	0	A (II, III)	A (I, II)	0	A (I–II)	475 (III, IV)	C (III, IV)
10 + 24 h	0	A (II, III)	0	0	0	0	532 (III, IV)	C (III, IV)
11 + 24 h	96 <sup>2</sup> (I, II)	C <sup>2</sup> (I, II)	0	0	0	0	427 (II–IV)	C (III, IV, P)
12 + 24 h	0	0	0	0	0	0	398 (III, IV, P)	C (IV, P)
13 + 24 h	0	A (III, IV)	A (II, III)	A (II–IV)	A (II, III)	A (I–III)	403 (II–IV)	C (III, IV)
14 + 24 h	0	0	0	0	0	0	469 (III, IV)	C (IV, P)
15 + 24 h	44 (II, III)	A (II)	A (I, II)	0	B (I, II)	B (II)	396 (II–IV)	C (III, IV)
16 + 24 h	0	0	0	0	0	0	415 (III, IV, P)	C (III, IV, P)
17 + 24 h	33 <sup>2</sup> (I–III)	B <sup>2</sup> (I, II)	B <sup>2</sup> (I–III)	C (II, III)	C (II, III)	C (I–II)	176 (III, IV)	C (III, IV)
18 + 24 h	0	0	0	0	0	0	196 (III, IV, P)	C (III, IV, P)
19 + 24 h	0	B <sup>2</sup> (I)	0	A (II)	A (II)	0	179 (III, IV)	B (II, III)
20 + 24 h	0	0	0	0	0	0	193 (III, IV, P)	C (III, IV)
21 + 24 h	0	0	0	0	0	0	151 (II, III)	C (II, III)
22 + 24 h	0	0	0	0	0	A (II–III)	178 (III, IV)	C (III, IV)

<sup>1</sup> From June 27 to September 5.

<sup>2</sup> Treated with a double dose (a whole tablet).

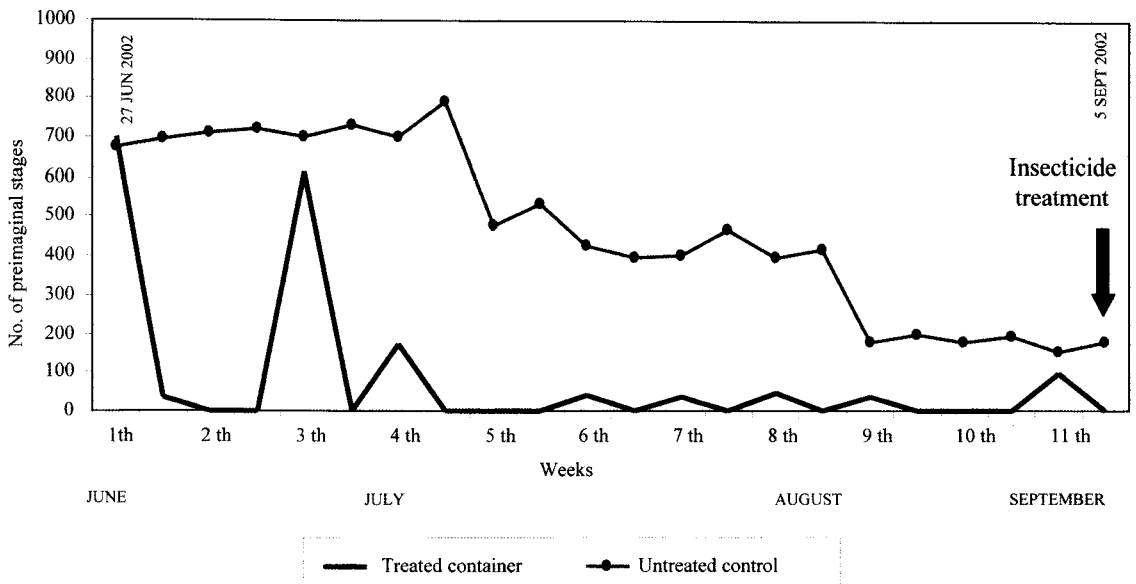


Fig. 2. Number of larval and pupal *Aedes albopictus* in 2 experimental containers during an 11-wk field assay with Vectobac® DT, carried out in Rome between June and September 2002

of their active ingredient (e.g., tablets, briquets, or granules). In most of the studies adopting these types of formulations, the duration of larvicidal activity in the control of *Ae. aegypti* was reported to be longer than the duration of activity observed in our study (Novak et al. 1985, Ali et al. 1994, Batra et al. 2000, de Melo-Santos et al. 2001). In particular, de Melo-Santos et al. (2001) reported that, under simulated field conditions, a *Bti* tablet formulation (C4P1) showed long-term larvicidal activity (>70% mortality) against larval *Ae. aegypti* in experimental containers exposed to sunlight (larvicidal activity lasting 13–35 days) and shade (larvicidal activity lasting 40–54 days). Considering that in some cases Vectobac DT provided a residual control for 2 wk in our experimental containers (Table 1), results more similar to ours were obtained by Batra et al. (2000), who reported that Vectobac tablets provided residual control of larval *Ae. aegypti* for 2 and 3 wk, respectively, in desert coolers and scrap tires.

Nevertheless, it should be considered that the duration of the residual effect of slow-release *Bti* formulations in containers is strongly influenced by the presence of an organic substrate (Ali et al. 1994, Sulaiman et al. 1997) and by the absence of other biotic and abiotic factors that may occur in natural habitats (Becker et al. 1992, Nayar et al. 1999). In our study, the organic material that had settled at the bottom of the containers probably accounted for the brief residual effect observed. The tablets, whether half or whole, probably sank very quickly, and the active ingredient settled among the decaying organic material and out of the feeding area of the larvae. However, this would not explain

why in approximately 20% of the cases the larvicidal activity seems to have lasted for about 1–2 wk, although the amount of organic substrate was about the same in all of the containers.

In light of these observations, and considering the absence of late 4th-stage larvae and pupae in the treated buckets, we may conclude that, under these experimental conditions, Vectobac DT is highly effective against larval *Ae. albopictus*. However, to obtain permanent mosquito control in these habitats, treatment would need to be performed at least every 8–10 days.

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