Comparison Between Diflubenzuron and a *Bacillus thuringiensis israelensis*– and *Lysinibacillus sphaericus*–Based Formulation for the Control of Mosquito Larvae in Urban Catch Basins in Switzerland

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COMPARISON BETWEEN DIFLUBENZURON AND A *BACILLUS THURINGIENSIS ISRAELENSIS*– AND *LYSINIBACILLUS SPHAERICUS*–BASED FORMULATION FOR THE CONTROL OF MOSQUITO LARVAE IN URBAN CATCH BASINS IN SWITZERLAND

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ABSTRACT. A field test was conducted to evaluate a commercial biolarvicide based on *Bacillus thuringiensis* var. *israelensis* and *Lysinibacillus sphaericus* to control mosquitoes breeding in catch basins in southern Switzerland. The efficacy and residual activity of the microbial mosquito larvicide applied at the recommended rate of 10 g per catch basin was compared to the currently used larvicide diflubenzuron. Both products provided a very good control activity (>97% of reduction) of late instars (3rd and 4th instars) and pupae for 4 wk. However, only the microbial formulation controlled immature stages during the whole period of the trial, with >98% of larval reduction. A single application of the microbial larvicide applied at 10 g per catch basin significantly reduced the number of immature mosquitoes for at least 70 days. The quantity of rainfall in the 48-h period before each sampling and the water temperature did not influence the efficacy of the treatments. Under the environmental conditions encountered in southern Switzerland, the larvicide tested may be a valid alternative to diflubenzuron to control mosquitoes in urban catch basins. The long-lasting control by the microbial larvicide further reduces the number of treatments required to keep the population of mosquitoes at low levels.

KEY WORDS *Bacillus thuringiensis* var. *israelensis*, *Lysinibacillus sphaericus*, residual activity, catch basins, mosquito larvae

INTRODUCTION

Mosquitoes are a major cause of public health concerns. They are a nuisance and they may play an important role as potential vectors of human diseases, such as malaria, filariasis, and arboviral diseases (dengue fever, chikungunya, West Nile fever, and yellow fever) (WHO 2004). In urban areas of southern Switzerland, the most widespread mosquito species is *Culex pipiens* L. (Flacio, personal communication). In 2003, however, the invasive species *Aedes albopictus* (Skuse), the Asian tiger mosquito, was recorded for the 1st time in southern Switzerland (Flacio et al. 2004), and since 2007 it has established itself, threatening northern regions of Europe (Flacio et al. 2004, Wymann et al. 2008). Both species are competent vectors for arboviruses (WHO 2004, Randolph and Rogers 2010, Medlock et al. 2012) that have recently colonized Europe (Angelini et al. 2007, Barzon et al. 2010, La Ruche et al. 2010, Danis et al. 2011, Gjenero-Margan et al. 2011). *Aedes albopictus* has a diurnal biting behavior and may cause great disturbance to the local population (Flacio et al. 2004). To avoid nuisance and to prevent introduction and spreading of mosquito-borne diseases in Switzerland, great efforts are made to maintain the population of mosquitoes below a critical level. *Aedes albopictus* is currently controlled using essentially chemical products, such as pyrethroids (permethrin and cypermethrin) against adults and the insect growth regulator (IGR) diflubenzuron (DFB) to fight immature aquatic stages. To a lesser extent, a biological formulation based on *Bacillus thuringiensis* var. *israelensis* de Barjac (*Bti* strain AM65-52) is used against larval stages in confined breeding sites. Diflubenzuron has been widely used for the control of mosquitoes, given its low acute toxicity towards fish, birds, mammals, and most aquatic nontarget organisms (WHO 2006). Nevertheless, DFB and other chitin synthesis inhibitors are not specific against mosquitoes. All chitin-producing organisms show a certain level of susceptibility (IPCS 1996).

The use of entomopathogenic bacteria for the control of immature larval stages is a valid and environmentally friendly alternative to chemicals. *Bacillus thuringiensis* var. *israelensis* and *Lysinibacillus sphaericus* Meyer and Neide (*Lsph*) are commonly used in mosquito control programs (Lacey 2007). *Bacillus thuringiensis* var. *israelensis* possesses a complex arsenal of mosquitocidal toxins, preventing the emergence of resistance (Georghiou and Wirth 1997). Although *Bti* is very effective and has a rapid onset of action by killing larvae within a few hours from the ingestion of the protoxins, its residual activity, especially in polluted water, is generally very short (Silapanuntakul et al. 1983). *Lysinibacillus*

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sphaericus has the advantages to survive well in polluted water, to be less sensitive to ultraviolet light, and to provide a higher residual activity than *Bti*, due to its capacity to reproduce in mosquito cadavers (Lacey 2007). On the other hand, *Lsph* is less active than *Bti* against some species of mosquito such as *Aedes* spp., and resistance has been reported in some field populations of *Cx. quinquefasciatus* Say and *Cx. pipiens* (Mulla et al. 2003, Su and Mulla 2004, Lacey 2007). Development of resistance to *Lsph* was prevented, delayed, and partially reverted by using mixtures of *Bti* and *Lsph* (Zahiri et al. 2002, Mulla et al. 2003). A novel commercially available biological mosquito larvicide (BML) consists of microparticles containing a specific ratio of *Bti* and *Lsph*. This formulation combines the properties of *Bti* and *Lsph*, providing rapid action and long-term efficacy, enhanced toxicity due to synergism between toxins, expanded host range, and reduction of possible resistance development by mosquitoes (Wirth et al. 2004).

In urban areas, *Cx. pipiens* and *Ae. albopictus* mosquitoes coexist in medium-sized containers (10–50 liters) such as catch basins (Carrieri et al. 2003). Catch basins represent suitable sites for the development of immature stages of mosquitoes (Su et al. 2003). Species of mosquitoes predominantly found in catch basins of southern Switzerland are *Cx. pipiens*, and to a lesser extent *Ae. albopictus* (Flacio and Engeler, personal communication). The control of mosquitoes in such breeding sites is challenging because of the variable and changing level of water that may dilute the active ingredient, and the pollution of the water (Su 2008).

The current study aimed to determine the efficacy and long-term residual activity of the microbial BML as compared to the currently used DFB formulation for the control of mosquitoes in urban catch basins under the environmental conditions encountered in southern Switzerland.

MATERIALS AND METHODS

Mosquitocidal products

The formulations tested were the microbial BML (*Bti* serotype H14, strain AM65-52 and *Lsph* serotype H5a5b, strain 2362; granules; 50 *Lsph* ITU/mg [VectoMax™ CG; Valent BioSciences Corp., Libertyville, IL]) and a commercial product containing 15% DFB (Device SC 15®; Crompton Europe Ltd., Slough, UK).

Experimental design

Field tests were carried out between August and October 2011 in the municipality of Chiasso (Ticino, southern Switzerland). The test site was divided into 2 sections that were treated with the microbial BML or the chemical DFB. Presamples were collected to identify a total of 49 suitable catch basins with consistent mosquito larval populations, prior to the randomly assignment of treatments. Diflubenzuron and BML were tested in 16 and 17 replicates per catch basin, respectively (Fig. 1). The BML was applied at a rate of 10 g per catch basin, as recommended by the manufacturer, by direct application as granular formulation into the basin. The IGR formulation was applied at a dosage of 0.6 mg DFB per liter, corresponding to 0.2 ml of product per catch basin with an estimated volume of 50 liters. In both sections, 8 catch basins served as untreated controls (UTCs) (Fig. 1). The treatments were carried out on August 8, 2011.

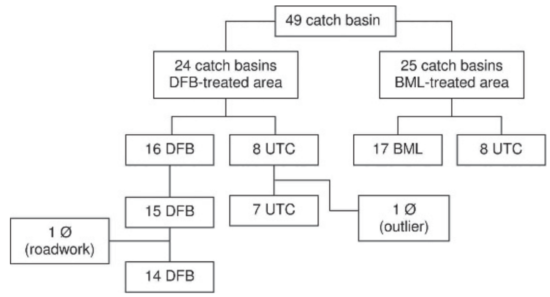

Fig. 1. Schematic presentation of the experimental design. DFB, diflubenzuron; BML, biological mosquito larvicide (*Bacillus thuringiensis* var. *israelensis* + *Lysinibacillus sphaericus*); UTC, untreated control.

Sampling

Prior to treatment, on August 3, 2011, baseline sampling was carried out to record the number of immature mosquitoes in both experimental and control sites (WHO 2005). Larvae and pupae were collected with a 0.2- to 0.3-mm mesh aquarium net (13 × 10 cm; Catch net—fine netting; JBL GmbH & Co. KG, Neuhofen, Germany) by 3 consecutive net sweeps. The immature stages captured were separated according to their stage. Larvae were defined as early (1st and 2nd instars) or late (3rd and 4th) instars. The total number of late-stage larvae and pupae was recorded. The number of early-stage larvae were estimated as a range (i.e., 0, 1–10, 11–100, 101–1,000, >1,000) to simplify counting. For the DFB-based formulation, deformed and bad-swimming larvae were considered as treatment-affected and not included in the counting. After counting, larvae and pupae were returned to the catch basins.

Posttreatment samples were taken by the same method as described above on day 2 and then weekly until the efficacy of the products against 3rd- and 4th-stage larvae had fallen below 80% for 3 consecutive samplings.

Water temperature and rainfall

During the whole test period, the abiotic parameters (such as rainfall and water temperature) that may influence the efficacy of the product were monitored (WHO 2005). For every catch basin, the water temperature was measured at each sampling between 9:00 a.m. and 12:00 p.m., using an outdoor thermometer. Hourly rainfall data were obtained from the nearby agricultural weather station (Mezzana: http://www.agrometeo.ch).

Data analysis

A larval score was assigned to each range of early instars (1st and 2nd instars) as follows: 0 = 0 larvae; 1 = 1–10 larvae; 2 = 11–100 larvae; 3 = 101–1,000 larvae; 4 = >1,000 larvae. Mean number of late instars (3rd and 4th instars) and pupae collected from replicate basins assigned to each treatment on each sample day were calculated. Differences in the number of pupae and of late instars counted at every sampling were tested for significance using the Wilcoxon test (α set to 0.05). Percentage of reduction (%R) in late-stage larvae and pupal densities were calculated using Mulla's formula (Mulla et al. 1971), which takes into account that the natural changes in the mosquito larval populations are occurring at the same level and rate in both treated and untreated sites: $\%R = 1 - [(C1 \times T2)/(T1 \times C2)]$ (where C1 is the abundance in untreated basins during pretreatment; C2 in untreated basins during treatment; T1 in treated basins during pretreatment; and T2 is the abundance in treated basins during treatment). We tested the null hypothesis that there was no difference between the period of residual activity of the IGR (DFB) and BML. The period of activity of the treatment is defined as the time between treatment and the last observation with <20% healthy late-stage larvae.

Percentages of reduction were transformed to arcsine values and differences between treatments were compared using the Wilcoxon signed-rank test (α set to 0.05).

Total rainfalls (mm) were calculated for a 48-h period before each larval sampling. A multiple linear regression analysis was performed to detect the influence of the water temperature and the 48-h-period rainfall on the percentage of larval and pupal reduction (arcsine-transformed).

If not otherwise stated, data are presented as mean ± SE. All statistical analyses were carried out using SPSS version 17.0.1 for Windows (SPSS Inc., Chicago, IL).

RESULTS

The design of the study is presented in Fig. 1. Among the 16 untreated catch basins, 1 was omitted from the analysis because the number of larvae counted during the pretreatment sampling deviated too much from the average (outlier). Moreover, from day 49 posttreatment onwards, 1 catch basin treated with DFB was excluded from the analysis because sampling was hindered by road works. On 2 sampling days (21 and 70 d posttreatment), 1 UTC and 1 BML-treated catch basin dried out. The corresponding data for these 2 basins were omitted from the analysis.

Early instars

The number of early (1st and 2nd) instars estimated in control catch basins fluctuated for the whole length of the trial, with larval scores ranging from a minimum of zero to a maximum of 33 on day 14 posttreatment. The same trend was observed for the number of early instars in the DFB-treated basins, with maximal larval scores of 24 on days 35 and 49 posttreatment. The population of early instars in BML-treated catch basins declined to zero immediately after the treatment.

Late instars

The number of late instars observed in UTC catch basins showed an increasing trend until August 22, with 110.87 ± 30.62 larvae per sample. Subsequently, the density of larvae declined progressively to low numbers, with 12.73 ± 5.83 larvae at the end of the trial (Table 1). Counts in UTC basins give an indication of the natural fluctuations of the mosquito population. The number of larvae in the BML-treated catch basins decreased to zero soon after the treatment and the counts remained significantly lower than those in UTCs throughout the test period. In DFB-treated basins, the number of larvae declined significantly only 1 wk after the treatment. From day 56 posttreatment onwards, no significant differences in the number of late instars were observed between DFB-treated and UTC basins (Table 1). Larval reduction of the 2 larvicides tested was significantly different (Wilcoxon signed-rank test, $P < 0.05$). Long-term efficacy was observed for the microbial larvicide BML, with percentages of reduction of 98.4% to 100% for the whole test period (Fig. 2). In contrast, reduction in larval density in DFB-treated catch basins fell below 80% on day 42 posttreatment, to increase again on day 49 to 86.2%. From day 56 posttreatment, the efficacy of DFB remained below 80% until the end of the study (Fig. 2). From day 42 posttreatment, the difference in efficacy between the 2 larvicides tested was significant. The monitoring ended on day 70 posttreatment, after 3 consecutive samplings with <80% of larval reduction in DFB-treated catch basins. In addition, the mosquito

Table 1. Average number of late-stage mosquito larvae sampled in both treated and untreated control catch basins at weekly intervals during August through October 2011, in southern Switzerland. The biological mosquito larvicide (BML) contained *Bacillus thuringiensis* var. *israelensis* (*Bti*) + *Lysinibacillus sphaericus* (*Lsph*).

		Average no. late larvae (± SE)		
Date	Day	Diflubenzuron (DFB)	*Bti* + *Lsph* (BML)	Untreated control (UTC)
Aug. 3, 2011	−5	59.31 ± 14.12	47.94 ± 13.38	52.00 ± 9.98
Aug. 10, 2011	2	25.31 ± 5.67[1]	0.00 ± 0.00[1,3]	38.00 ± 7.24[3]
Aug. 16, 2011	7	1.69 ± 0.94[1,2]	0.00 ± 0.00[1,3]	69.73 ± 23.55[2,3]
Aug. 22, 2011	14	0.31 ± 0.18[2]	0.00 ± 0.00[3]	110.87 ± 30.62[2,3]
Aug. 29, 2011	21	0.00 ± 0.00[2]	0.06 ± 0.06[3]	63.35 ± 15.05[2,3]
Sept. 5, 2011	28	0.13 ± 0.09[2]	0.53 ± 0.27[3]	35.07 ± 9.35[2,3]
Sept. 12, 2011	35	3.50 ± 2.38[2]	0.12 ± 0.08[3]	22.47 ± 6.91[2,3]
Sept. 19, 2011	42	2.94 ± 1.09[1]	0.12 ± 0.12[1,3]	9.80 ± 6.51[3]
Sept. 26, 2011	49	3.87 ± 1.90[1,2]	0.18 ± 0.13[1,3]	24.47 ± 7.29[2,3]
Oct. 3, 2011	56	16.93 ± 5.18[1]	0.06 ± 0.06[1,3]	39.00 ± 10.49[3]
Oct. 10, 2011	63	10.60 ± 5.38[1]	0.00 ± 0.00[1,3]	24.93 ± 8.80[3]
Oct. 17, 2011	70	5.13 ± 3.27[1]	0.00 ± 0.00[1,3]	12.73 ± 5.83[3]

[1] DFB and BML are significantly different (Wilcoxon test, $P < 0.05$).
[2] DFB and UTC are significantly different (Wilcoxon test, $P < 0.05$).
[3] BML and UTC are significantly different (Wilcoxon test, $P < 0.05$).

season was almost over and the number of larvae in UTCs was correspondingly low (Table 1).

Pupae

In UTCs, the number of pupae was low, ranging from 0.25 ± 0.38 to 8.33 ± 3.33 throughout the whole test period (Table 2). Pupal counts in both treatments decreased almost immediately after the application. Three and 7 pupae were still recovered 2 days after BML and DFB treatments, respectively, in the totality of the catch basins tested. The number of pupae remained at very low levels in BML-treated catch basins during the whole trial. This was in accordance with data recorded for late instars. In DFB-treated catch basins the average number of pupae increased again after 56 days posttreatment, with no significant differences with the untreated control for the last 2 samplings (Table 2). Percent reduction of the pupal population was 100% for both formulations tested until day 28 posttreatment, followed by a fluctuation between 80.2% and 100% for BML and 43.4% and 100% for DFB until the end of the experiment (Fig. 3).

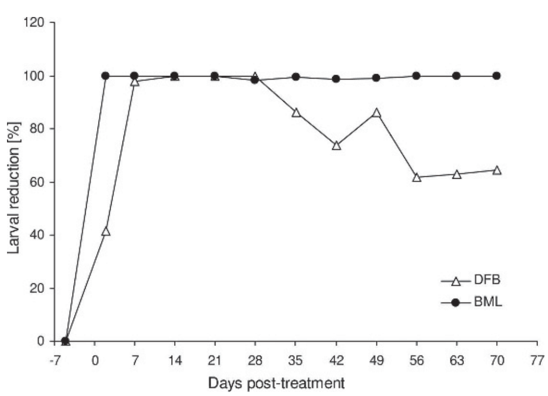

Fig. 2. Percentages of larval reduction (late instar larvae). DFB, diflubenzuron; BML, biological mosquito larvicide (*Bacillus thuringiensis* var. *israelensis* + *Lysinibacillus sphaericus*). Day 0 is the treatment date.

Water temperature and rainfall

Average water temperatures ranged from 15.02 ± 0.24°C to 26.02 ± 0.34°C during the test period (Fig. 4). During the entire trial, from August 3 to October 17, 2011, the total rainfall was 270.6 mm. The water temperature and the 48-h-period rainfall before sampling did not affect the efficacy of the BML ($R^2 = 0.30$, $P = 0.20$ for late instars and $R^2 = 0.24$, $P = 0.30$ for pupae) and DFB ($R^2 = 0.19$, $P = 0.39$ for late instars and $R^2 = 0.04$, $P = 0.84$ for pupae) treatments.

DISCUSSION

The BML showed an improved and longer lasting control of immature stages of mosquitoes in catch basins as compared to the IGR (15% DFB) under the environmental conditions encountered in Ticino. The reduction rate of late-stage larvae was high in both treatments and lasted for 56 d with DFB and at least 70 days in the case of BML. Soon after the BML application, 100% efficacy was reached and high levels of reduction (above 98%) were maintained for the entire duration of the experiment. The significant decrease in larval numbers after the treatment with BML was caused by the rapid killing action of *Bti* and *Lsph* toxins. The 3 pupae recovered 2 d after the BML application in 2 catch basins were probably at the initial stage of pupation at the time of the treatment. The efficacy and residual

Table 2. Average number of mosquito pupae observed in treated and control catch basins at weekly intervals during August through October 2011, in southern Switzerland. The biological mosquito larvicide (BML) contained *Bacillus thuringiensis* var. *israelensis* (*Bti*) + *Lysinibacillus sphaericus* (*Lsph*).

		Average no. pupae (± SE)		
Date	Day	Diflubenzuron (DFB)	*Bti* + *Lsph* (BML)	Untreated control (UTC)
Aug. 3, 2011	−5	4.19 ± 2.47	2.18 ± 1.26	4.27 ± 2.66
Aug. 10, 2011	2	0.44 ± 0.22	0.18 ± 0.13[3]	6.27 ± 3.44[3]
Aug. 16, 2011	7	0.00 ± 0.00[2]	0.00 ± 0.00[3]	4.80 ± 2.35[2,3]
Aug. 22, 2011	14	0.00 ± 0.00[2]	0.00 ± 0.00[3]	7.07 ± 1.79[2,3]
Aug. 29, 2011	21	0.00 ± 0.00[2]	0.00 ± 0.00[3]	6.07 ± 2.24[2,3]
Sept. 5, 2011	28	0.00 ± 0.00	0.00 ± 0.00	1.53 ± 1.03
Sept. 12, 2011	35	0.88 ± 0.88[2]	0.24 ± 0.18[3]	2.33 ± 0.73[2,3]
Sept. 19, 2011	42	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.20
Sept. 26, 2011	49	0.40 ± 0.21[2]	0.24 ± 0.24[3]	4.67 ± 2.95[2,3]
Oct. 3, 2011	56	1.00 ± 0.29[1,2]	0.06 ± 0.06[1,3]	8.33 ± 3.33[2,3]
Oct. 10, 2011	63	1.47 ± 0.70[1]	0.06 ± 0.06[1,3]	2.67 ± 0.98[3]
Oct. 17, 2011	70	2.73 ± 1.65[1]	0.00 ± 0.00[1,3]	5.73 ± 1.95[3]

[1] DFB and BML are significantly different (Wilcoxon test, $P < 0.05$).
[2] DFB and UTC are significantly different (Wilcoxon test, $P < 0.05$).
[3] BML and UTC are significantly different (Wilcoxon test, $P < 0.05$).

activity of *Lsph* and *Bti* in combination have been investigated in previous studies (Cetin et al. 2007, Su 2008, Anderson et al. 2011, Dritz et al. 2011). Combinations of commercial formulations of *Bti* and *Lsph* provided >90% control for 28 d against *Culex pipens* in septic tanks (Cetin et al. 2007). Anderson and collaborators (2011) tested 4 commercial products containing *Bti*, *Lsph*, or a combination of both for the control of mosquitoes in catch basins in Connecticut. They found that a single application of the formulation containing both *Bti* and *Lsph* as active ingredients significantly reduced the number of late instars and healthy pupae for 3 wk. In assays with simulated catch basins, a *Bti*- and *Lsph*-based formulation was reported to be effective against *Culex* mosquitoes, with a reduction of late instars of >90% over a period of 203 d (Su 2008). Our results are in line with this study, obtaining a reduction of the pupal population by 94.2–100% during the 1st 70 days of posttreatment. The good efficacy and long-term control of immature mosquito stages with BML in catch basins must be based on the synergistic action between *Bti* and *Lsph* toxins (Wirth et al. 2004), and on the ability of *Lsph* to recycle in dead larvae (Nicolas et al. 1987, Lacey 2007).

The insecticidal activity of DFB reached high levels of mortality (93%) at day 2 posttreatment only against pupae. Seven days after the treatment larvae and pupae had declined by 98% and 100%, respectively. This was to be expected because DFB is a chitin synthesis inhibitor and interferes with the molting of immature stages (WHO 2006). In a field study carried out in catch basins against *Cx. pipiens* and *Ae. albopictus*, the application of the DFB formulation (15% DFB) at a dosage of 0.28 ml per basin resulted in the inhibition of adult emergence of >89.4% over a 5-wk period, with a complete inhibition during the first 3 wk (Bellini et al. 2009).

During the whole test period, early instars were present in catch basins treated with DFB; this was likely due to the delayed action of the growth regulator. Microbial larvicides, on the other hand, kill mosquitoes already in the early stages. Thus, the number of early instars in BML-treated basins was reduced to zero soon after the treatment and until the end of the experiment. In control catch basins, the number of early-stage larvae, as well as pupae, fluctuated during the whole test period. The number of pupae was very low, probably due to natural mortality (Su 2008). The low and variable number of pupae in the controls renders the evaluation of efficacy against pupae for both treatments less reliable from a statistical point of view.

The population density of 3rd and 4th instars is a sensitive indicator for the evaluation of the efficacy of a microbial larvicide (Knepper and Walker 2004, Su 2008). For an insect growth

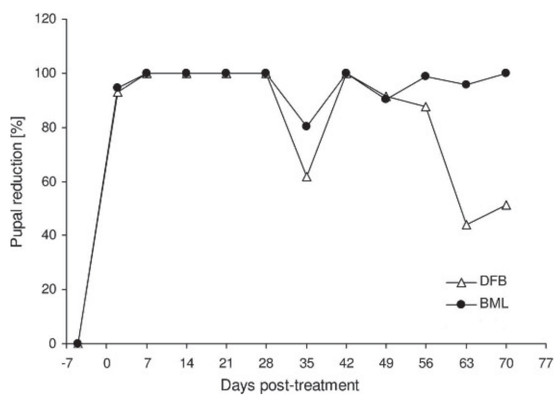

Fig. 3. Percentages of pupal reduction. DFB, diflubenzuron; BML, biological mosquito larvicide (*Bacillus thuringiensis* var. *israelensis* + *Lysinibacillus sphaericus*). Day 0 is the treatment date.

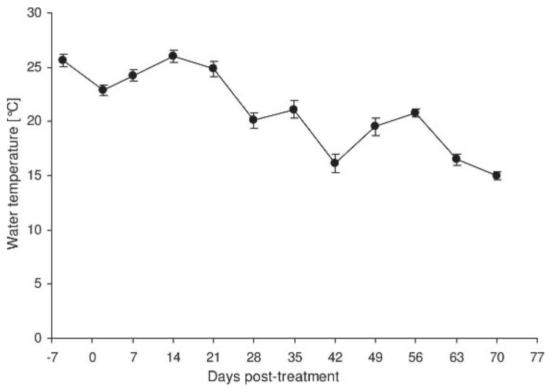

Fig. 4. Evolution of water temperature in catch basins (average ± SE).

regulator such as DFB, the evaluation of efficacy should also consider the inhibition of adult emergence, to exclude individuals that survive until the pupal stage but fail to emerge as adults. However, Amin and White (1984) found that when early instars were exposed to DFB, the main effects were induced before the 4th larval stage. Since all immature stages, including pupae, were returned to catch basins to avoid experimental biases and interferences with the mosquito populations, the inhibition of adult emergence following DFB treatment could not be evaluated.

The duration of the effect of a microbial insecticide can be reduced by precipitations, since the microbial agent may be diluted or flushed away (Mulla et al. 1999). The water temperature can also influence the efficacy of a microbial larvicide, with a reduced effectiveness due to low temperatures (Becker et al. 1992, Jat et al. 1999, Katbeh-Bader et al. 1999, Christiansen et al. 2004). We found that in our field assay none of the treatments were influenced by rainfall and water temperature.

While we could demonstrate the high efficacy and long residual activity of BML against all species of mosquitoes breeding in catch basins in southern Switzerland, the present study does not provide any indication on the efficacy and long-term activity on single species of the products tested. The immature stages of mosquitoes in the catch basins in southern Switzerland are dominated by *Cx. pipiens* (Flacio and Engeler, personal communication). In our pretreatment samples *Culex* spp. larvae accounted for 94.14% (95% confidence interval [CI]: 88.5–99.78%) of the total number. The frequency of *Ae. albopictus* larvae represented a minority, with changing numbers (5.86%, 95% CI: 0.22–11.50%) due to the limited flying range of the adults. Both early and late instars of *Ae. albopictus* were found in the catch basins, but their frequency (mean number of late-stage tiger mosquitoes in untreated control catch basins: 1.16 ± 0.29) was too low for statistical evaluation. Despite their very low number in both treated and control catch basins, there are indications that BML and DFB can control the immature stages of *Ae. albopictus*. During the entire posttreatment period, the total number of *Ae. albopictus* late instars in untreated control basins was 201, compared to 3 and 5 larvae observed in BML- and DFB-treated basins, respectively.

Under the environmental conditions characteristic for southern Switzerland, a single application of the microbial larvicide BML can control immature stages of mosquitoes breeding in catch basins for at least 70 days. The longer residual activity of BML compared to the currently used chemical growth regulator, DFB, indicates that this larvicide may be a valid alternative, allowing for reduced number of treatments required to keep the population of mosquitoes at low levels.

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REFERENCES CITED

Amin AM, White GB. 1984. Resistance potential of *Culex quinquefasciatus* against the insect growth regulators methoprene and diflubenzuron. *Entomol Exp Appl* 36:69–76.

Anderson JF, Ferrandino FJ, Dingman DW, Main AJ, Andreadis TG, Becnel JJ. 2011. Control of mosquitoes in catch basins in Connecticut with *Bacillus thuringiensis israelensis*, *Bacillus sphaericus*, and spinosad. *J Am Mosq Control Assoc* 27:45–55.

Angelini R, Finarelli AC, Angelini P, Po C, Petropulacos K, Macini P, Fiorentini C, Fortuna C, Venturi G, Romi R, Majori G, Nicoletti L, Rezza G, Cassone A. 2007. An outbreak of chikungunya fever in the province of Ravenna, Italy. *Euro Surveill* 12:pii=3260.

Barzon L, Pacenti M, Cusinato R, Cattai M, Franchin E, Pagni S, Martello T, Bressan S, Squarzon L, Cattelan A. 2010. Human cases of West Nile Virus Infection in north-eastern Italy, 15 June to 15 November 2010. *Euro Surveill* 16:pii=19949.

Becker N, Zgomba M, Ludwig M, Petric D, Rettich F. 1992. Factors influencing the activity of *Bacillus thuringiensis* var. *israelensis* treatments. *J Am Mosq Control Assoc* 8:285–289.

Bellini R, Albieri A, Carrieri M, Colonna R, Donati L, Magnani M, Pilani R, Veronesi R, Chiot G, Lanza N. 2009. Efficacy and lasting activity of four IGRs formulations against mosquitoes in catch basins of northern Italy. *Eur Mosq Bull* 27:33–46.

Carrieri M, Bacchi M, Bellini R, Maini S. 2003. On the competition occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. *Environ Entomol* 32:1313–1321.

Cetin H, Dechant P, Yanikoglu A. 2007. Field trials with tank mixtures of *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus* formulations against *Culex pipiens* larvae in septic tanks in Antalya, Turkey. *J Am Mosq Control Assoc* 23:161–165.

Christiansen JA, McAbee RD, Stanich MA, DeChant P, Boronda D, Cornel AJ. 2004. Influence of temperature and concentration of VectoBac® on control of the salt-marsh mosquito, *Ochlerotatus squamiger*, in Monterey County, California. *J Am Mosq Control Assoc* 20:165–170.

Danis K, Papa A, Papanikolaou E, Dougas G, Terzaki I, Baka A, Vrioni G, Kapsimali V, Tsakris A, Kansouzidou A. 2011. Ongoing outbreak of West Nile virus infection in humans, Greece, July to August 2011. *Euro Surveill* 16:pii=19951.

Dritz DA, Lawler SP, Evkhanian C, Graham P, Baracosa V, Dula G. 2011. Control of mosquito larvae in seasonal wetlands on a wildlife refuge using Vectomax™ CG. *J Am Mosq Control Assoc* 27:398–403.

Flacio E, Lüthy P, Patocchi N, Guidotti F, Tonolla M, Peduzzi R. 2004. Primo ritrovamento di *Aedes albopictus* in Svizzera. *Boll Soc Tic Sci Nat* 92:141–142.

Georghiou GP, Wirth MC. 1997. Influence of exposure to single versus multiple toxins of *Bacillus thuringiensis* subsp. *israelensis* on development of resistance in the mosquito *Culex quinquefasciatus* (Diptera: Culicidae). *Appl Environ Microbiol* 63:1095–1101.

Gjenero-Margan I, Aleraj B, Krajcar D, Lesnikar V, Klobučar A, Pem-Novosel I, Kurečić-Filipović S, Komparak S, Martić R, Đuričić S, etica-Radić L, Okmadžić J, Vilibić-Čavlek T, Babić-Erceg A, Turković B, Avšić-Županc T, Radić I, Ljubić M, Šarac K, Benić N, Mlinarić-Galinović G. 2011. Autochthonous dengue fever in Croatia, August-September 2010. *Euro Surveill* 16:pii=19805.

IPCS [International Programme on Chemical Safety]. 1996. *Environmental Health Criteria 184: diflubenzuron* [Internet]. Geneva, Switzerland: IPCS INCHEM [accessed June 18, 2012]. Available from: http://www.inchem.org/.

Jat K, Knight JW, Arshad ALI, Douglas B. 1999. Laboratory evaluation of biotic and abiotic factors that may influence larvicidal activity of *Bacillus thuringiensis* serovar. *israelensis* against two Florida mosquito species. *J Am Mosq Control Assoc* 15:32–42.

Katbeh-Bader A, Khyami-Horani H, Mohsen ZH. 1999. Effect of temperature on the susceptibility of *Culiseta longiareolata* (Macquart) (Dipt., Culicidae) to two standard strains of biocontrol bacteria. *J Appl Entomol* 123:629–631.

Knepper R, Walker N. 2004. *Efficacy of a novel, Bti/Bs combination formulation (VBC60035) against* Culex *larvae in stormwater catch basins in Saginaw County, Michigan* [Internet]. Saginaw, MI: Saginaw County Mosquito Abatement Commission [accessed June 18, 2012]. Available from: http://www.scmac.org/research.htm.

La Ruche G, Souarès Y, Armengaud A, Peloux-Petiot F, Delaunay P, Desprès P, Lenglet A, Jourdain F, Leparc-Goffart I, Charlet F. 2010. First two autochthonous dengue virus infections in metropolitan France, September 2010. *Euro Surveill* 15:pii=19676.

Lacey LA. 2007. *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. *J Am Mosq Control Assoc* 23:133–163.

Medlock JM, Hansford KM, Schaffner F, Versteirt V, Hendrickx G, Zeller H, Bortel WV. 2012. A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. *Vector Borne Zoonotic Dis* 12:435–447.

Mulla MS, Norland LEER, Fanara DM, Darwazeh HA, McKean DW. 1971. Control of chironomid midges in recreational lakes. *J Econ Entomol* 64:300–307.

Mulla MS, Su T, Thavara U, Tawatsin A, Kongngamsuk W, Phan-Urai P. 1999. Efficacy of new formulations of the microbial larvicide *Bacillus sphaericus* against polluted water mosquitoes in Thailand. *J Vector Ecol* 24:99–110.

Mulla MS, Thavara U, Tawatsin A, Chompsori J, Su T. 2003. Emergence of resistance and resistance management in field populations of tropical *Culex quinquefasciatus* to the microbial control agent *Bacillus sphaericus*. *J Am Mosq Control Assoc* 19:39–46.

Nicolas L, Dossou-Yovo J, Hougard J-M. 1987. Persistence and recycling of *Bacillus sphaericus* 2362 spores in *Culex quinquefasciatus* breeding sites in West Africa. *Appl Microbiol Biotechnol* 25:341–345.

Randolph SE, Rogers DJ. 2010. The arrival, establishment and spread of exotic diseases: patterns and predictions. *Nat Rev Microbiol* 8:361–371.

Silapanuntakul S, Pantuwatana S, Bhumiratana A, Charoensiri K. 1983. The comparative persistence of toxicity of *Bacillus sphaericus* strain 1593 and *Bacillus thuringiensis* serotype H-14 against mosquito larvae in different kinds of environments. *J Invertebr Pathol* 42:387–392.

Su T. 2008. Evaluation of water-soluble pouches of *Bacillus sphaericus* applied as prehatch treatment against *Culex* mosquitoes in simulated catch basins. *J Am Mosq Control Assoc* 24:54–60.

Su T, Mulla MS. 2004. Documentation of high-level *Bacillus sphaericus* 2362 resistance in field populations of *Culex quinquefasciatus* breeding in polluted water in Thailand. *J Am Mosq Control Assoc* 20:405–411.

Su T, Webb JP, Meyer RP, Mulla MS. 2003. Spatial and temporal distribution of mosquitoes in underground storm drain systems in Orange County, California. *J Vector Ecol* 28:79–89.

WHO [World Health Organization]. 2004. *The vector-borne human infections of Europe: their distribution and burden on public health* [Internet]. Copenhagen, Denmark: World Health Organization, Regional Office for Europe [accessed June 18, 2012]. Available from: http://www.euro.who.int.

WHO [World Health Organization]. 2005. *Guidelines for laboratory and field testing of mosquito larvicides* [Internet]. Geneva, Switzerland: World Health Organization [accessed June 18, 2012]. Available from: http://www.who.int/whopes/guidelines/en/.

WHO [World Health Organization]. 2006. *Pesticides and their application: for the control of vectors and pests of public health importance* [Internet]. Geneva, Switzerland: World Health Organization [accessed June 18, 2012]. Available from: http://www.who.int/whopes/recommendations/en/.

Wirth MC, Jiannino JA, Federici BA, Walton WE. 2004. Synergy between toxins of *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus*. *J Med Entomol* 41:935–941.

Wymann MN, Flacio E, Radczuweit S, Patocchi N, Lüthy P. 2008. Asian tiger mosquito (*Aedes albopictus*)—a threat for Switzerland? *Euro Surveill* 13:pii=8058.

Zahiri NS, Su T, Mulla MS. 2002. Strategies for the management of resistance in mosquitoes to the microbial control agent *Bacillus sphaericus*. *J Med Entomol* 39:513–520.