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# Spinosad as an effective larvicide for control of *Aedes albopictus* and *Aedes aegypti*, vectors of dengue in southern Mexico

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## Abstract

**BACKGROUND: Field trials were conducted during the wet and dry seasons in periurban and semi-rural cemeteries in southern Mexico to determine the efficacy of a suspension concentrate formulation of spinosad (Tracer 480SC) on the inhibition of development of *Aedes albopictus* L. and *Ae. aegypti* Skuse. For this, oviposition traps were treated with spinosad (1 or 5 mg $L^{-1}$), *Bacillus thuringiensis israelensis* (*Bti*, VectoBac 12AS), a sustained release formulation of temephos and a water control.**

**RESULTS: *Ae. albopictus* was subordinate to *Ae. aegypti* during the dry season, but became dominant or codominant during the wet season at both sites. The two species could not be differentiated in field counts on oviposition traps. Mean numbers of larvae + pupae of *Aedes* spp. in *Bti*-treated containers were similar to the control at both sites during both seasons. The duration of complete absence of aquatic stages varied from 5 to 13 weeks for the spinosad treatments and from 6 to 9 weeks for the temephos treatment, depending on site, season and product concentration. Predatory *Toxorhynchites theobaldi* Dyar and Knab suffered low mortality in control and *Bti* treatments, but high mortality in spinosad and temephos treatments. Egg counts and percentage of egg hatch of *Aedes* spp. increased significantly between the dry and wet seasons, but significant treatment differences were not detected.**

**CONCLUSION: Temephos granules and a suspension concentrate formulation of spinosad were both highly effective larvicides against *Ae. aegypti* and *Ae. albopictus*. These compounds merit detailed evaluation for inclusion in integrated control programs targeted at *Ae. aegypti* and *Ae. albopictus* in regions where they represent important vectors of human diseases.**

**Keywords:** *Aedes albopictus*; dengue vector; Mexico; spinosad; temephos; *Toxorhynchites theobaldi*

## 1 INTRODUCTION

The prevalence of serotypes of dengue virus, in particular the hemorrhagic form of dengue fever in humans, has become a major public health issue in Mexico and many other parts of Latin America, as well as in tropical and subtropical regions elsewhere in the world.[1,2] In Mexico, the principal vector, *Aedes aegypti* L., is sympatric over much of its range with the invasive Asian tiger mosquito, *Ae. albopictus* Skuse. The latter species has been firmly implicated as a vector of this virus and represents an emerging public health threat in the tropical and subtropical Americas.[3–5]

Control measures against these species are based on the elimination of aquatic habitats for the development of the immature stages and the application of a granular formulation of temephos to domestic water tanks and other potential larval development sites that cannot be drained or removed. During dengue outbreaks, these actions are often complemented by peridomiciliar, street-level fogging or aerial application of pyrethroid insecticides, although the efficacy of these actions remains questionable.[6,7] Moreover, resistance to temephos has been reported in container-inhabiting mosquito species from many regions of Asia and Latin America.[8] To promote the development of alternative mosquito control measures, the World Health Organization (WHO) has identified the advancement of effective biological or biorational larvicides as a priority.[9,10]

Spinosad is a naturally derived insecticide produced by the fermentation of a soil actinomycete.[11] It is a mixture of two macrolide lactone molecules, spinosyns A and D, that are neurotoxic to a restricted range of insects, particularly Diptera, Lepidoptera and Thysanoptera, and is considered to be one of the most selective products available for the conservation of insect predators in agriculture.[12] Spinosad has a very favorable ecotoxicological profile, with low toxicity to fish and virtually no toxicity to birds and mammals. Because of this, spinosad has been classified by the United States Environmental Protection Agency as a low-risk material.

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A number of laboratory studies have identified spinosad as being highly toxic to mosquito larvae.[13] Similarly, semi-field and field studies in tropical regions of Latin America and southern Asia have confirmed that suspension concentrate or granular formulations of this product can provide effective control of *Ae. aegypti* and *Culex* spp. during periods similar to that of temephos granules.[13–16] The WHO pesticide evaluation scheme (WHOPES) recently approved a granular formulation, a tablet and an emulsifiable concentrate of spinosad for field testing.[17] Caged tests have also indicated that spinosad is not repellent to ovipositing *Ae. aegypti*.[15] However, field studies on the effectiveness of spinosad as a larvicide targeted at control of *Ae. albopictus* have not been performed. Given the vector importance of this species, and the favorable results of spinosad-based control measures targeted at *Ae. aegypti*, the authors considered it important to examine the effectiveness of spinosad against *Ae. albopictus* in natural situations where it co-occurs with *Ae. aegypti*.

In the present study, which predates the WHOPES decision, the inhibition duration of development of mosquito larvae in spinosad-treated oviposition traps placed in a semi-urban and a semi-rural cemetery in southern Mexico was determined. Specifically, the hypothesis that spinosad treatments could provide effective control of the immature stages of *Ae. albopictus* and *Ae. aegypti* in different sites and seasons was tested. The efficacy of two concentrations of spinosad (suspension concentrate) was compared with that of temephos granules and a liquid formulation of *Bacillus thuringiensis israelensis* (*Bti*). Given that spinosad is considered to be a biorational compound with a selectivity profile that contrasts with the broad spectrum activity of the organophosphate temephos, an examination was also made of the impact of each of these insecticides on the abundance of immature stages of *Toxorhynchites theobaldi* Dyar and Knab, which are natural predators of *Ae. albopictus* and *Ae. aegypti* larvae, in oviposition traps.

## 2 MATERIALS AND METHODS

### 2.1 Insecticides

Spinosad was obtained as a 480 g $L^{-1}$ suspension concentrate (Tracer® 480SC; Naturalyte Insect Control, Dow Agrosciences LLC). *Bti* was obtained as a liquid suspension (VectoBac 12AS®; Valent Biosciences Corp.) that contained 12 000 international toxicity units (ITU) $mL^{-1}$. Temephos was obtained as a granular mineral-based formulation containing 10 g AI $kg^{-1}$, employed by the Mexican Ministry of Health (Secretaría de Salud) for control of the aquatic stages of dengue vectors in Mexico.[18]

### 2.2 Field sites

Identical field trials were performed using oviposition traps placed in two cemeteries, once during the rainy season and once during the dry season. The first cemetery (cemetery 1), named the Garden Cemetery (Panteón Jardín) owing to its abundance of trees and ornamental plants, which covered ∼70% of the ground area, was located in a periurban zone (14° 53′ N; 92° 14′ W) covering an area of 340 × 473 m (altitude 165 m) on the outskirts of the city of Tapachula, Chiapas, Mexico. Cemetery 2 was the Mazatán Municipal Cemetery, which covered a trapezoid area of 200 × 150 m at its widest point (altitude 40 m), with ∼25% tree cover, located in a semi-rural coastal zone (14° 52′ N; 92° 26′ W) on the outskirts of the village of Mazatán, Chiapas, Mexico, approximately 24 km from cemetery 1 (direct point-to-point distance).

### 2.3 Experimental procedures

Circular black plastic containers of 10 cm diameter and 20 cm height were placed beside tombs and monuments, which provided protection from rainfall. Each container was assigned to one of the following five treatments (all comprising a 1 L volume of dechlorinated tap water): (i) 1 mg $L^{-1}$ spinosad; (ii) 5 mg $L^{-1}$ spinosad; (iii) 0.1 g temephos granules; (iv) 13 µL *Bti* suspension (VectoBac AS12); (v) control (water alone). The quantity of *Bti* applied was based on the manufacturer's recommended rate, whereas the quantity of temephos applied was based on the recommendations of the Mexican government's public health legislation for vector control.[19]

Temephos granules were placed in a well-perforated plastic microcentrifuge tube that allowed the granules to be removed during monitoring procedures. A total of 20 containers were assigned to each treatment and arranged in modified Latin square designs. In cemetery 1 this consisted of 10 rows × 10 columns, each row with ten containers (two for each treatment), with a distance of 20–25 m between adjacent containers. In the trapezoid-shaped cemetery 2, containers were arranged in four rows of 15 containers per row (three for each treatment) and four rows of ten containers per row (two for each treatment), with a distance of 10–12 m between containers. All containers were clearly marked to reduce interference by members of the public; nonetheless, between 0 and 13 containers were lost during the course of the experiments (details in Table 1), which meant that sampling effort changed during the course of each experiment.

The first experiment ran for 13 weeks from 14 March to 14 June 2006, a period that began in the dry season and finished at the beginning of the wet season. The second experiment was performed during the wet season from 4 July to 4 October 2006. Weekly counts were performed to determine the number of living larvae and pupae present in each container. All insects (living and dead) were removed at each sample time. Water that had evaporated during the previous week was replaced with dechlorinated tap water in all treatments. As it was not possible to differentiate between *Ae. albopictus* and *Ae. aegypti* in the immature stages, oviposition trap samples were placed in centrifuge tubes inside insulated boxes and taken to the laboratory, where a minimum sample of ten larvae or pupae were reared from each container and identified to species following emergence of adults. For this, field-collected insects were placed in plastic trays containing dechlorinated water in a controlled temperature room at 27 ± 1 °C and supplied *ad libitum* with powdered dog biscuits as diet.

Oviposition was monitored by placing a strip of filter paper (Whatman No. 2) on a flat wooden spatula placed in the water and resting upright against the side of the container. Filter papers were replaced during the weekly monitoring procedures and taken to the laboratory, where eggs were counted and placed in water and emerging larvae were reared to adulthood and identified to species. Other aquatic invertebrates, including the predatory mosquito *Tx. theobaldi*, were also quantified during the weekly container inspections, and the water temperature of the containers, the air temperature (shade) and the relative humidity were determined on each sampling occasion using glass laboratory thermometers (−10 to 50 °C range) and a portable electronic hygrometer (GE Sensing Inc., Billerica, MA) respectively.

### 2.4 Statistical analysis

As numbers of mosquito larvae and pupae were too low in insecticide-treated containers for most of the period of the study

**Table 1.** Mean numbers of larvae + pupae of *Aedes albopictus* and *Ae. aegypti* (*Aedes* spp.), prevalence of *Ae. albopictus* determined by laboratory rearing of field-collected samples and mortality of predatory *Toxorhynchites theobaldi* registered in oviposition traps treated with different insecticides in two cemetery sites during the dry and wet seasons in southern Mexico[a]

| Season, site and treatment | Number of oviposition traps at start and finish of experiment | Mean *Aedes* spp. (larvae + pupae) per container per week (± SE) | Percentage prevalence of *Ae. albopictus* (number of laboratory-reared insects) | Percentage of mortality of *Toxorhynchites theobaldi* (total number observed) |
|---|---|---|---|---|
| **(A) Dry season** | | | | |
| *Cemetery 1* | | | | |
| Control | 20–19 | 4.78 (±0.65) a | 29.8 (506) | 0 (18) |
| *Bti* | 20–9 | 5.27 (±0.70) a | 41.4 (425) | 0 (12) |
| 1 mg $L^{-1}$ spinosad | 20–7 | 0.21 (±0.08) bc | 44.2 (138) | 0 (5) |
| 5 mg $L^{-1}$ spinosad | 20–8 | 0.03 (±0.02) c | 57.9 (19) | 0 (4) |
| Temephos | 20–15 | 0.94 (±0.26) b | 11.9 (285) | 0 (9) |
| **Totals:** | | **2363** | **31.5 (1373)** | |
| *Cemetery 2* | | | | |
| Control | 20–16 | 5.93 (±0.79) a | 22.9 (996) | 0 (1) |
| *Bti* | 20–14 | 8.15 (±0.98) a | 19.7 (1077) | 0 (1) |
| 1 mg $L^{-1}$ spinosad | 20–15 | 0.76 (±0.20) b | 25.0 (216) | 0 (0) |
| 5 mg $L^{-1}$ spinosad[b] | 20–13 | 0.00 (±0.00) (n/a) | 22.4 (85) | 0 (1) |
| Temephos | 20–19 | 1.64 (±0.33) b | 19.1 (423) | 0 (0) |
| **Totals:** | | **3748** | **21.2 (2797)** | |
| **(B) Wet season** | | | | |
| *Cemetery 1* | | | | |
| Control | 20–17 | 3.68 (±0.49) a | 60.8 (825) | 1 (141) |
| *Bti* | 20–12 | 3.90 (±0.70) a | 55.8 (764) | 2 (63) |
| 1 mg $L^{-1}$ spinosad | 20–14 | 0.94 (±0.21) b | 55.2 (315) | 75 (200) |
| 5 mg $L^{-1}$ spinosad | 20–10 | 0.86 (±0.36) bc | 42.6 (317) | 68 (251) |
| Temephos | 20–17 | 0.12 (±0.05) c | 24.6 (427) | 50 (125) |
| **Totals:** | | **2188** | **50.7 (2648)** | |
| *Cemetery 2* | | | | |
| Control | 20–19 | 9.00 (±0.74) a | 58.8 (1308) | 0 (2) |
| *Bti* | 20–19 | 6.56 (±0.65) a | 49.8 (1423) | 0 (14) |
| 1 mg $L^{-1}$ spinosad | 20–19 | 2.94 (±0.40) b | 56.2 (793) | 75 (8) |
| 5 mg $L^{-1}$ spinosad | 20–19 | 1.28 (±0.33) c | 56.1 (601) | 62 (29) |
| Temephos | 20–20 | 0.25 (±0.09) d | 46.6 (811) | 100 (8) |
| **Totals:** | | **5118** | **53.5 (4936)** | |

[a] Critical level of significance corrected for multiple comparisons by the Bonferroni procedure ($\alpha = 0.005$ for cemetery 1; $\alpha = 0.008$ for cemetery 2) shown in bold type.

[b] The 5 mg $L^{-1}$ spinosad treatment was eliminated from the analysis owing to the complete absence of immature mosquitoes in this treatment.

to allow separate analyses to be performed, and because both larval and pupal stages are of public health interest in larviciding programs, weekly counts of larvae and pupae in each container were pooled and subjected to repeated-measures generalized linear modeling using the GENMOD procedure in SAS (SAS Institute Inc., Cary, NC). For this, a negative binomial error distribution was specified that was found to be appropriate for discrete count data involving a high number of zero values (containers without mosquitoes). The aptness of this error distribution was verified by examination of the magnitude and distribution of residuals for both the fitted model and the corresponding correlation structure. Treatments in which no living mosquitoes were observed during the entire course of the experiment were excluded from the analysis. Critical levels of significance corrected for multiple comparisons by the Bonferroni procedure were $\alpha = 0.005$ for cemetery 1 and $\alpha = 0.0088$ for cemetery 2 during the dry season, and $\alpha = 0.005$ for both cemeteries during the wet season.

Given marked seasonal differences in the abundance of suitable sites for mosquito development, the number of containers that proved positive for *Tx. theobaldi* on each sampling occasion was compared by logistic regression in SAS with a binomial error structure based on the presence/absence of *Tx. theobaldi* to estimate the effect of the specified covariables on the presence of this predator.

To determine potential adverse effects of insecticides on *Tx. theobaldi* populations owing to toxicity or starvation in insecticide-treated containers, a multivariate ANOVA was conducted on the basis of the numbers of living and dead *Tx. theobaldi*, the numbers of living mosquito larvae prey and sampling effort (number of containers sampled in each treatment); the significance of treatment effects was determined by examination of *F*-values generated by Pillai's trace. The contribution of each variable to the overall *F*-value was determined by examining the magnitude of the standardized canonical coefficients. Sampling effort was

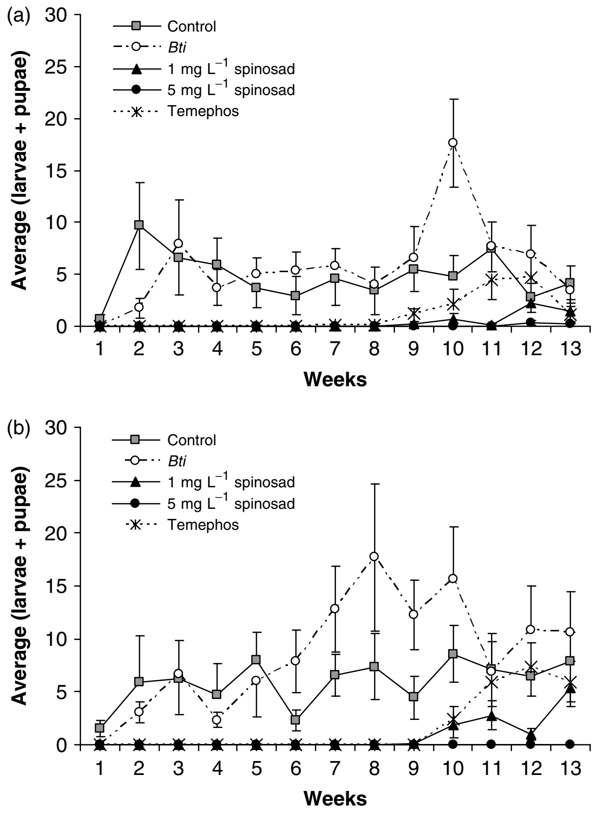

**Figure 1.** Dry-season dynamics of average larvae + pupae counts on *Aedes* spp. registered in insecticide-treated oviposition traps placed in (A) periurban cemetery 1, in which *Ae. albopictus* represented 31.5% of overall captures, and (B) semi-rural cemetery 2, in which *Ae. albopictus* represented 21.2% of overall captures. Vertical bars indicate SD based on 7–20 replicates, depending on sample time (see Table 1). For some points, only half the error bar is shown for clarity.

included in the model, as the number of containers sampled varied during the course of the experiment (Table 1).

Egg counts and percentage of egg eclosion (= emergence of adult from pupal case) of *Aedes* spp. were normalized by $\ln(x+1)$ and arcsine $\sqrt{p}$ transformation, respectively, and subjected to two-way ANOVA with season and treatment as factors.

## 3 RESULTS

### 3.1 Efficacy of insecticides on inhibition of mosquito development

#### 3.1.1 *Dry season*

The average (± SD) water temperature of containers at the moment of sampling was 26.7 ± 1.7 °C, whereas mean air temperature was 30.4 ± 2.5 °C and relative humidity was 65.2 ± 13.1% in cemetery 1, compared with water 25.7 ± 2.3 °C, air 30.8 ± 2.1 °C, and humidity 72.8 ± 2.1% in cemetery 2.

The total number of *Aedes* spp. observed developing in experimental containers during the dry season experiments was 2363 in cemetery 1 and 3748 in cemetery 2 (Table 1A). Laboratory rearing indicated that *Ae. albopictus* comprised 31.5 and 21.2% of the *Aedes* populations at sites 1 and 2 respectively, whereas the remaining individuals were all *Ae. aegypti*.

At both sites the mean numbers of immature *Aedes* spp. were highest in the control and *Bti* treatment, followed by the temephos treatment and 1 mg L$^{-1}$ spinosad treatment, and lowest in the 5 mg L$^{-1}$ spinosad treatment. *Bti* provided 1 week of complete inhibition (Figs 1A and B), and the overall abundance of *Aedes* spp. did not differ significantly from that of the control during the course of the experiment in cemetery 1 ($\chi^2 = 0.59$, df = 1, $P = 0.4416$) and cemetery 2 ($\chi^2 = 1.10$, df = 1, $P = 0.2944$). Temephos granules provided 6 weeks of absolute inhibition in cemetery 1 and 9 weeks of inhibition in cemetery 2, which represented significant reductions in mosquito numbers compared with the control (site 1: $\chi^2 = 49.1$, df = 1, $P < 0.0001$; site 2: $\chi^2 = 111.0$, df = 1, $P < 0.0001$) and *Bti* treatments (site 1: $\chi^2 = 62.4$, df = 1, $P < 0.0001$; site 2: $\chi^2 = 106.7$, df = 1, $P < 0.0001$). The 1 mg L$^{-1}$ spinosad treatment provided 8 weeks of absolute inhibition at both sites and was similar in control efficacy to the temephos treatment (site 1: $\chi^2 = 5.98$, df = 1, $P = 0.0145$; site 2: $\chi^2 = 1.79$, df = 1, $P = 0.1814$). Finally, the 5 mg L$^{-1}$ spinosad treatment provided 11 weeks of absolute inhibition in cemetery 1 and the entire 13 weeks of the experiment in cemetery 2, and was more effective than temephos (although the complete absence of immature mosquitoes in the 5 mg L$^{-1}$ spinosad treatment in cemetery 2 meant that the results from this treatment were excluded from the analysis).

Other culicids were observed in small numbers in oviposition traps (total of 13 individuals). Laboratory rearing of field-collected samples revealed these to be mostly *Haemagogus equines* Theobald (98.1%) and *Culex coronator* Dyar and Knab (percentages based on 156 laboratory-reared insects). Low numbers of other invertebrates, mostly chironomids, were also observed in oviposition traps, a total of 58–81 individuals, depending on the cemetery, but in insufficient numbers for analysis.

#### 3.1.2 *Wet season*

The average (± SD) water temperature of containers at the moment of sampling was 27.7 ± 1.3 °C, whereas the mean air temperature was 32.6 ± 1.8 °C and the relative humidity was 60.4 ± 9.6% in cemetery 1, compared with water and air temperatures of 27.5 ± 1.6 °C and 32.9 ± 2.2 °C, respectively, and a relative humidity of 69.5 ± 9.0% in cemetery 2. The total numbers of immature *Aedes* spp. observed in the wet season were 2188 in cemetery 1 and 5118 in cemetery 2 (Table 1B). Laboratory rearing indicated that *Ae. albopictus* comprised 50.7 and 53.5% of the *Aedes* populations at sites 1 and 2 respectively, which represented a marked increase in the prevalence of *Ae. albopictus* compared with dry-season observations; all the remaining individuals were *Ae. aegypti*.

The mean numbers of immature mosquitoes varied significantly between treatments, being most abundant in the control and *Bti* treatment, followed by the spinosad treatments, and lowest in the temephos treatment. Larvae were observed in the control and *Bti* treatments from the first week of the experiment (Figs 2A and B), and their abundance did not differ significantly between these treatments during the course of the experiment at either site (site 1: $\chi^2 = 0.61$, df = 1, $P < 0.4341$; site 2: $\chi^2 = 2.45$, df = 1, $P < 0.1172$). Spinosad treatment at 1 mg L$^{-1}$ resulted in complete inhibition for 5 weeks at both sites, and the abundance of immature *Aedes* spp. was significantly reduced compared with the control (site 1: $\chi^2 = 51.2$, df = 1, $P < 0.0001$; site 2: $\chi^2 = 117.0$, df = 1, $P < 0.0001$) and *Bti* treatment (site 1: $\chi^2 = 37.3$, df = 1, $P < 0.0001$; site 2: $\chi^2 = 88.3$, df = 1, $P < 0.0001$). Spinosad at 5 mg L$^{-1}$ provided absolute control for 7 and 10 weeks at sites 1 and 2 respectively, and was as effective as 1 mg L$^{-1}$ spinosad in reducing overall abundance of *Aedes* spp. in cemetery 1 ($\chi^2 = 0.95$, df = 1, $P < 0.3306$), but significantly more effective than 1 mg L$^{-1}$ spinosad in cemetery 2 ($\chi^2 = 10.54$,

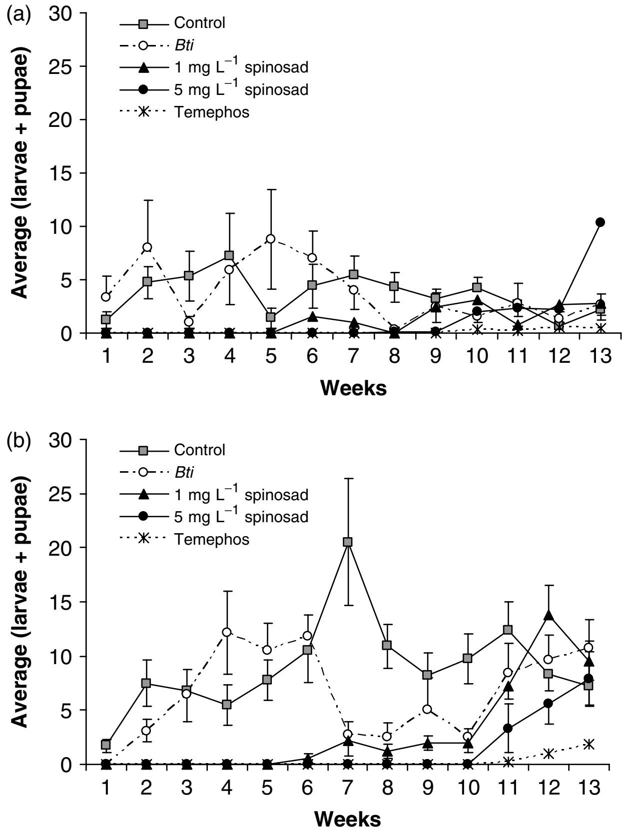

**Figure 2.** Wet-season dynamics of average larvae + pupae counts on *Aedes* spp. registered in insecticide-treated oviposition traps placed in (A) periurban cemetery 1, in which *Ae. albopictus* represented 50.7% of overall captures, and (B) semi-rural cemetery 2, in which *Ae. albopictus* represented 53.5% of overall captures. Vertical bars indicate SD based on 7–20 replicates, depending on sample time (see Table 1). For some points, only half the error bar is shown for clarity.

df = 1, $P < 0.0012$), where the total numbers of developing mosquitoes was much higher than at site 1. Finally, temephos granules provided absolute inhibition of *Aedes* spp. development for 9 weeks in both cemeteries. This treatment was similar to 5 mg L$^{-1}$ spinosad at site 1 ($\chi^2 = 5.44$, df = 1, $P < 0.0197$), but more effective than 5 mg L$^{-1}$ spinosad at site 2 ($\chi^2 = 10.00$, df = 1, $P < 0.0016$).

Other culicids were observed in very small numbers (a total of 18 individuals). Only 15 insects were observed as non-*Aedes* spp. during laboratory rearing of field-collected samples, of which ten were *H. equinus* and four were *Cx. coronator*. Low numbers of other invertebrates, mostly chironomids, were also observed in oviposition traps, a total of 255 at site 1 and 114 at site 2, but in insufficient numbers for analysis.

### 3.2 Insecticide effects of the abundance of *Toxorhynchites theobaldi*

During the dry season, *Tx. theobaldi* was present in low numbers (total 48 larvae across all treatments) in cemetery 1 but virtually absent in cemetery 2 (Table 1). During the wet season, an increase in the abundance of *Tx. theobaldi* was observed at both sites compared with that of the dry season ($\chi^2 = 333.0$, df = 3, $P < 0.0001$), suggesting that populations of this predator are correlated with seasonal fluctuations in the abundance of mosquito development sites. The highest numbers of *Tx. theobaldi* were recorded in spinosad treatments, but the majority of these were dead insects (Table 1). The low number of *Tx. theobaldi* recorded from the temephos treatment were all dead insects in the wet season. Examination of MANOVA standardized canonical coefficients (Table 2), indicating the magnitude of the contribution made by each variable to the overall correlation present in the model, revealed that the number of living and dead *Tx. theobaldi* and the sampling effort were the most influential variables in generating the observed differences between treatments. In contrast, the contribution of the number of mosquito larva prey available to canonical coefficient values was unimportant. Multiple comparison of treatments revealed that these effects could be grouped into two categories: control and *Bti* treatments differed significantly from the spinosad and temephos treatments, with the exception of the comparison of *Bti* and temephos, which was not significant following Bonferroni correction ($\alpha = 0.005$). The sampling effort was identified as a defining variable suggesting that the presence or absence of living and dead *Tx. theobaldi* in experimental containers was dependent on the size of the population, i.e. it was more likely to detect treatment differences when this species was common than when it was rare.

### 3.3 Insecticide effects on oviposition and egg eclosion

Egg counts were consistently higher in cemetery 2 than in cemetery 1 in both the wet season and the dry season (Table 3). Total egg counts in each treatment were in the range 799–1442 at site 1 and 956–2707 at site 2. Egg counts increased by approximately 30–50% between the dry season and the wet season, which was significant at both site 1 ($F_{1,194} = 8.2$, $P = 0.005$) and site 2 ($F_{1,194} = 27.5$, $P < 0.0001$). However, egg counts did not differ significantly between treatments at either site after correction for multiple comparison procedures (site 1: $F_{4,194} = 0.79$, $P < 0.53$; site 2: $F_{4,194} = 2.45$, $P = 0.05$).

During the dry season, a total of 1562 (31.7%) and 2223 (25.2%) eggs were found to have hatched in the period between the preceding and the current sample date at sites 1 and 2 respectively (Table 3). In contrast, overall percentage of egg hatch across all treatments was 34.1% at site 1 and 30.9% at site 2 and was significantly higher than that observed during the dry season at both sites 1 ($F_{1,194} = 8.18$, $P = 0.005$) and 2 ($F_{1,194} = 11.52$, $P = 0.001$). However, percentage of egg hatch did not differ significantly between treatments at either site (site 1: $F_{4,194} = 0.68$, $P = 0.6$; site 2: $F_{4,194} = 1.8$, $P = 0.13$).

## 4 DISCUSSION

A suspension concentrate formulation of spinosad and a mineral-based granular formulation of temephos were both highly effective at preventing the development of the aquatic stages of *Ae. aegypti* and *Ae. albopictus* in water containers located in two types of cemetery habitat in southern Mexico, a region where dengue fever is endemic. The duration of complete absence of mosquito aquatic stages provided by these compounds varied from 5 to 13 weeks for the spinosad treatments and from 6 to 9 weeks for the temephos treatment, depending on site, season and product concentration, whereas a *Bti*-based product provided just 1–2 weeks of larvicidal activity.

As in other tropical and subtropical regions of the world,[20] the warm humid climate, plentiful nectar sources, an abundance of water-filled flower vases, shade trees and tomb structures make the cemeteries of southern Mexico ideal habitats for the development

**Table 2.** Multivariate analysis of variance of the presence of *Toxorhynchites theobaldi* in insecticide-treated oviposition traps (data pooled for both sites and seasons)[a]

| | Variables | | | |
|---|---|---|---|---|
| Source, comparison | *Tx.* alive | *Tx.* dead | Prey items | Sampling effort |
| Standardized canonical coefficients | −0.4824 | −1.6128 | −0.0824 | 1.3372 |
| | Pillai's trace | *F*-value | df | *P** |
| Experiment | 0.801830 | 3.01 | 16, 192 | **0.0002** |
| Control versus *Bti* | 0.095248 | 1.18 | 4, 45 | 0.3306 |
| Control versus 1 mg $L^{-1}$ spinosad | 0.464855 | 9.77 | 4, 45 | <**0.0001** |
| Control versus 5 mg $L^{-1}$ spinosad | 0.427650 | 8.41 | 4, 45 | <**0.0001** |
| Control versus temephos | 0.334945 | 5.67 | 4, 45 | **0.0009** |
| *Bti* versus 1 mg $L^{-1}$ spinosad | 0.311365 | 5.09 | 4, 45 | **0.0018** |
| *Bti* versus 5 mg $L^{-1}$ spinosad | 0.332029 | 5.59 | 4, 45 | **0.0010** |
| *Bti* versus temephos | 0.237124 | 3.50 | 4, 45 | 0.0144 |
| 1 mg $L^{-1}$ versus 5 mg $L^{-1}$ spinosad | 0.126535 | 1.63 | 4, 45 | 0.1833 |
| 1 mg $L^{-1}$ spinosad versus temephos | 0.130349 | 1.69 | 4, 45 | 0.1698 |
| 5 mg $L^{-1}$ spinosad versus temephos | 0.062543 | 0.75 | 4, 45 | 0.5629 |

[a] Critical level of significance corrected for multiple comparisons by Bonferroni procedure ($\alpha = 0.005$) shown in bold type.

**Table 3.** Total egg counts and percentage of egg hatch from oviposition traps treated with different insecticides at two cemetery sites during the dry and wet seasons

| | Cemetery 1 | | Cemetery 2 | |
|---|---|---|---|---|
| Season, treatment | Total | Eclosion (%) | Total | Eclosion (%) |
| *Dry season* | | | | |
| Control | 1442 | 32.7 | 2707 | 29.6 |
| *Bti* | 729 | 31.4 | 2155 | 24.1 |
| 1 mg $L^{-1}$ spinosad | 902 | 30.6 | 1340 | 21.9 |
| 5 mg $L^{-1}$ spinosad | 799 | 27.0 | 956 | 23.1 |
| Temephos | 1003 | 36.8 | 1412 | 27.3 |
| | $\sum = 4875$ | Mean: 31.7 | $\sum = 8570$ | Mean: 25.2 |
| *Wet season* | | | | |
| Control | 1441 | 41.3 | 1827 | 32.7 |
| *Bti* | 1729 | 34.6 | 2390 | 24.9 |
| 1 mg $L^{-1}$ spinosad | 1670 | 36.5 | 2757 | 31.9 |
| 5 mg $L^{-1}$ spinosad | 1854 | 26.2 | 1913 | 34.4 |
| Temephos | 1455 | 32.0 | 3227 | 30.6 |
| | $\sum = 8149$ | Mean: 34.1 | $\sum = 12114$ | Mean: 30.9 |

of *Ae. albopictus* and *Ae. aegypti*. In terms of duration of absolute control and average numbers of larvae + pupae observed in oviposition traps, granular temephos and spinosad suspension were both effective as larvicides in periurban and semi-rural cemetery sites. Both these insecticides provided absolute control for periods that were equal or several weeks longer in the semi-rural cemetery 2 compared with the periurban cemetery 1. The reasons for this are unclear but may be related to differences in solar UV exposure or precipitation between the sites. Greater UV exposure at cemetery 1 seems unlikely given that this site had been planted with many trees and shrubs and had a greater total vegetation cover than the semi-rural cemetery 2. The abundance of shade trees is likely to reduce oviposition trap exposure to UV radiation which can degrade both spinosad[15] and temephos.[21] The alternative possibility of rainfall-related differences in the rate of dilution of insecticide treatments applied to oviposition traps may be more likely given that site 1 was located by the foothills of a volcano and ∼200 m higher than site 2 which was located at ∼20 m above sea level. Rainfall tends to start earlier and in greater amounts with increasing altitude in this region, although meteorological data to support this idea were not available for site 2.

The authors observed important seasonal differences in the abundance of *Ae. albopictus* that suggest that this species could be of greater public health importance during the wet season in southern Mexico; a finding that deserves further study. The decline of this mosquito during the dry season is likely due to the poor ability of its eggs to withstand desiccation,[22] whereas during the wet season populations can recover quickly and exploit food resources more efficiently than *Ae. aegypti*,[23] and may, on occasion, competitively exclude *Ae. aegypti* from certain localities.[24]

The performance of spinosad as a larvicide differed according to season. The product lost efficacy at both sites more rapidly in the wet season than in the dry season, presumably owing to rainfall, which is torrential and accompanied with strong winds, and which may have diluted the active ingredient in the spinosad-treated containers, in spite of their sheltered locations. In contrast, the concentration of temephos in containers was less likely to have been affected by wind-blown rainfall owing to the sustained-release nature of the granular formulation, which continues to liberate active ingredient during periods in which the insecticide present in solution may have become diluted. The granular formulation of temephos has an established record as an effective larvicide against *Ae. aegypti* in many parts of the world, and its larvicidal activity usually remains high for periods of 2–3 months post-treatment.[25–28] One of the main findings of the present study is that temephos granules were highly effective against *Ae. albopictus* and are likely to prove valuable in the control of this species in many parts of the world. However, the incidence of resistance to this insecticide is increasing worldwide,[8,29,30] so that the use of temephos should form one part of a larger integrated strategy of dengue vector control if it is to remain of value as a larvicidal product.

The bacterial insecticide *Bti* is toxic to both *Ae. aegypti* and *Ae. albopictus*[31,32] and has been used in control programs targeting vectors of dengue.[33] However, in the present study, *Bti* provided only a brief period of control of mosquito aquatic stages. This is likely due to the rapid degradation of this product in the environment, particularly when exposed to sunlight.[34] To overcome this, mosquito larviciding programs often apply *Bti* in tablet, granule or briquette formulations which provide sustained release of the insecticide over a period of weeks.[33] The main constraint to increased use of *Bti* in developing countries with limited public health budgets is its greater cost compared with cheap chemical alternatives such as temephos.

Spinosad has rapidly attracted attention as a potential mosquito larvicide[13] since the first laboratory and semi-field demonstration of its larvicidal effects in 2004.[14] Spinosad has a number of advantages for mosquito control, not least its unique mode of action and its very low toxicity to mammals, low toxicity to fish and selective toxicity to invertebrates.[11,12] It has been pointed out that the suspension formulations designed for agricultural use, such as those used here, may not be the most suitable for use in mosquito control programs and may underrepresent spinosad's true potential as a larvicide.[13] Accordingly, specialized formulations have been developed and are being commercialized for mosquito control. Widescale testing of these new formulations is required to establish their effectiveness across a range of habitats and the geographical variation in susceptibility to spinosad in different mosquito populations and across a range of species. That said, the present study represents the first evidence that spinosad is an effective larvicide against *Ae. albopictus* under natural conditions.

Larval mortality of the predatory mosquito *Tx. theobaldi* was observed in both spinosad and temephos treatments. This may have been due to the toxicity of these compounds to the developing predators, or due to the contamination of their prey with insecticide residues. Alternatively, many of the predatory larvae may have died in spinosad and temephos treatments owing to a paucity of prey items in these treatments. This issue requires further study.

The influence of different insecticides on the oviposition behavior of mosquitoes is relevant to their use in control programs, as gravid females will likely avoid oviposition in containers treated with substances that are repellent to mosquitoes. In this respect, oviposition and egg hatching were not reduced in containers treated with spinosad, *Bti* or temephos. Oviposition site selection in mosquitoes is influenced by a diversity of olfactory, chemical and visual factors[35] that can be exploited for mosquito management purposes.[36,37] Previous studies have reported no repellent effects of concentrations of 5 and 20 mg $L^{-1}$ spinosad against *Ae. aegypti*.[15] Similarly, granular formulations of temephos are not repellent to ovipositing females,[38] whereas suspensions of *Bti* may even be attractive to certain species.[39] However, as observed previously,[15] spinosad residues that contaminated the oviposition substrate were sufficient to kill a high proportion of newly hatched larvae, resulting in low numbers of insects from the spinosad treatments in the present laboratory rearing studies, whereas no such effects were observed in the temephos treatment.

In conclusion, the present results provide strong support for previous studies in which spinosad was shown to possess effective larvicidal activity against *Ae. aegypti*. These findings have been built upon by demonstrating that the suspension concentrate formulation of spinosad and a granular sustained-release formulation of temephos were both effective in preventing the development of *Ae. albopictus* in tropical cemetery conditions of southern Mexico, whereas a suspension of *Bti* rapidly lost activity. The larvicidal activity of spinosad and temephos against *Ae. albopictus* was maintained even during the wet season, when populations of this vector increased markedly. The authors believe that both these compounds are likely to prove useful in integrated vector management programs targeted at *Ae. aegypti* and *Ae. albopictus*. Confirming this belief will require detailed additional studies on the efficacy, cost effectiveness and impact on non-target organisms of each of these insecticides in the regions where these mosquitoes represent important vectors of human diseases.

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