Spinosad, a Naturally Derived Insecticide, for Control of *Aedes aegypti* (Diptera: Culicidae): Efficacy, Persistence, and Elicited Oviposition Response

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ABSTRACT The naturally derived insecticide spinosad is a reduced-risk material that is neurotoxic to Diptera. The 24-h 50% lethal concentration by laboratory bioassay in third instars of *Aedes aegypti* (L.) (Diptera: Culicidae) (Rockefeller strain) was estimated at 0.026 ppm. Two identical field trials were performed in an urban cemetery in southern Mexico during the dry and wet seasons. Water containers treated with 1 or 5 ppm spinosad suspension concentrate (Tracer, Dow Agrosciences) were as effective in preventing the development of *Aedes* spp. (mostly *Ae. aegypti*) as temephos granules during both trials, whereas the bacterial insecticide VectoBac 12AS performed poorly. The half-life of aqueous solutions of spinosad (10 ppm) placed in a warm sunny location was 2.1 d, compared with 24.5 d for solutions in a shaded location. Spinosad, temephos, and VectoBac were not repellent to gravid *Ae. aegypti* at the concentrations tested, and no ovicidal properties were observed. The 24-h survival of neonate larvae but was reduced by 94–100% in the presence of residues carried over from the spinosad treatments, but it was not affected by residues of temephos or VectoBac. The toxicological properties of spinosad, combined with its favorable environmental profile, should encourage the detailed evaluation of spinosad as a mosquito larvicide in domestic and urban environments.

KEY WORDS *Aedes aegypti*, half-life, larvicide, oviposition response, spinosad

Current measures for control of *Aedes aegypti* (L.) (Diptera: Culicidae), the principal vector of dengue and yellow fever in Mexico and many other countries of Latin America, are based on the physical elimination of larval development sites and the application of organophosphate granules (temephos) to domestic and urban water sources. Specific outbreaks of vector-borne disease also are controlled by space spraying of urban areas with pyrethroid insecticides (Secretaría de Salud 2003). As an alternative to the use of temephos, biological larvicides have been developed based on the bacterial endotoxin of *Bacillus thuringiensis israelensis* de Barjac (Bti), in liquid or slow-release pellet or briquette formulations. Such products clearly present a low risk to human health (Becker and Margalit 1993), but they often have low persistence and can be too expensive for wide-scale use in developing countries (Federici et al. 2003).

The naturally derived insecticide spinosad (Dow Agrosciences LLC) represents a new generation of biorational products developed for the agricultural industry that have a reduced spectrum of toxicity compared with the synthetic insecticides that were developed previously (Williams et al. 2003). Spinosad is a mixture of two neurotoxic macrolide compounds: spinosyn A and spinosyn D that are active mainly by ingestion. Spinosyns are produced by fermentation of the actinomycete *Saccharopolyspora spinosa* Mertz and Yao isolated from a Caribbean soil sample (Bret et al. 1997). Spinosyns A and D are highly toxic to Diptera, Lepidoptera, Thysanoptera, and some species of Coleoptera, but they have extremely low toxicity for mammals; therefore, spinosad is classified by the U.S. Environmental Protection Agency as a reduced-risk material (Thompson et al. 2000). Spinosad acts on the postsynaptic nicotinic acetylcholine and GABA receptors, resulting in tremors, paralysis, and death.

Spinosad was shown to be highly toxic to *Ae. aegypti* and *Anopheles albimanus* Weidemann in the laboratory, and it completely suppressed the development of *Ae. aegypti*, *Culex* spp., and chironomid larvae in semi-natural field conditions for periods of 8 to >22 wk, depending on concentration (Bond et al. 2004). Additional studies have reported the larvicidal properties of spinosad in this and other mosquito species (Liu et al. 2004a, 2004b; Cetin et al. 2005; Darriet et al. 2005; Darriet and Corbel 2006; Romi et al. 2006), or as an adulticide in a sugar bait formulation (Müller and Schlein 2006).

The suitability of larvicidal compounds for use in insect vector control programs depends on a variety of properties and characteristics, including their persis-

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tence in the environment and the behavioral responses of insect vectors that come into contact with the compound. The aim of the current study was to evaluate the efficacy of spinosad as a larvicide of *Ae. aegypti* in an important urban habitat (Vezzani 2007), namely, the water-filled containers used for flowers in a local cemetery in southern Mexico. We also examined the persistence of spinosad solutions exposed to sunlight or shaded conditions and compared the oviposition responses of gravid females and progeny survival when exposed to solutions of spinosad, temephos, and Bti.

Materials and Methods

Mosquitoes and Insecticides. Eggs and adults of *Ae. aegypti* (insecticide-susceptible Rockefeller strain) were obtained from a laboratory colony maintained in the Centro Regional de Investigación en Salud Pública, Tapachula, Chiapas, Mexico. This colony was maintained at 27 ± 1°C, 70 ± 10% RH, and a photoperiod of 12:12 (L:D) h, by using powdered rabbit food as the larval diet, whereas adults were allowed to feed on restrained rabbits.

Spinosad was obtained in a suspension concentrate formulation (Tracer Naturalyte Insect Control, Dow Agrosciences LLC, Indianapolis, IN) containing 480 g/liter active ingredient (AI). Bti was obtained as a liquid concentrate formulation (VectoBac 12AS, Valent BioSciences Corp., Libertyville, IL) containing 1,200 international toxicity units (ITU) per mg. Temephos was obtained as a sand granule formulation containing 1% (wt:wt) (AI) that is used extensively in Mexico and was supplied by the Secretaría de Salud (Mexico City, Mexico).

Laboratory Bioassay. To confirm that mosquito susceptibility to spinosad and technical procedures were in agreement with those reported previously by our laboratory (Bond et al. 2004), a bioassay was performed using a methodology adapted from the Elliot larval test (WHO 1975). Briefly, groups of 25 late third and early fourth instars of *Ae. aegypti* were placed in 150-ml plastic cups containing a solution of spinosad at one of the following concentrations: 0.001, 0.003, 0.01, 0.03, and 0.1 ppm (AI), where 1 ppm is equivalent to 1 mg (AI)/liter. Four groups of larvae were assigned to each treatment. An additional cup containing dechlorinated tap water was used as a control. After 1-h exposure, larvae were transferred to cups containing 100 ml of untreated dechlorinated water. Approximately 2 to 3 mg of powdered soybean and yeast was added to each cup as food. Mortality responses were recorded 24 h later. A larva was classified as dead if it did not move when gently touched with the point of a toothpick. The assay was performed four times on different dates. Results were subjected to logit regression in the Generalized Linear Interactive Modeling (GLIM) program with a binomial error distribution specified (Numerical Algorithms Group 1993). The error distribution was scaled to account for overdispersion in mortality results.

Field Trials in a Cemetery. The efficacy of spinosad for control of *Ae. aegypti* was evaluated in a local cemetery, representing a typical urban habitat for the development of this species. For this, black plastic containers (10 cm in diameter and 20 cm in height) were placed in sheltered positions beside tombs and graves over an area of 330 by 150 m in a municipal cemetery in the town of Tapachula (14° 54′ N, 92° 16′ W), Chiapas, Mexico, at an altitude of ≈160 m above sea level. One liter of one of the following five treatments was placed in each container: 1) 1 ppm spinosad, 2) 5 ppm spinosad, 3) 1 μl of VectoBac (≈1,200 ITU), 4) 0.1 g of temephos granules contained inside a multiply-perforated 1.5-ml plastic microcentrifuge tube to facilitate handling, and 5) dechlorinated water control. The concentrations of VectoBac and temephos represent recommended field rates, whereas the chosen concentrations of spinosad had proved effective in previous field studies in seminatural conditions (Bond et al. 2004). Twenty containers, each representing a single replicate, were prepared for each treatment and distributed among 20 rows consisting of five containers. The distance between containers within a row was 20–25 m (depending on the presence of a suitable tomb or grave), whereas the distance between rows was ≈15 m.

Each container was carefully inspected at weekly intervals. All living aquatic insects were counted and removed, including any that were dead at the time of sampling. Immature mosquitoes were classified visually to genus, and other aquatic insects were classified to family. Subsamples of mosquito larvae and pupae were taken to the laboratory for rearing to confirm identifications made in the field. Water lost through evaporation was replaced with clean dechlorinated water. The experiment began on 13 March 2006, toward the end of the dry season, and was terminated 14 wk later at the beginning of the rainy season. The entire experiment was repeated beginning on 3 July 2006, halfway through the rainy season, and it was terminated 14 wk later at the end of the rainy season. These trials are referred to as dry season and wet season experiments. Meteorological data for each experimental period were obtained from a Health Enviromonitor digital weather logger (Davis Instruments Corp., Hayward, CA) located on the grounds of ECOSUR 2.2 km away from the municipal cemetery.

The numbers of larvae and pupae of *Ae. aegypti* counted weekly were pooled into 2-wk intervals to meet the requirements for sphericity of the variance-covariance matrix for repeated measures mixed model analysis of variance (ANOVA) with a general correlation matrix structure specified (Brown and Prescott 1999). The suitability of this model was verified by inspection of residuals. Other mosquito or chironomid species were rarely observed in containers and were not subjected to statistical analysis. Containers that were lost or were tampered with by members of the public (e.g., used as flower vases) during the course of the experiment were considered as missing data points. Data from the second trial were normalized by $\ln(x + 1)$ transformation before mixed model ANOVA

with a Toeplitz correlation structure specified. The suitability of this model was verified by inspection of residuals. To control the increased probability of type I errors during multiple comparisons, treatment means were compared by least significant difference (LSD) tests with a Bonferroni-corrected critical value of $\alpha = 0.005$ instead of the usual $\alpha = 0.05$ (Sokal and Rohlf 1981).

Persistence of Spinosad in Sunny and Shaded Conditions. The half-life of spinosad in aqueous habitats in sunny and shaded situations was estimated in an experiment using a solution of 10 ppm spinosad in distilled water. One-liter volumes of the solution were placed in circular brown plastic bowls (18.5 cm in diameter and 9 cm in height) with a total capacity of 1.5 liters, and they were subjected to one of the following treatments: 1) full exposure to the sun in an open area outside the laboratory, 2) shaded conditions on the bench of a shack with a galvanized sheet metal roof supported by metal struts (without walls) located outside the laboratory, or 3) darkened section of the laboratory at a temperature of 25 ± 2°C. Each treatment involved four fully independent replicates, i.e., the solution from each bowl was sampled only once.

The toxicity of spinosad in each treatment was determined at the start of the experiment (day 0) and after 1-, 2-, 5-, 10-, 20-, 30-, 60-, and 90-d exposure to each treatment. For this, each exposed sample was diluted to produce four concentrations between 1.0 and 0.003 ppm (designed to span the estimated LC_{50} concentration) that were used in insect bioassays, described above. Groups of 30 third and fourth instars of *Ae. aegypti* were exposed to each solution for 1 h, transferred to clean water, and fed with powdered diet. Mortality was evaluated 24 h later. Control larvae were treated identically, but they were exposed to dechlorinated water alone. The LC_{50} value for each replicate was estimated by the proportional distance method of Reed and Muench (1938) by using the formula log (50% lethal concentration) $= A + (\log h \times D)$, where A is log (dilution above 50% mortality), h is the dilution factor (3.3 in our bioassay), and D is the interpolated 50% mortality concentration calculated from the proportional distance formula $D =$ (% mortality above 50% − 50%) / (% mortality above 50% − % mortality below 50%). This method was chosen over probit analysis, because we were only interested in major changes in spinosad toxicity over time and because the proportional distance method only requires that one dilution tested results in >50% mortality and one dilution results in <50% mortality, representing an important reduction in the logistical requirements (e.g., number of larvae, setup times) of each bioassay, and a corresponding increase in the number of samples that could be processed simultaneously. The proportion of original toxicity remaining in each sample was calculated by dividing the estimated LC_{50} by the initial LC_{50} value at the start of the experiment (day 0).

Proportional values were subjected to linear regression against duration of exposure or against the cumulative dose of UV-A and UV-B radiation (wavelength 290–390 nm) measured by the Health Enviromonitor weather station located beside the plastic bowls exposed to direct sunlight. Water temperatures were measured daily using a laboratory thermometer at 1400 hours, the warmest time of the day. No rainfall occurred during the period in which spinosad solutions were exposed to direct sunlight, whereas the metal roof of the shack protected bowls placed in the shaded treatment during periods of rainfall late in the experiment.

Oviposition Response and Ovicidal Properties. Gravid nulliparous 5–6-d-old *Ae. aegypti* that had fed on rabbits 2–3 d previously were placed in groups of 20 in wood-framed cages (50 by 50 by 50 cm) with nylon mesh walls. Each cage contained two plastic cups (11 cm in diameter by 7.5 cm in height with a capacity of 500 ml containing 350 ml of dechlorinated water) and two identical cups containing a similar volume of one of the following treatments: 1) 5 ppm spinosad, 2) 20 ppm spinosad, 3) 35 mg of temephos granules (equivalent to 0.1 g/liter), and 4) 0.35 μl of VectoBac (equivalent to 1 μl/liter). The highest concentration of spinosad was chosen to increase the likelihood of detecting an ovipositional response or ovicidal effect in this treatment. A filter paper strip (3 cm in width by 35 cm in length) was placed around the inner circumference of each cup as an oviposition substrate. Control and treatment cups were placed in opposing corners of the cage. Four cages, one cage from each treatment, were placed outside the laboratory under the roof of the shack described in the previous experiment. The experiment commenced at 1700 hours, and 60 min later, the number of mosquitoes resting on the inside or on the lip of each cup was recorded. Cages remained undisturbed for 24 h after which time the paper strips were removed, and number of eggs was counted under a binocular microscope. One hundred eggs were selected randomly at five to six locations along the length of each paper strip and placed in plastic trays containing dechlorinated water and powdered diet. The number of eggs that hatched and the number of living larvae was recorded 3 d later. The entire experiment was performed on 12 occasions during the rainy season (6 July–1 September 2006).

The average number of eggs per cup and average number of mosquitoes observed inside, or on the lip, of each cup 60 min after the start of the experiment were subjected to paired t-test for each treatment and its respective control. Percentage of egg hatch and larval survival could not be normalized by arcsine or $\log(x + 1)$ transformation; thus, each treatment and control was compared using the nonparametric Mann–Whitney test (Sokal and Rohlf 1981).

Results

Laboratory Bioassay. The LC_{50} of spinosad was estimated at 0.026 ppm (95% CL, 0.022–0.031; scale parameter, 3.7) with a slope ± SE of 1.832 ± 0.152.

Cemetery Field Trials. In the dry season experiment, 2,814 aquatic insects in total were observed in

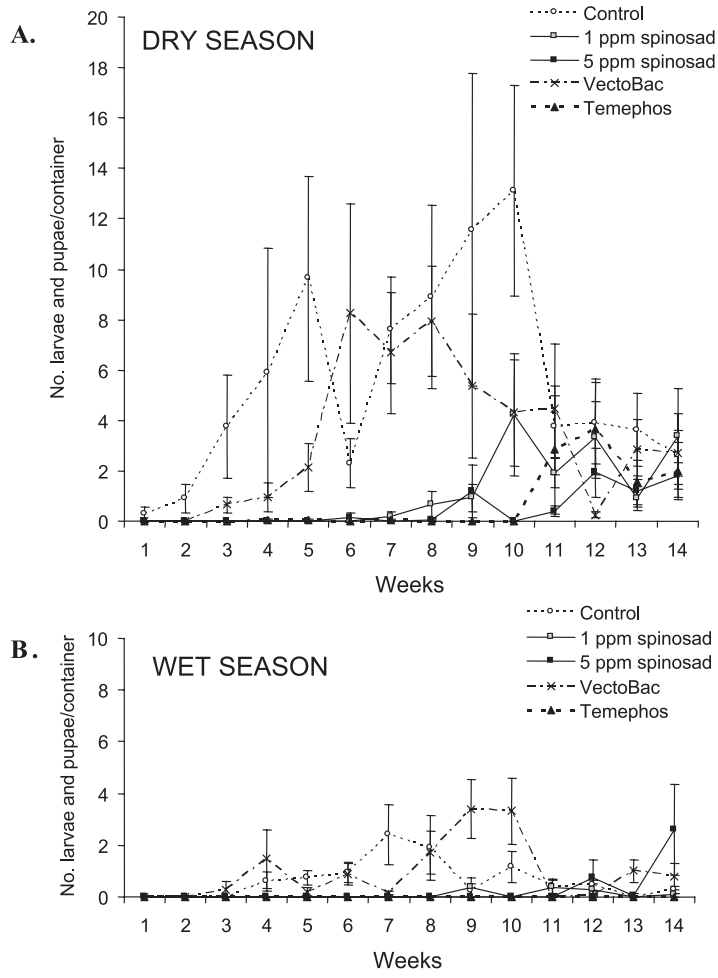

Fig. 1. Average number of larvae and pupae of *Aedes* spp. observed in water containers placed in a cemetery during 14-wk periods of (A) dry season (B) rainy season in southern Mexico. Vertical bars indicate SEM.

water containers, of which 93% were larvae and pupae of *Aedes* species. The remaining collection was comprised of *Toxorhynchites theobaldi* (Dyar & Knab) (3.4%), chironomid species (2.3%), *Culex* spp. (1.2%), and *Pantala* spp. (0.07%). Identification of laboratory-reared samples of collected *Aedes* larvae ($n = 775$) indicated that 94.4% were *Ae. aegypti* and 5.6% were *Ae. albopictus* (Skuse).

The average number of larvae and pupae observed in control containers rose steadily from 1 to 10 wk after the start of the trial but decreased from week 11 when the rainy season began (Fig. 1A). Before week 11, average weekly precipitation was 9.8 ± 4.2 mm, whereas from week 11 onward average weekly precipitation was 88.2 ± 25.0 mm. Insecticides had a significant effect on the numbers of developing *Aedes* spp. (time × treatment interaction: $F_{24, 95} = 6.36$; $P < 0.001$).

Applications of 1 and 5 ppm spinosad or temephos resulted in complete control of mosquito development for 6, 8, and 10 wk, respectively (Fig. 1A). The average number of mosquitoes observed over the course of the experiment did not different significantly among these three treatments (Table 1). In contrast, VectoBac resulted in complete control of mosquito development for 2 wk, but it did not differ significantly from the control in any sample or taken as an average over the entire period of the experiment (Table 1). Chironomid larvae seemed to be particularly sensitive to spinosad, and they were never observed developing in the 5 ppm spinosad treatment.

In the wet season experiment, the total number of developing mosquitoes ($n = 527$) was less than observed in the dry season trial. Of these, 89% were *Aedes* spp. and the remaining 11% were *Tx. theobaldi.* Laboratory rearing of collected larvae, most of which were early instars, resulted in high levels of mortality so that a reliable estimate of the proportion of each *Aedes* species present was not possible. *Culex* spp. or chironomid larvae were not observed in any treatment. Insecticides had a significant effect on the numbers of developing *Aedes* spp. (time × treatment interaction: $F_{24, 470} = 5.25$; $P < 0.001$).

Table 1. Differences between mean of number of *Aedes* spp . (combined larvae and pupae) observed in different treatments applied to water containers in an urban cemetery in trials performed during the dry and wet seasons in southern Mexico

Treatment comparison	Difference between means	SE of difference	df	*t* value
Dry season trial				
Control vs. 1 ppm spinosad	1.44	0.26	95	5.45***
Control vs. 5 ppm spinosad	1.80	0.26	95	6.98***
Control vs. VectoBac	0.32	0.27	95	1.21
Control vs. temephos	1.84	0.26	95	7.16***
1 ppm spinosad vs. 5 ppm spinosad	0.35	0.27	95	1.32
1 ppm spinosad vs. VectoBac	−1.12	0.28	95	−4.07***
1 ppm spinosad vs. temephos	0.39	0.27	95	1.47
5 ppm spinosad vs. VectoBac	−1.47	0.27	95	−5.50***
5 ppm spinosad vs. temephos	0.04	0.26	95	0.15
VectoBac vs. temephos	1.51	0.27	95	5.66***
Rainy season trial				
Control vs. 1 ppm spinosad	0.32	0.08	95	3.74***
Control vs. 5 ppm spinosad	0.28	0.08	95	3.38***
Control vs. VectoBac	−0.10	0.08	95	−1.22
Control vs. temephos	0.38	0.08	95	4.44***
1 ppm spinosad vs. 5 ppm spinosad	−0.03	0.09	95	−0.39
1 ppm spinosad vs. VectoBac	−0.42	0.09	95	−4.91***
1 ppm spinosad vs. temephos	0.06	0.09	95	0.67
5 ppm spinosad vs. VectoBac	−0.39	0.09	95	−4.56***
5 ppm spinosad vs. temephos	0.09	0.09	95	1.07
VectoBac vs. temephos	0.48	0.09	95	5.61***

Numbers of larvae and pupae per container were pooled over 14-d intervals for mixed model analysis of variance.
*** $P \leq 0.001$ (the Bonferroni-corrected critical value was $\alpha = 0.005$).

The average number of *Aedes* spp larvae and pupae per container fluctuated between 0 and 2.4 in the control over the course of the study (Fig. 1B). The average weekly precipitation during the experimental period was 77.8 ± 21.1 mm. Control of immature stages was very similar to that observed in the dry season trial. Spinosad applied at 1 or 5 ppm completely controlled mosquito development for 8 and 11 wk, respectively. Temephos completely controlled development for 14 wk, whereas VectoBac completely eliminated mosquito development for 2 wk. The overall average number of larvae and pupae did not differ significantly among spinosad or temephos treatments (Table 1). The treatment involving VectoBac did not differ significantly from the control.

Persistence of Spinosad in Sunny and Shaded Conditions. Spinosad solutions placed in direct sunlight experienced a rapid exponential loss of toxicity (Fig. 2A). After 20-d exposure, the proportion of original toxicity remaining (OAR) was 0.003, equivalent to a 99.7% loss of toxicity or a half-life of 2.1 d. The mean air temperature during this period was 28.6 ± 0.3°C, ranging from a daily maximum average of 34.3 ± 0.19°C to a daily minimum average of 23.8 ± 0.21°C. Maximum solution temperatures registered at 1400 hours averaged 38.45 ± 0.56°C in this treatment. The rate of toxicity loss in solutions placed in the shade was considerably less than that in the sun, with a proportion of 0.08 OAR after 90 d, equivalent to a 92% loss of toxicity, representing a half-life of 24.5 d. The average maximum daily temperature of the solution maintained in the shade was 29.5 ± 0.19°C. In contrast, spinosad solutions held in shaded conditions the laboratory at 25°C retained 50% of the OAR after 90 d.

A comparison of OAR against cumulative dose of UV incident radiation showed a clear negative exponential relationship ($r^2 = 0.945$) (Fig. 2B). Overall, spinosad solutions placed in warm sunny locations lost toxicity ≈10-fold faster than equivalent solutions placed in shaded conditions.

Oviposition Response and Ovicidal Properties. The number of female *Ae. aegypti* observed visiting treatment cups 60 min after the start of the experiment was significantly greater in the 20 ppm spinosad treatment compared with its control (Fig. 3), whereas the number of females visiting the cups of the remaining insecticide treatments did not differ significantly from their respective controls. The number of eggs laid in each cup during the 24-h exposure period and egg hatch did not differ significantly between control and treatments for any of the insecticides tested (Table 2). However, the number of larvae that survived 72 h posthatching was severely reduced in the 5 ppm spinosad treatment compared with its control and was completely eliminated in the 20 ppm spinosad treatment, presumably due to the presence of spinosad residues on the filter paper used as an oviposition substrate. In contrast, 72-h survival of larvae that hatched from filter papers exposed to the VectoBac (89%) and temephos (79%) treatments was high but did not differ from that of their respective controls.

Discussion

Our results showed that spinosad is highly toxic to *Ae. aegypti* in agreement with previous studies in our laboratory (Bond et al. 2004), and by others that tested this species (Darriet et al. 2005, Darriet and Corbel

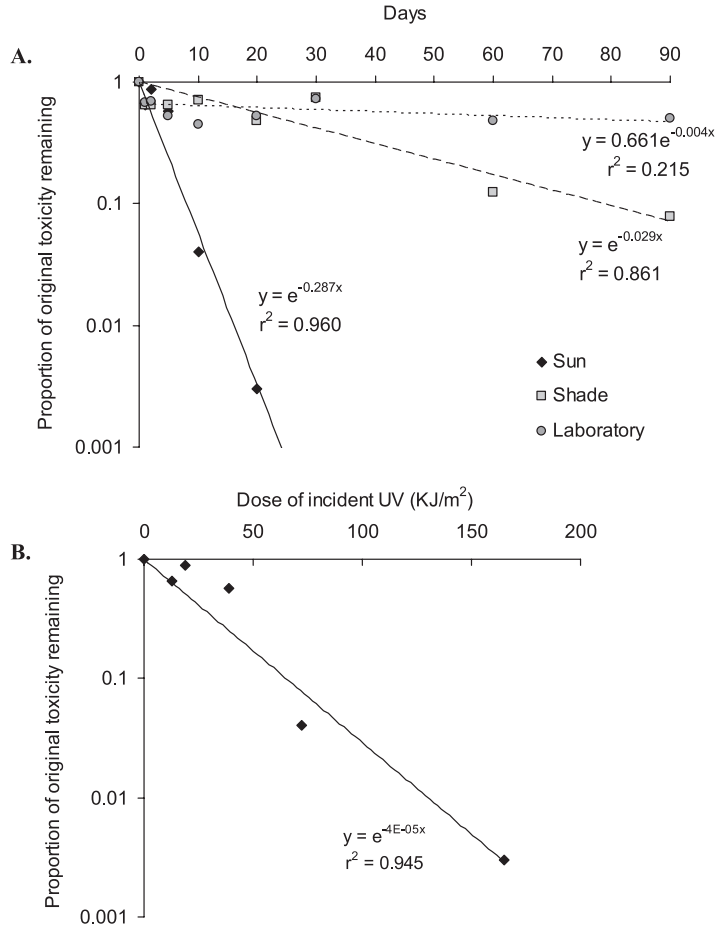

Fig. 2. Linear regression of proportion of original toxicity remaining (A) over time in spinosad solutions placed in direct sunlight (solid line), in shaded conditions (dashed line) or in the laboratory (dotted line) in southern Mexico. (B) Correlation between proportion of original toxicity remaining and cumulative dose of solar UV in the treatment involving exposure to sun.

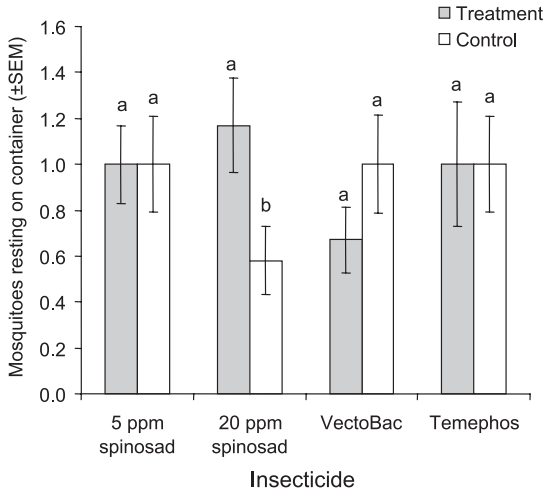

Fig. 3. Mean number of female *Ae. aegypti* observed resting on insecticide treatment or control cups 60 min after the start of the experiment.

2006, Romi et al. 2006), and other mosquito species (Cetin et al. 2005), including populations with known resistance to synthetic insecticides (Liu et al. 2004b).

In our field trials, both Tracer applications performed similarly to temephos. These results compare favorably to those of Bond et al. (2004). In contrast, spinosad treatment of septic tanks in Turkey at rates of 25–200 g (AI)/ha provided control of *Culex pipiens* L. for just 1 or 2 wk, presumably due to continuous dilution and high levels of microbial degradation in such environments (Cetin et al. 2005).

In evaluating the persistence of spinosad in sunny and shaded locations, it was not possible to separate the effect of UV radiation and the heating effect of infrared (IR) radiation. In nature, exposure to sunlight is invariably accompanied by both UV and IR irradiation. However, it was clear that spinosad solutions placed in warm, sunny locations lost toxicity ≈10-fold faster than solutions placed in the shade. Because *Ae. aegypti* preferentially oviposits in shaded habitats (Fay and Eliason 1966, Vezzani et al., 2005), the ability

Table 2. Oviposition of caged gravid *Ae. aegypti*, egg hatch, and larval survival from treatments involving different insecticides and a water control

Variable and treatment	Means ± SEM		*P*
	Treatment	Control	
Mean no. of eggs/cup			
5 ppm spinosad	245.6 ± 21.8	273.3 ± 31.1	0.346[a]
20 ppm spinosad	274.4 ± 29.6	241.8 ± 24.1	0.204[a]
VectoBac	236.1 ± 32.6	274.8 ± 19.3	0.170[a]
Temephos	248.6 ± 31.1	255.9 ± 24.6	0.827[a]
Mean prevalence of eclosion (%)			
5 ppm spinosad	37.7 ± 7.5	48.7 ± 6.8	0.225[b]
20 ppm spinosad	41.7 ± 6.2	39.5 ± 5.9	0.839[b]
VectoBac	38.7 ± 6.1	41.2 ± 6.7	0.795[b]
Temephos	39.7 ± 7.8	44.0 ± 8.4	0.686[b]
Mean 72 h survival of hatched larvae (%)			
5 ppm spinosad	5.8 ± 4.8	86.9 ± 8.0	<0.001[b]
20 ppm spinosad	0.0 ± 0.0	92.4 ± 2.5	<0.001[b]
VectoBac	89.3 ± 3.9	94.1 ± 2.8	0.148[b]
Temephos	69.7 ± 9.3	79.5 ± 8.3	0.165[b]

Means were based on 12 replicate cages, each containing 20 gravid females. Samples of eggs from each treatment were placed in clean water and monitored for hatching and survival of larvae.

[a] Significance based on pairwise two-tailed *t*-test. The Bonferroni-corrected critical value was $\alpha = 0.0083$.

[b] Significance based on Mann–Whitney test. The Bonferroni-corrected critical value was $\alpha = 0.0083$.

of spinosad to persist for weeks or months in the shade favors the suppression of mosquito development for periods that extend over the annual peaks of vectorial activity and that often coincide with seasonal fluctuations in rainfall in tropical regions. The insignificant mammalian toxicity and favorable environmental profile of spinosad, involving degradation by photolysis and microbial action (Cleveland et al. 2002, Thompson et al. 2002, Liu and Li 2004), means that bioaccumulation and related ecological problems that arise from persistent xenobiotic compounds are highly unlikely for this product.

High concentrations of organophosphate and pyrethroid insecticides tend to be deterrent for oviposition (Moore 1977, Verma 1986), whereas other compounds, such as methoprene or granular formulations of temephos, are not repellent (Mather and DeFoliart 1983, Beehler and Mulla 1993, Pates and Curtis 2005). In our study, a weak but significant attraction to visit cups containing spinosad was observed at a concentration of 20 ppm but not at 5 ppm. Spinosad has a distinctive aroma of damp earth, characteristic of the presence of actinomycetes, that may have proved attractive to gravid females. However, it did not result in an increase in the number of eggs laid in the spinosad treatments, or any other of the treatments that we tested. This finding could have been influenced by the response of *Ae. aegypti* to conspecific eggs (Allan and Kline 1998) or by the skip oviposition behavior shown by some, but not all populations, of this species (Corbet and Chadee 1993, Harrington and Edman 2001), and the limited possibility to disperse eggs over various oviposition sites in our caged experiments.

None of the insecticides we tested exhibited ovicidal properties. The ovicidal activity of spinosad depends on the target insect, concentration of active ingredient (Adán et al. 1996, Medina et al. 2001, Bloem et al. 2005), and solvent used to apply the compound (Pineda et al. 2004). However, larvae that emerged from eggs collected from the spinosad treatments suffered very high levels of mortality, presumably due to the presence of spinosad residues on the egg surface and the filter paper oviposition substrate, whereas residues of temephos and Bti were insufficient to cause neonate mortality.

Agrochemical retailers in Mexico currently supply Tracer at a cost of US$444 per liter. This would be sufficient to treat 96,000 liters of water with a concentration of 5 ppm (AI) at a cost of US$0.46 per liter. In comparison, the cost of 25 kg of temephos granules bought by the Secretaria de Salud for public health use in Mexico is US$228. This is sufficient to treat 250,000 liters of water at a cost of US$0.09 per liter. Although currently more expensive than temephos treatment, considerable savings could be accrued by the purchase of large quantities of spinosad directly from the manufacturer, making spinosad based mosquito control a feasible option in countries such as Mexico, where limited government budgets necessitate the use of low-cost vector control products.

We conclude that spinosad was as effective as temephos granules in eliminating the immature stages of *Aedes* spp., mostly *Ae. aegypti*, in an urban cemetery during the wet and dry seasons in southern Mexico. Persistence and oviposition response studies indicated that spinosad could retain its insecticidal properties for periods of several months in shaded conditions preferred by *Ae. aegypti* and it was not repellent for mosquito oviposition. The combination of the toxicological properties and favorable environmental profile means that spinosad deserves detailed evaluation as a mosquito larvicide in domestic and urban vector control programs.

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