Research Article

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Paradoxical effects of sublethal exposure to the naturally derived insecticide spinosad in the dengue vector mosquito, *Aedes aegypti*

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Abstract

BACKGROUND: Recent studies have indicated that spinosad, a mixture of two tetracyclic macrolide compounds produced during the fermentation of a soil actinomycete, may be suitable for controlling a number of medically important mosquito species, including the dengue vector, *Aedes aegypti* L. The authors determined the effects of a 1 h exposure to a 50% lethal concentration (LC_{50}) of spinosad in the larval stage on the wing length, longevity and reproductive capacity of the adult survivors.

RESULTS: The LC_{50} of spinosad for a wild-caught population of *Ae. aegypti* from Chiapas, southern Mexico, was estimated to be 0.06 mg AI L^{-1} in late third instars. Paradoxically, the female survivors of exposure to this concentration were significantly larger (as determined by wing length) laid more eggs, but were slightly less fertile than control females. This was probably due to elimination of the smaller and more susceptible fraction of mosquito larvae from the experimental population following spinosad treatment. Male survivors, in contrast, were significantly smaller than controls. No significant differences were detected in the adult longevity of treated and control insects of either sex.

CONCLUSIONS: The increase in reproductive capacity of spinosad-treated females did not compensate for mortality in the larval stage and would be unlikely to result in population increase in this mosquito under the conditions that were employed. Sustained-release formulations would likely assist in minimizing the occurrence of sublethal concentrations of this naturally derived product in mosquito breeding sites.

Keywords: adult longevity; *Aedes aegypti*; reproductive capacity; spinosad; sublethal effect; wing length

1 INTRODUCTION

Chemical-based control represents a key strategy in the management of populations of insect vectors of medical and veterinary importance.[1] However, the need to develop novel products that have a low impact on human health and the environment is well established.[2] The naturally derived insecticide spinosad (Dow Agrosciences LLC, Indianapolis) is produced during fermentation of the soil actinomycete *Saccharopolyspora spinosa* Mertz & Yao. This product has been classified by the United States Environmental Protection Agency as a reduced-risk material owing to its very low mammalian toxicity and favourable ecotoxicological profile.[3]

Spinosad is a mixture of two tetracyclic macrolide neurotoxins, spinosyns A and D, that target the nicotinic acetyl-choline and GABA receptors of the insect's nervous system, leading to paralysis and death. Spinosad is currently used in agriculture to control dipteran, lepidopteran, thysanopteran and some coleopteran pest species in a diversity of crops worldwide. Recent studies have identified spinosad as a potentially valuable tool for control of several important mosquito species,[4–7] including *Aedes aegypti* L.,[4] the mosquito that transmits dengue and yellow fever. Spinosad treatment of water containers in urban habitats prevented the development of *Ae. aegypti* larvae over periods of many weeks.[4,8] Such observations highlight the potential of this product as a biorational larvicide in tropical regions such as Mexico and Central America, where dengue fever represents a public health priority.

As the concentration of the toxicant declines over time, on account of the processes of environmental degradation (mainly photolysis in the case of spinosad), some larvae may be exposed to sublethal concentrations of the toxicant during the course of their development. Sublethal effects on mosquito development, reproduction and longevity have been observed in the insects that survive exposure to synthetic insecticides,[9,10] insect hormone analogues,[11,12] pathogenic microorganisms[13,14] and a number of botanical extracts.[15,16]

In the present study, the consequences of exposing *Ae. aegypti* larvae to a 50% lethal concentration of spinosad were examined. This involved comparing measurements of adult body

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size, reproductive capacity and longevity in the survivors of the spinosad treatment with those of control insects. The authors also examined correlations between the different variables, looking for evidence that sublethal exposure to this naturally derived product could affect mosquito life history parameters of direct relevance to its role as a vector of disease.

2 MATERIALS AND METHODS

2.1 Mosquitoes and spinosad

Adult mosquitoes of *Ae. aegypti* were collected in the wild, mated and maintained in laboratory conditions in the Centro Regional de Investigación en Salud Pública in Tapachula, Chiapas, Mexico. The eggs laid by these females were used in this study. The responses observed in the first laboratory generation therefore represented those of a natural population of this species. Assays were performed in the laboratories of El Colegio de la Frontera Sur, Tapachula, Chiapas, Mexico, from October 2006 to August 2007. Laboratory conditions were 27±0.2 °C and 75±5% RH with a 12 : 12 h light : dark photoperiod. Mosquitoes were reared to adulthood by incubating approximately 4000 eggs in 5 L of dechlorinated tap water in a 20 × 30 × 20 cm plastic tray. Larvae were fed *ad libitum* during the early stages of development with powdered rabbit food before being transferred to experimental recipients. A commercial 480 g L^{-1} spinosad SC (Tracer® Naturalyte Insect Control, Dow Agrosciences LLC, Indianapolis) was purchased locally. Dilutions of this spinosad formulation were prepared in dechlorinated water immediately prior to each experiment. Experimental dilutions were not exposed to sunlight, and excess volumes of each dilution were discarded within 24 h of having been prepared.

2.2 Determination and confirmation of the LC_{50} value

To estimate the LC_{50} value, groups of 25 late third-instar larvae were transferred to 200 mL plastic cups filled with 150 mL of either dechlorinated tap water (control) or one of the following concentrations of spinosad: 0.001, 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0 mg AI L^{-1} in dechlorinated tap water. After 1 h exposure, each group was carefully transferred into new identical cups containing 150 mL of water free of spinosad. The 1 h exposure time was briefer than the 24 h period of exposure currently recommended by the WHO for toxicity tests on insecticidal compounds.[17] However, this period was selected to be consistent with the United States Environmental Protection Agency (EPA) protocol and previously published studies[4,8] based on the long-established Elliot bioassay method.[18] Treated larvae were fed with ∼1 mg of powdered rabbit food every 2 days. At 24 h after exposure, mortality was scored by gently touching every larva with a wood toothpick. Any larvae that did not respond to this stimulus were considered dead. The bioassay, which involved two replicate groups of 25 insects per concentration, was performed on six different dates, giving a total of 300 insects per concentration. The results of concentration–mortality assays were subjected to logit regression in GLIM.[19] Overdispersion in the mortality results was taken into account by scaling the error distribution.[20]

To confirm the reproducibility of the results, groups of 25 late third instars were exposed to dechlorinated water (control) or a single concentration of spinosad, representing the LC_{50} concentration calculated in the previous experiment. Conditions were identical to those of the previous bioassay, and mortality was assessed at 24 h post-exposure. The experiment was performed 24 times on different dates, giving a total of 24 replicates for both treatment and control. Mortality results were analysed by comparing the observed mortality with the expected 50% mortality value by *t*-test.

2.3 Sublethal effects of exposure to spinosad

Groups of 25 late third-instar larvae were exposed for 1 h to an LC_{50} concentration of spinosad or a dechlorinated water control following the procedures described in the bioassay experiment. Dead larvae were counted and removed 24 h post-exposure. Surviving larvae were reared to adulthood on a diet of powdered rabbit food provided *ad libitum*. Adult mosquitoes were transferred individually to 200 mL plastic cups covered with a mesh lid and fed with 5% sucrose solution for the duration of their lifetime.

Each female was allowed to mate with a single control male inside the plastic cup. Females were then moved to a new cup containing 10 mL of water and an oviposition substrate comprising strips (3 cm width × 15 cm length) of Whatman grade 1 filter paper. Oviposition was monitored every day, and, if eggs were observed, the paper strip was replaced with a new strip. Ovipositing females were offered blood meals and were allowed to feed until satiated. The number of eggs laid by each female in her lifetime and the number of batches of eggs were counted. Eggs were incubated in clean water to determine fertility (percentage of hatching). The adult survival time was recorded for both sexes. On death, one wing of each individual was selected at random and the length from the axial vein to the outmost extreme of the R1 vein was measured using a microscope and calibrated graticule. The experiment was performed 20 times on different dates, giving a total of 20 replicates for both treatment and control. Longevity, wing length, fecundity and fertility results of untreated and spinosad-treated groups were not normally distributed and were subjected to Mann–Whitney *U*-tests. Additionally, possible correlations between biological parameters were examined using Spearman's rank correlation.

3 RESULTS

3.1 Determination and confirmation of the LC_{50} value

Logit regression of mortality at 24 h post-treatment against $\log_e$ [concentration] gave an estimated LC_{50} value of 0.060 mg AI L^{-1} (range of 95% confidence limits 0.045–0.079) following correction for overdispersion in the mortality results (scale parameter 6.5, $n = 300$ larvae per concentration). Slope and intercept values (± SE), given in terms of the $\log_e$ odds ratio (p/q), were slope 1.283 ± 0.137, intercept 3.618 ± 0.431.

In the experiment to confirm the reproducibility of the LC_{50}, an average of 12.2 ± 4.6 (mean ± SD) individuals died in each group, representing 48.8% mortality, which was not significantly different from the expected 50% mortality (12.5 deaths/group) ($t = 0.313$, df = 23, $P = 0.757$). No mortality was observed in control larvae.

3.2 Sublethal effects of exposure to spinosad

Groups of insects used in the sublethal effects experiment suffered an average of 51.7% mortality following 1 h exposure to the LC_{50} concentration of spinosad. The mortality of larvae between the 24 h post-exposure period (when dead larvae were removed) and pupation was zero, whereas a small number of pupae (<3%) did not emerge as adults and were discarded. Average wing length of exposed females (Table 1) was significantly greater than that of unexposed females ($U = 3452$, $P < 0.001$). Exposed males, on

Table 1. Effects of spinosad treatment on the survivors of a 1 h exposure to an LC_{50} concentration in the late third instar. Values are means (± SD) based on 20 replicates[a]

	Females		Males	
Parameter	Treated	Control	Treated	Control
Wing length (mm)	2.47 (±0.22) a	2.29 (±0.28) b	1.89 (±0.13) a	2.04 (±0.13) b
Longevity (days)	42.9 (±16.3) a	40.9 (±17.1) a	38.1 (±14.9) a	40.2 (±18.2) a
Fecundity				
Number of egg masses	6.2 (±2.4) a	5.5 (±2.7) b	–	–
Total number of eggs	276.2 (±111.2) a	205.2 (±121.5) b	–	–
Fertility (% egg hatch)	72.6 (±4.9) a	84.9 (±6.5) b	–	–
Total progeny production	200.1 (±81.5) a	174.1 (±102.9) b	–	–

[a] Means followed by identical letters do not differ significantly for comparisons of treatment and control groups for each sex (Mann–Whitney, $P > 0.05$).
Calculation of SD values and statistical comparisons were performed using mean values calculated from each of 20 replicates. The total number of insects of each sex in each treatment varied from 118 to 152.

the other hand, were significantly smaller than their unexposed counterparts ($U = 3763$, $P < 0.001$).

Exposure to spinosad did not significantly affect adult longevity; treated and control mosquitoes of each sex had similar mean lifespans (females: $U = 8087$, $P = 0.168$; males: $U = 8757$, $P = 0.749$). On average, exposed females laid a greater number of egg batches during their lifetime than control females ($U = 3543$, $P = 0.020$). The total number of eggs laid by exposed females during their lifetime was also significantly greater than that of unexposed females ($U = 5742$, $P < 0.001$). Fertility, measured as the percentage of eggs that hatched, was on average significantly higher in the unexposed than in the exposed females ($U = 1349$, $P < 0.001$). However, the absolute number of eggs that hatched was higher in the exposed females ($U = 7123$, $P < 0.01$). Exposed females thus laid more viable eggs than unexposed females.

There were no significant correlations between experimental variables within groups, except in the group of unexposed females, for which wing length was positively correlated with total number of eggs produced (Spearman's $r_s = 0.238$, $P < 0.01$).

4 DISCUSSION

Initial studies have indicated that spinosad may be a promising new biorational insecticide for control of vectors of medical importance,[4–8,21] but in situations where lethal concentrations are not achieved, such as when the toxicant degrades over time, a number of immature mosquitoes will be able to develop in the presence of sublethal concentrations of spinosad. This is an issue of concern because the progeny of such insects are likely to inherit traits that permit them to survive in the presence of spinosad residues; this in turn, may allow the development of mosquito populations with increasing resistance to spinosad-based products. In the present study the authors examined the effects of exposure to an LC_{50} concentration of spinosad in the late third instar on the reproductive capacity, body size (wing length) and longevity of *Ae. aegypti* adults.

The 1 h LC_{50} concentration in third instars was estimated at 0.060 mg AI L^{-1}, which is higher than the previous value (0.026 mg AI L^{-1}) estimated in the authors' laboratory;[4,8] the difference is probably due to the wild-caught population employed in the present study, as opposed to the laboratory-reared Rockefeller strain that was used previously. What does 1 h LC_{50} mean? The 1 h period of exposure to spinosad was selected on the basis of EPA guidelines and previous studies by the authors, whereas in natural habitats the exposures to sublethal concentrations of a toxicant are likely to be both longer in duration and highly variable between sites owing to environmental heterogeneity, making accurate quantification of such exposure highly problematic.

Adult females that had been exposed to spinosad as larvae were significantly larger (as measured by wing length), produced more eggs and produced more offspring, although the percentage of fertility of eggs was significantly but slightly reduced, compared with control females. Wing length is a reliable, albeit conservative, indicator of body size in aedine mosquitoes,[22] and is known to be closely correlated with the size of blood meals, the duration of the gonotrophic cycle and the number of eggs produced.[23] Detailed morphometric studies have recently confirmed the usefulness of wing measurements for estimating body size in this species.[24] For adult males, the exposed group had significantly shorter wings than control males. Adult longevity was not affected by exposure to spinosad in either sex.

Exposure to spinosad appears to have selectively eliminated smaller, possibly weaker, females from the experimental population, leaving the larger and more fecund females to develop to the adult stage. This is probably why the correlation between wing length and fecundity was only significant in the control group, because many of the smaller females had been eliminated in the spinosad-treated group. The development period between treatment and pupation was too brief (~24 h) to evaluate accurately. The brief period of post-exposure development and the *ad libitum* supply of food mean that the reduction in larval density observed following treatment with spinosad was unlikely to be the cause of increased size of female survivors compared with controls.[24] In contrast, in situations where food is very limited, reductions in larval densities following larviciding can actually result in an increase in the number of emerging adult mosquitoes on account of reduced competition.[25] However, the possibility cannot be ruled out that increased interference during feeding or increased concentrations of metabolic waste products may have affected the growth of female mosquitoes in the control treatment compared with spinosad-treated females that experienced lower rearing densities during the final instar.

Exposed females produced 15% more offspring than control females, but, as 50% of the larval population had been eliminated

by spinosad treatment, the increased reproductive capacity of spinosad-treated females failed to compensate for mortality in the immature stages and would be unlikely to result in a population increase in the mosquito, at least under the conditions that were employed. Males responded differently, and survivors of spinosad treatment tended to be smaller than controls, although the reasons for this result are not clear.

Other insecticides also affect the reproductive capacity and lifespan of surviving mosquitoes. Sublethal exposure to organophosphates or pyrethroids can result in changes in fecundity and immature development times.[9,10,26] Similarly, reductions in wing length, fecundity, egg size, glycogen reserves and the longevity and feeding capabilities of adult females have been reported in the survivors of juvenile hormone analogue treatments,[9,11,12] whereas treatment with mosquito pathogens or botanical extracts can result in extended development times and reductions in body size, fecundity, the number of gonotrophic cycles and adult longevity.[14,27,28] It seems that sublethal effects are particular to each larvicide and depend on the mode of action, life stage treated, duration of exposure, concentration and environmental factors such as temperature and food supply.

In conclusion, the female survivors of exposure to an LC_{50} concentration of spinosad showed increased reproductive capacity, probably owing to the elimination of the smaller and more susceptible fraction of the mosquito population. This result underlines the need to maintain concentrations of spinosad at lethal levels in larval habitats, a requirement that is particularly challenging in situations where exposure to strong sunlight,[8] or continuous water flow, degrade or dilute the toxicant. Sustained-release formulations of spinosad may greatly assist in overcoming this problem, but are not yet widely available for testing.

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REFERENCES

1 Curtis CF and Davies CR, Present use of pesticides for vector and allergen control and future requirements. *Med Vet Entomol* **15**:231–235 (2001).
2 Hemingway J, Beaty BJ, Rowland M, Scott TW and Sharp BL, The Innovative Vector Control Consortium: improved control of mosquito-borne diseases. *Trends Parasitol* **22**:308–312 (2006).
3 Thompson GD, Dutton R and Sparks TC, Spinosad – a case study: an example from a natural products discovery programme. *Pest Manag Sci* **56**:696–702 (2000).
4 Bond JG, Marina CF and Williams T, The naturally derived insecticide spinosad is highly toxic to *Aedes* and *Anopheles* mosquito larvae. *Med Vet Entomol* **18**:50–56 (2004).
5 Liu H, Cupp EW, Guo A and Liu N, Insecticide resistance in Alabama and Florida mosquito strains of *Aedes albopictus*. *J Med Entomol* **41**:946–952 (2004).
6 Darriet F, Duchon S and Hougard JM, Spinosad: a new larvicide against insecticide-resistant mosquito larvae. *J Am Mosq Contr Assoc* **21**:495–496 (2005).
7 Romi R, Proietti S, Di Luca M and Cristofaro M, Laboratory evaluation of the bioinsecticide spinosad for mosquito control. *J Am Mosq Contr Assoc* **22**:93–96 (2006).
8 Pérez CM, Marina CF, Bond JG, Rojas JC, Valle J and Williams T, Spinosad, a naturally derived insecticide, for control of *Aedes aegypti* (Diptera: Culicidae): efficacy, persistence, and elicited oviposition response. *J Med Entomol* **44**:631–638 (2007).
9 Robert LL and Olson JK, Effects of sublethal dosages of insecticides on *Culex quinquefasciatus*. *J Am Mosq Contr Assoc* **5**:239–246 (1989).
10 Shaalan EA, Canyon DV, Younes MW, Abdel-Wahab H and Mansour AH, Effects of sub-lethal concentrations of synthetic insecticides and *Callitris glaucophylla* extracts on the development of *Aedes aegypti*. *J Vect Ecol* **30**:295–298 (2005).
11 Sawby R, Klowden MJ and Sjogren RD, Sublethal effects of larval methoprene exposure on adult mosquito longevity. *J Am Mosq Contr Assoc* **8**:290–292 (1992).
12 Ritchie SA, Asnicar M and Kay BH, Acute and sublethal effects of (*S*)-methoprene on some Australian mosquitoes. *J Am Mosq Contr Assoc* **13**:153–155 (1997).
13 Hare SGF and Nasci RS, Effects of sublethal exposure to *Bacillus thuringiensis* var *israelensis* on larval development and adult size in *Aedes aegypti*. *J Am Mosq Contr Assoc* **2**:325–328 (1986).
14 Marina CF, Ibarra JE, Arredondo-Jimenez JI, Fernandez-Salas I, Liedo P and Williams T, Adverse effects of covert iridovirus infection on life history and demographic parameters of *Aedes aegypti*. *Entomol Exp Appl* **106**:53–61 (2003).
15 Jeyabalan D, Arul N and Thangamathi P, Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, *Anopheles stephensi* Liston. *Biores Technol* **89**:185–189 (2003).
16 Nathan SS, Kalaivani K and Murugan K, Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Acta Trop* **96**:47–55 (2005).
17 World Health Organization Pesticide Evaluation Scheme, 10th report of the WHOPES working group WHO/CDS/NTD/WHOPES/20071, Geneva, Switzerland (2007).
18 *Manual on Practical Entomology in Malaria. Part II*. World Health Organization, Geneva, Switzerland, pp. 148–152 (1975).
19 *The GLIM System: Release 4 Manual*, ed. by Francis B, Green M and Payne C. Numerical Algorithms Group, Clarendon Press, Oxford, UK (1993).
20 Aitkin M, Anderson D, Francis B and Hinde J, *Statistical Modelling in GLIM*. Clarendon Press, Oxford, UK (1989).
21 Darriet F and Corbel V, Laboratory evaluation of pyriproxyfen and spinosad, alone and in combination, against *Aedes aegypti* larvae. *J Med Entomol* **43**:1190–1194 (2006).
22 Siegel JP, Novak RJ, Lampman RL and Steinly BA, Statistical appraisal of the weight–wing length relationship of mosquitoes. *J Med Entomol* **29**:711–714 (1992).
23 Briegel H, Metabolic relationship between female body size, reserves and fecundity of *Aedes aegypti*. *J Insect Physiol* **36**:165–172 (1990).
24 Jirakanjanakit N, Leemingsawat S, Thongrungkiat S, Apiwathnasorn C, Singhaniyom S, Bellec C, *et al*, Influence of larval density or food variation on the geometry of the wing of *Aedes* (*Stegomyia*) *aegypti*. *Trop Med Internat Health* **12**:1354–1360 (2007).
25 Agudelo-Silva F and Speilman A, Paradoxical effects of simulated larviciding on production of adult mosquitoes. *Am J Trop Med Hyg* **33**:1267–1269 (1984).
26 Reyes-Villanueva F, Juarez-Eguia M and Flores-Lea A, Effects of sublethal dosages of Abate upon adult fecundity and longevity of *Aedes aegypti*. *J Am Mosq Contr Assoc* **6**:739–741 (1990).
27 Hongyu Z, Changju Y, Jingye H and Lin L, Susceptibility of field populations of *Anopheles sinensis* (Diptera: Culicidae) to *Bacillus thuringiensis* subsp *israelensis*. *Biocontr Sci Technol* **14**:321–325 (2004).
28 Sakthivadivel M and Thilagavathy D, Larvicidal and chemosterilant activity of the acetone fraction of petroleum ether extract from *Argemone mexicana* L seed. *Biores Technol* **89**:213–216 (2003).

