# Laboratory Evaluation of Pyriproxyfen and Spinosad, Alone and in Combination, Against *Aedes aegypti* Larvae

FREDERIC DARRIET AND VINCENT CORBEL

Institut de Recherche pour le Développement, Laboratoire de Lutte contre les Insectes Nuisibles, 911 Avenue Agropolis, BP 64501, 34934 Montpellier Cedex 5, France

J. Med. Entomol. 43(6): 1190–1194 (2006)

**ABSTRACT** In this study, the efficacy of pyriproxyfen and spinosad, alone and in combination, was evaluated against the dengue vector *Aedes aegypti* (L.). Larval bioassays were carried out on susceptible mosquito larvae to determine the concentration–mortality responses of mosquitoes exposed to each insecticide alone and in mixture. Synergism between pyriproxyfen and spinosad was determined by the calculation of a combination index (CI) by using the isobologram method. For pyriproxyfen, $LC_{50}$ and $LC_{95}$ were $1.1 \times 10^{-4}$ ($1.0 \times 10^{-4}$–$1.1 \times 10^{-4}$) and $3.2 \times 10^{-4}$ ($2.9 \times 10^{-4}$–$3.6 \times 10^{-4}$) mg/liter, respectively. Pyriproxyfen acted at very low concentrations by inhibiting the adult emergence of *Ae. aegypti* (97% inhibition rates at $3.3 \times 10^{-4}$ mg/liter). Spinosad activity was ≈500 times lower than that of pyriproxyfen against the Bora strain, with $LC_{50}$ and $LC_{95}$ values estimated at 0.055 (0.047–0.064) and 0.20 (0.15–0.27) mg/liter, respectively. A binary mixture of pyriproxyfen and spinosad was realized at the ratio 1:500 by considering the values of the $LC_{50}$ obtained for each product. The $LC_{50}$ and $LC_{95}$ of the mixture were 0.019 (0.016–0.022) and 0.050 (0.040–0.065) mg/liter, respectively. The mixture combined both the larvicidal activity of spinosad and the juvenoid action of pyriproxyfen. From the $LC_{70}$ to $LC_{99}$, a significant synergism effect was observed between the two insecticides (CI ranged from 0.74 to 0.31). This strong synergism observed at high concentrations allows a reduction by five and nine-fold of pyriproxyfen and spinosad amounts to kill almost 100% mosquitoes. Combination of pyriproxyfen and spinosad may then represent a promising strategy to improve mosquito control in situations with insecticide-resistant *Aedes* dengue vectors.

**KEY WORDS** pyriproxyfen, spinosad, mixture, synergism, *Aedes aegypti*

*Aedes aegypti* (L.) is the principal epidemic vector of dengue virus in the world, responsible for a viral infection causing between 50 and 100 million cases and thousands of deaths every year (Gubler 2002). Because vaccines are still under development, dengue prevention depends entirely on vector control. However, it remains extremely difficult to implement sustainable vector control programs, because it requires a large budget, commitment, and active community participation (Gubler and Kuno 1997). The most effective way for controlling dengue vectors is larval source reduction, i.e., elimination or cleaning of water-holding containers that serve as the larval habitats for *Ae. aegypti* in the domestic environment. The application of insecticides in containers that cannot be eliminated also is considered as a priority during interepidemic periods. Treatments have to deal with the diversity and multiplicity of larval habitats, the dose and the choice of pesticides to apply and insecticide resistance issues. Insecticides should present low risk to humans and other nontarget organisms and should exhibit a good residual activity to prevent the colonization of breeding sites between two applications.

During the past 30 yr, temephos (mammalian toxicology: acute oral, $LD_{50}$ for rates between 4,204 and 10,000 mg/kg) has been widely used for the control of *Ae. aegypti* larvae in domestic and peridomestic environments despite a relatively low persistence (1–2 mo) in the field (Pinheiro and Tadei 2002, Thavara et al. 2004). Unfortunately, the occurrence of high level of temephos-resistance in natural mosquito populations in many parts of the world (Rawlins 1998, Paeporn et al. 2003) has underlined the need to use new candidates for vector control (WHO 2000). Alternative pesticides should belong to nonconventional chemical classes as intensive use of both larvicides and adulticides in the past has resulted in the emergence of resistance to organophosphorus (Karunaratne and Hemingway 2001, Rodriguez et al. 2001), carbamate (Vaughan et al. 1998), and pyrethroid insecticides (Mebrahtu et al. 1997, Brengues et al. 2003).

Among suitable alternatives for dengue prevention, *Bacillus thuringiensis israelensis* H-14 (*Bti*) is strongly advocated for the treatment of drinking water (WHO 1999) because it shows high insecticidal activity against mosquito larvae (Lee et al. 1996), yet causing little or no harm to humans, beneficial insects, and

0022-2585/06/1190–1194$04.00/0 

other nontarget organisms (Tabashnik 1994). However, *Bti* tends to settle at the bottom of the water containers soon after application and thus requires frequent applications (Gubler and Kuno 1997). The low residual activity of *Bti* in larval habitats (Lee et al. 2005) may then represent an obstacle for long-term control of *Ae. aegypti* in the future.

To achieve more efficient mosquito control and avoid the development of resistance, the World Health Organization (WHO) also recommends the use of insecticides sharing different modes of action either in mixtures, rotations, or mosaics (WHO 2003). The application of temephos in association with *Bti* has shown promising results against *Culex quinquefasciatus* Say under both simulated and field conditions (Andrade 1989). Similarly, Chung et al. (2001) showed that mixture of pirimephos methyl and *Bti* dispersed in thermal fog was very effective against adults and larvae of *Ae. aegypti*, causing almost complete mortality as well as long-term efficacy in treated containers. Wirth et al. (2000) demonstrated strong insecticidal activity of *Bacillus sphaericus* and *Bti* combinations against *Ae. aegypti*, efficacy mainly explained by the high level of synergism exhibited between the two components of the association.

Recent laboratory and field trials have shown good persistence and efficacy of controlled release formulations of pyriproxyfen (a juvenile hormone mimic) in terms of adult emergence inhibition against *Ae. aegypti* (Nayar et al. 2002). Spinosad also established a new standard for low environmental and human risks (Williams et al. 2003) and offers interesting approaches to integrated pest management and insecticide resistance management (Salgado 1997, Darriet et al. 2005).

In this context, we investigated under laboratory conditions the performances of pyriproxyfen and spinosad, alone and in combination, against the dengue vector *Ae. aegypti.* Larval bioassays were first carried out with the susceptible Bora strain to determine the concentration–mortality responses of mosquitoes exposed to each insecticide alone then in mixture. Finally, the existence of synergism between pyriproxyfen and spinosad was tested by the calculation of a combination index (CI) by using the isobologram method described by Chou and Talalay (1984).

## Materials and Methods

**Biological Material.** The susceptible Bora strain of *Ae. aegypti*, originally from French Polynesia, was used for the bioassays. The Bora strain was maintained for >10 yr under laboratory conditions (27 ± 2°C and 80% RH) and is free of any detectable insecticide resistance mechanisms.

**Insecticides.** The technical grade (98.7%) of pyriproxyfen [4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy)propyl ether] used in our study was provided by Sumitomo Chemical Co., Ltd. First, Tokyo, Japan. Pyriproxyfen mimics the action of the juvenile hormones on a number of physiological processes and is a potent inhibitor of embryogenesis, metamorphosis, and adult formation (Ishaaya and Horowitz 1992). The acute oral $LD_{50}$ of pyriproxyfen for rats is >5,000 mg/kg (Tomlin 2000). The technical grade (90.4%) of spinosad [mixture of 50–95% of (2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-14-methyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione, and 50–5% (2*S*,3a*R*,5a*S*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-4,14-dimethyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione] was provided by Dow AgroSciences (Indianapolis, United Kingdom). This compound is a mixture of two natural metabolites, spinosynes A and D, produced by the fermentation of the soil actinomycete *Saccharopolyspora spinosa.* This compound shares a unique mode of action involving the postsynaptic nicotinic acetylcholine and γ-aminobutyric acid receptors (Salgado 1998). The acute oral $LD_{50}$ of spinosad for rats ranges from 3,800 to 5,000 mg/kg (Tomlin 2000).

**Larval Bioassays.** Larval bioassays were carried out using technical grades of pyriproxyfen and spinosad dissolved in ethanol. Each bioassay was repeated three times using third instars of the Bora strain. Groups of 25 larvae were placed in 99 ml of distilled water, with 1 ml of insecticide solution (or 1 ml of insecticide mixture). Four lots per concentration ($n = 100$) and a minimum of five concentrations per replicate, providing mortality within a range of 0–100%, were used for each replicate. The long duration of the test requires that the larvae should be provided every day with a small amount of food (dry cat food) at a concentration of 100 mg/liter until the first pupae occurred in the cups. The larvae in the control were fed in the same manner as those in the treated batches. The temperature was maintained at 27°C throughout the test, and the larval, pupal, and adult mortalities were recorded every day until emergence. Data were analyzed according to the method of Finney (1971) by using Probit Software (Raymond et al. 1997). This software uses iterative method of maximum likelihood to fit a regression between logarithm of concentration and the probit mortality. This software provides an estimation of $LC_{50}$ and $LC_{95}$ with their 95% confidence intervals.

**Analysis of Synergism.** The existence of synergism between pyriproxyfen and spinosad was determined by the calculation of a combination index (CI) according to the method of Chou and Talalay (1984) by using CalcuSyn software (Chou and Hayball 1996). This isobologram-based method is particularly well adapted to analyze multiple drugs effects (Tallarida 2002). The CI gives a quantitative measure of the interactions (synergism, antagonism, and summation) occurring between insecticides. For two insecticides with independent modes of action (as for pyriproxyfen and spinosad), the CI is calculated for the mortality $x$ by the following formula:

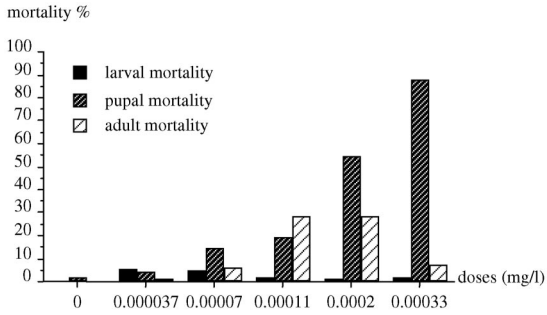


**Fig. 1.** Mortalities induced by pyriproxyfen on the larvae, pupae, and adults of *Ae. aegypti*.

$$CI_x = \frac{LC_x^{\text{pyriproxyfen (m)}}}{LC_x^{\text{pyriproxyfen}}} + \frac{LC_x^{\text{spinosad (m)}}}{LC_x^{\text{spinosad}}} + \frac{LC_x^{\text{pyriproxyfen (m)}} \times LC_x^{\text{spinosad (m)}}}{LC_x^{\text{pyriproxyfen}} \times LC_x^{\text{spinosad}}}$$

where $LC_x^{\text{pyriproxyfen (m)}}$ and $LC_x^{\text{spinosad (m)}}$ designate the doses of pyriproxyfen and spinosad, respectively, inducing an *x* mortality in mixture and $LC_x^{\text{pyriproxyfen}}$ and $LC_x^{\text{spinosad}}$ designate the doses of pyriproxyfen and spinosad inducing the same *x* mortality when used alone. A CI = 1, <1, and >1 indicates an additive effect, a synergistic effect, and an antagonistic effect, respectively.

## Results

For each test, the mortality recorded in the control batches was <5%. For pyriproxyfen, $LC_{50}$ and $LC_{95}$ values were $1.1 \times 10^{-4}$ ($1.0 \times 10^{-4}$–$1.1 \times 10^{-4}$) and $3.2 \times 10^{-4}$ ($2.9 \times 10^{-4}$–$3.6 \times 10^{-4}$) mg/liter, respectively. Figure 1 showed that pyriproxifen acted at very low doses by inhibiting the adult emergence of *Ae. aegypti* (97% inhibition rates at $3.3 \times 10^{-4}$ mg/liter). The $LC_{50}$ and $LC_{95}$ values of spinosad were 0.055 (0.047–0.064) and 0.20 (0.15–0.27) mg/liter, respectively, indicating that spinosad activity was ≈500 times lower than those of pyriproxyfen against the Bora strain (Fig. 2).

A binary mixture of pyriproxyfen and spinosad was realized at the ratio 1:500 by considering the values of the $LC_{50}$ obtained for each product. The $LC_{50}$ and $LC_{95}$ values of the mixture were 0.019 (0.016–0.022) and 0.050 (0.040–0.065), respectively. The mixture combined both the larvicidal action of spinosad and the juvenoid effect of pyriproxyfen (Fig. 3). At the lower ranges of concentrations (from $LC_{10}$ to $LC_{60}$), the mixture induced only an additive effect on mortality (Table 1). From the $LC_{70}$ to $LC_{99}$, a significant synergism effect was exhibited between the two insecticides (CI ranged from 0.74 to 0.31). Because of a strong synergism between the two products, the concentrations of pyriproxyfen and spinosad can be reduced by five- and nine-fold, respectively, in combination to eliminate almost 100% mosquitoes (dose reduction indexes were given by the Calcusyn software).

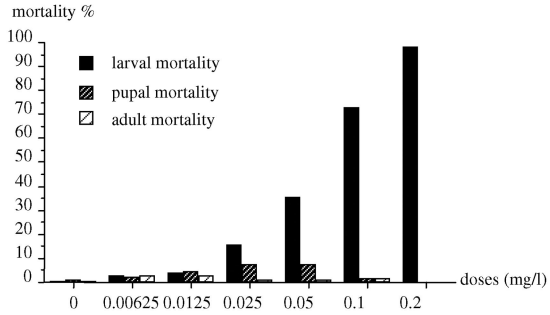


**Fig. 2.** Mortalities induced by spinosad on the larvae, pupae, and adults of *Ae. aegypti*.

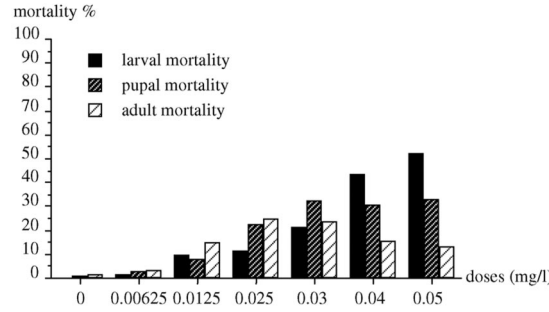


**Fig. 3.** Mortalities induced by pyriproxyfen and spinosad mixture (1:500) on the larvae, pupae, and adults of *Ae. aegypti*.

## Discussion

Currently, vector control strategies are directed toward the use of nonconventional chemicals with strong insecticidal properties, good persistence, and low toxicity for humans and nontarget organisms. Pyriproxyfen is an insect growth regulator with typical juvenoid action that has proved promising for con-

**Table 1. CI of the pyriproxyfen and spinosad mixture on the susceptible strain (Bora-Bora) *Ae. aegypti***

| % mortality (lethal dose level) | Dose of 1:500 insecticide mixture (mg/liter) | CI | Interactions |
|---|---|---|---|
| 10 | 0.011 | 1.61 (0.87–2.35)[a] | Additive effect |
| 20 | 0.014 | 1.30 (0.81–1.79) | Additive effect |
| 30 | 0.016 | 1.13 (0.75–1.51) | Additive effect |
| 40 | 0.017 | 1.01 (0.69–1.33) | Additive effect |
| 50 | 0.019 | 0.91 (0.63–1.19) | Additive effect |
| 60 | 0.021 | 0.83 (0.58–1.08) | Additive effect |
| 70 | 0.024 | 0.74 (0.51–0.97) | Moderate synergism effect |
| 80 | 0.027 | 0.65 (0.44–0.86) | Synergism effect |
| 90 | 0.033 | 0.54 (0.34–0.74) | Synergism effect |
| 95 | 0.040 | 0.45 (0.26–0.64) | Synergism effect |
| 99 | 0.059 | 0.31 (0.14–0.48) | Strong synergism effect |

[a] Range in parentheses shows 95% confidence limits.

trolling vector mosquitoes such as *Culex*, *Anopheles*, and *Aedes* species (Kamimura and Arakawa 1991, Okazawa et al. 1991, Lee et al. 2005). Pyriproxyfen acts at very low doses against mosquito larvae and persists several months in larval habitats (Yapabandara and Curtis 2004, Sihuincha et al. 2005). In this study, the strong efficacy of pyriproxyfen has been confirmed against susceptible mosquito larvae of *Ae. aegypti.* However, unlike chemical larvicides, pyriproxyfen inhibits adult emergence but does not kill the larvae. Although pyriproxyfen seems to show no cross-resistance with conventional insecticides (Ishaaya et al. 2005), its mode of action may pose some operational challenges, because householders may perceive the continued presence of larvae as a treatment failure.

Conversely, spinosad showed high toxicity against mosquito larvae but no direct action on pupae and adults. Spinosad is considered as a promising bioinsecticide for public health, because it shows good insecticidal activity against target pests and low risk to humans and the nontarget fauna (Williams et al. 2003). It acts rapidly against a broad range of mosquito species and shows no cross-resistance with commonly used insecticides such as organophosphates, carbamates, and pyrethroids (Darriet et al. 2005). A recent field trial demonstrated that spinosad, at 10 mg/liter, prevented *Ae. aegypti* breeding in larval habitats for >5 mo (Bond et al. 2004).

Considering the good performances and the complementary action of spinosad and pyriproxyfen against the dengue vector *Ae. aegypti*, a mixture of both compounds was tested against third instars of the Bora strain. The insecticide mixture showed very good efficacy against *Ae. aegypti* in terms of larvicidal effect and adult emergence inhibition. Similarly, Lee et al. (2005) have recently demonstrated that addition of pyriproxyfen to *Bti* formulations enhanced larval control and prevented adult emergence. In our study, the rapid killing effect of spinosad on mosquito larvae allows to overcome the slow and specific action of pyriproxyfen on pupae and adults. Larvae that emerged later and had not been affected by spinosad were subsequently killed at the pupal stage by the pyriproxyfen. This combined action of pyriproxyfen and spinosad probably explains the strong synergism observed with the mixture at high concentrations (from $LC_{70}$ to $LC_{99}$). Such phenomenon may then improve the efficacy of the treatments in the field while substantially reducing the cost and toxicity as a result of a reduction of insecticide amounts.

So far, synergism between chemicals and/or biological agents has been demonstrated in literature (Wirth et al. 2000; Corbel et al. 2002, 2003; Darriet et al. 2003) and is considered as an important parameter for controlling resistant pests and for slowing down the evolution of resistance (Curtis 1985). Because *Ae. aegypti* populations are becoming more and more resistant to conventional insecticides, alternative strategies have to be rapidly implemented in the future for dengue vector control. The combination of insecticides such as spinosad and pyriproxyfen may be promising for controlling *Ae. aegypti* mosquito larvae provided that treatments persist at least during the whole dengue transmission season. Investigations should now be conducted under simulated field conditions to assess the efficacy and residual activity of such combinations in natural breeding sites of *Ae. aegypti*, as for example in domestic water storage containers, which represent a primary larval habitat for *Ae. aegypti* in many countries affected by the disease.

## Acknowledgments

We are very grateful to Sumitomo Chemical Co. Ltd. and Dow AgroSciences for providing the active ingredients of pyriproxyfen and spinosad.

## References Cited

**Andrade, C.F.S. 1989.** Ecologica de supressão de populações de culicideos e simulideos. Tese de detourado-Instituto de Biologia da UNICAMP, Campinas, Brazil.

**Bond, J. G., C. F. Marina, and T. Williams. 2004.** The naturally derived insecticide spinosad is highly toxic to *Aedes* and *Anopheles* mosquito larvae. Med. Vet. Entomol. 18: 50–56.

**Brengues, C., N. J. Hawkes, F. Chandre, L. Mc Carroll, S. Duchon, P. Guillet, S. Manguin, J. C. Morgan, and J. Hemingway. 2003.** Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. Med. Vet. Entomol. 17: 87–94.

**Chou, T. C., and P. Talalay. 1984.** Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv. Enzyme Regul. 22: 27–55.

**Chou, T. C., and M. P. Hayball. 1996.** CalcuSyn®: Windows software for dose effect analysis. Biosoft, Ferguson, MO.

**Chung, Y. K., S. G. Lam-Phua, Y. T. Chua, and R. Yatiman. 2001.** Evaluation of biological and chemical insecticide mixture against *Aedes aegypti* larvae and adults by thermal fogging in Singapore. Med. Vet. Entomol. 15: 321–327.

**Corbel, V., F. Darriet, F. Chandre, and J. M. Hougard. 2002.** Insecticide mixtures for mosquito net impregnation against malaria vectors. Parasite 9: 255–259.

**Corbel, V., M. Raymond, F. Chandre, F. Darriet, and J. M. Hougard. 2003.** Efficacy of insecticide mixtures against larvae of *Culex quinquefasciatus* (Say) (Diptera: Culicidae) resistant to pyrethroids and carbamates. Pest Manag. Sci. 60: 375–380.

**Curtis, C. F. 1985.** Theoretical models of the use of insecticide mixtures for management of resistance. Bull. Entomol. Res. 75: 259–265.

**Darriet, F., V. Corbel, and J. M. Hougard. 2003.** Efficacy of mosquito nets treated with a pyrethroid-organophosphorous mixture against *Kdr*− and *Kdr*+ malaria vectors (*Anopheles gambiae*). Parasite 10: 359–362.

**Darriet, F., S. Duchon, and J. M. Hougard. 2005.** Spinosad: a new larvicide against insecticide resistant mosquito larvae. J. Am. Mosq. Control Assoc. 21: 495–496.

**Finney, D. J. 1971.** Probit analysis. Cambridge University Press, Cambridge, United Kingdom.

**Gubler, D. J., and G. Kuno. 1997.** Dengue and dengue hemorrhagic fever. CAB International, New York.

**Gubler, D. J. 2002.** Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol. 10: 100–103.

**Ishaaya, I., and A. R. Horowitz. 1992.** Novel phenoxy hormone analog (pyriproxyfen) suppresses embryogenesis

and adult emergence of sweet potato whitefly. J. Econ. Entomol. 85: 2113–2117.

**Ishaaya, I., S. Kontsedalov, and A. R. Horowitz. 2005.** Biorational insecticides: mechanism and cross-resistance. Arch. Insect Biochem. Physiol. 58: 192–199.

**Kamimura, K., and R. Arakawa. 1991.** Field evaluation of an insect growth regulator, pyriproxyfen, against *Culex pipiens pallens* and *Culex tritaeniorhychus.* Jpn. J. Sanit. Zool. 42: 249–254.

**Karunaratne, S. H., and J. Hemingway. 2001.** Malathion resistance and prevalence of the malathion carboxylesterase mechanism in populations of mosquito vectors of disease in Sri Lanka. Bull. World Health Organ. 79: 1060–1064.

**Lee, H. L., E. R. Gregorio, M. S. Khadri, and P. Seleena. 1996.** Ultralow volume application of *Bacillus thuringiensis* ssp. *israelensis* for the control of mosquitoes. J. Am. Mosq. Control Assoc. 12: 651–655.

**Lee, Y. W., J. Zairi, H. H. Yap, and C. R. Adanan. 2005.** Integration of *Bacillus thuringiensis* H-14 formulations and pyriproxyfen for the control of larvae of *Aedes aegypti* and *Aedes albopictus.* J. Am. Mosq. Control Assoc. 21: 84–89.

**Mebrahtu, Y. B., J. Norem, and M. Taylor. 1997.** Inheritance of larval resistance to permethrin in *Aedes aegypti* and association with sex ratio distortion and life history variation. Am. J. Trop. Med. Hyg. 56: 456–465.

**Nayar, J. K., A. Al, and M. Zaim. 2002.** Effectiveness and residual activity comparison of granular formulations of insect growth regulators pyriproxyfen and S-methoprene against Florida mosquitoes in laboratory and outdoor conditions. J. Am. Mosq. Control Assoc. 18: 196–201.

**Okazawa, T., B. Bakote'e, H. Suzuki, H. Kawada, and N. Kere. 1991.** Field evaluation of an insect growth regulator, pyriproxyfen, against *Anopheles punctulatus* on north Guadalcanal, Solomon Islands. J. Am. Mosq. Control Assoc. 7: 604–607.

**Paeporn, P., N. Komalamisra, V. Deesin, Y. Rongsriyam, and S. Thongrungkiat. 2003.** Temephos resistance in two forms of *Aedes aegypti* and its significance for the resistance mechanism. Southeast Asian J. Trop. Med. Public Health 34: 786–792.

**Pinheiro, V. C., and W. P. Tadei. 2002.** Evaluation of the residual effect of temephos on *Aedes aegypti* (Diptera, Culicidae) larvae in artificial containers in Manaus, Amazonas state, Brazil. Cad Saude Publica 18: 1529–1538.

**Rawlins, S. C. 1998.** Spatial distribution of insecticide resistance in Caribbean populations of *Aedes aegypti* and its significance. Rev. Panam. Salud Publica 4: 243–251.

**Raymond, M., G. Prato, and D. Ratsira. 1997.** Probit and logit analysis program, version 2.0. Praxème, Biometric, Centre National de la Recherche Scientifique, Montpellier, France.

**Rodriguez, M. M., J. Bisset, D. M. de Fernandez, L. Lauzan, and A. Sauca. 2001.** Detection of insecticide resistance in *Aedes aegypti* (Diptera: Culicidae) from Cuba and Venezuela. J. Med. Entomol. 38: 623–628.

**Salgado, V. L. 1997.** The modes of action of spinosad and other insect control products. Down Earth 52: 35–43.

**Salgado, V. L. 1998.** Studies on the mode of action of spinosad: insect symptoms and physiological correlates. Pestic. Biochem. Physiol. 60: 91–102.

**Sihuincha, M., E. Zamora-Perea, W. Orellana-Rios, J. D. Stancil, V. Lopez-Sifuentes, C. Vidal-Ore, and G. J. Devine. 2005.** Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru. J. Med. Entomol. 42: 620–630.

**Tabashnik, B. J. 1994.** Evolution of resistance to *Bacillus thuringiensis.* Annu. Rev. Entomol. 39: 47–79.

**Tallarida, R. J. 2002.** The interaction index: a measure of drug synergism. Pain 98: 163–168.

**Thavara, U., A. Tawatsin, W. Kong-Ngamsuk, and M. S. Mulla. 2004.** Efficacy and longevity of a new formulation of temephos larvicide tested in village-scale trials against larval *Aedes aegypti* in water-storage containers. J. Am. Mosq. Control Assoc. 20: 176–182.

**Tomlin, C. 2000.** The pesticide manual, 12th ed. British Crop Protection Council, London, United Kingdom.

**Vaughan, A., D. D. Chadee, and R. Ffrench Constant. 1998.** Biochemical monitoring of organophosphorus and carbamate insecticide resistance in *Aedes aegypti* mosquitoes from Trinidad. Med. Vet. Entomol. 12: 318–321.

**[WHO] World Health Organization. 1999.** *Bacillus thuringiensis* Environmental Health Criteria 217. World Health Organization, Geneva, Switzerland.

**[WHO] World Health Organization. 2000.** Strengthening Implementation of the Global Strategy for Dengue Fever/Dengue Haemorrhagic Fever Prevention and Control. Document WHO/CDS/(DEN)/IC/2000.1. World Health Organization, Geneva, Switzerland.

**[WHO] World Health Organization. 2003.** Guidelines on the management of public health pesticides. Document WHO/CDS/WHOPES/2003.7. Geneva, Switzerland.

**Williams, T., J. Valle, and E. Viñuela. 2003.** Is the naturally-derived insecticide spinosad® compatible with insect natural enemies? Biocontrol Sci. Technol. 13: 459–475.

**Wirth, M. C., B. A. Federici, and W. E. Walton. 2000.** Cyt1A from *Bacillus thuringiensis* synergizes activity of *Bacillus sphaericus* against *Aedes aegypti.* Appl. Environ. Microbiol. 66: 1093–1097.

**Yapabandara, A. M., and C. F. Curtis. 2004.** Control of vector and incidence of malaria in an irrigated settlement scheme in Sri Lanka by using the insect growth regulator pyriproxyfen. J. Am. Mosq. Control Assoc. 20: 395–400.

*Received 22 November 2005; accepted 21 April 2006.*