FORUM

The Macrocyclic Lactone "Spinosad," a Promising Insecticide for Tsetse Fly Control

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J. Med. Entomol. 41(5): 814–818 (2004)

ABSTRACT The susceptibility of tsetse flies (Diptera : Glossinidae), *Glossina palpalis gambiensis* (Vanderplank), and *G. m. morsitans* (Westwood) to topically applied spinosad, a mixture of insecticidal molecules from the actinomycete *Saccharopolyspora spinosa*, is almost as high as to deltamethrin. However, susceptibility to spinosad does not differ significantly between teneral and gravid flies, contrary to deltamethrin. Spinosad might be a promising candidate for future tsetse control by the sequential aerial technique.

KEY WORDS *Glossina* spp., tsetse control, susceptibility, insecticide, spinosad

HUMAN AND ANIMAL TRYPANOSOMOSIS constitutes a severe threat to livestock development and human health in a large part of sub-Saharan Africa (Bourn et al. 2001). For many years, the control of the diseases has concentrated on the control of its main vector, the tsetse fly (Diptera Cyclorrapha: Glossinidae) (Allsopp 2001). Despite considerable efforts, large areas remain infested by the fly. Furthermore, international funding organizations lost interest in this disease, and trypanosomosis joined the list of neglected diseases. However, in July 2000, at their assembly in Lomé, Togo, the Heads of State of the Organization of African Unity gave new driving force to the battle against tsetse by declaring that all member states and possible partners would join forces to eradicate tsetse flies from the African continent.

Irrespective of the scale of the operation to eradicate the tsetse fly, the control of tsetse currently depends and will continue to depend largely on the use of insecticides. The range of insecticides that are being used to control tsetse is, unfortunately, very limited (Allsopp 2001). This is a consequence of the stringent set of conditions an insecticide must meet before it can be accepted for tsetse control. The identification of new products that could be used as alternatives to the currently used insecticides is thus of considerable importance. One possible candidate might be spinosad.

Spinosad is a metabolite from the aerobic fermentation of the soil actinomycete *Saccharopolyspora spinosa* (Sparks et al. 1999). This insecticide, belonging to the naturalyte class of insecticides, consists of a mixture of two macrolides (spinosyns A and D) that exert their insecticide action mainly through the activation of nicotinic acetylcholine receptors (Salgado 1998). Spinosad also affects gamma-aminobutyric acid receptor function, and this may contribute further to its insecticide activity (Watson 2001). This unique mode of action is coupled with a high degree of selective toxicity toward the insect orders Lepidoptera, Diptera, and Thysanoptera, as well as toward some species of Coleoptera and Orthoptera that consume large amounts of foliage. In view of this insecticide spectrum and low toxicity to many beneficial arthropods (Thompson et al. 2000), it seemed worthwhile to test the effect of spinosad on tsetse flies.

To determine if spinosad could be an alternative insecticide for use in tsetse control, laboratory bioassays were conducted. As insecticide susceptibility differs strongly among *Glossina* spp. and among flies of different physiological status, toxicity of spinosad was evaluated to two species of tsetse [*Glossina morsitans morsitans* (Westwood) and *G. palpalis gambiensis* (Vanderplank)], and to flies of different physiological status (teneral and pregnant flies).

Materials and Methods

Insecticide Formulations and Tsetse Fly Species Used. Male and female *G. m. morsitans* and *G. p. gambiensis* were obtained from the tsetse-breeding colony of the Institute of Tropical Medicine of Antwerp (Elsen et al. 1993). Two suspension concentrates of spinosad (DOW Agrosciences, Indianapolis, IN) were used. In the first experiment, teneral *G. p. gambiensis* flies were treated with a suspension concentrate containing 11.6% active ingredient; for the second experiment, pregnant *G. m. morsitans* and *G. p. gambiensis* females were treated topically using a 48% suspension concentrate. Topically applied spinosad was dissolved in acetone, and all insecticide solutions were replaced within 6 d.

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Table 1. Susceptibility of tsetse flies to topically applied spinosad

	No.	Slope (SE)	LD50[a] (95% confidence limit)	LD90 (95% confidence limit)	χ^2
Teneral *G. p. gambiensis*	750	0.913 (0.748)	2.500 (2.497–2.502)	3.908 (3.787–4.029)	173.40
Gravid *G. p. gambiensis*	1500	1.476 (0.068)	2.180 (2.110–2.250)	3.051 (2.930–3.172)	773.03
Gravid *G. m. morsitans*	1200	1.698 (0.105)	1.117 (1.046–1.187)	1.874 (1.753–1.995)	381.58

[a] LD50, dose of spinosad in nanograms per microliter that kills 50% of the flies 48 h after treatment.

Topical Applications of Spinosad to the Tsetse Fly. The same method used by the authors to evaluate the susceptibility of teneral and pregnant tsetse to deltamethrin (De Deken et al. 1998) was also used to assess the susceptibility to spinosad. Flies were immobilized with nitrogen gas. Using an Arnold hand microapplicator (Burkard Manufacturing, Rickmansworth, UK), a microdrop (1 μl) containing the active ingredient dissolved in acetone was applied to the mesonotum of individual flies. Control flies were treated with the solvent (acetone) only. After treatment, flies were put in plastic cups and kept for 72 h in an incubator at 25°C and between 70 and 85% RH. During this period, flies received no feeding. Mortality (absence of the slightest movement) was recorded at 24, 48, and 72 h after application. Mortality in the treated group was corrected for mortality in the control group according to the formula of Abbott (1925). The lethal dose for 50% (LD_{50}) or 90% (LD_{90}) of the population 48 h after the application was obtained through probit analysis (Stata Corp 2001) on the corrected mortality figures.

Experiment 1: Topical Treatment of Teneral Tsetse. Batches of 40–210 1-d-old teneral male or female *G. p. gambiensis* were treated topically with a microdrop (1 μl) of different spinosad dilutions containing between 0.8 and 3.5 ng spinosad/μl. A total of 750 teneral flies was used in this experiment.

Experiment 2: Topical Treatment of Pregnant Females. Dilutions of spinosad (48% SC) varying between 0.48 and 3.84 ng (AI)/μl were applied topically to batches of 135 gravid *G. m. morsitans* or *G. p. gambiensis* with a mean age of 5 wk. Only flies with a visible second- or third-stage larva were used in this experiment. A total of 1,500 pregnant *G. p. gambiensis* and 1,200 *G. m. morsitans* were used in this experiment. All flies were offered a blood meal 24 h before treatment.

Statistical Methodology. Probit analysis was used to analyze the results of the tsetse mortality 48 h after treatment. Two independent variables are used to predict the cumulative normal probability of 50 or 90% of the flies dying 48 h after treatment: the dose of spinosad and the type of fly (gravid *G. m. morsitans*, gravid *G. p. gambiensis*, or teneral *G. p. gambiensis*).

Results

Experiment 1: Topical Application of Spinosad to Teneral Tsetse. For each of six spinosad dilutions, batches of 40–210 teneral *G. p. gambiensis* were treated topically. No differences in spinosad susceptibility were observed between teneral males or females of *G. p. gambiensis*. Therefore, lethal doses for 50 and 90% of teneral *G. p. gambiensis* 48 h after treatment were determined on the basis of combined results for males and females (Table 1; Fig. 1).

The toxic action of spinosad on tsetse flies was characterized by excitation of the insect nervous system leading to involuntary muscle contractions, prostration with tremors, paralysis, and death.

Experiment 2: Topical Treatment of Pregnant Females. The LD_{50} and LD_{90} of spinosad for female *G. m. morsitans* and *G. p. gambiensis* carrying a late second- or third-stage larva were determined by topical treatment of batches of 135 gravid flies with 8 and 10 different spinosad dilutions. Fly mortality was assessed 48 h after treatment, and results are summarized in Table 1 and Figure 1.

Probit analysis of the results of experiments 1 and 2 revealed a significant effect of dose ($P < 0.001$) on fly mortality after 48 h. With regard to the type of fly, mortality was lower in *G. p. gambiensis* than in *G. m. morsitans* ($P < 0.001$), but the probit model using the type of fly and dose as explanatory variables and mortality as response variable showed no significant difference between teneral and gravid *G. p. gambiensis* ($P = 0.20$).

Discussion

Innate susceptibility of tsetse to insecticides, expressed as LD_{50}, varies significantly between *Glossina* species and can vary significantly between flies of the same species according to their physiological status (Table 2).

The experiments carried out in this study showed the excellent toxicity of spinosad to *Glossina* sp., compared (especially for gravid tsetse flies) with the toxicity of deltamethrin (Table 2). The bioassays revealed also that knock down and mortality caused by spinosad occurs slower than after pyrethroid intoxication. Therefore, it may be preferable to assess mortality 72 h instead of 48 h after treatment.

Gravid tsetse flies are reported to be more tolerant than nonpregnant tsetse to organochlorines and most pyrethroids (Burnett 1962, Hadaway 1972, Riordan 1987). Diversion of liposoluble insecticides into lipid components of the uterine milk and inert storage of toxicant in the larva were believed to contribute to the increased tolerance observed in the gravid tsetse (Irving 1968, Kwan et al. 1982). In this study, teneral *G. p. gambiensis* seemed to be more tolerant than gravid flies, because the confidence limits of the LD50s for these flies did not overlap. However, assuming that the

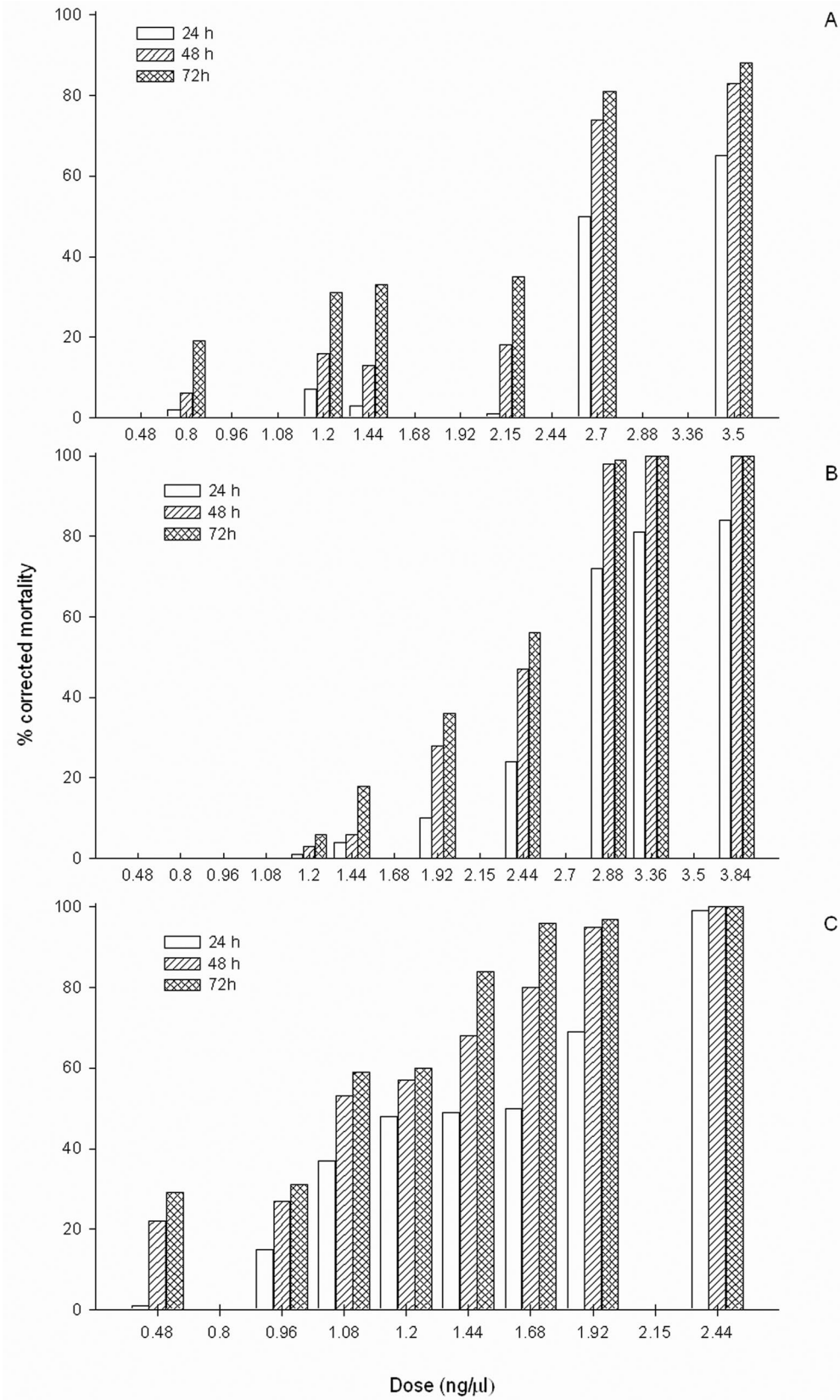

Fig. 1 Corrected mortality (%) of teneral *G. p. gambiensis* (A), pregnant *G. p. gambiensis* (B), and pregnant *G. m. morsitans* (C) after topical application with spinosad.

Table 2. Toxicity values of several insecticides to gravid or teneral tsetse flies

Susceptibility (LD50) of tsetse 48 h after topical treatment	Spinosad (ng/fly)	Deltamethrin (ng/fly)	Endosulfan (ng/fly)
Gravid *G. m. morsitans*	1.12	0.40[a]	
Teneral *G. m. morsitans*		0.06[a]	4.5[b]
Gravid *G. p. gambiensis*	2.18	4.19[a]	42.2 (gravid *G.p.palpalis*)[c]
Teneral *G. p. gambiensis*	2.50	0.67[a]	7.7 (teneral*G.p.palpalis*)[b]

[a] De Deken et al. 1998.
[b] Hadaway 1976.
[c] Riordan 1987.

mortality curves for insecticide dose have the same shape for all *G. p. gambiensis*, the probit model showed no difference between gravid and teneral *G. p. gambiensis* ($P = 0.20$).

It is not clear why gravid tsetse flies showed no higher insecticide tolerance to spinosad than young teneral females, but this constant toxicity level certainly is an advantage of spinosad over other insecticides. During past tsetse control operations, the levels of tolerance of gravid females to some insecticides (e.g., endosulfan) were sufficiently important to necessitate a reduced interval between successive aerial sprayings (Kwan et al. 1982). Such adaptations may not be required when spinosad is used as insecticide.

Spinosad is far less toxic to honey bees than to tsetse, and in this respect, compares favorably with the commonly used deltamethrin. The topical dose of spinosad causing acute toxicity in honey bees (<1 μg/bee) is still >200 times the dose required to kill tsetse. Besides, when spinosad residues have dried completely, toxicity to foraging bees is considered negligible (Thompson et al. 2000). Furthermore, spinosad's toxicity is low to mammals and birds, moderate to fish, and nonexistent to plants (Cleveland et al. 2002).

Compared with sequential aerial spraying of nonresidual, ultra–low-volume insecticide solutions, the application of residual insecticides on baits (target screens or tsetse-feeding hosts) is nowadays often preferred to control tsetse populations. Because chronic toxicology tests in mammals have shown that spinosad is not carcinogenic, teratogenic, mutagenic, or neurotoxic (Schoonover and Larson 1994), application of the product on hosts of tsetse could be considered. Effectiveness of spinosad applied to the host to control tsetse will depend largely on the proportion of flies coming into contact with the treated host and the persistence of the spinosad. Degradation of spinosad in the environment occurs rather rapidly, primarily through photodegradation and microbial decomposition, and therefore, residual activity is not guaranteed. No specific spinosad formulation was available for treatment of the feeding host, but some bioassays with spinosad-treated guinea pigs showed that spinosad had no repellent effect on tsetse. The aqueous solution of 0.019% spinosad applied to the feeding host caused high mortality (>80%), but for a period not exceeding 5 d. Therefore, application of spinosad to livestock to control tsetse will require the development of more persistent formulations.

In conclusion, spinosad is extremely toxic to tsetse and may perhaps become an alternative to commonly used insecticides such as endosulfan or deltamethrin for the control of tsetse by aerial spraying.

Acknowledgments

The authors thank D. Kelili for critical review of the manuscript and Dow Agrosciences for kindly providing the spinosad formulations.

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Received 22 August 2003; accepted 5 April 2004.