SPINOSAD BAIT FOR THE CARIBBEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The establishment of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), in Florida resulted in the need for quarantine treatments of citrus for shipment to certain states and countries. The state of Florida has established a fly-free protocol that permits shipment from areas in compliance without further treatment. One option of the protocol calls for the use of a toxic bait cover spray; however, sprays containing malathion are required to contain 190,000 ppm active ingredient. Thus, alternative pesticides are needed because of environmental, human health, and property damage concerns with malathion. Spinosad, an extract of a bacterial broth, is a contact and stomach poison for target pests. It was combined with a sugar-yeast hydrolysate mixture and tested as a bait spray on colony-reared adult flies in a no-choice test. The EC_{99} values were estimated to be 9.4 and 5.8 ppm for sexually mature females and males, respectively. These relatively low values indicate that spinosad is an excellent candidate for field testing.

Key Words: Pesticide, *Anastrepha suspensa*, citrus, quarantine

RESUMEN

El establecimiento en la Florida de la mosca de la fruta del Caribe., *Anastrepha suspensa* (Loew), ha hecho que las frutas cítricas enviadas a ciertos estados y países reciban tratamientos cuarentenarios. El estado de la Florida ha establecido un protocolo para áreas libres de moscas que permite el envio de frutas de las áreas que cumplan con el mismo, sin requerir tratamiento. Una opción del protocolo es la de cubrir las frutas con un cebo tóxico. Sin embargo, es requerido que la cubierta contenga 190,000 ppm de malathion como ingrediente activo. Por lo tanto, se necesitan otras alternativas en cuanto a insecticidas porque el uso del malathion es dañino al ambiente y la salud humana. Spinosad, un extracto de caldo de bacterias, es un veneno de contacto y estomacal para esta plaga. El Spinosad fue combinado con una mezcla de azúcar y levadura hidrolizada y probado como cebo de aspersión en moscas adultas de una colonia de laboratorio, en una prueba sin opción. Los valores de EC_{99} fueron estimados en 9.4 y 5.8 ppm respectivamente para hembras y machos maduros sexualmente. Estos valores relativamente bajos indican que el Spinosad es un excelente candidato para pruebas de campo.

Florida citrus exported to foreign countries and domestic citrus producing areas must meet certain quarantine requirements to prevent the spread of the Caribbean fruit fly, *Anastrepha suspensa* (Loew)(Diptera:Tephritidae). The Florida caribbean fruit fly protocol is a body of regulations that allows fresh citrus fruit to be certified free of the Caribbean fruit fly and shipped to Japan, California, Texas, Bermuda, and Hawaii. One option of the certification procedure requires that a bait spray, composed of 20% technical grade malathion (typically 95% purity) and 80% Nulure® by volume (190,000 ppm malathion), be aerially applied to groves every 7-10 days during harvest to eradicate adults (Riherd et al. 1994). The use of malathion bait in Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), eradication programs has met with much criticism by the public because of concerns about damage to automobile finishes in Florida (Anonymous 1992) and human health risks in California (Kahn et al. 1992, Russell et al. 1994). These problems with the usage of malathion indicate that an alternative bait is needed.

Spinosad is the common name of a mixture of Spinosyn A and Spinosyn D with CAS Registry numbers of 131929-60-7 and 131929-63-0, respectively. The spinosyns are a naturally-derived group of molecules from a bacteria, Actinomycetes: *Saccharopolyspora spinosa (*Mertz & Yao 1990).

MATERIALS AND METHODS

The methodology generally followed is that previously reported for Caribbean fruit fly bioassays of abamectin in bait (Hennessey & King 1996). The flies were from a colony which has been maintained since 1971 at the Miami ARS laboratory (24-27°C, 70-85% RH) and protected from exposure to insecticides. The flies used in this study were reared as larvae on agar-based diet (Hennessey 1994) and fed as adults on a mixture consisting of 25% yeast hydrolysate enzymatic powder (ICN Biochemicals, Inc., Cleveland, OH) and 75% sugar. Two-week-old males and females were removed from a stock cage with a vacuum hose and placed in a test cage (0.16 m^3, 9-12 flies per cage). Female flies at two weeks of age were considered to be fertilized and ready to oviposit. Tests were carried out over 48 h in an environmental chamber (28-30°C, 85-95% RH, photoperiod 14:10 (L:D), fluorescent light). Flies were deprived of food, but not water (agar-water gel), 1-2 h before testing. During testing, flies had access to agar-water gel and spinosad bait in no-choice tests.

A sample (55 ml, Lot #B721-24, formulation NAF-85, DowElanco, Indianapolis, IN) of spinosad, which was supplied by the manufacturer and specified to contain 45.9% active ingredient, was used in all tests. A stock solution (2.50 mg/ml) was prepared for each test by dilution of 544.7 mg of the above sample with 95% ethanol to 100 ml in a volumetric flask. Appropriate dilutions were prepared using 95% ethanol such that 0.1 ml added to 5 g bait would give the desired test dose levels of 50, 25, 10, 7, 4, 2, 1, 0.5 and 0.1 ppm. The bait was essentially the same as the food used for feeding adult flies in the colony except for water content. Stock bait was prepared by combining 25 ml distilled water and 40 g sucrose in a 250 ml beaker and heating with stirring until the sucrose dissolved. After cooling the solution to ambient temperature, 10 g yeast hydrolysate was added to the solution and manually mixed with a stirring rod until a homogeneous mixture was formed. For each dose level to be tested, 5 g of the stock bait was weighed into a 16 ml vial and 0.1 ml of test solution was added using a dispensing pipette. The vial was sealed with a Teflon-lined cap and the contents of the vial were mixed by manual shaking and also by stirring just prior to spotting.

Bait was manually applied to the bottom of 15 cm glass petri dishes using disposable dropping pipets (20-30 droplets, 2-3 mm diam, 0.15 to 0.16 g total per dish). Two dishes were prepared for each dose level (0, 0.1,0.5, 1, 2, 4, 7, 10, 25, and 50 ppm). The bait was allowed to dry at ambient temperature for 24 h before testing. At the beginning of each test, a petri dish was placed in each cage (droplet side down) and supported by three small clothespins 2 cm above the floor of the cage. The top side of the dish was covered with translucent brown masking tape to inhibit flies walking under the dish from flying upward and getting stuck in the bait. Cages were randomly distributed within the incubator with respect to dose.

Irreversible knockdown followed by death (assessed visually) of the flies after 24 and 48 h exposure was the criterion used to determine the effective concentration (EC). Five replications of the experiment, each conducted on a different date, with two replicates of each spinosad concentration per date, were done for each sex. Sexes were tested separately. Each test utilized a different generation (batch) of flies from colony production. All test apparatus was thoroughly washed between tests. Values of EC_{50}s, EC_{99}s, and 95% fiducial limits (FL) were calculated using probit analysis (SAS Institute 1992). The LOG10 option was used because it gave the best fit of the data.

RESULTS AND DISCUSSION

Adults were observed to feed on the bait droplets at all concentrations of spinosad with no apparent repellency even at the maximum dose tested. It has been previously documented that two-week-old Caribbean fruit flies consume sugar and protein at a high rate and that protein feeding was higher at that age for females than for males (Landolt & Davis-Hernandez 1993).

Mean percentages of female flies knocked down ranged from 2 to 99% at 24 h and 5 to 100% at 48 h for bait containing 0 to 50 ppm spinosad, respectively (Table 1). For male flies knockdown ranges were 1 to 100% and 3 to 100% for exposures of 24 and 48 h, respectively (Table 1).

The EC_{50} and EC_{99} predicted from probit analysis for females at 24 h were 4.6 and 23.8 ppm, respectively (Table 2). At 48 h the EC_{50} and EC_{99} were reduced to 2.6 and 9.4 ppm, respectively. The fiducial limits were also reduced from 3.3-5.9 ppm and 14.3-81.5 ppm at 24 h to 1.7-3.4 ppm and 5.9-43.5 ppm at 48 h for the EC_{50} and EC_{99}, respectively (Table 2). At 24 h the EC_{50} (3.4 ppm) for males was slightly lower than for females and the EC_{99} (27.1 ppm) was slightly higher but the fiducial limits overlapped in both cases (Table 2). Previous results obtained using a similar bait with abamectin showed that a lower dosage was required for an EC_{50} and EC_{99} for females than males at 24 and 48 h (Hennessey & King 1996). At 48 h the values of both the EC_{50} (1.2 ppm) and EC_{99} (5.8 ppm) for males were slightly lower than the corresponding values for fe-

TABLE 1. MEAN PERCENTAGES (±SEM) OF CARIBBEAN FRUIT FLIES IRREVERSIBLY KNOCKED DOWN IN CAGE TESTS WITH SPINOSAD IN BAIT AT 10 CONCENTRATIONS.

Concentration, ppm	Females		Males	
	24 h	48 h	24 h	48 h
0	2.0±1.3	5.0±2.1	3.0±1.4	4.0±1.5
0.1	4.9±2.9	6.8±3.1	1.0±0.9	3.1±1.5
0.5	2.9±2.7	7.0±2.7	1.0±0.9	6.0±3.8
1	3.8±1.5	11.0±1.7	7.0±3.5	41.8±7.3
2	7.0±2.8	30.0±5.3	21.2±5.3	78.5±4.6
4	50.9±5.6	88.8±2.7	70.4±4.8	98.0±1.3
7	75.7±6.2	95.9±1.6	77.7±3.7	99.0±0.9
10	94.1±1.5	100±0	96.3±2.7	100±0
25	97.0±1.4	99.0±0.9	95.5±2.7	100±0
50	99.0±0.9	100±0	100±0	100±0

TABLE 2. EC^1_{50} AND EC_{99} OF SPINOSAD IN BAIT AT 24 H AND 48 H TO FEMALE AND MALE CARIBBEAN FRUIT FLIES IN CAGE TESTS.

	Females	
	24 h	48 h
EC_{50}	4.6	2.6
95% FL	3.3-5.9	1.7-3.4
EC_{99}	23.8	9.4
95% FL	14.3-81.5	5.9-43.5
	Males	
	24 h	48 h
EC_{50}	3.4	1.2
95% FL	2.5-4.3	1.1-1.4
EC_{99}	27.1	5.8
95% FL	15.9-75.3	4.6-8.1

[1]Effective concentration, ppm.

males (Table 2). The probit curves of predicted knockdown versus the logarithm (base 10) of concentration exhibits an unusually high slope and indicates that spinosad concentrations in the low ppm range will be suitable at 24 or 48 h for >99% mortality (Fig. 1).

Compared to the present protocol which requires 190,000 ppm of malathion in bait in the Caribbean fruit fly-free spray program, spinosad offers a very promising alternative since a dose of only 100 ppm is an order of magnitude greater than that required for an EC of 99% for females or males. Spinosad (NAF-85) has been shown to have LC_{50} levels of >200 ppm when tested as a contact and stomach poison against selected hemipteran, coleopteran, neuropteran, and acarine beneficial species (Schoonover & Larson, 1995). Additional data, provided by DowElanco (Spinosad Technical Guide), indicates that the effects of spinosad, relative to the environment, mammals, birds, fish, various aquatic organisms, and beneficial insects, are favorable. The acute oral toxicity for mice, rats, bobwhite quail, and mallard ducks, based on LD_{50} values, were all >2000 mg/kg. The acute dermal toxicity for rabbits was also >2000 mg/kg. Spinosad is highly toxic to honey bees when administered as a topical application, but when toxicity was evaluated in field-sprayed apple blossoms no statistical difference in mortality was observed between the treated and control groups. Assessments of potential exposures and risks to workers using spinosad to spray cotton at a level of 202.3 g active ingredient per acre indicated a margin of exposure of 1,800 for the worst case, i.e. the mixer and loader for aerial spraying. The application of 12 ounces of spray bait with 100 ppm active ingredient, which is 10 times the maximum required to achieve an LD_{99} mortality observed in this study, requires only 0.034 g spinosad per acre and the safety factor is even greater. These considerations, plus the low dosage requirements, justify field tests to determine more precisely those concentrations of spinosad that will be effective for fruit fly control under grove conditions.

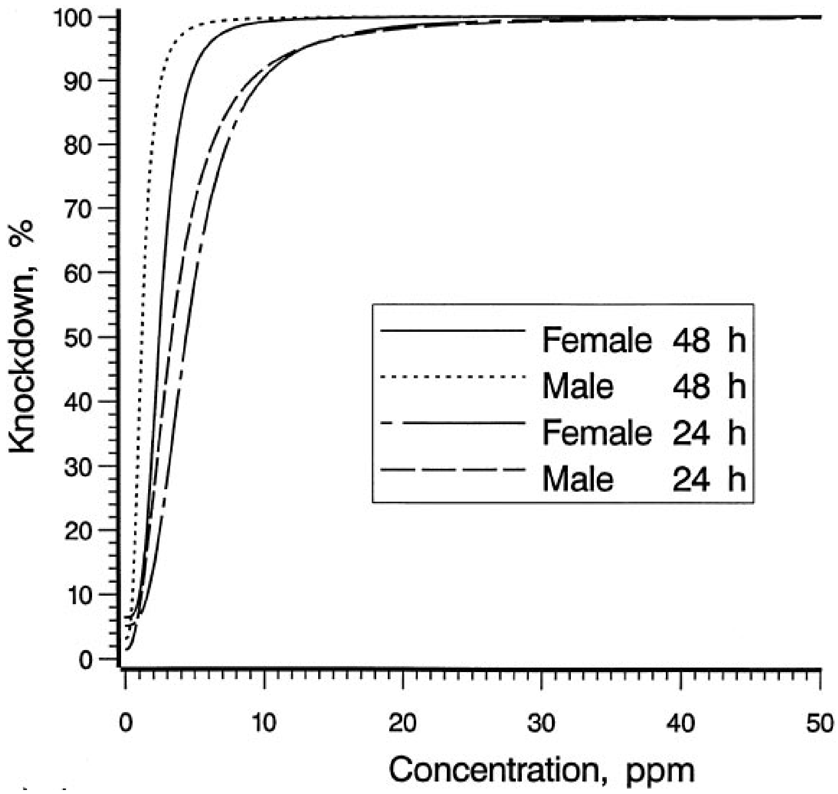

Fig. 1. Probit curve of predicted percentage knockdown and death of Caribbean fruit flies at various concentrations of spinosad after exposure times of 24 and 48 h.

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