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A REFEREED PAPER

TOXICITY OF INSECTICIDE-TREATED SPHERES TO CARIBBEAN FRUIT FLY, *ANASTREPHA SUSPENSA* AND MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE)

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Abstract. **The Caribbean fruit fly (CFF), *Anastrepha suspensa* (Loew), and the Mediterranean fruit fly (MFF), *Ceratitis capitata* (Wiedemann), are major tephritid pests that attack a wide range of tropical and subtropical plants. The potential for establishment of these fruit fly species in major U.S. fruit-producing areas (i.e., California, Florida and Texas) has demanded the need for the development of effective reduced-risk pest management tactics to control these flies without the use of broad-spectrum toxic insecticide sprays. In laboratory studies, we evaluated the use of toxic bait stations for control of *A. suspensa* and *C. capitata.* Flies were exposed to five treatments in no-choice tests and evaluated at 2, 4, 24, 48 and 72 hours. Treatments included: 1) a new sphere design treated with 1% Spinosad, 2) an old sphere design treated with 1% Spinosad, 3) old sphere design treated with 2% imidacloprid, 4) an untreated new sphere design (control), 5) an untreated old sphere design (control). Experimental design was completely randomized block with 6 and 5 replicates for CFF and MFF, respectively. During the first 24 hours, the only treatment that significantly reduced the survival *A. suspensa* below the control was the old sphere design with 2% imidacloprid. However, at 48 and 72 hours, respectively, significantly more *A. suspensa* survived in both controls compared with other treatments. There were no significant differences at 48 and 72 hours between any of the insecticide-treated spheres. Similar results were recorded for *C. capitata*. The results indicate the potential for using our new sphere design treated with 1% Spinosad for controlling *A. suspensa* and *C. capitata.***

In Florida, the ever present Caribbean fruit fly (CFF), *Anastrepha suspensa* (Loew), and the occasional invasive Mediterranean fruit fly (MFF), *Ceratitis capitata* (Wiedemann), have become serious pests of many tropical and subtropical fruits (Weems, 1967). To protect fruit producing areas in the U.S. and abroad, rigid agricultural quarantines have been established (Anonymous, 1992). Various post-harvest treatments must be adopted as sanitary control measures prior to the export of fruits and vegetables. This might include bait-sprays, vapor heat, hot air or hot water immersion, followed by cold storage or methyl bromide fumigation of a limited range of citrus varieties (Sharp, 1993). Pre-harvest strategies are also approved and are included in the "Fly Free Zone" concept (Simpson, 1993).

In addition to infesting commercial plantings, fruit flies are typically present in wild and residential plants (Norrbom and Kim, 1988), and commercial control in these environments has generated extreme concern (Headrick and Goeden, 1996). The typical means of eradicating invasive fruit fly populations involve repeated aerial applications of broad-spectrum bait-sprays followed by the release of sterile males (Sterile Insect Technique = SIT). These strategies have garnished criticism from urban populations and conservationists concerned with the effects of broad-spectrum insecticides on non-target organisms (Clark et al., 1996). A potential alternative to the application of broad-spectrum insecticides in residential areas would be the deployment of an attract-and-kill device where fruit flies would either come into contact with or be attracted to a sucrose/bait/toxin combination (bait station) (Liburd et al., 1999, 2004).

The concept of bait stations for tephritid fruit fly control including, *Bactrocera* spp., *Rhagoletis pomonella* (Walsh) and *Toxotrypana curvicauda* Gerstaecker is not new (Aluja, 1996; Landolt et al., 1988; Prokopy, 1975; Sivinski and Calkins, 1986). More recently, Liburd et al. (2004) demonstrated the potential use of imidacloprid-treated spheres for control of *A. suspensa* in areas where it may be difficult to apply broad-spectrum insecticides. However, prior to field testing imidacloprid-treated spheres, the pesticide manufacturer elected not to pursue licensing for use in citrus. Consequently, we selected a bio-pesticide, Spinosad (SpinTor 2SC) (Dow Agro Sciences, Indianapolis, Ind.) formulated from a naturally occurring soil bacterium, *Saccharopolyspora spinosad*, as the toxicant for our bait station study against *A. suspensa* and *C. capitata*. The authors chose this bio-pesticide based on its environmental/safety attributes (Thompson et al., 1999). Also, Spinosad efficacy against *A. suspensa* and *C. capitata* has been previously demonstrated in laboratory and field tests with little or no effects on the parasitoids of either species (Burns et al., 2001; King and Hennessey, 1996). Spinosad is presently registered for use in aerial application over commercial citrus but not approved to be applied aerially over residential areas.

Materials and Methods

Experiments to evaluate the toxicity of insecticide-treated spheres to control *A. suspensa* and *C. capitata* were conducted in the Small Fruits and Vegetable Integrated Pest Management Laboratory, University of Florida, Gainesville, Fla. A total of five sphere treatments were evaluated. Treatments

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included 1) a sphere treated with 2% (a.i.) imidacloprid (standard), 2 and 3) two spheres treated with 1% (a.i.) Spinosad, and 4 and 5) two spheres that were untreated (controls). For Spinosad sphere treatments two designs were evaluated (old and new). Spinosad treated spheres were obtained from Pest Management Innovations, LLC, Harpers Ferry, W.V. One sphere containing 1% Spinosad had a sucrose cap attached to the top of the sphere to stimulate feeding (old design) (Fig. 1). The other sphere with 1% Spinosad had the sucrose cap built into the sphere design (new design) (Fig. 2). Spinosad treated spheres were painted the same yellow color as imidacloprid and control spheres.

Spheres preparation. Imidacloprid-treated sphere design was similar to the one used by Liburd et al. (2004). Briefly, spheres were brush painted with yellow enamel paint ([4CI-3 Behr Flat Yellow Cluster], Home Depot, Gainesville, Fla.), and treated with 2% Admire 2 (Bayer, Research Triangle Park, N.C.), and 20% sucrose solution. The two control spheres used in the study were the same as the old and new Spinosad spheres but they were not treated with pesticides. The experimental design was a completely randomized block with six replicates for CFF and five replicates for MFF.

Source of insects. Puparium of *A. suspensa* were obtained from colonies maintained at the Florida Department of Agriculture and Consumer Service's Division of Plant Industry in Gainesville, Fla. (Burns, 1995). The puparium were sterilized by gamma irradiation with 10Krad. Irradiated puparium was treated with 4 $g \cdot L^{-1}$ of powdered fluorescent dye (Dayglo Color Corporation, Cleveland, Ohio) to mark the adults on emergence.

Fig. 1. Old model sphere containing 1% Spinosad with a sucrose cap attached to the top of the sphere to stimulate feeding.

Fig. 2. New model sphere with 1% Spinosad with the sucrose cap built into the sphere design.

Ceratitis capitata puparium were received from Moscamed rearing facility, Guatemala. Similar to *A. suspense*, the puparia were sterilized by gamma irradiation with 10Krad (100 gray). Irradiated puparium was treated with 4 $g \cdot L^{-1}$ of powdered fluorescent dye to mark the adults on emergence.

Both *A. suspensa* and *C. capitata* puparium were held in a 60 × 60 cm Plexiglas cage. Newly emerged flies were maintained on a diet of sugar (sucrose), protein (yeast hydrolysate) and water. For each treatment, water was placed in one soufflé cup (59.2 mL) with a dental wick (1 cm diameter) protruding through the lid to allow flies easy access to water. Flies became sexually mature 3-10 d after emergence. Males and females were then separated according to sex and 25 females and 25 males were released into each of five cages (30 × 30 cm) (BioQuip, Rancho Dominguez, Calif.) containing spheres for the experiments. Temperature and humidity were maintained at 26.8 ± 0.4°C and 92.5 ± 11.3%, respectively. A photoperiod of 16:8 (L:D) was provided with three grow lamps with the aid of timers.

Sampling. The mortality rate of *A. suspensa* and *C. capitata* was recorded in intervals of 2, 4, 24, 48 and 72 h post-exposure to treatments by counting the number of male and female flies killed after coming in contact with the spheres.

Statistical analysis. Male and female data were initially pooled together to examine the overall effects of the treatments on *A. suspensa* and *C. capitata*. Finally, data were sepa-

Table 1. Mean ± SEM number of *A. suspensa* killed.

Treatment	Hours post-treatment				
	2	4	24	48	72
Control (old)	0.0 ± 0.0 b	0.2 ± 0.2 b	0.7 ± 0.2 b	1.8 ± 0.5 b	1.8 ± 0.5 b
Spinosad 1% (old)	0.6 ± 0.5 ab	6.2 ± 3.9 ab	12.5 ± 3.9 a	25.7 ± 5.3 a	33.2 ± 4.9 a
Spinosad 1% (new)	1.2 ± 0.6 ab	5.0 ± 3.3 ab	13.0 ± 4.8 a	24.2 ± 4.6 a	32.0 ± 5.2 a
Control (new)	0.5 ± 0.3 ab	0.5 ± 0.3 ab	1.2 ± 0.6 b	3.2 ± 0.8 b	5.3 ± 2.4 b
Imidacloprid 2% (old)	3.0 ± 0.8 a	6.3 ± 1.8 a	15.3 ± 4.6 a	24.7 ± 4.2 a	30.0 ± 4.6 a

Means within columns followed by the same letter are not significantly different ($P < 0.05$, LSD test, SAS Institute, Inc., 2001).
For 2 h, $F = 4.58$; df = 4, 20; $P = 0.0087$; for 4 h, $F = 3.48$; df = 4, 20; $P = 0.0259$; for 24 h, $F = 16.56$; df = 4, 20; $P = < 0.0001$; for 48 h, $F = 30.55$; df = 4, 20; $P = < 0.0001$; for 72 h, $F = 33.60$; df = 4, 20; $P = < 0.0001$.

Table 2. Mean ± SEM number of *C. capitata* killed.

Treatment	Hours post-treatment				
	2	4	24	48	72
Control (old)	0.0 ± 0.0 a	0.2 ± 0.2 a	0.4 ± 0.2 c	3.0 ± 0.8 c	5.2 ± 1.6 c
Spinosad 1% (old)	0.2 ± 0.2 a	2.0 ± 1.1 a	32.6 ± 3.3 a	45.4 ± 1.8 a	47.8 ± 1.2 a
Spinosad 1% (new)	0.0 ± 0.0 a	0.8 ± 0.6 a	20.2 ± 3.4 b	39.2 ± 1.9 ab	46.0 ± 1.5 a
Control (new)	0.0 ± 0.0 a	0.0 ± 0.0 a	1.2 ± 0.6 c	4.4 ± 1.5 c	10.6 ± 2.7 b
Imidacloprid 2% (old)	1.2 ± 0.8 a	2.0 ± 1.3 a	11.4 ± 1.9 b	30.4 ± 1.2 b	42.8 ± 1.1 a

Means within columns followed by the same letter are not significantly different ($P < 0.05$, LSD test, SAS Institute, Inc., 2001).
For 2 h, $F = 2.14$; df = 4, 16; $P = 0.1236$; for 4 h, $F = 2.16$; df = 4, 16; $P = 0.1206$; for 24 h, $F = 70.52$; df = 4, 16; $P = < 0.0001$; for 48 h, $F = 185.22$; df = 4, 16; $P = < 0.0001$; for 72 h, $F = 119.79$; df = 4, 16; $P = < 0.0001$.

rated according to sex in order to determine the toxicity of treatments to males and females independently. All data were subjected to Analysis of Variance (ANOVA) followed by mean separation by using the least significant difference (LSD) test (SAS Institute, 2001). The results were considered significant if $P < 0.05$.

Results and Discussion

Anastrepha suspensa. At 2 and 4 h, only imidacloprid-treated spheres (2% a.i.) killed significantly more *A. suspensa* than the old control. However, fly mortality data from imidacloprid-treated spheres were not significantly different from Spinosad-treated spheres or the new control (Table 1).

Results from the 24, 48 and 72 h observation periods were similar. These results were different from those recorded at 2 and 4 h in that all insecticide sphere treatments killed significantly more *A. suspensa* flies compared with both controls. There was no significant difference between any of the insecticide-treated spheres (Table 1).

In the new Spinosad sphere design, flies are attracted to the visual 'fruit type' stimulus and can be seen alighting on all parts of the sphere. This was not the case with the older Spinosad sphere design. In the older Spinosad sphere version flies spent considerable time on the sphere but did not feed as much on the sucrose cap with the toxicant. This was probably due to the change in the shape of the sphere in the old design.

Ceratitis capitata. At 2 and 4 h, there was no significant difference among any of the treatments. However, at 24 h the highest mortality was recorded in the old Spinosad treatments, which was significantly higher than all other treatments. There was no significant difference between the new Spinosad treatment and imidacloprid-treated spheres. These two treatments were significantly higher than the controls (Table 2).

The results at 48 h were similar to those observed at 24 h. However, the Spinosad treatments (old and new) were not significantly different. At 72 h there were no significant differences between the old and new Spinosad treatments. Data collected on fly mortality were also not significantly different to imidacloprid-treated sphere treatments. All spheres treated with insecticides killed significantly more *C. capitata* than the controls (Table 2).

Susceptibility of male and female. Overall, there was no significant treatment difference between male and female *A. suspensa* and *C. capitata.* Also, there were no significant differences among insecticide-sphere treatments for female and male for *A. suspensa.* However, all insecticide sphere treatments killed significantly more *A. suspensa* (males and females) compared with controls (Table 3). Similar results were obtained for *C. capitata*, as all insecticide sphere treatments killed significantly more males and females compared with controls (Table 4). There was no significant difference among Spinosad sphere treatments (Table 4). Among the insecticide sphere treatments, imidacloprid-treated spheres

Table 3. Mean ± SEM male and female *A. suspensa* killed.

Treatment	Female	Male
Control (old)	1.2 ± 0.4 b	0.7 ± 0.2 b
Spinosad 1% (old)	16.5 ± 2.5 a	17.0 ± 2.8 a
Spinosad 1% (new)	17.2 ± 2.8 a	14.8 ± 2.6 a
Control (new)	3.8 ± 1.4 b	1.5 ± 0.9 b
Imidacloprid 2% (old)	18.7 ± 2.0 a	11.3 ± 3.6 a

Means within columns followed by the same letter are not significantly different ($P < 0.05$, LSD test, SAS Institute, Inc., 2001). For all females, $F = 19.09$; df = 4, 20; $P = < 0.0001$; for all males, $F = 23.78$; df = 4, 20; $P = < 0.0001$. For all treatments $n = 150$.

Table 4. Mean ± SEM male and female *C. capitata* killed with different treatments.

Treatment	Female	Male
Control (old)	3.4 ± 0.9 b	1.8 ± 0.6 d
Spinosad 1% (old)	21.8 ± 1.2 a	26.0 ± 0.8 a
Spinosad 1% (new)	22.4 ± 1.2 a	23.6 ± 2.0 ab
Control (new)	5.6 ± 2.1 b	5.0 ± 1.0 c
Imidacloprid 2% (old)	23.4 ± 0.8 a	19.4 ± 0.5 b

Means within columns followed by the same letter are not significantly different ($P < 0.05$, LSD test, SAS Institute, Inc., 2001). For all females, $F = 51.75$; df = 4, 16; $P = < 0.0001$; for all males, $F = 126.76$; df = 4, 16; $P = <0.0001$. For all treatments $n = 125$.

were significantly less effective in killing *C. capitata* males than the old design of Spinosad-treated spheres (Table 4). Our results show that the toxicity of the new Spinosad design was just as effective as previous sphere models.

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