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EFFICACY OF SPINOSAD BAIT SPRAYS TO CONTROL MEDITERRANEAN AND CARIBBEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE) IN COMMERCIAL CITRUS IN FLORIDA

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ABSTRACT

A serious infestation of Mediterranean fruit fly in Florida in 1997 and 1998 led to the widespread aerial and foliar application of malathion-bait sprays. Public concerns over property damage, environmental impact and public health led to the immediate need and acceptability of alternative pesticide/bait combinations. Preliminary work with spinosad, a derivative of a soil microorganism developed by Dow AgroSciences, in combination with a new bait (Sol-Bait) showed promise. To ensure that this product would be effective in Florida for fruit fly control, three field tests were conducted using aerial and/or foliar applications. Results indicated that sprays with spinosad-SolBait provided comparable and significant control levels for sterile Mediterranean and Caribbean fruit flies in comparison to standard malathion with NU-LURE® or SolBait treatments by aerial or foliar application. In one test, honey bees and hives were exposed to sprays in the treatment area and no significant treatment differences were observed in hive condition or brood. Insufficient data on effects of treatments on naturally occurring and introduced beneficial insects were collected for statistical analysis but it appears no harmful effects were observed.

Key Words: Spinosad, Bait spray, Mediterranean fruit fly, Caribbean fruit fly

RESUMEN

Durante los años 1997 y 1998 ocurrió en Florida una infestación de impotancia de la mosca del Mediterráneo, que trajo como consecuencia una amplia aspersión aérea y foliar de la mezcla malathion-cebo. Debido a la preocupación pública por daños a la propiedad privada, el impacto ambiental, y la salud pública fue posible la aceptación necesaria e inmediata de alternativas a las combinaciones de pesticida/cebo. Los trabajos iniciales con Spinosad, el cual es un derivado de microorganismos del suelo, con un cebo nuevo (SolBait) y producido por la compañía Dow AgroScience fueron prometedores. Para asegurar la efectividad del producto en el control de la mosca de la fruta en Florida, se efectuaron tres pruebas de campo usando aspersión aérea y foliar. Los resultados indicaron que las aspersiones de Spinosad combinado con SolBait produjeron un nivel de control de las moscas del Mediterráneo y del Caribe, comparable y significativo en comparación con los estándares de los tratamientos usando Malathion con NU-LURE® o SolBait aplicados por aspersión aérea o foliar. En una de las pruebas, colmenas de abejas fueron expuestas a las aspersiones en el (rea de tratamiento y no se observó diferencia significativa en las condiciones de las colmenas o las crías. Los datos acerca del efecto de los tratamientos en los insectos benéficos fueron insuficientes, pero no se observaron efectos dañinos.

Since 1956, malathion-bait sprays have been used extensively for the control of Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) and, more recently, Caribbean fruit fly (Caribfly), *Anastrepha suspensa* (Loew) (Steiner et al. 1961). Ten serious infestations of Medfly were eradicated successfully in Florida using malathion-bait spray mixtures applied by ground and/or air (Clark et al. 1996). The same strategy was used to eradicate Medfly from Brownsville, Texas in 1966 (Stephenson & McClung 1966). California has had a simi-

lar history of Medfly infestations since 1975 and has used malathion-bait spray in all or part of their eradication efforts (Carey et al. 1999).

In 1997, the detection of 749 Medflies in a five-county area in Florida led to the widespread aerial and ground application of the malathion-bait spray mixture over a heavily populated urban environment. Subsequent reports of vehicle paint discoloration, fish kills and public complaints about the use of organophosphate insecticides led to increased emphasis on identifying

alternative insecticides and attractants with reduced environmental and public health impact.

Several materials with insecticidal properties in conjunction with an improved bait attractant have been tested against several species of economic tephritid fruit flies. Phloxine B is a photoactive dye which is toxic to certain insect species (Heitz 1987, 1995). Moreno and Mangan (1995) and Liquido et al. (1995) reported that phloxine B added to an attractant bait could evoke a high degree of mortality in Medfly and other fruit fly species.

Shortly thereafter, a new species of actinomycetes, *Saccharopolyspora spinosa* (Mertz and Yao 1990) was shown to produce compounds, primarily spinosyns A and D, which had insecticidal properties. Spinosad, a mixture of spinosyns A and D, has shown activity against several insect orders including Diptera. It acts as a stomach and contact poison and degrades rapidly in the environment. Preliminary laboratory tests by King and Hennessey (1996) and Adan et al. (1996) indicated mortality of Caribfly and Medfly fruit flies at low concentrations.

An initial field test by Moreno et al. (2000) with spinosad and other compounds in a new bait material, SolBait (Moreno and Mangan 2000), against sterile, dyed Mediterranean fruit flies, showed efficacy comparable to the standard, malathion-NU-LURE®. This test in Florida indicated that aerial sprays by helicopter and foliar spot sprays of spinosad-SolBait in commercial citrus groves were equal to or better than malathion-NU-LURE® in reducing fruit fly populations. However, additional field trials were needed to confirm the efficacy of spinosad-SolBait against Caribfly and Medfly using aerial and ground application techniques.

Non-target effects of materials tested were evaluated by releasing the fruit fly parasitoid *Diachasmimorpha longicaudata* (Ashmead), and by placing honey bee hives in treatment blocks during the first test.

MATERIALS AND METHODS

Three replicated field tests were conducted in commercial orange groves in Florida in spring and fall, 1999 and spring, 2000. Test 1 consisted of ground applications of foliar sprays of spinosad and malathion in SolBait at higher volumes compared to the malathion-NU-LURE® standard of 20% malathion ULV with 80% NU-LURE® at 12 oz of mix per acre. Test 2 was also a ground application of foliar sprays of spinosad-SolBait versus malathion-NU-LURE® plus water at the standard dosage but at the same volume per acre as spinosad-SolBait. Test 3 compared aerial application of spinosad-SolBait at two rates to the malathion-NU-LURE® standard and to ground application of spinosad-SolBait. Tests 1 and 3 were conducted against both sterile Caribflies

and Medflies while Test 2 was conducted against sterile Medfly only.

Treatments

Test 1 (22 March 1999-03 May 1999). Each treatment was applied to 3.2 ha plots replicated four times in a commercial orange grove in DeSoto County, Florida. Blocks had about 309 trees per ha. Treatment blocks were separated by a minimum buffer of 91.4 m. Treatments were also replicated in time by separation of applications at two week intervals for three applications. The treatments in the experiment were (1) a check (SolBait only) at 21.5 l/ha, (2) spinosad at 80 ppm (0.28 g AI/ha) in SolBait at 21.5 l/ha, and (3) malathion (Fyfanon® 96.8%) (208.3 g AI/ha) at 175.4 ml/ha in SolBait at 21.3 l/ha; and (4) malathion (Fyfanon® 96.8%) (208.3 g AI/ha) at 175.4 ml/ha, was applied with NU-LURE® bait at 700.9 ml/ha as spot sprays on 30 trees per ha at 30 ml per tree. Each of the first three treatments was applied as a foliar spot spray at a rate of 60 ml per tree. Foliar spot sprays for treatments 1, 2, and 3 were applied to about a one square meter area of each tree at about $\frac{2}{3}$ tree height using Spraying Systems Co.® 5500 cone jet nozzles attached to a 0.6 m wand and a GunJet #43 hand held spray gun. Working pressure of 862.5 kPa was generated by a Hypro D-30 diaphragm pump and Honda GX 160 5.5 hp engine mounted on a John Deere "Gator™" (4 × 2, 10 hp) utility vehicle. Treatment four was applied by using 11.4-l (3 gallon) hand held pump up sprayers on every 10th or 12th tree of each row.

Test 2 (08 November 1999-06 December 1999). Three treatments were applied to four 4.0 ha blocks of mature orange trees in a commercial grove in Highlands County, Florida. Blocks had about 309 trees per ha and were separated by a minimum buffer of 91.4 m. The application was repeated one time with a two-week interval between applications. Treatments consisted of (1) a control (untreated), and foliar spot sprays at 60 ml per tree of either (2) spinosad 80 ppm (1.57 g AI/ha) in SolBait at 18.7 l/ha or (3) malathion 5 EC (MICRO FLO® 56%) (187.2 g AI/ha) at 312.5 ml/ha with NU-LURE® at 700.9 ml/ha and water at 17.7 l/ha. Foliar spot spray was applied to trees at about $\frac{2}{3}$ tree height using hand held nozzles attached to the same pressure spray equipment described in Test 1.

Test 3 (20 March 2000-08 May 2000). Treatments were applied to 24, 4.0 ha plots in a commercial orange grove in Hendry County, Florida. A minimum buffer of 122 m separated treatment blocks. Treatments were replicated four times and replicated in time by separation at two-week intervals for three treatment dates. Treatments consisted of (1) a control (untreated), (2) a SolBait check applied aerially at 3.5 l/ha, (3) malathion

(Fyfanon® 96.8%) (208.3 g AI/ha) at 175.4 ml/ha with NU-LURE® at 700.9 ml/ha applied aerially, (4) spinosad 80 ppm (0.13 g AI/ha) in SolBait applied aerially at 1.75 l/ha, (5) spinosad 80 ppm (0.28 g AI/ha) in SolBait applied aerially at 3.5 l/ha, and (6) spinosad 80 ppm (3.37 g AI/ha) in SolBait applied as a foliar spot spray at 90 ml per tree (40.2 l/ha) using hand held nozzles attached to the same pressure spray equipment described in Tests 1 and 2.

For the first and third treatment dates, all treatments were applied. For the second treatment date, no check treatment was included.

Insects

Test 1. Sterile, dyed Medfly pupae were received from the El Pino rearing facility in Guatemala at the USDA Preventative Release Program facility at MacDill AFB, Tampa, Florida. The flies were eclosed in plastic adult rearing containers (PARC), held for five days, and transported to the test site in an air-conditioned van. The dyed Caribfly pupae were shipped from the Caribfly rearing facility in Gainesville, Florida to Ft. Pierce for eclosion one week before release. Flies were also transported in air-conditioned vans to the test site. Sterile, dyed Medflies and Caribflies with about equal numbers of males and females were released at the rate of 17,300 flies/ha in each treatment block the day before treatment. Flies were released statically at two equidistant release points along the central row of each test plot.

The braconid parasitoid, *Diachasmimorpha longicaudata* (Ashmead) was released at the rate of 10,000 per treatment plot one day after treatment application for one test date only. Nylon stocking exposure traps containing sterile larvae of Caribfly in larval diet were placed in host trees for 5 days to monitor *D. longicaudata* activity.

Brood and hive condition of the honey bee, *Apis mellifera* L. was observed by placing two hives in the center of each treatment block one week before the first treatment. Hive condition was rated individually by experienced apiary personnel on a scale of 1-5 (dead-strong) and recorded as the average of two hives in each block. Brood was measured by counting the number of frames of brood for each hive and averaging the results from the two hives in each block. Hives were evaluated three times at two-week intervals.

Test 2. Sterile, dyed Medflies with about equal numbers of males and females were released at the rate of 23,700 flies/ha in each treatment block the day before treatment. Flies were released statically from three equidistant release points along the central row of each block.

Test 3. Sterile, dyed Caribflies with about equal numbers of males and females were released at the rate of 17,300/ha in each treatment block for the first treatment date. Medflies and Caribflies

were released at 17,300/ha for the second and third treatment dates. Medflies were released statically from two central release points and Caribflies were released from three release points along the central row of each treatment block.

Data Collection and Analysis

In test 1, 10 plastic International Pheromone (South Wirral, UK) IPM (Liquibaitor®) traps with Concept™'s Medfly Biolure® (ammonium acetate, putrescine, trimethylamine) were placed in each plot for the first treatment date. Ten Jackson traps containing trimedlure (TML) plugs were added for subsequent treatments to attract male flies in addition to the predominantly female attractive IPM trap with Biolure®. Traps were placed about 72 h after treatment application, checked and removed 6 d after application. Data were collected on the number of flies trapped by species and treatment for each date interval. For test 2, 10 Multi-Lure® traps with the Medfly Biolure® were placed in each plot for each treatment date. Traps were managed as in Test 1. For test 3, 10 Multi-Lure® traps were placed in each plot for each treatment date. For the first treatment date in which only Caribflies were released, traps contained a prepared solution of torula yeast and borax (4-5 g tablets/500 ml water). For succeeding treatment dates in which Caribflies and Medflies were released, a Concept™'s trimethylamine lure was added to each trap. Traps were managed as in test 1.

Environmental monitoring of water and soil was done for all tests but most extensively for Test 3. Pre and post-treatment water and soil samples were taken as well as 4" × 4" swab cards for aerial and ground application. Direct samples of all mixed treatment materials were taken before application. Split samples were evaluated by the Division of Agricultural Environmental Services, FDACS and the USDA, APHIS, PPQ laboratory in Gulfport, MS.

A randomized complete block experimental design (Little and Hills 1975) consisting of four, three or five treatments, with four replicates was used for tests 1, 2, and 3, respectively. Fly recoveries were combined where two trap types occurred at trap locations. Data were analyzed by dates using Statistix® ANOVA, and means separated using Tukey's HSD ($P = 0.05$).

RESULTS

Large plot sizes of 3-4 ha repeated over space and time were used to reduce the variability inherent in field trials. Buffers of 91-122 m minimized migration of flies between plots. No difference was indicated by statistical analysis among application dates for either Caribfly or Medfly for each of the three tests.

In test 1, there was a difference between the check and the three ground application pesticide treatments for both Caribfly ($F = 5.73$, $df = 3,42$, $P = 0.0022$) and Medfly ($F = 11.22$, $df = 3,42$, $P < 0.0001$). There were no differences among insecticide treatments (Fig. 1) although the malathion-SolBait treatment had the lowest mean number of flies trapped for both fly species (Table 1). For the average of the three test dates, spinosad-SolBait, malathion-NU-LURE® and malathion-SolBait reduced Medfly populations by 80, 76 and 91%, respectively compared to the check. Likewise, Caribflies were reduced 87, 94 and 91% for the same treatments.

No adverse or measurable effect was shown by any of the treatments on the number of frames of honey bee brood (Table 2). No significant differences in hive condition were observed (Table 3).

Due to the small numbers of recaptures of established beneficial insects in test plots including the checks, the data could not be analyzed statistically. Similarly, few recaptures of adult *D. longi-*

caudata and small numbers of larval-traps resulted in insufficient data but suggest that pesticide treatments had no effect.

In test 2, spinosad-SolBait was compared to an equal volume of malathion, NU-LURE® and water mixture applied with ground application equipment. Only sterile Medflies were used in this test. Treatment effects were observed ($F = 29.69$, $df = 2,20$, $P < 0.0001$) and comparison of means (Fig. 2) indicates that spinosad-SolBait and the malathion-NU-LURE®-water mixture provided comparable control (Table 1). Medfly populations were reduced by about 90 and 89% for the spinosad-SolBait and malathion-NU-LURE®-water treatments, respectively.

Aerial and foliar spot spray applications of spinosad-SolBait at two different rates were compared to the malathion-NU-LURE® standard by aerial application in test 3. For Medfly, there were treatment effects ($F = 18.90$, $df = 6,34$, $P < 0.0001$). The SolBait check and untreated control were different from the insecticide treat-

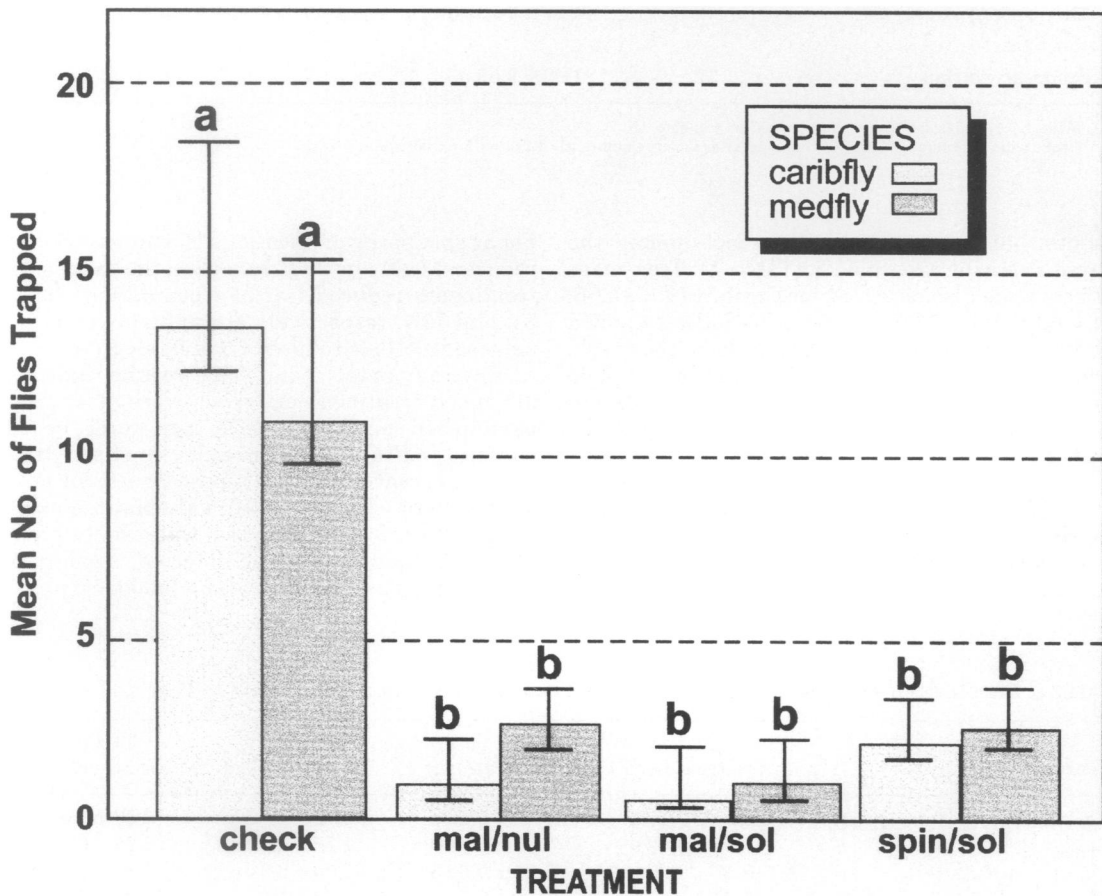


Fig. 1. Mean numbers (\pm SE) of sterile Caribflies and Medflies recovered following ground applications in Test 1. For each species, bars with the same letter are not significantly different from each other according to Tukey's HSD ($P = 0.05$).

TABLE 1. EFFECT OF SPINOSAD AND MALATHION TREATMENTS ON STERILE RELEASED CARIBBEAN AND MEDITERRANEAN FRUIT FLIES.

Treatments	Mean no. Caribfly/trap		Mean no. Medfly/trap	
	n	x \pm SE	n	x \pm SE
Test 1 ¹ —Foliar Spot				
Check	12	13.75 \pm 5.16 a ²	12	12.33 \pm 2.89 a
Spinosad/SolBait	12	2.16 \pm 0.90 b	12	2.50 \pm 0.71 b
Malathion/NuLure	12	1.08 \pm 0.56 b	12	3.00 \pm 0.66 b
Malathion/SolBait	12	0.67 \pm 0.40 b	12	1.08 \pm 0.45 b
Test 2—Foliar Spot				
Control		not released	8	115.38 \pm 17.75 a
Spinosad/SolBait			8	10.78 \pm 3.10 b
Malathion/NuLure			8	11.38 \pm 5.23 b
Test 3				
Control	12	937.83 \pm 108.81 a	7	109.14 \pm 24.42 a
Aerial				
Check	8	759.38 \pm 155.26 ab	3	149.00 \pm 72.67 a
Spinosad/SolBait-1.8 l/ha	12	468.08 \pm 141.18 bc	7	4.29 \pm 1.06 b
Spinosad/SolBait-3.5 l/ha	12	233.83 \pm 66.01 cd	7	2.14 \pm 0.83 b
Malathion/NuLure	12	44.17 \pm 32.97 d	7	0.00 \pm 0.00 b
Foliar Spot				
Spinosad/SolBait	12	21.92 \pm 9.69 d	7	0.14 \pm 0.14 b

¹Mean separation is distinct for each species and test date.²Means within a column followed by the same letter are not significantly different (P = 0.05, Tukey's HSD).

ments, but there was no difference among the three pesticide treatments (Table 1). Treatment effects were also observed for Caribfly (F = 14.66, df = 6,63, P = <0.0001). Spinosad-SolBait applied at 1.8 l/ha by air was not different from the check, while only the 3.5 l/ha spinosad-SolBait applied by air was statistically similar to the malathion-NU-LURE® standard. Figure 3 illustrates the treatment differences for both species.

Compared to the untreated control, Medfly populations aerally treated with malathion-NU-LURE® at 0.876 l/ha, spinosad-SolBait at 1.8 l/ha, and spinosad-SolBait at 3.5 l/ha reduced Medfly populations by 100, 96, and 99% respectively.

Foliar spot spray applications of spinosad-SolBait reduced Medfly by 99%. Likewise, the same aerial treatments reduced Caribfly populations by 95, 54, and 73%, respectively. Ground applications of spinosad-SolBait reduced Caribfly by 97%.

Environmental monitoring results indicated all mixed treatment materials were at or below established percentages. No pre-water or soil samples indicated detectable levels of any treatment material. The highest post-treatment levels of treatment materials were: soil-malathion = 640 ppb, spinosad = not detected; water-malathion = 0.65 ppb, spinosad = not detected; swab-malathion = not sampled, spinosad = 0.00015 μ g/cm².

TABLE 2. BROOD NUMBERS OF HONEY BEES EXPOSED TO SPINOSAD/MALATHION TREATMENTS IN TEST 1.

Treatments	n	24 h pre-treatment ¹	14 d post 1st treatment	14 d post 2nd treatment	14 d post 3rd treatment
Check	8	6.25 \pm 0.56 a ^{2,3}	7.00 \pm 0.68 a	6.12 \pm 0.72 a	5.62 \pm 0.86 a
Spinosad/SolBait	8	7.25 \pm 0.62 a	7.88 \pm 0.74 a	7.25 \pm 1.08 a	6.75 \pm 0.80 a
Malathion/NuLure	8	6.25 \pm 0.66 a	7.25 \pm 0.59 a	7.00 \pm 0.91 a	5.88 \pm 1.19 a
Malathion/SolBait	8	7.62 \pm 0.65 a	6.50 \pm 0.76 a	6.12 \pm 1.09 a	6.25 \pm 1.25 a

¹Successive treatments were cumulative.²Mean and SE of avg. no. of frames of brood.³Means within a column followed by the same letter are not significantly different (P = 0.05, Turkey's HSD).

TABLE 3. HIVE CONDITION OF HONEY BEES EXPOSED TO SPINOSAD/MALATHION TREATMENTS IN TEST 1.

Treatments	n	24 h pre- treatment ¹	14d post 1st treatment	14d post 2nd treatment	14d post 3rd treatment
Check	8	3.88 ± 0.40 a ^{2,3}	3.62 ± 0.42 a	3.75 ± 0.37 a	3.50 ± 0.19 a
Spinosad/SolBait	8	4.12 ± 0.40 a	4.25 ± 0.25 a	4.38 ± 0.38 a	3.75 ± 0.31 a
Malathion/NuLure	8	3.75 ± 0.53 a	3.75 ± 0.37 a	3.75 ± 0.41 a	3.50 ± 0.33 a
Malathion/SolBait	8	4.25 ± 0.41 a	3.62 ± 0.42 a	3.75 ± 0.45 a	3.25 ± 0.37 a

¹Successive treatments were cumulative.²Mean and SE of avg. no. of frames of brood.³Means within a column followed by the same letter are not significantly different (P = 0.05, Tukeys HSD).

DISCUSSION

Aerial applications of a spinosad-SolBait material at the rate of 3.5 l/ha compared to either a bait only check or an untreated control and the standard malathion-NU-LURE® mix provided acceptable control of sterile Medflies and Caribflies released in commercial citrus groves. Similar results were reported by Moreno et al. (2000) comparing spinosad-SolBait to 10 and 20% rates of malathion in NU-LURE®. Likewise, comparable results have been observed for wild Medflies in a recent eradication program in Guatemala and Mexico (Rendon et al. 2000).

Spinosad-SolBait at 1.8 l/ha applied by air in test 3 did not provide an adequate measure of control for Caribflies possibly due to differences in foraging behavior. The reduction in Caribfly population was 54% compared to the control and a 96% reduction in Medflies. The aerially applied 3.5-l/ha rate of spinosad-SolBait was significantly different than the control and reduced Caribflies by 73% compared to 99% for Medflies.

Applications of spinosad-SolBait applied as foliar spot sprays on individual trees in a com-

mercial grove setting at 90 ml of mix per tree provided excellent reduction of both Medflies and Caribflies. There were no differences observed among Spinosad-SolBait treatments and malathion-NU-LURE® treatments at 876.8 ml/ha or at 90 ml per tree. Application of 90 ml per tree is equivalent to about 21.5 l of mix per ha. Lower recapture rates of flies in this test compared to tests 2 and 3 may be attributed to a prior pesticide application in this grove.

Honey bees exposed to foliar spot sprays in test 1 exhibited no effects either in number of frames of brood produced or in overall hive condition. SolBait has been shown to be repellent to the European honey bee (Tarshis Moreno 2001), which may explain the absence of any effect of the foliar spot sprays. Likewise, the larval-trap data from release of the braconid parasitoid, *Diachasmimorpha longicaudata*, suggests that neither of the insecticide treatments affects parasitism though the number of replicates for this measure was inadequate (J. M. Sivinski and T. C. Holler, pers. comm.).

Costs of aerial application and materials were compared at the 3.5 l/ha rate for spinosad-SolBait and 876.8 ml/ha for the standard malathion-NU-LURE® bait mix. Spinosad-SolBait derived from

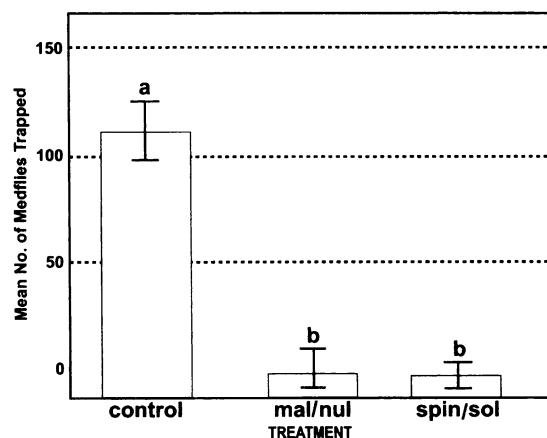


Fig. 2. Mean numbers (±SE) of Medflies recovered following ground applications in Test 2. Bars with the same letter are not significantly different from each other according to Tukey's HSD (P = 0.05).

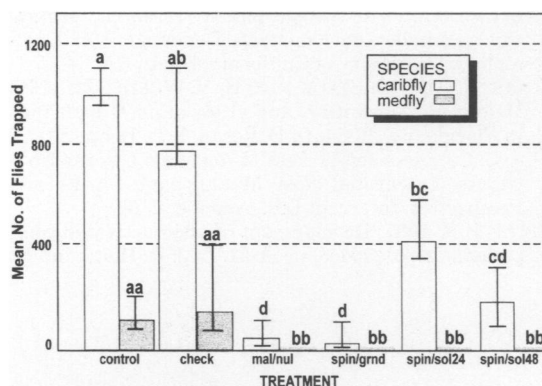


Fig. 3. Mean numbers (±SE) of sterile Caribflies and Medflies recovered following ground (spin/grnd) and aerial applications in Test 3. For each species, bars with the same letter are not significantly different from each other according to Tukey's HSD (P = 0.05).

the GF 120 formulation (Dow AgroSciences) would cost about \$18.53/ha (\$7.50/acre) based on current private applicator costs in Florida. Malathion-NU-LURE® costs are \$12.36/ha (\$5.00/acre). Due to variability of equipment used and rates of material for ground application, no cost estimates were determined.

The results of this series of tests indicate that spinosad-SolBait bait materials may be used as an alternative tool for control of fruit fly pests in Florida. Further studies are needed on relative response of fruit fly species to the SolBait-based toxins, application techniques and equipment, dosage of spinosad, rate of application, incorporation into bait stations and any effect on non-target organisms in commercial citrus groves.

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