CROP PROTECTION

Effect of Spinosad Bait against *Ceratitis capitata* (Wied.) and *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae) in Laboratory

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Efeito de Isca Tóxica à Base de Spinosad Contra *Ceratitis capitata* (Wied.) e *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae) em Laboratório

RESUMO - Estudos de laboratório foram conduzidos para determinar as mortalidades causadas por spinosad em *Ceratitis capitata* (Wied.) e *Anastrepha fraterculus* (Wied.). Isca formulada à base de spinosad foi comparada com iscas tóxicas preparadas com malatiom, triclorfom, fentiom e deltametrina. O tempo letal aumentou em adultos de *C. capitata* (mosca-do-mediterrâneo) alimentados com menores concentrações de spinosad. O tempo letal (TL50) foi estimado em 106, 126 e 154 min. para adultos de *C. capitata* expostos a 80, 8 e 4 ppm de spinosad, respectivamente. Não houve diferenças nos TL50 para as idades de 2-3; 3-5 e 5-7 dias de idade de *C. capitata* expostas a 80 ppm de spinosad. Ambas as espécies de moscas-das-frutas apresentaram padrão similar de sobrevivência quando tratadas com spinosad a 80 ppm. Em geral, fentiom e triclorfom apresentaram TL50 menores que spinosad para diferentes idades de *C. capitata*. Após 180 minutos de início de exposição à isca com spinosad, as taxas de mortalidade de 2-3 e 3-5 dias de idade de *C. capitata* foram semelhantes àquelas obtidas para fentiom. O TL50 para *A. fraterculus* foi estimado em 85,9 min., ligeiramente menor que para *C. capitata*, quando expostas a 8 ppm de spinosad. Os TL50 para adultos da mosca-do-mediterrâneo expostos a concentrações iguais ou inferiores a 0,4 ppm de spinosad foram superiores a 12h. As folhas mantidas no campo até dois dias após sua pulverização com iscas à base de spinosad, malatiom, fentiom e deltametrina causaram altos níveis de mortalidade de adultos de *C. capitata*.

PALAVRAS-CHAVE: Insecta, mosca-das-frutas, inseticida, mortalidade

ABSTRACT - Laboratory studies were conducted to determine the mortalities caused by spinosad in *Ceratitis capitata* (Wied.) and *Anastrepha fraterculus* (Wied.). Spinosad formulated bait, diluted in water, was compared with baits of malathion, trichlorfon, fenthion and deltamethrin. The lethal time increased for *C. capitata* (medfly) adults fed in lower concentrations of spinosad. The LT50 values for *C. capitata* were estimated in 106, 126 and 154 min for 80, 8 and 4 ppm, respectively. No differences at LT50 were observed for different ages, 2-3d, 3-5d and 5-7d old *C. capitata* exposed to 80 ppm. Both fruit fly species presented similar pattern of survival when treated with spinosad at 80 ppm. In general, fenthion and trichlorfon showed LT50 values lower than spinosad for different ages of medfly. After 180 min from initial exposure to spinosad bait, the mortality rates of 2-3d and 3-5d old *C. capitata* were similar to that by fenthion. The LT50 for *A. fraterculus* was estimated in 85.9 min, slightly shorter than for *C. capitata* when both species were exposed to 8 ppm of spinosad. Medfly adults exposed to concentrations equal or inferior to 0.4 ppm of spinosad showed LT50s longer than 12 h. Leaves treated and kept in the field with baits of spinosad, malathion, fenthion and deltamethrin, caused high mortalities of medfly adults until two days after treatment.

KEY WORDS: Insecta, fruit fly, insecticide, mortality

Ninety four *Anastrepha* species and medfly - *Ceratitis capitata* (Wied.) have been reported in Brazil. South American fruit fly - *Anastrepha fraterculus* (Wied.) and medfly are the most polyphagous species infesting Brazilian fruits, where are known 66 and 58 hosts, respectively (Zucchi 2000, 2001).

Fruit flies need sugars and proteinaceous sources to their survival and sexual maturity. In the field several foods are available for adults, such as bird dung, plant sap exuding and rotting fruits caused by the feeding of insects, by diseases or mechanical damage (Mariconi & Iba 1955, Christenson & Foote 1960, Bateman 1972, Orlando & Sampaio 1973). Bait efficacy depends on its particular capacity to compete with those materials in attracting fruit flies. Furthermore, the bait needs to have aceptable and phagostimulating qualities to be consumed by the flies. The methods used to develop a specific bait (Solbait) were described in Moreno and Mangan (2002), whose base formulation was 2% AI Solulys in 15% invert sugar, water, 0.4% xanthan gum, 1% ammonium acetate, 1% polysorbate 60, 1% polythyelene glycol 200 and 0.25% soybean oil. Solbait can be used with a wide variety of insecticides such as thiamethoxam, imidacloprid, abamectin, emamectin, milbemectin, cyromazine, fipronil, indoxacarb, spinosad and malathion. Those authors concluded that SolBait is more acceptable and synergistically more toxic to *Anastrepha ludens* (Loew) than Mazoferm bait.

Historically, bait and insecticide sprays to control fruit flies have been used since the beginning of the 20th century (Moreno & Mangan 2000). In 1952, protein-hydrolisate baits using organophosphate (parathion) plus brown sugar were first tested in guavas to control *Bactrocera dorsalis* (Hendel) and medfly in Hawaii (Steiner 1952). Even now the most common practice for fruit fly control is an insecticide bait spray (Calkins & Malavasi 1995).

In the state of São Paulo, malathion and other organophosphate insecticides have been used since 60's (Orlando & Sampaio 1973). Nowadays, eight organophosphate and two pyrethroids are available to control fruit fly populations in Brazil in cover spray and toxic bait.

The main reason for the widespread use of malathion is its low mammalian toxicity and low price (Moreno & Mangan 2000), however, that insecticide can affect the biological control (Ehler *et al.* 1984, Hoy & Dahlsten 1984). Therefore, alternative baits have been demanded by IPM and eradication fruit fly programs due to tactics of surveillance of beneficial insects and inundative release of parasitoids.

The first active ingredient in the Naturalyte class, spinosad is a mixture of spinosyn A and spinosyn D. A spinosad protein bait, sold in many countries as GF-120 by Dow AgroSciences, would be safer to humans because it combines both protein and insecticide in the commercial product. GF-120N is also registered by USDA and most European organic registry organizations as an organic insecticide. The objective of this study was to measure the mortality of *C. capitata* and *A. fraterculus* adults exposed to spinosad protein bait.

Material and Methods

Colonies. The flies were originated from a colony maintained at the Instituto Biológico, in Campinas, SP, since 1993 with introduction of wild specimens every year. Prior to the tests, the medfly larvae were reared in artificial media (Raga *et al.* 1996) and the South American fruit fly larvae were reared in papaya fruits. The emerged adults were fed on a mixture of 1:3 yeast extract and sugar. About 12h before the beginning of the experiments the flies were deprived of food and water. During testing, only baits were available to the flies. Tests were carried out in laboratory under 25 ± 1ºC, 70 ± 10% RU and 14h photophase.

Trials. Spinosad bait (GF-120) contains 0.02% spinosad and 99.98% water, sugar and attractants. Commercial products diluted in distilled water for medfly consisted of spinosad bait (4, 8 and 80 ppm), trichlorfon (1,500 ppm) and fenthion (750 ppm) both in mixture with protein (provided by Dow AgroSciences – blank formulation without spinosad) at 3% and, fenthion (750 ppm) added to the protein Aumax® at 3%. Adults from the control plots were fed on protein Dow (3%). To determine the mortality, five females and five males of 2-3-, 3-5- and 5-7d old *C. capitata* were placed in each of ten replicate small plastic cages (260 cc). Aproximately 0.2 ml of bait was disposed daily through the Teflon® gutter situated on the upper third of the cage.

Spinosad doses used against *A. fraterculus* were the same as for medfly. This bait was compared with malathion (1,000 ppm), fenthion (750 ppm) and deltamethrin (12.5 ppm), all added to protein Aumax® at 3%. Adults from the control plots were fed on protein Aumax® (3%). Four females and four males of 1-3-, 3-5- and 5-7d-old *A. fraterculus* were placed in each of five replicate plastic cages (1400 cc). About 1 ml of bait was disposed daily through the cotton inside the 2.7 cm plastic dishes, placed on the cage ground. Evaluations of adult survival were carried out at 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min after initial exposure.

Low-Dose Trial. To determine the effects of low doses, five females and five males of 2-3d-old medflies were exposed to 0.40, 0.33, 0.29, 0.25 and 0.20 ppm of spinosad in ten replicate plastic cages (1400 cc). The control and all low doses contained 5% of protein Bio Anastrepha. About 1 ml of bait was disposed daily through the cotton inside the 2.7 cm plastic dishes, placed on the cage ground. Adult survival was evaluated at 1, 2, 4, 6, 8, 10, 12, 21, 24, 28, 30 and 34h after the initial of exposure.

Residual Bait Assay. Diluted baits and protein (control) were manually sprayed on the upper surface of *Citrus* sp. foliage in a field near the laboratory until complete covering. Baits were allowed to dry at environmental temperature before testing. One leaf was collected at intervals of 0, 1, 2, 4, 7 and 10 days after spraying and put in one of the ten replicate cages (1400 cc), containing five females and five males of 2-3d-old *C. capitata*. New adults were exposed at each residual assay. Treated leaves were disposed on the ground of the similar cages used for *A. fraterculus*.

Treatments consisted of spinosad bait (4, 8 and 80 ppm), malathion (1,000 ppm), fenthion (750.0 ppm) and deltamethrin (12.5 ppm), all added to the protein Aumax® (3%). During the experiment the average temperature in the field was 19.4ºC and the rainfall reached 44 mm.

Statistical Analysis. A completely randomized design was used for selection of flies and treatments. Data were analyzed by ANOVA, and means separated using Tukey´s Test ($P < 0.05$). Irreversible knockdown followed by death of the adults were the criteria to determine the mortality.

Results and Discussion

No differences at LT_{50} were detected for the different ages of *C. capitata* when treated with spinosad at 8 ppm. Similar results were obtained for 2-3- and 3-5d-old adults at 80 ppm. However, for spinosad at 80 and 4 ppm, higher LT_{50} values were observed for 5-7d-old adults of *C. capitata* in comparison to flies with inferior ages (Table 1).

Considering the average age of the insects, the lethal time increased for medflies fed on lower concentrations of spinosad. The LT_{50} values for *C. capitata* were estimated in 106, 126 and 154 min for 80, 8 and 4 ppm, respectively. Fenthion and trichlorfon showed LT_{50} values significantly lower than those for spinosad, except for this compound at 4 ppm for 3-5d-old flies. The values obtained in this treatment with spinosad were similar to those with fenthion plus Aumax® allowed for 5-7d-old adults. The lowest LT_{50} values were observed for trichlorfon, with mean value of 12 min (Table 1).

The organophosphate insecticides fenthion and trichlorfon (acetylcholinesterase inhibitors) presented LT_{50} values significantly lower than those for spinosad (based on the non overlapping of 95% confidential limit values of LT_{50}), for the different ages of *C. capitata*. Only the treatment with spinosad at 4 ppm for 3-5d-old flies presented LT_{50} value similar to that with fenthion (mixed with Aumax®) for 5-7d-old flies. This relatively low LT_{50} value of spinosad at the concentration of 4 ppm (for 3-5d-old flies) may be related to a higher consumption of the bait by the flies influenced by a possible lower repellency of the chemical in such a low concentration (Table 1, Fig. 1). In the case of organophosphates, low LT_{50} values can also be attributed to the contact action of these compounds.

Both *A. fraterculus* and *C. capitata* presented similar survival pattern when treated with spinosad at 80 ppm (Fig. 1). LT_{50}s were similar in *A. fraterculus* and *C. capitata* for spinosad at concentrations of 80 and 4 ppm. In the case of spinosad at 8 ppm, the LT_{50} was slightly shorter in *A. fraterculus* [85.9 min (95% CL: 73.2 - 100 min)] than in *C. capitata* [126 min (95% CL: 117 - 138 min)].

The survival patterns for fenthion were slightly different from those observed for spinosad, for both species of fruit

Table 1. Median lethal time of insecticides (bait) against *C. capitata* in laboratory conditions.

Treatment	Age (days)	LT_{50}	Slope	χ^2	DF
Spinosad 80 ppm	2 – 3	83.4 (74.4 – 94.5)	2.40 ± 0.20	2.35	3
	3 – 5	75.9 (71.9 – 80.3)	6.03 ± 0.46	7.47	3
	5 – 7	191 (178 – 206)	3.99 ± 0.41	4.85	3
	Average	106 (97.1 – 117)	2.71 ± 0.22	5.82	5
Spinosad 8 ppm	2 – 3	132 (121 – 146)	3.16 ± 0.34	5.60	3
	3 – 5	125 (114 – 138)	2.78 ± 0.29	6.48	6
	5 – 7	118 (111 – 126)	4.25 ± 3.52	2.10	7
	Average	126 (117 – 138)	3.20 ± 0.27	6.06	5
Spinosad 4 ppm	3 – 5	54.2 (46.2 – 62.7)	1.47 ± 0.09	3.02	6
	5 – 7	234 (218 – 255)	3.92 ± 0.22	2.14	4
	Average	154 (128 – 195)	1.35 ± 0.12	4.96	6
Fenthion + protein (Aumax®)	3 – 5	16.7 (13.3 – 19.8)	2.25 ± 0.23	6.20	4
	5 – 7	49.1 (44.0 – 54.7)	2.35 ± 0.10	1.14	4
	Average	28.9 (24.3 – 33.3)	1.96 ± 0.06	0.48	4
Fenthion + protein (Dow)	2 – 3	25.6 (22.1 – 29.1)	2.44 ± 0.26	7.99	4
Trichlorphon + protein (Dow)	2 – 3	12.2 (9.62 – 14.3)	3.69 ± 0.51	1.00	2

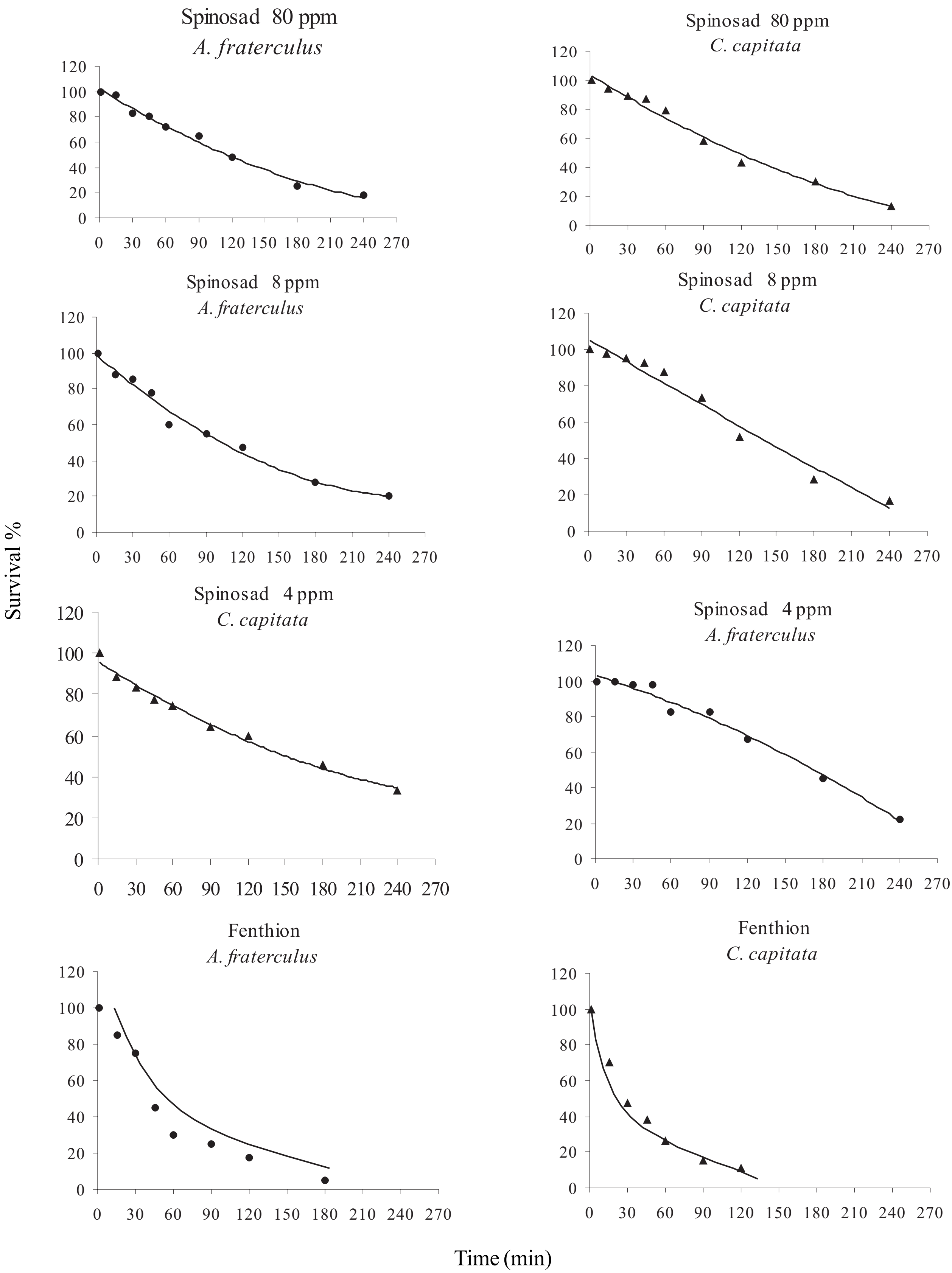

Figure 1. Comparative survival rate between *C. capitata* and *A. fraterculus* exposed to different dilutions of spinosad bait and fenthion bait.

Table 2. Cumulative mortality of *C. capitata* adults (n = 10) exposed to different baits in laboratory cages.

Treatment	2-3d-old				3-5d-old				5-7d-old			
	60 min	120 min	180 min	240 min	60 min	120 min	180 min	240 min	60 min	120 min	180 min	240 min
Spinosad 4 ppm	-	-	-	-	5.0 b	6.9 ab	7.5 a	8.6 a	0.2 b	1.2 b	3.3 b	4.8 c
Spinosad 8 ppm	1.1 c	3.8 b	6.8 a	8.2 a	1.5 c	5.1 bc	6.9 a	7.9 a	1.1 b	5.6 a	7.7 a	8.9 ab
Spinosad 80 ppm	3.2 b	6.6 ab	8.0 a	9.5 a	2.0 bc	8.5 ab	9.2 a	9.6 a	1.0 b	1.9 b	3.8 b	6.9 bc
Triclorphon + protein Dow	10.0 a	10.0 a	10.0 a	10.0 a	-	-	-	-	-	-	-	-
Fenthion + protein Dow	7.8 a	9.6 a	10.0 a	10.0 a	8.7 a	9.9 a	10.0 a	10.0 a	6.0 a	7.9 a	10.0 a	10.0 a
Control (protein Dow)	1.1 c	1.7 c	1.8 b	3.6 b	2.8 bc	2.9 cd	2.9 b	4.1 b	0.2 b	0.5 b	0.7 c	1.3 d
Control (protein Aumax®)	-	-	-	-	1.1 c	1.9 d	1.9 b	2.0 b	0.3 b	1.2 b	1.2 c	1.3 d
Control (water)	1.7 bc	1.7 c	2.3 b	2.4 b	-	-	-	-	-	-		-
CV (%)	22.6	18.7	17.2	16.8	27.4	21.9	16.0	15.5	19.5	23.6	16.6	14.1

Means followed by the same letter, in the columns, are not significantly different at 0.05% by Tukey's test.

Table 3. Cumulative mortality of *A. fraterculus* adults (n = 8) exposed to different baits in laboratory cages.

Treatment	1-3d-old				3-5d-old				5-7d-old			
	60 min	120 min	180 min	240 min	60 min	120 min	180 min	240 min	60 min	120 min	180 min	240 min
Spinosad 4 ppm	2.4 b	3.0 c	5.2 bc	7.0 a	1.4 bc	2.6 c	4.4 b	6.2 b	1.4 bcd	2.0 b	4.4 b	5.8 b
Spinosad 8 ppm	2.0 b	3.4 c	5.2 bc	7.2 a	3.2 ab	4.2 bc	5.8 ab	6.4 ab	1.0 cd	1.6 bc	3.6 b	6.2 b
Spinosad 80 ppm	2.6 b	4.2 bc	4.8 c	6.8 a	2.2 bc	4.2 bc	6.0 ab	6.6 ab	1.4 bcd	2.0 b	4.2 b	6.0 b
Fenthion +Aumax®	5.4 a	6.6 a	7.0 a	8.0 a	5.6 a	6.6 ab	7.6 a	7.8 ab	3.0 abc	5.8 a	7.0 a	8.0 a
Deltamethrin + Aumax®	5.2 a	6.4 a	7.0 a	7.8 a	5.6 a	7.0 a	7.6 a	7.8 ab	4.0 a	6.6 a	7.6 a	8.0 a
Malathion + Aumax®	5.0 a	6.2 ab	6.8 ab	8.0 a	6.0 a	7.2 a	7.4 a	8.0 a	3.6 ab	5.6 a	7.2 a	8.0 a
Control (protein Aumax®)	0.0 c	0.2 d	0.2 d	0.2 b	0.4 c	0.6 d	0.6 c	0.8 c	0.0 d	0.0 c	0.0 c	0.0 c
CV (%)	9.4	9.4	6.7	4.5	15.6	11.7	8.8	5.9	18.8	11.1	6.4	5.2

Means followed by the same letter, in the columns, are not significantly different at 0.05% by Tukey's test.

flies (Fig. 1). The patterns for fenthion were also different between species. In the case of *A. fraterculus*, the LT_{50} of fenthion was 43.8 min (95% CL: 36.9 - 50.7 min)]. This value was higher than that for *C. capitata* [LT_{50} of fenthion = 28.9 min (95%CL: 24.3 -33.3 min)], but smaller than the LT_{50} values observed for spinosad (concentrations ≤ 80 ppm) in both species (LT_{50}s > 83.4 min).

In general, after 90-120 min exposure to spinosad the mortality rate of medfly adults was similar to that of fenthion (Table 2, Fig. 1). Mortalities caused by spinosad baits were similar to the fenthion ones, 180 min after exposure of 2-3- and 3-5d-old medfly adults. However, 5-7d-old adults were less affected by spinosad at 4 and 80 ppm than by fenthion, even after 240 min.

Only 240 min after beginning the experiment, 1-3- and 3-5d-old adults of *A. fraterculus*, exposed to different doses of spinosad, showed similar mortalities to those of fenthion and deltamethrin (Table 3). For both ages, no differences were detected between spinosad (at 8 and 80 ppm) and malathion at 240 min. As observed for *C. capitata*, 5-7d-old adults of *A. fraterculus* presented lower susceptibility to spinosad than to fenthion. Fenthion, deltamethrin and malathion caused similar and higher mortalities (> 70% after 120 min) to all ages of the South American fruit fly.

Higher LT_{50} values of spinosad in comparison to those of organophosphate compounds were expected because of the mode of action of spinosad. This insecticide normally kills the insects by the cessation of feeding and paralysis. The mode of action of spinosad is characterized by excitation of the insect nervous system, leading to involuntary muscle contractions, prostration with tremors, and paralysis. These effects are consistent with the activation of nicotinic acetylcholine receptors by a mechanism that is clearly novel and unique among known insect control products. Spinosad also affects GABA receptor function that may contribute to insect activity (Salgado 1998, Salgado *et al.* 1998).

Adults of *C. capitata* exposed to 0.25 to 0.40 ppm of spinosad showed high mortality 24h after beginning the exposure (Fig. 2). The LT_{50}s of spinosad at concentrations equal or inferior to 0.4 ppm were longer than 12h (or 720 min), therefore, much longer than the LT50 values observed for concentrations higher than 4 ppm (e.g. LT_{50}= 126 min for spinosad at 8 ppm). So, when *C. capitata* receives low doses of spinosad, a longer period of exposure is necessary for an effective control. In the field, baits are continuously available for flies, even after considerable insecticide degradation. Thus for spinosad bait, the time of exposure is essential to achieve optimum control.

Medfly adults exposed to treated leaves for 3h showed high mortalities until two days after application of the baits of spinosad, fenthion, malathion and deltamethrin. Except for malathion, the survival of medfly for the remaining baits 7 d after application were similar to the control (Fig. 3), probably due to the rainfall of 35 mm on the fourth day of the experiment. Ten days after application, rates of surviving from treated plots were similar to the control.

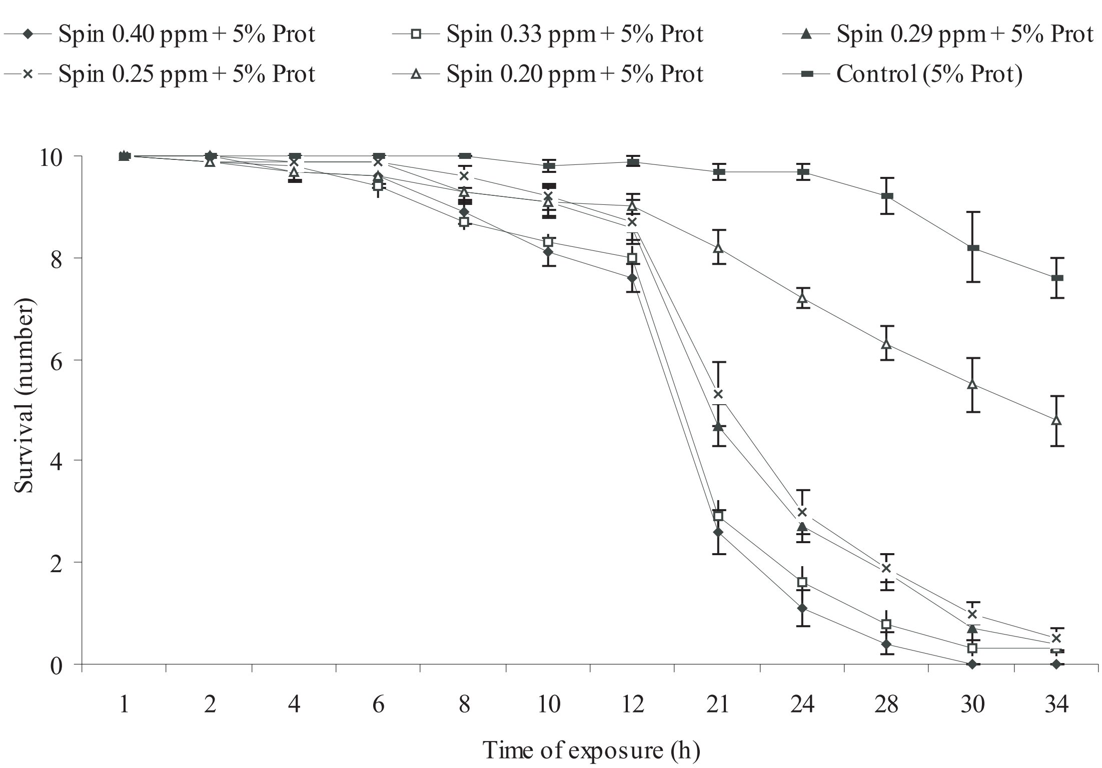

Figure 2. Survival (number) of 2-3d-old adults (females + males) of *C. capitata* exposed to low doses of spinosad baits in laboratory cages.

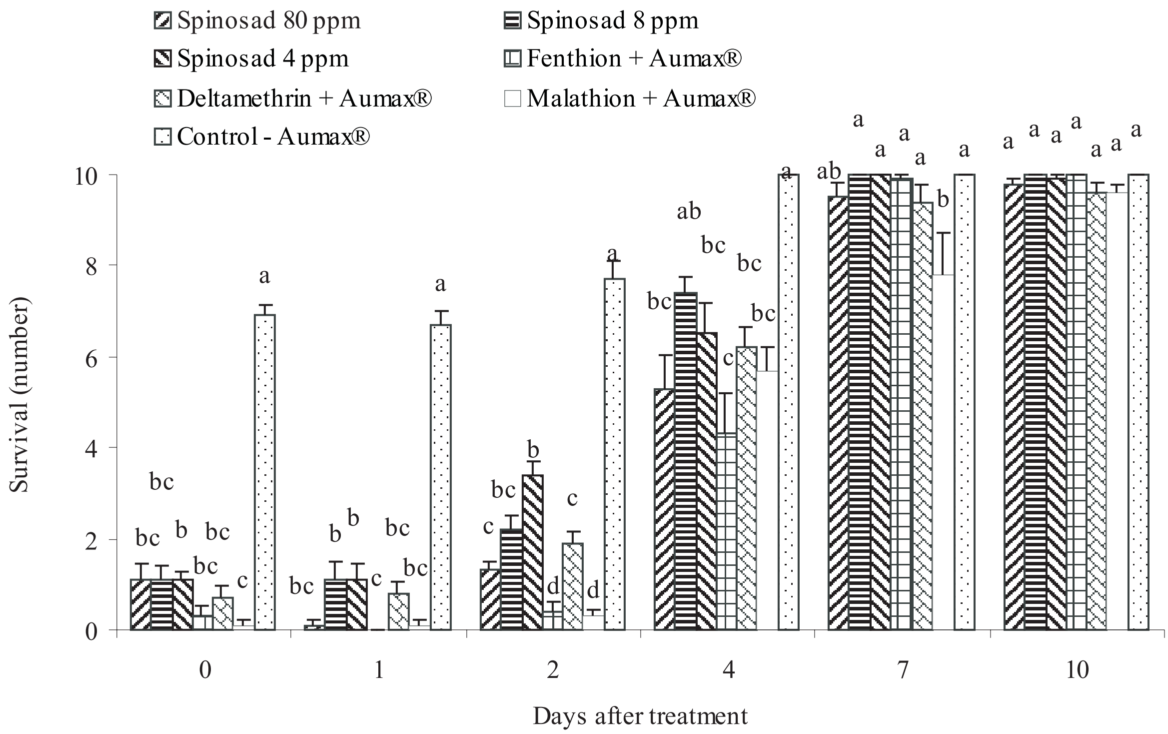

Figure 3. Survival (n = 10) of 2-3d-old adults of *C. capitata* exposed to leaves treated with different baits into cages. Bars (+ SE) within times followed by similar letter are not significantly different at 0.05% by Tukey´s test.

In laboratory, King & Henessey (1996) obtained mean mortalities of 50.9% and 88.8% when *Anastrepha suspensa* (Loew) females were exposed to 4 ppm of spinosad during 24h and 48h, respectively. For these females, estimated EC_{50}s were 4.6 ppm and 2.6 ppm respectively for both periods.

According to Burns *et al.* (2001), the susceptibility to spinosad bait in the field trial was not different between *A. suspensa* and *C. capitata*, and both spinosad bait and malathion - Nulure® - water mixture provided comparable control of the medfly.

Based on field cage studies, Vargas *et al.* (2002) verified that *C. capitata* females fed longer on spinosad bait than on malathion bait. Due to additional advantage of its selectivity to the braconid parasitoid *Fopius arisanus* (Sonan), the authors considered spinosad a viable alternative to substitute malathion on fruit fly suppression programs.

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