



Toxicity of Different Dilutions of Spinosad Bait against Two Fruit-fly Species (Diptera: Tephritidae)

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Authors' contributions

This work was carried out with the contribution of all authors. Authors FBB, SBS and LKSF contributed to the maintenance of fruit-fly colonies and development of the experiments. Author AR designed the study and wrote the first draft of the manuscript. Authors LTG and MES helped with the statistical analysis and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Toxic bait is an alternative to manage fruit flies (Tephritidae) in orchards and in wide-area programmes. The mortality caused by different dilutions of spinosad baits on adults of *Ceratitidis capitata* (Wied.) and *Anastrepha grandis* (Macquart) (Tephritidae) were compared with a hydrolysed protein (BioAnastrepha) and water in laboratory. Females and males of *C. capitata* and *A. grandis* were exposed to Success[®] 0.02CB (GF-120 NF Naturalyte fruit-fly, 0.02% spinosad) diluted in distilled water (v/v) at 1:1.5; 1:4.5; 1:9.0 and 1:18.0. Spinosad dilutions at 1: 1.5 and 1: 4.5 showed similar effects and provided 100% adult mortality 24 hours after exposure to the baits. *Anastrepha grandis* was more susceptible to spinosad baits than *C. capitata*. Females of *C. capitata* were less susceptible to spinosad diluted at 1: 1.5, 1: 4.5 and 1: 90, than *C. capitata* males and both sexes of *A. grandis*. The values of lethal times (LT₅₀) varied for the different dilutions of spinosad for both fruit-fly species; however, with different patterns for each species. The LT₅₀ values of the two highest concentrations (1: 1.5, 1: 4.5) of spinosad bait were similar for fruit flies of both sexes of

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each species, but a significant difference was observed between species, with higher LT_{50} values for *C. capitata*. In general, the cumulative mortalities of spinosad baits increased at 240, 360, 480 minutes and 24 hours after exposure. BioAnastrepha was shown to be toxic to both species, especially for *A. grandis*, killing 82% of females and 72% of males at 24 hours after exposure. Spinosad bait may be used in different dilutions to manage *C. capitata* and *A. grandis*, with similar toxicity for the two highest spinosad concentrations.

Keywords: *Diptera*; *Tephritidae*; *toxic bait*; *Ceratitidis capitata*; *Anastrepha grandis*.

1. INTRODUCTION

The Tephritidae fruit-fly *Ceratitidis capitata* (Wied.) and *Anastrepha grandis* (Macquart) cause tremendous economic losses to horticultural crops and limit international trade because of quarantine restrictions. In Brazil, *C. capitata* (medfly) was found in 93 botanical hosts [1], while *A. grandis* has been registered in nine Cucurbit hosts [2].

In the last two decades, a high number of synthetic insecticides have been banned and this fact has affected the fruit-fly (Diptera: Tephritidae) management programmes in several countries. The reduced availability of authorized chemicals has changed the current scenario of fruit-fly control with the use of insecticides in cover spray or toxic baits [3,4,5] in Brazil.

The organic insecticide spinosad is often used in many countries to manage fruit flies in organic and conventional crops [6]. Spinosyns initially cause muscle contractions and tremors by exciting neurons in the nervous system. After prolonged periods of hyperexcitation, insects show postural changes and became paralysed [7]. Spinosyn A, the main active ingredient of the insecticide spinosad, presents interaction with the calcium channel and shows a novel mode of action [8]. Spinosad bait (GF-120) is a combination attractant, feeding stimulant, and spinosad insecticide [9] for controlling many fruit-fly species [3,10,11,12,13,14,15,16,17]. Success[®] 0.02CB (spinosad bait) is registered for use in 10 fruit crops against *C. capitata*, *Anastrepha fraterculus* (Wied.), *A. obliqua* Macquart and *Bactrocera carambolae* Drew & Hancock in Brazil [18].

Although spinosad bait has been registered in Brazil for controlling fruit flies since 2006, few fruit growers use the bait station technology, probably due to the financial cost of application of GF-120 at 1 litre of commercial product diluted with 1.6 litre of water per hectare. The objective

of the present study was to evaluate the mortality of two fruit-fly species when treated with different dilutions of the commercial product of spinosad bait in the laboratory, aiming at reducing the cost of field applications.

2. MATERIALS AND METHODS

Adults of *C. capitata* and *A. grandis* were obtained in September 2018 from laboratory colonies that have been maintained at the Instituto Biológico, in Campinas, State of São Paulo (SP), Brazil, since 1993 and 2002, respectively. Both species were reared as described in earlier studies [19]. The average number of generations per year of *C. capitata* and *A. grandis* were of 16.6 and 5.4, respectively, in the laboratory. In the case of medfly, we introduced adults originated from coffee berries collected from unsprayed plants at least once a year to maintain the genetic diversity of the colony.

We tested Success[®] 0.02CB (GF-120 NF Naturalyte fruit-fly, 0.02% spinosad, Dow Agrosciences Industries Ltd.) diluted in distilled water (v/v) at 1:1.5, 1:4.5, 1:9.0 and 1:18.0 in comparison with a commercial hydrolysed protein at 5% (BioAnastrepha) and distilled water (untreated control). The pH, determined with a pH meter Alphalab (model PA 200, Piracicaba, SP, Brazil), showed values of 4.23; 4.16; 4.20; 4.27; 6.93 and 8.35, respectively.

Five females and five males of 4 - 5 day-old *C. capitata* and 20 - 23 day-old *A. grandis* were captured in glass tubes that were then closed with cotton wool. Prior to the exposure, the tubes were stored in refrigerator at approx. -15°C for 4 minutes, and the flies were transferred immediately to glass Petri dishes (150 mm diameter). The insecticide suspension (2 mL) was applied with volumetric pipettes into a plastic container filled with hydrophilic cotton (190 mg). Before the returned of fruit-fly activity, the cotton was transferred by tweezers to the middle of Petri dishes.

After the treatment, the flies were maintained at room temperature ($25 \pm 3^\circ\text{C}$) and ambient humidity ($50 \pm 10\%$). Evaluations of cumulative mortality were conducted at 15, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480 minutes and 24 h after initial exposure. Irreversible knockdown followed by the death of the adults was the criterion to determine mortality [20].

Each Petri dish was considered one replication per treatment (in total 10 replicates). We performed ANOVA (Sisvar, version 5.6) [21]. Three-factor ANOVA (fruit-fly species; sex; bait treatments) was used to compare the mortality of fruit flies. The LT_{50} (lethal time) values for each compound were estimated by Probit analysis [22] using the Polo Plus program [23].

3. RESULTS AND DISCUSSION

Anastrepha grandis was more susceptible to spinosad baits than *C. capitata* ($F = 9.91$; $P > 0.0018$). There was a significant difference between the control (water) and the remaining treatments ($F = 488.2$; $P < 0.0001$), irrespective to the fruit-fly species and sex. Spinosad dilutions at 1: 1.5 and 1: 4.5 showed a similar effect (Tukey's test) and provided 100% adult mortality 24 hours after exposure to the bait (Table 2).

The susceptibility of fruit flies differed by sex ($F = 45.99$; $P < 0.001$). Females of *C. capitata* were less susceptible to spinosad diluted at 1: 1.5, 1: 4.5 and 1: 9.0, than *C. capitata* males and both sexes of *A. grandis* (Table 1). In the case of spinosad diluted at 1: 18.0, males of both fruit-fly species were more susceptible than the respective females. For *Bactrocera zonata* Saunders, the lethal concentration of spinosad for males was lower than for females [24]. Females of *Rhagoletis indifferens* Curran (Diptera: Tephritidae) fed longer on spinosad bait than males, but the effect on the paralysis/mortality did not differ between sexes [25]. The mortality of *Rhagoletis pomonella* (Walsh) exposed to chlorantraniliprole bait (mixture of yeast protein hydrolysate and sugar) was significantly higher in males than in females [26]. The higher insecticide susceptibility of the males of different fruit-fly species corroborates the results obtained for *C. capitata* and *A. grandis* in the present study.

Although medfly females were less affected by the spinosad toxic bait under laboratory conditions, studies in the field (citrus grove surrounded by fig, grape, pear, mulberry,

pomegranate and olive trees) indicated that females disperse and feed more than males due to the fact that females require a substantial and varied diet to realize peak fecundity [27]. The availability of a solely food (spinosad bait) in the cage may have affected the food consumption, resulting in lower mortality rates for the females.

Differences in insecticide susceptibility between males and females were also reported for other insect species [e.g., *Costelytra zealandica* (White) (Coleoptera: Scarabaeidae), *Aedes albopictus* (Skuse) (Diptera: Culicidae)] by several authors [28,29]. For these species, it was assumed that the higher susceptibility of males was due to the smaller size and/or greater physiological susceptibility [28,29]. Significant differences in body weight between males (131 mg) and females (170 mg) of *A. fraterculus* were observed in a Brazilian population of the fruit fly; however, it was not detected any correlation between the body weight and the susceptibility of adults to fenthion, when the insects were exposed to the insecticide via residual contact [30]. In the present experiment, the adults of *C. capitata* and *A. grandis* were exposed to spinosad through ingestion, which may have contributed to the differences detected between the sexes.

The dilution of Success[®]0.02 CB at 1: 10 (v/v) was considered inadequate for *B. zonata* control in Israel [17]. In another study, more flies of *Bactrocera dorsalis* (Hendel) and *Bactrocera cucurbitae* (Coquillett) also responded to spinosad bait than to water, but the responses varied markedly between females of both species [31].

The medfly insects used in the present experiment were from a pesticide-susceptible population (S) (personal communication); thus, the formulated spinosad (Success[®]0.02 CB) at high insecticide concentrations may not have provided the appropriate feeding stimuli to induce the fruit-fly (*C. capitata* and *A. grandis*) insects to ingest enough toxic ingredient to cause mortality, considering the non-increasing mortality rates with the concentration increase in the toxic bait, mainly for the three lowest spinosad dilutions at 240 minutes after treatment (Table 2). This hypothesis should be elucidated in future studies because this effect on the medfly behaviour can favour the development of insecticide resistance, changes in biological parameters or induce population outbreaks [32,33,34], especially if bait stations are established in wide-area programmes.

Table 1. Number of dead adults (mean ± SEM) of fruit flies exposed to different dilutions of spinosad bait (Success 0.02 CB) in laboratory (n=5)

Treatment		<i>A. grandis</i>		<i>C. capitata</i>	
		Female	Male	Female	Male
Success	1 : 1.5	4.23 ± 0.92aA	4.45 ± 1.04aA	3.53 ± 1.96aB	4.38 ± 1.00aA
	1 : 4.5	4.18 ± 1.11aA	4.23 ± 1.21aA	3.50 ± 1.57aB	4.25 ± 1.50aA
	1 : 9.0	3.60 ± 1.48bB	4.23 ± 1.00aA	2.80 ± 1.90bC	3.90 ± 1.28aA
	1 : 18.0	1.65 ± 2.05cB	2.08 ± 1.87bA	1.65 ± 2.02cB	2.25 ± 1.75bA
BioAnastrepha		1.35 ± 1.73cA	1.38 ± 1.50cA	1.40 ± 1.68cA	1.75 ± 1.72bA
Water		0.20 ± 0.56dA	0.15 ± 0.36dA	0.00 ± 0.00dA	0.00 ± 0.00cA

Mean numbers (±SE) in the same column followed by the same lower case are not significantly different (one-way ANOVA; $P > 0.05$). Means within rows followed by the same upper case are not significantly different (one-way ANOVA; $P > 0.05$)

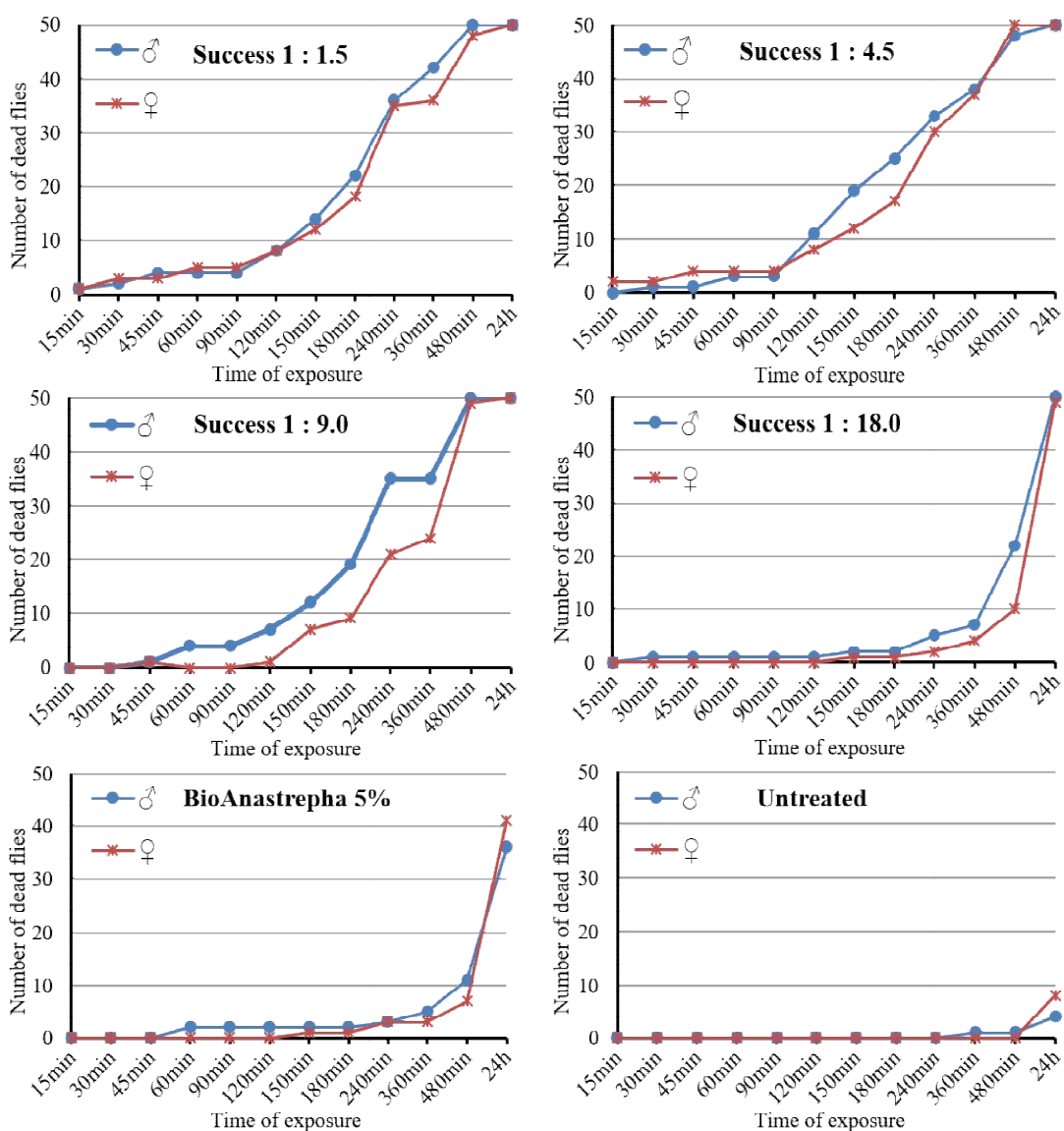


Fig. 1. Cumulative mortality of *Anastrepha grandis* per treatment (n=50) up to 24 hours of exposure in laboratory

Table 2. Cumulative mortality of *Anastrepha grandis* (Ag) and *Ceratitis capitata* (Cc) during four times of exposure to different dilutions of spinosad bait in laboratory (n=10)

Treatment		240 min		360 min		480 min		24 h	
		Ag	Cc	Ag	Cc	Ag	Cc	Ag	Cc
Success	1 : 1.5	7.10 ± 1.85aA	3.70 ± 1.25abB	7.80 ± 1.48aA	8.20 ± 1.99aA	9.90 ± 0.42aA	9.70 ± 0.67aA	10.00 ± 0.00aA	10.00 ± 0.00aA
	1 : 4.5	6.30 ± 2.41aA	4.40 ± 0.97aB	7.50 ± 1.96abA	7.80 ± 1.62aA	9.80 ± 0.63aA	8.80 ± 0.79abA	10.00 ± 0.00aA	10.00 ± 0.00aA
	1 : 9.0	5.60 ± 1.34aA	3.20 ± 1.13abB	5.80 ± 1.32bA	6.00 ± 2.17bA	9.80 ± 0.31aA	7.60 ± 1.58bA	10.00 ± 0.00aA	10.00 ± 0.00aA
	1 : 18.0	0.70 ± 0.82bB	2.80 ± 2.02abA	1.10 ± 0.74cB	2.80 ± 0.82dA	3.20 ± 1.14bA	2.80 ± 2.20cA	9.90 ± 0.32aA	9.30 ± 0.82aA
BioAnastrepha 5%		0.60 ± 0.70bB	2.4 ± 2.17bA	0.80 ± 0.88cB	2.90 ± 2.84cA	1.80 ± 1.23bcB	3.10 ± 2.81cA	7.70 ± 1.25bA	4.20 ± 2.6 bB
Water		0.00 ± 0.00bA	0.00 ± 0.00cA	0.00 ± 0.32cA	0.00 ± 0.00dA	0.10 ± 0.31cA	0.00 ± 0.00d	1.20 ± 1.23cA	0.00 ± 0.00cB

Mean numbers (\pm SE) in the same column followed by the same lower case are not significantly different (one-way ANOVA; $P > 0.05$). Mean numbers (\pm SE) within rows of respective transect followed by the same upper case are not significantly different (one-way ANOVA; $P > 0.05$)

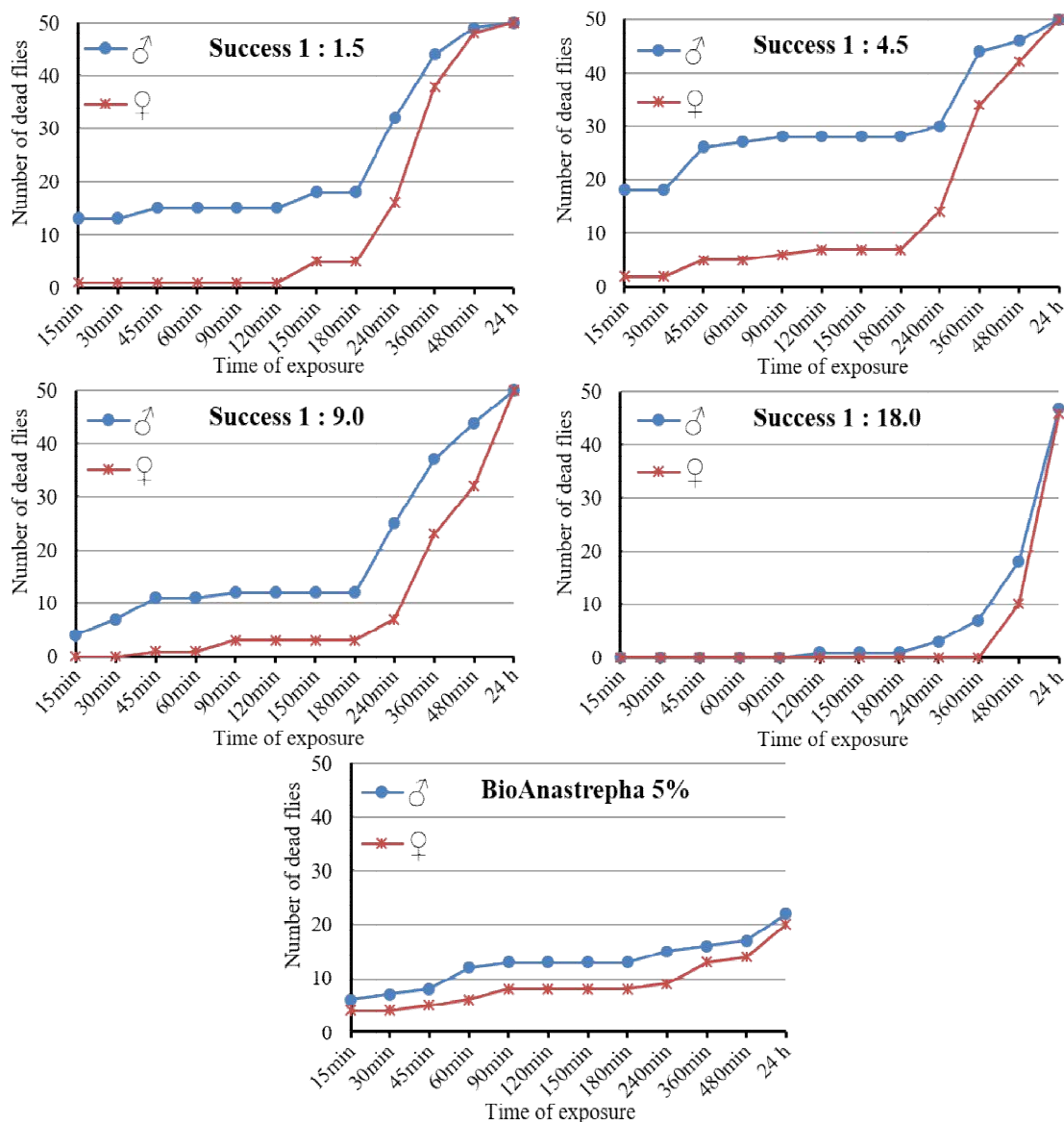


Fig. 2. Cumulative mortality of *Ceratitis capitata* per treatment (n=50) up to 24 hours of exposure in laboratory

In this aspect, the amount of food ingested by *Anastrepha suspensa* (Loew) was dependent on the concentration and class of the food (carbohydrate, protein, amino acid). All amino acids (as 0.25% solution in water) were phagostimulatory to males and all amino acids except asparagine were phagostimulatory to females. Cystine and hydroxyl-L-proline (as 0.25% solution + 4% sucrose) were highly inhibitory to females, and proline was high inhibitory to males. The flies ingested greater amount of sucrose at 8% than at 2, 4, 16 and 32% [35]. Therefore, the attractiveness of toxic

bait to fruit flies is dependent on the composition and concentration of each component of the bait, and on the insect sex [35,36,37].

For the control of *A. fraterculus*, a concentration twice high as those provided by Success®0.02 CB was required [38]. Although no medfly resistance was detected to spinosad bait in the field, selected strains of *B. dorsalis* were obtained in the laboratory after a few generations when spinosad was provided by topical applications [39].

Table 3. Cumulative mortality of *Anastrepha grandis* (Ag) and *Ceratitis capitata* (Cc) during four times of exposure to different dilutions of spinosad bait in laboratory (n=10)

Treatment	<i>Anastrepha grandis</i>				
		240min	360 min	480 min	24h
Success	1 : 1.5	7.10 ± 1.85 B	7.80 ± 1.48 B	9.80 ± 0.42 A	10.00 ± 0.00 A
	1 : 4.5	6.30 ± 2.41 B	7.50 ± 1.96 B	9.80 ± 0.63 A	10.00 ± 0.00 A
	1 : 9.0	5.60 ± 1.34 B	5.80 ± 1.32 B	9.90 ± 0.31 A	10.00 ± 0.00 A
	1 : 18.0	0.70 ± 0.82 C	1.10 ± 0.74 C	3.20 ± 1.14 B	9.90 ± 0.32 A
BioAnastrepha		0.60 ± 0.7 B	0.80 ± 0.8 B	1.80 ± 1.23 B	7.70 ± 1.25 A
Water		0.00 0.00 A	0.10±0.32 A	0.10 ± 0.31A	1.20 ± 1.23 A
<i>Ceratitis capitata</i>					
Success	1 : 1.5	3.70 ± 1.25 C	8.20 ± 1.99 B	9.70 ± 0.67 AB	10.00 ± 0.00 A
	1 : 4.5	4.40 ± 0.97 C	7.80 ± 1.62 B	8.80 ± 0.79 AB	10.00 ± 0.00 A
	1 : 9.0	3.20 ± 1.13 D	6.00 ± 2.17 C	7.60 ± 1.58 B	10.00 ± 0.00 A
	1 : 18.0	2.80 ± 2.02 B	2.80 ± 0.82 B	2.80 ± 2.20 B	9.30 ± 0.82 A
BioAnastrepha		2.40 ± 2.17 C	2.90 ± 2.84 AB	3.10 ± 2.81 AB	4.20 ± 2.61 A
Water		0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A

Mean numbers (±SE) within rows followed by the same upper case are not significantly different (one-way ANOVA; $P > 0.05$)

Table 4. Comparison of lethal times (LT₅₀) obtained for both sex of *Ceratitis capitata* (Cc) and *Anastrepha grandis* (Ag) exposed to insecticides under cover spray in laboratory

Treatment	Species	Sex	LT ₅₀ (min)	Slope ± SE	X ²	df
Success 1 : 1.5	Cc	Females	272.6 (253.8 – 294.3)	6.09 ± 0.55	2.54	4
	Ag	Females	210.8 (192.7 – 231.7)	3.90 ± 0.37	2.69	5
	Cc	Males	186.8 (168.9 – 204.9)	4.01 ± 0.45	2.95	4
	Ag	Males	192.2 (177.6 – 208.4)	4.69 ± 0.43	0.82	5
Success 1 : 4.5	Cc	Females	294.2 (268.9 – 324.6)	4.54 ± 0.50	2.11	4
	Ag	Females	214.0 (196.5 – 234.1)	4.20 ± 0.39	1.51	5
	Cc	Males	154.2 (118.6 – 181.9)	2.79 ± 0.46	1.40	4
	Ag	Males	193.4 (176.3 – 212.3)	3.80 ± 0.36	1.95	6
Success 1 : 9.0	Cc	Females	389.2 (352.1 – 439.2)	4.43 ± 0.49	1.57	4
	Ag	Females	282.3 (227.6 – 376.2)	4.74 ± 0.88	5.54	5
	Cc	Males	245.1 (221.0 – 271.1)	3.97 ± 0.48	1.01	4
	Ag	Males	207.4 (170.4 – 261.3)	3.56 ± 0.52	9.98	7
Success 1 : 18.0	Cc	Females	764.5 (674.2 – 884.9)	5.46 ± 0.60	3.04	2
	Ag	Females	649.7 (574.8 – 754.9)	4.80 ± 0.52	5.96	3
	Cc	Males	606.7 (531.8 – 712.1)	3.75 ± 0.36	4.23	5
	Ag	Males	518.0 (459.2 – 599.9)	3.99 ± 0.39	6.74	5
BioAnastrepha	Cc	Females	4526.7 (1719.4 – 34290.3)	0.63 ± 0.12	1.15	10
	Ag	Females	852.2 (726.7 – 1039.5)	3.36 ± 0.35	5.49	5
	Cc	Males	2770.3 (1059.3 – 25167.6)	0.50 ± 0.11	1.04	10
	Ag	Males	961.3 (768.0 – 1306.9)	2.25 ± 0.25	7.68	6

BioAnastrepha is a commercial hydrolysed protein for monitoring fruit-fly in Brazil. This product was shown to be toxic to both species, especially for *A. grandis*, killing 82% of females and 72% of males at 24 hours after initial exposure (Fig. 1). BioAnastrepha exhibited a difference with the control (water) at 24 hours, when the product was more toxic to *A. grandis* than to *C. capitata* (Table 2). No mortality of medfly was detected during the experiment, when the adults were exposed to distilled water.

We obtained interactions between species versus dilutions ($F = 3.92$; $P < 0.0016$), spinosad dilutions vs time of exposure ($F = 24.72$; $P < 0.0001$) and spinosad dilutions vs sex ($F = 4.24$; $P > 0.0008$).

Spinosad diluted at 1: 1.5, 1: 4.5 and 1: 9.0 caused similar mortalities to fruit flies of both species at 240 min, 360 min and 24 hours after exposure. No statistical differences in mortalities for both species were obtained only 24 hours

after exposure for spinosad diluted at 1: 18.0 (Table 2). In this evaluation, all spinosad dilutions exhibited a similar number of dead adults considering each isolated species or between species for each spinosad dilution.

The responses of both fruit-flies varied according to the time of exposure to baits ($F = 273.40$; $P < 0.0001$). The level of cumulative mortalities provided by spinosad dilutions increased during the different exposure periods (Figs. 1 and 2). In general, the cumulative mortalities increased at 240, 360, 480 minutes and 24 hours after exposure (Table 3).

The phagostimulatory components of GF-120 spinosad bait allowed the use of a low concentration of spinosad (80 ppm of AI) for the control of *Anastrepha ludens* (Loew). The active ingredients of the bait remained effective for up to 48 hours, but the toxic bait reduced or prevented feeding by *A. ludens* for the first 8 hours, inducing mortality rates below 45%. The mortality increased to 89% by 24 hours, and 99% by 48 hours [40].

The values of LT_{50} varied for the different dilutions of spinosad for both fruit-fly species; however, with different patterns for each species. In the case of *C. capitata* (females and males), the LT_{50} values of the two highest concentrations (1: 1.5 and 1: 4.5) were similar to each other, but differed from the remaining concentrations. The highest contrast (up to 4.1 times) was observed between the dilutions of 1: 1.5 (186.8 – 272.6 min) and of 1: 18.0 (764.5 – 606.7 min) (Table 4).

In the case of *A. grandis* (females and males), the LT_{50} values of the three highest concentrations (1: 1.5, 1: 4.5 and 1: 9.0) were similar each other, but, differed from the dilution of 1: 18:0. The highest contrast (up to 3.1 times) was also observed between the dilutions of 1: 1.5 (192.2 - 210.8 min) and of 1: 18.0 (518.0 - 649.7 min) (Table 4).

Differences between fruit-fly species and sexes were detected for the lethal times of spinosad, with higher values of LT_{50} for *C. capitata* females than those for *A. grandis* females, for the two highest concentrations of the toxic bait. The *C. capitata* males were more susceptible than the medfly females, presenting shorter lethal times, for the two highest concentrations of spinosad; however, in the case of *A. grandis*, similar values of LT_{50} were observed for both sexes (of the same species), for all evaluated dilutions of the toxic bait (Table 4).

The LT_{50} values observed for BioAnastrepha were much higher (≥ 5.9 times) than those observed for spinosad bait at any concentration for both sexes of medfly. For females of *A. grandis*, the LT_{50} values of BioAnastrepha were also higher (≥ 3.0 times) than those verified for spinosad bait, except for the dilution of 1: 18.

A minimum of 98% of *A. grandis* died before 480 minutes after being exposed to spinosad diluted at 1: 1.5, 1: 4.5 and 1: 9.0, while for *C. capitata*, for the same dilutions and period of time, the mortalities reached 97%, 88% and 76%, respectively (Table 3). Clearly, the highest dilutions required more time to kill the insects (Figs. 1 and 2, Table 4), probably due to the ingestion of lower quantities of spinosad.

BioAnastrepha was less toxic to *C. capitata* than to *A. grandis*, but the lethal effect was clearly visible only at 24 hours after exposure (Table 3). This product is derived from corn hydrolysed protein and also contains fructose, glucose and sucrose and stabilizers to increase the active lifespan in the field. The cause of the toxicity of BioAnastrepha to fruit flies is still unknown, but this effect may increase the efficacy of toxic baits (formulated with BioAnastrepha) in the field.

Considering the insecticide concentration, an advantage of higher dilutions of spinosad (like 1: 4.5 and 1: 9.0) is related to the increasing number droplets per tree, providing more spots for fruit flies for feeding on the toxic bait in the canopy. However, other insects of local fauna, like other Diptera (predators and parasites), parasitoids (Hymenoptera), and ants, may feed on spinosad bait [41] and higher insecticide dilutions may cause a lower impact on non-target organisms in the field. Probably, spinosad treatments do not irritate or repel flies [42] of different species and may cause toxic effect on them, depending on the concentration.

Spinosad bait (89.0 ppm AI) killed females of *R. indifferens* before they oviposit in cherry fruits [11]. However, this strategy may be affected when natural food sources or fruit exudate is available [36]. Thus, the possibility of using different dilutions of Success 0.02CB for killing fruit flies, and consequently, to protect the fruits from oviposition in the field, deserves further investigation.

4. CONCLUSION

Spinosad bait may be used in different dilutions to manage *C. capitata* and *A. grandis*

populations, with similar toxicity for the two highest spinosad concentrations (1: 1.5 and 1: 4.5), for both fruit-fly species. Higher dilutions of spinosad bait may provide similar efficacy against to fruit flies (Tephritidae), and also may reduce the risk of repellency and application costs. Further field research is needed to confirm the effectiveness of GF-120 dilutions against fruit-fly adults under different edaphic-climatic conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Zucchi RA, Moraes RCB. Fruit flies in Brazil - host and parasitoids of the Mediterranean fruit fly; 2012. Available: <http://www.lea.esalq.usp.br/ceratitis>. Acesso em 30 de novembro de 2018.
- Zucchi RA, Moraes RCB. Fruit flies in Brazil - *Anastrepha* species, their host plants and parasitoids, 2008. Available: <http://www.lea.esalq.usp.br/anastrepha> (Accessed: 30 Nov. 2018).
- Raga A, Sato ME. Effect of spinosad bait against *Ceratitis capitata* (Wied.) and *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae) in laboratory. Neotropical Entomology. 2005;34(5):815-822.
- Raga A, Sato, ME. Time-mortality for fruit flies (Diptera: Tephritidae) exposed to insecticides in laboratory. Arquivos do Instituto Biológico. 2006;73(1):73-77.
- Raga A, Sato, ME. Controle químico de moscas-das-frutas. Technical Document. 2016;20:14.
- Yee WL. Spinosad versus spinetoram effects on kill and oviposition of *Rhagoletis indifferens* (Diptera: Tephritidae) at differing fly ages and temperatures. Journal of Insect Science. 2018;18(4):1-10.
- Salgado VL. Pesticide Biochemistry and Physiology. 1998;60:91-102.
- Orr N, Shaffner AJ, Richey K, Crouse GD. Novel mode of action of spinosad: Receptor binding studies demonstrating lack of interaction with known insecticidal target sites. Pesticide Biochemistry and Physiology. 2009;95:1-5.
- Mangan RL, Moreno DS, Thompson GD. Bait dilution, spinosad concentration, and efficacy of GF-120 based fruit fly sprays. Crop Protection. 2006;25:125-133.
- King JR, Hennessey MK. Spinosad bait for the Caribbean fruit fly (Diptera: Tephritidae). Florida Entomologist. 1996;79(4):526-530.
- Yee WL, Alston DG. Effects of spinosad, spinosad bait, and chloronicotinyl insecticides on mortality and control of adult and larval Western cherry fruit fly (Diptera: Tephritidae). Journal of Economic Entomology. 2006;99(5):1722-1732.
- Flores S, Gomez LE, Montoya P. Residual control and lethal concentrations of GF-120 (spinosad) for *Anastrepha* spp. (Diptera: Tephritidae). Journal of Economic Entomology. 2011;104(6):1885-1891.
- Ekesi S, Mohamed S, Tanga CM. Comparison of food-based attractants for *Bactrocera invadens* (Diptera: Tephritidae) and evaluation of Mazoferm-spinosad bait spray for field suppression in mango. Journal of Economic Entomology. 2014;107(1):299-309.
- Hafsi A, Abbes K, Harbi A, Rahmouni R, Chermiti B. Comparative efficacy of malathion and spinosad bait sprays against *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) in Tunisian citrus orchards. Journal of Entomology and Zoology Studies. 2015;3(6):246- 49.
- Varikou K, Garantonakis N, Birouraki A. Residual attractiveness of various bait spray solutions to *Bactrocera oleae*. Crop Protection. 2015;68:60-66.
- Smaili MC, Bakri A, Gaboune F, Bouharroud R, Blenzar A. Comparison of the effect of spinosad, kaolin and protein bait spray on *Ceratitis capitata* (Diptera: Tephritidae) in citrus orchards in the Garb (Morocco). International Journal of the Research in Agricultural Sciences. 2016;3(4):197-205.
- Gazit Y, Akiva R. Toxicity of malathion and spinosad to *Bactrocera zonata* and *Ceratitis capitata* (Diptera: Tephritidae). Florida Entomologist. 2017;100(2):385-389.
- MAPA. Agrofit – Sistema de Agrotóxicos Fitossanitários; 2018. Available: http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. (Accessed 09 Nov 2018).
- Raga A, Galdino LT, Silva SB, Baldo FB, Sato ME. Comparison of insecticide

- toxicity in adults of the fruit flies *Anastrepha fraterculus* (Wied.) and *Anastrepha grandis* (Macquart) (Tephritidae). Journal of Experimental Agriculture International. 2018;25(2):1-8.
20. Raga A, Sato ME. Toxicity of neonicotinoids to *Ceratitis capitata* and *Anastrepha fraterculus* (Diptera: Tephritidae). Journal of Plant Protection Research. 2011;51:413-419.
 21. Ferreira DF. Sisvar: A Guide for its Bootstrap procedures in multiple comparisons. Ciência e Agrotecnologia. 2014;38(2):109-112.
 22. Finney, DJ, Probit Analysis, third Ed. Cambridge University Press, London; 1971.
 23. Le Ora Software. A user's guide to probit or logit analysis. In: Robertson JL, Preisler HK, Russel RM (Eds.) Berkeley, CA, USA: LeOra Software. 2003;7-11.
 24. El-Aw MAM, Draz KAA, Hashem AG, El-Gendy IR. Mortality comparison among spinosad-, Actara-, Malathion-, and Methomyl- containing baits against peach fruit fly, *Bactrocera zonata* Saunders under laboratory conditions. Journal of Applied Sciences. 2008;4(2):216-223.
 25. Yee WL. Insecticide, sugar, and diet effects on feeding and mortality in *Rhagoletis indifferens* (Dipt., Tephritidae). Journal of Applied Entomology. 2009; 133: 297-306.
 26. Teixeira LAF, Gut LJ, Wise JC, Isaacs R. Lethal and sublethal effects of chlorantraniliprole on three species of *Rhagoletis* fruit flies (Diptera: Tephritidae). Pest Management Science. 2009;65:137-143.
 27. Hendrichs, J, Katsoyannos BI, Papaj DR, Prokopy RJ. Sex differences in movement between natural feeding and mating sites and tradeoffs between food consumption, mating success and predator evasion in Mediterranean fruit flies (Diptera: Tephritidae). Oecologia. 1991;86:223-231.
 28. Elliott, R, Perrott DCF. Response to insecticides in *Costelytra zealandica* (White) in relation to sex and weight. New Zealand Journal of Agricultural Research. 1965;8(1):142-155.
 29. Boubidi SC, Rossignol M, Chandre F, Tounsi R, Lagneau C, Fontenille D, Reiter P. Gender bias in insecticide susceptibility of *Aedes albopictus* is solely attributable to size. Journal of the American Mosquito Control Association. 2016;32(3):251-253.
 30. Humeres E, Cruz IBM, Oliveira AK age and time exposure-related toxicity of fenthion to male and female *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae). Anais da Sociedade Entomológica do Brasil. 1999;28(2):285-292.
 31. Barry JD, Miller NW, Piñero JC, Tuttle A, Mau RFL, Vargas RI. Effectiveness of protein baits on melon fly and oriental fruit fly (Diptera: Tephritidae): Attraction and feeding. Journal of Economic Entomology. 2006;99(4):1161-1167.
 32. Morse JG. Agricultural implications of pesticide-induced hormesis of insects and mites. Human & Experimental Toxicology. 1998;17:266-269.
 33. Khan HAA. Spinosad resistance affects biological parameters of *Musca domestica* Linnaeus. Scientific Reports. 2018;8: 14031.
 34. Yee WL. Spinosad versus spinetoram effects on kill and oviposition of *Rhagoletis indifferens* (Diptera: Tephritidae) at differing fly ages and temperatures. Journal of Insect Science. 2018;18(4):1-10.
 35. Sharp JL, Chambers DL. Consumption of carbohydrates, proteins, and amino acids by *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) in the laboratory. Environmental Entomology. 1984;3(3): 768-773.
 36. Cangussu JA, Zucoloto FS. Effect of protein sources on fecundity, food acceptance and sexual choice by *Ceratitis capitata* (Diptera, Tephritidae). Revista Brasileira de Biologia. 1997;5:611-618.
 37. Plácido-Silva MC, Silva Neto AM, Zucoloto FS, Joachim-Bravo IS. Effects of different protein concentrations on longevity and feeding behavior of two adult populations of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). Neotropical Entomology. 2006;35(6):747-752.
 38. Schutze IX, Baronio CA, Baldin MM, Loek AE, Botton M. Toxicity and residual effects of toxic baits with spinosyns on the South American fruit fly. Pesquisa Agropecuária Brasileira. 2018;53(2):144-151.
 39. Vontas J, Hernández-Crespo P, Margaritopoulos JT, Ortego F, Feng HT, Mathiopoulou KD, Hsu J. Insecticide resistance in Tephritid flies. Pesticide Biochemistry and Physiology. 2011;100: 199-205.
 40. Mangan RL. Effects of bait age and prior protein feeding on cumulative time-

- dependent mortality of *Anastrepha ludens* (Diptera: Tephritidae) exposed to GF-120 spinosad baits. Journal of Economic Entomology. 2009;102(3):1157-1163.
41. Gazit Y, Gavriel S, Akiva R, Timar D. Toxicity of baited spinosad formulations to *Ceratitis capitata*: from the laboratory to application. Entomologia Experimentalis et Applicata. 2013;147:120-125.
42. Yee WL. Temperature and food availability effects on spinosad and malathion against *Rhagoletis indifferens* (Diptera: Tephritidae) in the laboratory. Phytoparasitica. 2017;45(5):673-682.

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