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Source: Florida Entomologist, 98(1) : 202-208

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.098.0135

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Toxicities and residual effects of toxic baits containing spinosad or malathion to control the adult *Anastrepha fraterculus* (Diptera: Tephritidae)

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Abstract

An important objective of Integrated Pest Management (IPM) is to reduce chemical contamination of the environment and food; for example by replacing broadcast sprays with selective toxic baits. The objective of the study was to evaluate the toxicity and residual effects of the a ready-for-use commercial bait Success* 0.02 CB®, which contains 0.24 g a.i. L^{-1} of spinosad, and to compare it's performance to a few other formulations with spinosad and malathion mixed either in hydrolyzed corn protein (Biofruit® 3%) or in sugarcane molasses (7%) on adults *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) in the laboratory, greenhouse and field conditions. In the laboratory, formulations with spinosad caused mortality equivalent to malathion-based toxic baits 96 h after exposure of the insects, regardless of the attractive substance used. In the greenhouse, Success* 0.02 CB®, resulted in mortality of 81.9% of *A. fraterculus* adults 7 days after application of treatment; being significantly superior to either standard spinosad or malathion treatments (mortality between 44.1 to 62.1%) in the same evaluation period. In field, in the absence of rain, Success* 0.02 CB® and spinosad formulations with Biofruit® 3% or sugarcane molasses (7%) caused mortalities from 70.0 to 83.0% up to 7 DAT, not differing statistically from the malathion treatments (mortality of 100%) during this time. However, at 10 DAT only malathion formulations with Biofruit® 3% or sugarcane molasses (7%) substantial mortalities, i.e., 73.3% and 76.7%, respectively, which were superior to formulations with spinosad (mortality < 45%). However, at 14 DAT all tested formulations caused less than 40% mortality of *A. fraterculus* adults. One day after a rain (3.8 mm), the formulations with malathion caused mortalities between 56.7 and 81.8%, which were statistically superior to the formulations with spinosad (mortality < 20%). However, after the occurrence of an additional 0.4 mm of rain, all formulations caused mortality lower than 15%. Biofruit® 3% can be used as a replacement for sugarcane molasses (7%) in formulating toxic baits and Success* 0.02 CB® and other formulations with spinosad may be used to replace malathion to manage populations of *A. fraterculus*. In practical field operations, the effectiveness of toxic bait formulations may be extended by applying them to the lower canopy where they are partially protected from rain.

Key Words: attract and kill control; South American fruit fly; insecticide; integrated pest management

Resumo

Um dos principais objetivos do Manejo Integrado de Praga (MIP) é a redução da contaminação do ambiente e alimentos. Desta forma, o objetivo deste estudo foi avaliar a toxicidade e o efeito residual da formulação comercial Success* 0.02 CB® e formulações com inseticida espinosade e malation misturados com soluções aquosas de proteina hidrolisada de milho (Biofruit® 3%) ou melaço de cana-de-açúcar (7%) sobre adultos de *A. fraterculus* em condições de laboratório, casa de vegetação e campo. Em laboratório, a toxicidade das formulações com inseticida espinosade causou mortalidade equivalente as formulações com inseticida malation 96 horas após a exposição dos insetos, independentemente da substância atrativa usada. Em casa de vegetação, a formulação Success* 0.02 CB®, resultou em mortalidade de 81,9% dos adultos de *A. fraterculus* 7 DAT, sendo significativamente superior a mortalidade observa com tratamentos com espinosade ou malation (mortalidade entre 44 a 62,1%) no mesmo período de avaliação. Em campo, na ausência de chuva, a formulação Success* 0.02® CB e as formulações com Biofruit® 3% e melaço de cana de açúcar (7%) misturadas com espinosade causaram mortalidade de 70,0 a 83,0%, 7 DAT, não diferindo estatisticamente do tratamento com malation (100% de mortalidade) neste tempo. No entanto, 10 DAT, as formulações Biofruit® 3% e melaço de cana-de-açúcar (7%) misturadas com malation causaram mortalidade de 73,3 e 76,7, respectivamente, sendo superior estatisticamente das formulações com espinosade (mortalidade < 45%). Contudo, 14 DAT, todas as formulações testadas proporcionaram mortalidade menor que 40% de adultos de *A. fraterculus*. Na presença de chuva (3,8 mm), as formulações com malation proporcionaram mortalidade superior a 55%, 3 DAT, sendo superior estatisticamente das formulações com espinosade (mortalidade < 20%). Contudo, após a ocorrência de mais 0,4 mm

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de chuva (5 DAT), todas as formulações causaram mortalidade inferior a 15%. A proteína hidrolisada de milho (Biofruit® 3%) pode ser usada em substituição ao melaço de cana-de-açúcar (7%) nas formulações de isca tóxicas e a formulação comercial Success* 0.02 CB® e as formulações com inseticidas espinosade em iscas tóxicas pode ser usadas para substituir o inseticida malation para o manejo da população de *A. fraterculus.*

Palavras Chave: Controle atrai e mata; mosca das frutas Sul Americana; inseticidas; Manejo Integrado de Pragas

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) is a major pest of fruit production in the Americas (Scoz et al. 2004) (Härter et al. 2010; Nava & Botton 2010). This species ranges from southern United States to northern Argentina, and it has been associated with 97 native and exotic host plant species from 20 botanical families (Zucchi 2014). *Anastrepha fraterculus* has significant fruit-damaging potential given that females lay their eggs in the fruit, and in which the larvae subsequently open galleries (Nava & Botton 2010). In late-maturing cultivars, losses can reach 100% if control measures are not adopted (Rupp et al. 2006).

In Brazil, the primary *A. fraterculus* management strategy has been to apply organophosphate insecticides (Scoz et al. 2004; Härter et al. 2010). However, this strategy is associated with the resurgence of pests targeted for control, outbreaks of secondary pests and mortality of their natural enemies (Scoz et al. 2004; Nondillo et al. 2007; Barbosa-Negrisoli et al. 2009). Given these problems and the growing consumer demand for products free from toxic residues, organophosphate insecticides are being removed from the market or undergoing use restrictions, which limit the pest control (Scoz et al. 2004).

Toxic baits are a pest management tool to reduce fruit fly populations without requiring the broadcast application of insecticides (Navarro-Llopis et al. 2012). Although effective, the lack of information regarding the efficiency of bait attractancy and the efficacy of various active ingredients as replacements for organophosphates in pest control remain primary obstacles preventing the adoption of this toxic baits (Härter et al. 2010). In Brazil, sugarcane molasses (a by-product of the sugar manufacturing process that contains reducing sugars and non-crystallized sucrose) has been the most commonly used attractant in toxic bait formulations (Raga et al. 2006). However, its use has caused variability in fruit fly control in several regions due to the lack of standardization, which has tended to invalidate this technique for pest management (Raga et al. 2006). Therefore the use of Biofruit 3% has been recommended to replace sugarcane molasses. However, little information is currently available regarding the use of Biofruit 3% in baits in the control of *A. fraterculus* adults (Raga et al. 2006). Currently, the malathion, an organophosphate, is the principal insecticide used in toxic baits (Scoz et al. 2004; Raga & Sato 2011). Spinosad, an insecticidal product derived from the fermentation of the soil bacterium *Saccharopolyspora spinosa* (Mertz and Yao), has been used in fruit fly control programs in several countries (Chueca et al. 2007; Piñero et al. 2011; Gazit et al. 2013; Manrakhan et al. 2013). In addition to its high efficiency on tephritids, spinosad has low toxicity to mammals and fish, and it has been reported to exert relatively minimal effects on beneficial insects (Tomas & Mangam 2005; Ruiz et al. 2008; Mangan & Moreno 2009; Urbaneja et al. 2009).

In Brazil, spinosad is available in a concentrated suspension formulation (Tracer® 480 SC, with 480 g a.i. L^{-1} of spinosad) and as a ready-for-use toxic bait (Success* 0.02 CB® , which contains 0.24 g a.i. L^{-1} of spinosad) (Agrofit 2014). Currently, Success* 0.02 CB® is registered for the control of *Anastrepha obliqua* (Macquat), *Bactrocera carambolae* (Drew & Hancock) and *Ceratitis capitata* (Wiedemann) on citrus and mango crops (Agrofit 2014). In other countries, this Success* 0.02 CB® known as GF-120, and it is recommended for use in organic production by the United State Department of Agriculture (USDA 2010). Raga & Sato (2005) reported that this formulation is highly toxic to *A. fraterculus* adults in the laboratory. However, little information is available regarding its biological activity and residual effects on adult South American fruit flies. Thus, the objective of the study was to evaluate the toxicity and residual effects of the a ready-for-use commercial bait Success* 0.02 CB®, which contains 0.24 g a.i. L^{-1} of spinosad, and to compare it's performance to a few other formulations with spinosad and malathion mixed either in hydrolyzed corn protein (Biofruit® 3%) or in sugarcane molasses (7%) on adults *A. fraterculus* in the laboratory, greenhouse and field conditions.

Materials and Methods

INSECTS

Peach fruits (*Prunus persica* L.) Batsch. (Rosaceae) infested with *A. fraterculus* larvae were collected in orchards in a commercial area in Pelotas, Rio Grande do Sul State, Brazil (31.7719° S, 52.3425° W) and taken to the laboratory to obtain adult fruit flies. After emergence, the insects were identified, transferred to breeding cages (41.0 × 29.5 × 30.0 cm) and fed with water and a solid mixture of soybean protein, wheat germ, and brewer's yeast (at a ratio of 3:1:1) (Machota-Júnior et al. 2010). Papaya fruits (*Carica papaya* L.) were used as substrates for egg laying and larval development. The rearing was performed in climatized room at 25 ± 2 °C, 60 ± 10% RH and 12:12 h L:D.

DESCRIPTION OF THE TREATMENTS

For bioassays, 2 food baits were used, i.e., Biofruit® 3% commercial product (based on 3% hydrolyzed corn protein) (Biocontrole, São Paulo, Brazil) and the 7% sugarcane molasses. These food baits were used in formulating toxic baits with the insecticides Tracer® 480 SC (spinosad 0.096 g a.i. L^{-1}) and Malation® 500 EC (malathion 1.0 g a.i. L^{-1}) (Cheminova Ltd., São Paulo, Brazil). The treatments were as follows:

T1, commercial toxic bait Success* 0.02 CB® (mixture of hydrolyzed corn protein, invert sugar, oil, gum, potassium sorbate, ammonium acetate, and spinosad at 0.24 g a.i. L^{-1}) (Agrofit 2014);

T2, Biofruit® 3% + Tracer® 480 SC (spinosad 0.096 g a.i. L^{-1});

T3, Biofruit® 3% + Malation® 500 EC (malathion 1.0 g a.i. L^{-1});

T4, sugarcane molasses (7%) + Tracer® 480 SC (spinosad 0.096 g a.i. L^{-1});

T5, sugarcane molasses (7%) + Malation® 500 EC (malathion 1.0 g a.i. L^{-1});

T6, only Biofruit® 3%; and

T7, only sugarcane molasses (7%) without any insecticide as a negative control.

The treatments with Biofruit® 3% + Malation® 500 EC (malathion 1.0 g a.i. L^{-1}) and sugarcane molasses (7%) + Malation® 500 EC (malathion 1.0 g a.i. L^{-1}) were used as references for mortality (positive controls).

The treatments, T2, T3, T4 and T5, were prepared in 1 L of distilled water. However, Success* 0.02® was prepared by mixing one volume of it in 1.5 volumes of water.

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TOXICITY OF TOXIC BAITS TO ADULT *A. FRATERCULUS* IN LABORATORY

The toxicity of the baits was evaluated using 6–8-day old adult *A. fraterculus*. Adults were deprived of food 12 h prior to the bioassay in a laboratory at 25 ± 2 °C, 70 ± 10% RH and 12:12 h L: D. For this, 8 adults (4 females and 4 males) were transferred to 300 mL cages made with transparent plastic and inverted on acrylic plates (12.0 cm × 12.0 cm). A total of 10 holes (2 mm diam) were made on the top of each cage to allow gas exchange and avoid excess humidity. Treatments were applied to the adults through a plastic pipette tip attached to the center of each cage's upper face at a depth of 0.5 cm. A small piece of cotton soaked with a particular bait solution was inserted into each pipette tip. This methodology gave the insects access to the toxic bait only by ingestion. Toxic baits were made available to adults for 24 h. Then, the tips were replaced with new tips that contained a hydromel solution (2.5%), which served as food for the insects during the evaluation period of the experiment. The experimental design was completely randomized with 8 treatments and 8 repetitions (n = 8). Evaluations of the mortality of adults in the treatments were performed 24, 48, 72 and 96 h after exposure to treatments (HAET). Insects were considered dead when they failed to react to the touch of a fine brush. The toxicity of each treatment was calculated using the formula of Schneider-Orelli (1947).

EFFICACY OF TOXIC BAITS TO *A. FRATERCULUS* ADULTS IN GREENHOUSES

Cages (2.0 × 2.0 × 2.0 m) coated with a plastic screen (2.0 × 2.0 mm) and supported by 0.5 cm iron frames were used to trap *A. fraterculus* adults. Cages were set on 2-year-old peach cv. 'Eldorado' plants grown in 250 L plastic pots in a greenhouse at 25 ± 2 °C, RH 70 ± 10% RH and 12:12 h L:D. All treatments were applied on a peach branch approximately 2.0 cm in diam during the dormancy period of the crop (between Jun and Aug) by a manual pulverizer model 'Jacto' PJT Teejet of 20 L capacity equipped with a full cone nozzle (FL-5VS). Two h after the treatments were applied, 30 *A. fraterculus* adults (15 females and 15 males) at 6 to 8 days of age, food deprived for 12 h, were released in each cage. Plastic containers with cotton wool soaked in distilled water were fixed onto the inner walls of the cages during the evaluation period. The cage floor was lined with a white "voile" type fabric to facilitate the viewing and counting of dead insects. Evaluations were performed at 1, 3, 5 and 7 DAT. The experiment was completely randomized with 4 repetitions (n = 4). Evaluations of the mortality of adults in the treatments were performed 96 HAET. The efficacy of each treatment was calculated using the formula of Schneider-Orelli (1947).

RESIDUAL EFFECT OF TOXIC BAITS TO *A. FRATERCULUS* ADULTS IN THE FIELD

The residual effects in the field of all toxic bait treatments in the absence and presence of rain were evaluated. The first experiment was performed in the absence of rain (0.0 mm), while the second experiment was conducted with 3.8 mm of rain on 2 DAT and 0.4 mm of rain on 4 DAT. In both assays, the treatments were applied to the branches of peach cv. 'Chiripá' trees during the dormancy period (between Jun and Aug) using a manual pulverizer model 'Jacto' PJT Teejet of 20 L capacity equipped with a full cone nozzle (FL-5VS). A total of 6 peach plants with a 2 m height and 3 m canopy diam were used for each treatment. After, 1, 3, 5, 7, 10 and 14 DAT and 1, 3, and 5 DAT for the first and second experiment, respectively, one branch (1.5 cm diam and 5 cm length) was excised from each tree, taken to the laboratory, and fixed in the upper face of the each experimental unit (cage) made with transparent plastic containers (300 mL) as described above. In each cage, 6 *A. fraterculus* adults (3 females and 3 males) were placed. Treatments (toxic baits) were exposed to adults for 24 h. During this period, the insects were deprived of food but continued to receive water. After removing the treatments, a piece of cotton wool soaked in hydromel solution (2.5%) was added to each cage to feed the insects. The experimental design was completely randomized with 8 treatments and 6 repetitions (n = 6). Evaluations of the mortality of adults in the treatments were performed 96 HAET. Insects were considered dead when they exhibited no reaction to the touch of a fine brush. The residual effect of each treatment was calculated using the formula of Schneider-Orelli (1947).

DATA ANALYSIS

All data were submitted to the Bartlett Shapiro-Wilk normality test (PROC UNIVARIATE) (SAS Institute 2011). Thereafter, all data were transformed by $\sqrt{x} + 0.1$ and submitted to analysis of variance and the means were compared by Tukey's test ($P \leq 0.05$) (PROC GLM) (SAS Institute 2011).

Results

TOXICITY OF TOXIC BAITS IN THE LABORATORY

Exposure of adult *A. fraterculus* insects to the baits revealed that these insects are highly susceptible to malathion (mortality of 84.8%) during the first 24 HAET. However, the toxic effects of baits with spinosad were observed to be similar to the control treatment at 24 HAET and significantly different from the malathion treatment ($F_{6, 49}$ = 28.145, P < 0.0001) (Table 1). However, at 48 and 72 HAET, the Biofruit® 3% + Tracer® 480 SC and the sugarcane molasses + Tracer® 480 SC treatments showed increased mortality (ranging from 43.8 to 73.3%) (Table 1). However, mortalities in these spinosad treatments were significantly lower ($F_{6, 49}$ = 24.145, P < 0.0001) than the malathion treatment (mortality > 88%) at 72 HAET (Table 1). In contrast, the commercial spinosad bait, Success* 0.02 CB®, caused mortality similar ($F_{6, 49}$ = 24.145, P = 0.0801) to that observed using malathion baits (Table 1). On the final evaluation (96 HAET), the Biofruit® 3% + Tracer® 480 SC and sugarcane molasses + Tracer® 480 SC formulations showed an increase in mortality (74.6 to 100%), which were similar to the mortalities of Success* 0.02 CB® and the malathion baits ($F_{6, 49}$ = 40.956, P = 0.1150) (Table 1).

EFFICACY OF TOXIC BAITS IN THE GREENHOUSE

In the greenhouse after 3 days of insect exposure, the control efficiency of spinosad baits was similar to that obtained using malathion (Fig. 1). However, 5 days after releasing insects into the cages, an increase in mortality of adults exposed to Success* 0.02 CB® was observed, which was significantly greater than that of all other formulations and the control treatments ($F_{6, 21}$ = 15.924; P < 0.0001) (Fig. 1). At day 7 Success* 0.02 CB® was substantially more efficient (≈ 82% mortality) than other the toxic baits ($F_{6, 21}$ = 27.997; P < 0.0001) (Fig. 1). In this evaluation, toxic baits with Tracer® 480 SC had control efficiencies similar ($F_{6, 49}$ = 3.499, P = 0.0991) to the toxic baits with Malation® 500 EC, regardless of the attractive substance used in the formulation.

RESIDUAL EFFECT OF TOXIC BAITS IN THE FIELD

In the absence of rain, all of bait formulations – regardless of whether they contained spinosad or malathion – caused 70.8–100% up to 7 DAT; and all were statistically similar ($F_{10, 264}$ = 3.1076, P = 0.0006) during those days (Table 2). However at 10 DAT malathion formula-

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Table 1. Average numbers of live *Anastrepha fraterculus* adults (N ± SE) and percent mortalities (%M) at various times (h) after having been allowed 24 h to ingest each toxic bait in the laboratory. Mortality was assessed at 24, 48, 72 and 96 h after the 24-h ingestion period.

Treatment	Effect of bait residues assess various times (h) after a 24-h ingestion period							
	24		48		72		96	
	N ± SE[1]	% M[2]	N ± SE[1]	% M[2]	N ± SE[1]	% M[2]	N ± SE[1]	% M[2]
Success® 0.02 CB (spinosad 0.24 g a.i. L^{-1})	5.6 ± 0.65 Aa	27.4	2.0 ± 0.38 Bc	73.3	0.1 ± 0.13 Cc	98.3	0.0 ± 0.00 Cb	100
Biofruit 3% + Tracer® 480 SC (spinosad 0.096 g a.i. L^{-1})	6.6 ± 0.38 Aa	14.5	2.9 ±0.35 Bb	61.7	2.0 ± 0.65 Bb	73.3	1.9 ± 0.58 Cb	74.6
Biofruit 3% + Malation (malathion 1.0 g a.i. L^{-1})	0.8 ± 0.41 Ab	90.3	0.6 ± 0.38 Ac	91.7	0.6 ± 0.38 Ac	91.7	0.5 ± 0.27 Ab	93.2
Biofruit 3%	7.8 ± 0.25 Aa	0.0	7.5 ± 0.27 Aa	0.0	7.5 ± 0.27 Aa	0.0	7.4 ± 0.26 Aa	0.0
Sugarcane molasses (7%) + Tracer® 480 SC (spinosad 0.096 g a .i. L^{-1})	6.4 ± 0.32 Aa	13.6	3.8 ± 0.41 Bb	48.3	3.8 ± 0.41 Bb	50.0	1.8 ± 0.49 Cb	75.0
Sugarcane molasses (7%) + Malation (malathion 1.0 g a.i. L^{-1})	1.1 ± 0.40 Ab	84.8	1.1 ± 0.40 Ac	84.5	0.9 ± 0.40 Ac	88.3	0.9 ± 0.40 Ab	87.5
Sugarcane molasses (7%)	7.4 ± 0.26 Aa	0.0	7.2 ± 0.37 Aa	0.0	7.2 ± 0.37 Aa	0.0	7.0 ± 0.33 Aa	0.0

[1]Means followed by the same uppercase letter in the rows and lowercase letter in the columns do not differ significantly by Tukey test (α = 0.05).
[2]Mortality calculated by the formula of Schneider-Orelli (1947).

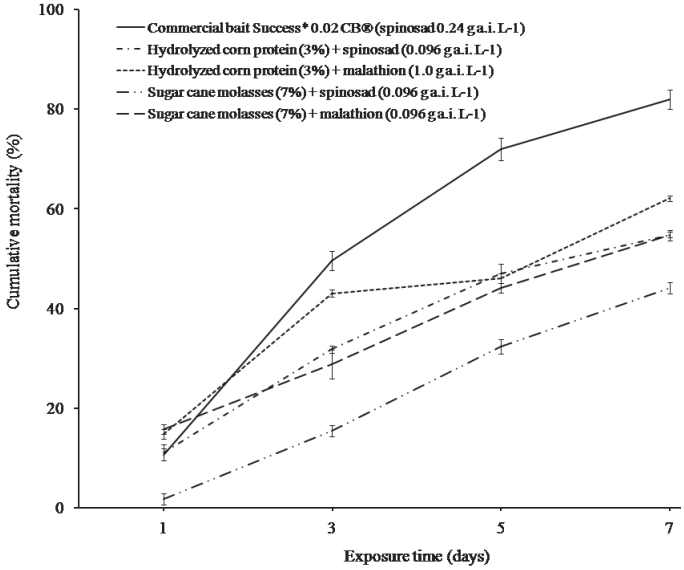


Fig. 1. The mortality of *Anastrepha fraterculus* adult after 1, 3, 5 and 7 days of exposure to toxic baits. (Vertical bars indicate the standard error of the mean). Mortality was calculated by the formula of Schneider-Orelli (1947).

tions with Biofruit® 3% or sugarcane molasses caused mortality rates of 73.3% and 76.7%, respectively; which were statistically greater ($F_{6, 49}$ = 187.41; $P < 0.0001$) than corresponding mortality rates with spinosad formulations (43.8% and 8.3%, respectively) (Table 2). At 14 DAT, the toxicities of the residues had declined further. Thus at 14 DAT the mortality of *A. fraterculus* adults exposed to malathion was 39.6%, which was similar to the 33.3% mortality obtained with Success* 0.02 CB® ($F_{6, 49}$ = 15.499, $P < 0.0001$). In contrast at 14 DAT Biofruit® 3% + Tracer® 480 SC and sugarcane molasses (7%) + Tracer® 480 SC baits no longer retained a useful level of toxicity (Table 2).

With 3.8 mm of rain on 2 DAT, only the malathion treatments caused substantial mortality (ranging from 56.7 to 81.8% up to 3 DAT), which were significantly better than the control and spinosad baits including Success* 0.02 CB® (16.0%) and the control (0.0%) ($F_{6, 49}$ = 13.803; $P <$ 0.0001) (Table 3). After an additional 0.4 mm of rain on 4 DAT, none of the tested formulations caused significant insect mortality ($F_{6, 49}$ = 2.950; P = 0.0531) (Table 3).

Discussion

The use of toxic baits has been an important alternative for the management the adult fruit fly populations (Navarro-Llopis et al. 2012). In the present study, the mortality data revealed that toxic bait containing the attractant Biofruit® 3% provided a level of mortality of *A. fraterculus* adults similar to baits with sugarcane molasses 7%. Härter et al. (2010) observed that the application of Biofruit® 3% + Malation® 500 CE 1.0 g a.i. L^{-1} toxic bait was effective in controlling *A. fraterculus* in peach orchards. Montes & Raga (2006) found that the hydrolyzed protein BioAnastrepha® 3% was 16.3 times more attractive to *C. capitata* adults than sugarcane molasses 7%. Therefore, the use of Biofruit® 3% as an attractive substance in toxic baits offers advantages over sugarcane molasses, because Biofruit® 3% has a standardized composition and can be used at lower concentrations than sugarcane molasses.

The exposure of *A. fraterculus* adults to toxic baits showed that spinosad formulations induce mortality levels similar to those induced by malathion, indicating that spinosad is highly toxic to *A. fraterculus*

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Table 2. Average numbers of live *Anastrepha fraterculus* adults (N ± SE) and percent mortalities (%M) at 96 h after a 24-h exposure to residues on peach branches of various toxic baits at 1, 3, 5, 7, 10 and 14 days after treatment (DAT) in the absence of rain.

	Residual effect at various days after treatment - DAT											
	1		3		5		7		10		14	
Treatment[1]	N ± SE[2]	% M[3]	N ± SE[2]	% M[3]	N ± SE[2]	% M[3]	N ± SE[2]	% M[3]	N ± SE[2]	% M[3]	N ± SE[2]	% M[3]
Success® 0.02 CB (spinosad 0.24 g a.i. L^{-1})	0.1 ± 0.13 Ab	97.9	0.4 ± 0.18 Ab	93.8	1.1 ± 0.35 Ab	80.9	1.0 ± 0.42 Ab	83.3	3.4 ± 0.38 Bb	43.8	4.0 ± 0.5 Bb	33.3
Biofruit 3% + Tracer® 480 SC (spinosad 0.096 g a.i. L^{-1})	0.5 ± 0.27 Ab	91.5	0.8 ± 0.25 Ab	87.5	0.9 ± 0.30 Ab	85.4	1.1 ± 0.30 Ab	81.3	5.5 ± 0.19 Ba	8.3	6.0 ± 0.0 Ba	0.0
Biofruit 3% + Malation (malathion 1.0 g a.i. L^{-1})	0.0 ± 0.00 Ab	100.0	0.0 ± 0.00 Ab	100.0	0.0 ± 0.00 Ab	100.0	0.0 ± 0.00 Ab	100.0	1.6 ± 0.32 Ac	73.3	3.6 ± 0.3 Bb	39.6
Biofruit 3%	5.9 ± 0.13 Aa	0.0	6.0 ± 0.00 Aa	0.0	6.0 ± 0.00 Aa	0.0	6.0 ± 0.00 Aa	0.0	6.0 ± 0.00 Aa	0.0	6.0 ± 0.0 Aa	0.0
Sugarcane molasses (7%) + Tracer® 480 SC (spinosad 0.096 g a .i. L^{-1})	1.1 ± 0.30 Ab	81.3	1.1 ± 0.30 Ab	81.3	1.8 ± 0.31 Ab	70.2	1.8 ± 0.37 Ab	70.8	5.5 ± 0.19 Ba	8.3	5.6 ± 0.2 Ba	6.3
Sugarcane molasses (7%) + Malation (malathion 1.0 g a.i. L^{-1})	0.0 ± 0.00 Ab	100.0	0.0 ± 0.00 Ab	100.0	0.4 ± 0.18 Ab	93.6	0.0 ± 0.00 Ab	100.0	1.4 ± 0.28 Ac	76.7	3.8 ± 0.8 Bb	37.5
Sugarcane molasses (7%)	6.0 ± 0.00 Aa	0.0	6.0 ± 0.00 Aa	0.0	5.9 ± 0.13 Aa	0.0	6.0 ± 0.00 Aa	0.0	6.0 ± 0.00 Aa	0.0	6.0 ± 0.0 Aa	0.0

[1]The experimental design was completely randomized with 6 repetitions/treatments.
[2]Means followed by the same uppercase letter in the rows and lowercase letter in the columns do not differ significantly by Tukey test (α = 0.05).
[3]Mortality calculated by the formula of Schneider-Orelli (1947).

Table 3. Average numbers of live *Anastrepha fraterculus* adults (N ± SE) and percent mortalities (%M) at 96 h after a 24-h exposure to residues on peach branches of various toxic baits at 1, 3, 5, 7, 10 and 14 days after treatment (DAT) with 3.8 mm of rain on 2 DAT and 0.4 mm of rain on 4 DAT.

	Residual effect at various days after treatment - DAT					
	1		3		5	
Treatment[1]	N ± SE[2]	% M[3]	N ± SE[2]	% M[3]	N ± SE[2]	% M[3]
Success® 0.02 CB (spinosad 0.24 g a.i. L^{-1})	0.4 ± 0.14 Ac	93.3	4.2 ± 0.60 Ba	16.0	5.5 ± 0.34 Aa	5.2
Biofruit 3% + Tracer® 480 SC (spinosad 0.096 g a.i. L^{-1})	1.4 ± 0.17 Ab	76.7	5.8 ± 0.17 Ba	0.0	6.0 ± 0.00 Aa	0.0
Biofruit 3% + Malation (malathion 1.0 g a.i. L^{-1})	0.6 ± 0.15 Ac	90.0	2.2 ± 0.48 Bb	56.7	5.0 ± 0.26 Ca	14.3
Biofruit 3%	6.0 ± 0.00 Aa	0.0	5.0 ± 0.26 Aa	0.0	5.8 ± 0.17 Aa	0.0
Sugarcane molasses (7%) + Tracer® 480 SC (spinosad 0.096 g a .i. L^{-1})	1.6 ± 0.21 Ab	70.0	5.0 ± 0.52 Ba	9.1	5.8 ± 0.17 Aa	2.8
Sugarcane molasses (7%) + Malation (malathion 1.0 g a.i. L^{-1})	0.8 ± 0.16 Ac	86.7	1.0 ± 0.37 Bb	81.8	5.5 ± 0.34 Ca	8.3
Sugarcane molasses (7%)	6.0 ± 0.00 Aa	0.0	5.5 ± 0.34 Aa	0.0	6.0 ± 0.00 Aa	0.0

[1]The experimental design was completely randomized with 6 repetitions/treatments.
[2]Means followed by the same uppercase letter in the rows and lowercase letter in the columns do not differ significantly by Tukey test (α = 0.05).
[3]Mortality calculated by the formula of Schneider-Orelli (1947).

adults and may be used as an alternative to organophosphates in toxic bait formulations. Scoz et al. (2004) and Raga & Sato (2005) observed mortalities above 90% in *A. fraterculus* adults exposed to spinosad. This level of mortality is equivalent to that obtained using the organophosphates fenthion and trichlorfon, both of which are unavailable on the Brazilian market. Toxicity similar to spinosad was also reported for *C. capitata* adults exposed to the organophosphates phosmet and malathion (Urbaneja et al. 2009; Manrakhan et al. 2013). In the present study, a few formulations of spinosad showed high levels of mortality over time. These results are similar to those obtained by Scoz et al. (2004) and Raga & Sato (2005), who reported initially lower mortalities and greater TL_{50} (time required to kill 50% of the insects exposed to the treatment) for spinosad baits than baits with the organophosphates fenthion and trichlorfon. This difference likely results from the mechanism of action of spinosad, which usually kills insects by inducing paralysis and preventing feeding. In contrast, organophosphates inhibit nerve transmission, which leads to insect death soon after contact or ingestion of the product (Raga & Sato 2005). The mode of action of spinosad insecticide is by ingestion, differently from malathion bait that also acts by contact.

Our results are important for integrated pest management because the spinosyns show little activity against natural enemies that assist in the biological control of fruit flies and other pests that attack fruit (Wang et al. 2005; Ruiz et al. 2008; Urbaneja et al. 2009).

One of the limitations associated with using toxic baits is the low persistence of formulations in the field, especially in regions with frequent rains (e.g., subtropical regions) (Revis et al. 2004). This limitation was confirmed in the present study, in which the residual effects of formulations were significantly reduced by rainfall. Raga & Sato (2005) had observed a low efficiency of Success* 0.02 CB® and Aumax® 3% + malathion 1.0 g a.i. L^{-1} formulations in controlling *C. capitata* adults after a 44 mm rainfall in 10 days. Prokopy et al. (2003) reported a low level of toxicity in *B. cucurbitae* adults exposed to the toxic bait GF-120® after an 8 mm rain. However, Mangan et al. (2006) observed a 14-day persistence of GF-120®. One of the explanations for the lower efficiencies of some formulations may be attributed to their low viscosity and to the application method (Mangan et al. 2006). When diluted in water, the toxic bait loses adherence to the plant and can be easily removed by the rain (Heath et al. 2009). In both bioassays in this study, the treatments were applied to the branches of peach during the crop's dormant period (between Jun and Aug) using a manual sprayer. However, the application should be directed to the bottom of the foliage, where the tephridids prefer to feed and where the material will be partially protected from rain (Mangan et al. 2006).

In the absence of rain, toxic baits with spinosad exhibit high mortality up to 7 DAT, while formulas with malathion retained high mortality rates up to 10 DAT. Flores et al. (2011) also reported reduced mortality of *A. ludens* (Loew), *A. obliqua* (Macquart) and *A. serpentina* (Wiedemann) after 7 DAT of the toxic bait GF-120® on mango (*Mangifera indica* L.) and melon (*Cucumis melo* L.) leaves. The shorter residual effect of the toxic baits with spinosad may be related to the environmental degradation of this product. Spinosad has a 7 day half-life, and photolysis is the main mode of its degradation (Revis et al. 2004, Gazit et al. 2013). These results indicate that toxic baits must be reapplied every 7 to 10 days or after rain.

The results of the present study reveal that the Biofruit® 3% may be used as a replacement for sugarcane molasses in formulating toxic baits. Tracer® 480 SC (spinosad 0.096 g a.i. L^{-1}) and Success* 0.02 CB® formulas can be alternatives to malathion for managing populations of *A. fraterculus* in Brazilian orchards, especially prior to harvesting the fruit because of spinosad's short pre-harvest waiting period.

Reason for the infrequent use of toxic baits in peach orchards for the control of fruit flies are the low cost of organophosphate insecticides in Brazil and the need to re-apply toxic baits at 7-day intervals because of rain. However, the spinosad baits hold promise to benefit the rural producer who previously lacked this option. In addition, consumers would benefit, because both the environment and the harvested would be free from toxic residues.

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