

Concentration and lethal time of toxic baits based on spinosyns on *Ceratitis capitata* and *Diachasmimorpha longicaudata*¹

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ABSTRACT

The use of toxic baits with spinosyns (spinosad and spinetoram), along with the parasitoid *Diachasmimorpha longicaudata*, is a sustainable alternative for the management of *Ceratitis capitata*. This study aimed to evaluate the lethal concentration (LC) and lethal time (LT) of spinosad and spinetoram, associated with the food lures sugarcane molasses at 7 %, Biofruit at 3 %, Ceratrap® at 1.5 %, Flyral® at 1.25 %, Isca Samaritá® and Samaritá Tradicional® at 3 %, on *C. capitata*, under laboratory conditions, as well as their effect, at the concentration of 96 mg L⁻¹, on *D. longicaudata*. For the lethal time data, mortality was assessed at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 36, 48, 60, 72, 84 and 96 h after the exposure to the toxic baits. The lowest lethal concentrations (LC₅₀ and LC₉₅), to spinetoram (0.5 mg L⁻¹ and 3.7 mg L⁻¹, respectively) and spinosad (0.8 mg L⁻¹ and 7.8 mg L⁻¹, respectively), corresponded to the association with Samaritá Tradicional® at 3 %. The lowest lethal time (TL₅₀), in hours, for the spinosad insecticide, corresponded to the formulation containing Biofruit at 3 % (6.6), and, to spinetoram, Samaritá Tradicional® at 3 % (7.9). For *D. longicaudata*, the formulations that caused the lowest mortality corresponded to the association of Biofruit® at 3 % with spinosad (4.7 %) and Samaritá Tradicional® at 3 % with espinetoram (3.5 %). The toxic baits formulated with spinosad and espinetoram, associated with Isca Samaritá® at 3 %, caused a mortality rate of more than 60 % to the parasitoid *D. longicaudata*.

KEYWORDS: Tephritidae; Mediterranean fruit fly; fruit fly parasitoids; hydrolyzed protein.

RESUMO

Concentração e tempo letal de iscas tóxicas à base de espinosinas sobre *Ceratitis capitata* e *Diachasmimorpha longicaudata*

O emprego de iscas tóxicas com espinosinas (espinosade e espinetoram), associadas ao parasitoide *Diachasmimorpha longicaudata*, é uma alternativa para o manejo de *Ceratitis capitata*. Objetivou-se avaliar a concentração letal (CL) e o tempo letal (TL) de espinosade e espinetoram, associados aos atrativos alimentares melaço de cana-de-açúcar a 7 %, Biofruit a 3 %, Ceratrap® a 1,5 %, Flyral® a 1,25 %, Isca Samaritá® e Samaritá Tradicional® a 3 %, sobre *C. capitata*, bem como seu efeito, na concentração de 96 mg L⁻¹, sobre *D. longicaudata*, em laboratório. Para os dados de tempo letal, a mortalidade foi avaliada em 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 36, 48, 60, 72, 84 e 96 h após a exposição às iscas tóxicas. As menores concentrações letais (CL₅₀ e CL₉₅) corresponderam, para espinetoram (0,5 mg L⁻¹ e 3,7 mg L⁻¹) e espinosade (0,8 mg L⁻¹ e 7,8 mg L⁻¹), à associação com Samaritá Tradicional® a 3 %. O menor tempo letal (TL₅₀), em horas, para o inseticida espinosade, correspondeu à formulação com Biofruit a 3 % (6,6) e, para espinetoram, Samaritá Tradicional® a 3 % (7,9). Para *D. longicaudata*, as formulações que causaram menor mortalidade corresponderam à associação de Biofruit® a 3 % com espinosade (4,7 %) e Samaritá Tradicional® a 3 % com espinetoram (3,5 %). As iscas tóxicas formuladas com espinosade e espinetoram, associados à Isca Samaritá® a 3 %, provocaram mortalidade superior a 60 %, ao parasitoide *D. longicaudata*.

PALAVRAS-CHAVE: Tephritidae; mosca-do-mediterrâneo; parasitoides de moscas-das-frutas; proteína hidrolisada.

INTRODUCTION

The Mediterranean fruit fly or medfly [*Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)], which originates from the tropical

Africa (Malacrida et al. 1998), is the most invasive and cosmopolitan species of fruit flies, with a capacity to develop in 361 hosts worldwide (Mcquate & Liquido 2017). The damage caused by *C. capitata* mainly results from punctures that lead

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to the development of larvae in the fruits, which then consume the pulp (Nava & Botton 2010).

The use of insecticides in cover spray has been the main form of control adopted by producers (Botton et al. 2016). However, it is important to note that the use of insecticides with deep action, mainly organophosphorus, is not authorized in several crops, due to the risks of toxic residues in fruits (Raga & Sato 2016). Thus, the integration of management practices, such as the use of toxic baits along with the action of parasitoids, has become an interesting alternative (Urbaneja et al. 2009).

Spinosyns (spinosad and spinetoram), neurotoxic insecticide agonists of acetylcholine, have received attention because they are more selective to beneficial insects, when compared to organophosphates (Crouse et al. 2001, Sparks et al. 2001, Galm & Sparks 2015, Schutze et al. 2018), and have, therefore, become alternatives for the management of the Mediterranean fruit fly. Spinosad is a naturally occurring metabolite that is formed through the anaerobic fermentation of the *Saccharopolyspora spinosa* Mertz & Yao bacterium, and is composed of two microcyclic lactones, spinosyn A and spinosyn D. This metabolite is the lethal agent employed in the commercial toxic bait Success® 0.02 CB (Hsu & Feng 2006, Akmoutsou et al. 2011, Markussen & Kristensen 2011, Vontas et al. 2011, Galm & Sparks 2015). Spinetoram is a semisynthetic molecule developed from spinosad, using a combination of molecular modifications resulting from the artificial neural network (RNA) (rhamnose-3'-O-ethylation) modeling and traditional quantitative structure-activity relationship (QSAR) (hydrogenation of the 5,6-double bond) represented by 3'-O-ethyl-5,6-dihydro-spinosyn J (main component) and 3'-O-ethyl spinosyn L (secondary component) (Galm & Sparks 2015).

Diachasmimorpha longicaudata (Ashmed) (Hymenoptera: Braconidae) is an important parasitoid of fruit flies worldwide, mainly due to its ease of rearing and the intensive foraging of females in the search of hosts (Garcia & Ricalde 2013, Garcia et al. 2017). This parasitoid originates from the Indo-Australian region (Carvalho & Nascimento 2002) and is considered to be highly effective for use in biological control programs against *Anastrepha* spp. and *C. capitata* (Garcia & Ricalde 2013, Garcia et al. 2017).

Due to the potential for the use of *D. longicaudata* in the biological control of *C. capitata* and spinosyns

as lethal agents in toxic bait formulations, the toxicity of spinosad and spinetoram, associated with food lures, was evaluated in medfly in the laboratory, as well as the effects of spinosyns on the parasitoid.

MATERIAL AND METHODS

The experiments were carried out at the entomology laboratory of the Embrapa Uva e Vinho, in Bento Gonçalves, Rio Grande do Sul State, Brazil (temperature of 25 ± 2 °C, relative humidity of 70 ± 10 % and photophase of 12 h), from September 2016 to January 2017. Adults of *C. capitata* were obtained from a breeding colony maintained in the laboratory, which originated from larvae collected from fruits of strawberry guava (*Psidium cattleianum* Sabine), in Pelotas, Rio Grande do Sul State, Brazil.

The rearing colony was maintained on the artificial diet for larval development described by Salles (1992) and modified by Nunes et al. (2013), and adults were fed with a solid diet used for the rearing of *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae), which is composed of soybean extract, wheat germ and brown sugar (3:1:1) (Machota-Junior et al. 2010), and were provided with water in polyurethane sponges placed in Petri dishes (9 cm in diameter), due to the ease of preparation and the excellent adaptability of the insects to the diets used.

Six food lures were used: a) Biofruit (3 %) (hydrolyzed corn protein); b) Ceratrap® (1.5 %) (enzymatic hydrolyzed protein of animal origin); c) Flyral® (1.25 %) (enzymatic hydrolyzed protein of animal origin); d) Isca Samaritá® (3 %) (hydrolyzed corn protein); e) Samaritá Tradicional® (3 %) (hydrolyzed protein of vegetal origin, reducing sugars and preservatives); and f) sugarcane molasses at 7 %. The concentrations of the food lures were defined by the manufacturers' recommendations and/or practical use experience. For the formulation of toxic baits, the food attractants were mixed with the insecticides spinosad (Tracer® 480 SC, 480 g L⁻¹ of active ingredient) and spinetoram (Delegate® 250 WG, 250 g L⁻¹ of active ingredient).

In the lethal time and toxicity experiments on adults of *D. longicaudata*, the toxic bait Success® 0.02 CB (0.24 g L⁻¹ of the active ingredient spinosad) was used as a reference toxic bait, which was diluted in water at a ratio of 1:1.5 (commercial product:water), according to the manufacturer's recommendations to

obtain a concentration of 96 mg L⁻¹ (Agrofit 2003, Barry et al. 2006). The evaluated treatments were as it follows: sugarcane molasses + spinosad; sugarcane molasses + spinetoram; sugarcane molasses; Biofruit + spinosad; Biofruit + spinetoram; Biofruit; Flyral® + spinosad; Flyral® + spinetoram; Flyral®; Ceratrap® + spinosad; Ceratrap® + spinetoram; Ceratrap®; Samaritá Tradicional® + spinosad; Samaritá Tradicional® + spinetoram; Samaritá Tradicional®; Isca Samaritá® + spinosad; Isca Samaritá® + spinetoram; Isca Samaritá®; Success® 0.02 CB; and distilled water.

To determine the lethal concentrations (LC₅₀ and LC₉₅), spinosad and spinetoram were diluted at eight concentrations of the ingredients (Table 2). The results are shown in Table 1. Table 2 shows the logarithmically spaced concentrations (250 mg L⁻¹, 130 mg L⁻¹, 70 mg L⁻¹, 40 mg L⁻¹, 20 mg L⁻¹, 6 mg L⁻¹, 2 mg L⁻¹, 0.1 mg L⁻¹ and 150 mg L⁻¹, 75 mg L⁻¹, 38 mg L⁻¹, 10 mg L⁻¹, 3 mg L⁻¹, 0.8 mg L⁻¹, 0.06 mg L⁻¹ and 0.005 mg L⁻¹, respectively) defined from preliminary experiments that resulted in adult mortality between 99 % and 10 %. Aiming to evaluate the effects of the previously defined concentrations, 5-8-day-old adults of *C. capitata* (post-emergence) were deprived of the food diet for 12 h. After this period, 10 adults (5 males and 5 females) were transferred to transparent plastic cages (300 mL) with a cut in the bottom and covered with *voile* fabric, receiving a drop of 40 µL of formulated toxic bait placed on 1 cm² polyethylene terephthalate (PET) coverslips, for 4 h. After the removal of the toxic baits, the *C. capitata* adults were fed a 10 % mead solution offered via capillarity in hydrophilic cotton. Adult mortality was evaluated at 24, 48, 72 and 96 h after exposure (HAE), considering the insects that did not respond to the touch of a fine brush as dead. The experimental design was completely randomized, with 12 replicates per treatment, with each cage containing five couples of *C. capitata* considered as an individual replicate.

To determine the lethal times (LT₅₀ and LT₉₅) of the toxic bait formulations, the lures were mixed with the insecticides spinosad and spinetoram, at a 96 mg L⁻¹ concentration, using the concentration of spinosad present in the standard ready-to-use toxic bait Success® 0.02 CB as a reference, and following the methodology described in the previous experiment. For comparison purposes, each lure was

evaluated in isolation or combined with spinosyns and compared with Success® 0.02 CB and a spinosyn-free control. Adult mortality was assessed at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 36, 48, 60, 72, 84 and 96 HAE.

For the determination of the lethal concentrations LC₅₀ and LC₉₅, the lethal times LT₅₀ and LT₉₅ and their respective confidence intervals (95 % CI), times and concentration-mortality data were submitted to Probit analysis, using the POLO-PC software (Leora Software 1987). A probability test (F-test) was conducted to evaluate the hypothesis that the lethal concentrations values were the same. When the hypothesis was rejected, pairwise comparisons were performed, and significance was assumed when there was no overlap of the confidence intervals.

Adults of *D. longicaudata* were obtained from cages used in a rearing colony, according to the methodology suggested by Carvalho et al. (1998). These cages were made from transparent plastic containers with a lid and a capacity of 500 mL. The lids had a circular cut at the bottom, where thin fabric of the *voile* type was attached to allow air to enter and prevent the escape of insects. For the bioassay, each cage contained 20 couples of *D. longicaudata* at 5 days of age and deprived of food for 12 h. After this period, 20 adults (10 couples) were transferred to transparent plastic cages (300 mL) with a cut in the bottom that was covered with *voile* fabric, receiving a drop of 40 µL of formulated toxic bait, according to the treatments described above and conditioned on 1 cm² PET coverslips, for 4 h.

After the removal of the toxic baits, the adults of *D. longicaudata* were fed with a solution of 10 % mead offered by capillarity in hydrophilic cotton. Adult mortality was evaluated up to 96 HAE, considering insects that did not react to the touch of a fine brush as dead. The experimental design was completely randomized, with five replicates per treatment and each replicate being composed of a cage containing 10 adult couples of *D. longicaudata*. The number of live insects was submitted to analysis of variance (Anova), and the means were compared using the Tukey test at 5 % of probability ($p \leq 0.05$), with the statistical software SPSS 24.0 (SPSS Inc., Chicago, IL, EUA). Adult survival data of *D. longicaudata* were transformed into percent of control by the equation proposed by Abbott (1925).

RESULTS AND DISCUSSION

All the evaluated formulations of toxic baits based on spinosyns were toxic to *C. capitata* adults (Table 1). The biological responses of *C. capitata* adults using the bioassay (toxic bait intake) were satisfactory, based on the Probit analysis (chi-square < 10), showing consistent results and an association between the mortality and dose data (Table 1). When comparing the LC₉₅ values, all the toxic baits formulated with the insecticide spinetoram presented a higher biological activity in adults of *C. capitata*, when compared to spinosad, except for Flyral® + spinosad, demonstrating a variation in the toxicity of the compounds according to the bait. The lowest LC₅₀ and LC₉₅ values were recorded for the Samaritá Tradicional® + spinetoram formulation, being 0.5 mg L⁻¹ and 3.7 mg L⁻¹, respectively. For the other toxic baits, the lowest LC₅₀ values were obtained with the formulations of sugarcane molasses + spinetoram (0.6 mg L⁻¹), Ceratrap® + spinetoram (0.7 mg L⁻¹) and Samaritá Tradicional® + spinosad (0.8 mg L⁻¹). The two evaluated spinosyns caused mortality in adults of *C. capitata*, and a lower dose was required for the insecticide spinetoram, when compared to spinosad.

The lowest doses found for the LC₉₅ values corresponded to the Samaritá Tradicional® + spinosad (7.8 mg L⁻¹) and Samaritá Tradicional® + spinetoram (3.7 mg L⁻¹) formulations, followed by Biofruit + spinetoram (10.7 mg L⁻¹), Isca Samaritá® + spinetoram (15.4 mg L⁻¹), Ceratrap® + spinetoram

(18.7 mg L⁻¹) and sugarcane molasses + spinetoram (19.4 mg L⁻¹). Similarly, both evaluated insecticides were effective in the control of adults of *C. capitata*, although generally at lower concentrations for the insecticide spinetoram.

Toxic baits must contain a combination of protein (attractive) with sugar (phagostimulant), thus allowing a stimulus to the search and ingestion of the formulations by the insects. Hydrolyzed proteins provide free amino acids for nutrition and reproduction, as well as the phagostimulatory action, which causes the rapid search for these substances (Vargas & Prokopy 2006).

The food lure Samaritá Tradicional® has in its composition hydrolyzed protein of corn, reducing sugars and preservatives (Samaritá). In this study, this lure gained prominence in the formulations of the toxic baits by overlapping in its confidence intervals for LC₉₅, when associated with spinetoram, with these formulations being equivalent only to its formulation with the insecticide spinosad (Table 1). Studies have shown that *C. capitata* is attracted to carbohydrates, such as fructose, glucose and sucrose. These are the compounds that most stimulate their neurosensory responses and promote the acquisition of energy for survival, mating, localization and oviposition in suitable hosts (Zucoloto 2000). This aspect is very important for the evaluation of the toxicity of toxic bait formulations, since one of the main factors that influence the efficiency of formulations is the phage-stimulating effect of the material supplied, which can result in more or less attractiveness (Nestel et al. 2004).

Table 1. Average lethal concentrations (LC₅₀ and LC₉₅; mg L⁻¹) of toxic bait formulations on *Ceratitis capitata* under laboratory conditions (temperature of 25 ± 2 °C, relative humidity of 70 ± 10 % and photoperiod of 12 h).

Lure	Insecticide (96 mg L ⁻¹)	Angular coefficient (± standard error)	LC ₅₀ (95 % CI) ^a	LC ₉₅ (95 % CI) ^b	χ ^{2c}
Sugarcane molasses (7 %)	spinosad	1.122 ± 0.090	2.9 (1.4-4.9)	84.8 (48.7-189.1)	9.9
	spinetoram	1.095 ± 0.094	0.6 (0.4-0.9)	19.4 (12.8-32.8)	5.6
Biofruit (3 %)	spinosad	1.052 ± 0.086	2.9 (1.2-5.2)	104.4 (55.2-269.5)	11.0
	spinetoram	1.351 ± 0.224	1.4 (1.0-1.8)	10.3 (7.3-16.9)	1.6
Isca Samaritá® (3 %)	spinosad	0.924 ± 0.076	1.1 (0.6-1.7)	65.2 (42.6-110.4)	5.1
	spinetoram	1.382 ± 0.185	1.0 (0.5-1.5)	15.4 (10.4-27.9)	5.3
Samaritá Tradicional® (3 %)	spinosad	1.628 ± 0.219	0.8 (0.4-1.2)	7.8 (5.5-12.5)	0.8
	spinetoram	1.788 ± 0.260	0.5 (0.3-0.7)	3.7 (2.6-6.4)	0.8
Flyral® (1.25 %)	spinosad	1.033 ± 0.086	0.9 (0.5-1.4)	36.0 (24.2-58.3)	2.5
	spinetoram	1.081 ± 0.089	1.1 (0.6-1.8)	35.7 (20.7-75.7)	7.1
Ceratrap® (1.5 %)	spinosad	1.118 ± 0.135	3.5 (1.6-5.8)	102.1 (65.7-187.5)	4.6
	spinetoram	1.150 ± 0.113	0.7 (0.3-1.4)	18.7 (9.4-51.7)	9.5

^a LC₅₀: lethal concentration responsible for causing 50 % of mortality in the population; ^b LC₉₅: lethal concentration responsible for causing 95 % of mortality in the population; ^c Chi-square (p < 0.05).

In terms of lethal time (LT), a significant variation in the *C. capitata* survival was observed when members of this species were exposed to formulations of toxic baits containing spinosad and spinetoram at the concentration of 96 mg L⁻¹ (Table 2). The lowest values of LT₅₀ and LT₉₅, in hours, were found for the toxic bait formulations of Biofruit + spinosad (6.6) and Success[®] 0.02CB (14.9), and the highest values of LT₅₀ and LT₉₅ corresponded to the toxic baits Flyral[®] + spinetoram (10.0) and Ceratrap[®] + spinosad (30.1), respectively (Table 2). Additionally, based on their overlapping confidence intervals (95 % CI, in hours) for the estimated LT₉₅ values, only the association of Biofruit + spinosad (14.2-18.0) was equivalent to the ready-to-use toxic bait Success[®] 0.02 CB (14.1-16.0). Success[®] 0.02 CB (= GF-120[®] NF) is marketed in the USA and Europe and is authorized for use in several crops in Brazil, for the management of *A. fraterculus* (Wiedemann 1830), *Anastrepha obliqua* (Macquart 1835), *Bactrocera carambolae* (Drew & Hancock 1994) and *C. capitata* (Agrofit 2003), and the components present in this toxic bait formulation (Dow Elanco 1994) can reduce the plant uptake by making the active ingredient available to insects for a longer period of time (Revis et al. 2004). Moreover, it is important to point out that such action is evidenced in the absence of rain over the applications, since the formulations are easily washed away (Borges et al. 2015, Harter et al. 2015).

In general, the baits formulated with the insecticide spinosad had an average mortality time lower than those formulated with spinetoram, except for the attractant Ceratrap[®] (Table 2). Raga & Sato (2005), using the concentrations of 80 mg L⁻¹, 8 mg L⁻¹ and 4 mg L⁻¹ of spinosad from dilutions of GF-120[®] NF against adults of *C. capitata*, estimated LT₅₀ values of 106, 126 and 154 min, respectively. These authors justified the low values for the lowest concentrations because they are associated with the high consumption of the baits by the medfly adults, demonstrating the high attractiveness of this toxic bait and the low repellency of spinosad and indicating a high consumption of the toxic bait, independently of the concentration of the insecticide.

As for the effects of the toxic baits on *D. longicaudata* adults, the formulations with the insecticides spinosad and spinetoram, associated with the food lure Isca Samaritá[®], showed a significant difference in mortality in the evaluations performed at 24 ($F = 22.01$; $DF = 99$; $p < 0.0001$), 48 ($F = 14.22$; $DF = 99$; $p < 0.0001$), 72 ($F = 11.55$; $DF = 99$; $p < 0.0001$) and 96 HAE ($F = 9.30$; $DF = 99$; $p < 0.0001$), with 67 % and 61%, respectively, when compared to the other toxic baits and the pattern represented by the ready-to-use formulation Success[®] 0.02 CB (10.6 %) (Figure 1).

Ruiz et al. (2008) evaluated the effects of the topical application of GF-120[®] NF (= Success[®] 0.02 CB) on *D. longicaudata*, in the laboratory and under

Table 2. Lethal times (LT₅₀ and LT₉₅), in hours, of toxic bait formulations, at a concentration of 96 mg L⁻¹, for the control of *C. capitata* under laboratory conditions (temperature of 25 ± 2 °C, relative humidity of 70 ± 10 % and photoperiod of 12 h).

Lure	Insecticide (96 mg L ⁻¹)	Angular coefficient (± standard error)	LT ₅₀ (95 % CI) ^a	LT ₉₅ (95 % CI) ^b	χ ^{2c}
Sugarcane molasses (7 %)	spinosad	4.401 ± 0.198	9.7 (7.8-11.5)	22.9 (18.3-34.1)	181.730
	spinetoram	4.047 ± 0.187	8.9 (8.4-9.4)	22.7 (21.1-24.7)	10.065
Biofruit (3 %)	spinosad	4.321 ± 0.207	6.6 (5.9-7.2)	15.8 (14.2-18)	31.615
	spinetoram	3.593 ± 0.160	8.8 (7.7-9.9)	25.3 (21.4-31.9)	65.257
Flyral [®] (1.25 %)	spinosad	4.303 ± 0.203	9.9 (9.4-10.4)	23.9 (22.3-26.1)	5.200
	spinetoram	3.565 ± 0.161	9.9 (9.0-10.9)	28.8 (25.2-34.2)	36.254
Ceratrap [®] (1.5 %)	spinosad	3.334 ± 0.147	9.6 (8.2-11.1)	30.0 (24.7-39.7)	79.421
	spinetoram	3.461 ± 0.157	9.1 (8.6-9.7)	27.4 (25.1-30.1)	10.865
Samaritá Tradicional [®] (3 %)	spinosad	4.657 ± 0.222	8.2 (7.7-8.8)	18.6 (17.1-20.5)	20.882
	spinetoram	4.142 ± 0.198	7.9 (7.5-8.4)	19.8 (18.4-21.5)	13.292
Isca Samaritá [®] (3 %)	spinosad	5.314 ± 0.249	9.3 (8.5-10)	18.9 (17-21.7)	44.557
	spinetoram	3.324 ± 0.147	9.1 (7.5-10.6)	28.3 (16.7-39.0)	99.985
Success [®] 0.02CB	spinosad	5.313 ± 0.259	7.3 (6.9-7.7)	14.9 (14.0-16)	9.992

^a LT₅₀: lethal time responsible for causing 50 % of mortality in the population; ^b LT₉₅: lethal time responsible for causing 95 % of mortality in the population; ^c Chi-square ($p < 0.05$).

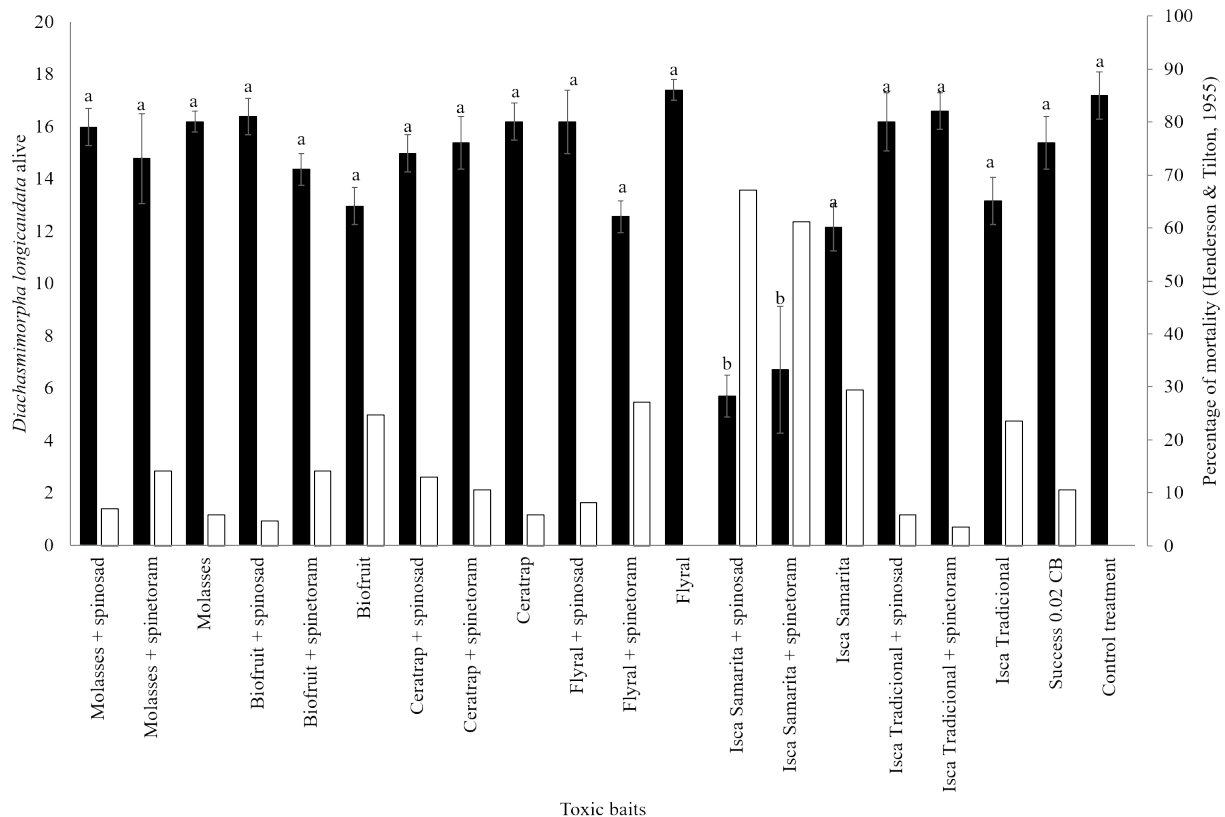


Figure 1. Number of insects alive ($N \pm SE$; black bars) and percentage of mortality (M%; white bars) in *Diachasmimorpha longicaudata*, at 96 h after the exposure to toxic baits under laboratory conditions (temperature of 25 ± 2 °C, relative humidity of 70 ± 10 % and photoperiod of 12 h). ¹Mean numbers of living *Diachasmimorpha longicaudata* adults indicated by the same lowercase letters do not differ from one another, based on the Tukey test ($p > 0.05$). ² Corrected mortality calculated by the equation by Abbott (1925).

semi-field conditions, at a concentration of 80 mg L^{-1} , and observed 95 % of mortality in the parasitoids, over a period of 5 days, when compared with 2 % of mortality in the control over the same period. The authors also found a high mortality rate when the parasitoids ingested a mixture of GF-120[®] NF with honey at 50 %. However, they report the possibility that the baits contained compounds that were repellent to the parasitoids, since *D. longicaudata* apparently showed resistance to feeding. The authors also found that sublethal effects, including decreased longevity and reproductive capacity, through contact with surfaces treated with GF[®]-120 NF, are dependent on the exposure time.

Similarly, formulations of toxic baits containing spinosad showed lower effects on the parasitoids *Fopius arisanus* (Sonan) and *Psytalia fletcheri* (Silvestri) (Hymenoptera: Braconidae), if compared to toxic baits formulated with the insecticide malathion (Stark et al. 2004), corroborating studies

carried out in Hawaii, where it was observed that the application of 11 sprays of toxic baits containing the insecticide spinosad (GF[®]-120 NF) did not affect a *F. arisanus* population in the field. However, notably, the toxicity of spinosyn-based insecticides depends on the form of application (ingestion or topical) (Biondi et al. 2012), dose and exposure time (Ruiz et al. 2008) and insect species (Takahashi et al. 2005).

The results of the present study show the effect of different formulations of toxic baits obtained in the laboratory, where the adults of *D. longicaudata* were forced to feed on the material offered. In the field, toxicity and side effects to *D. longicaudata* may be less damaging to the parasitoid. This possibility is associated with the low biological persistence of toxic baits, when applied under field conditions, as the active ingredient is degraded by the presence of constant rainfall (Revis et al. 2004, Flores et al. 2011, Harter et al. 2015). The laboratory results suggest that the Mediterranean fruit fly can be managed by

combining the use of toxic baits based on spinosyn and the use of *D. longicaudata*.

CONCLUSIONS

1. Based on the lethal concentration, the insecticide spinetoram presents a greater biological activity than spinosad against *C. capitata*;
2. Formulations of Biofruit at 3% + spinosad and Isca Samaritá® at 3% + spinetoram present the lowest lethal concentration and lethal time for *C. capitata*;
3. Spinetoram presents a lethal concentration of less than 36 mg L⁻¹ for *C. capitata*, while the concentration of spinosad needs to be increased to 104 mg L⁻¹, varying according to the attractant used in the mixture;
4. Except for Isca Samarita at 3%, all toxic bait formulations (associated with spinetoram or spinosad) have low toxic effects on the *D. longicaudata* parasitoid.

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