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PEST MANAGEMENT

Bioassay Method for Toxicity Studies of Toxic Bait Formulations to *Ceratitis capitata* (Diptera: Tephritidae)

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Abstract

The development of appropriate methodologies for bioassays is of paramount importance to study the methodological factors that may interfere in the experimental design. Thus, laboratory bioassays have become increasingly important for the determination of data that are realistic and replicable. The objective of the study was to develop a bioassay methodology with the ready-to-use toxic bait Success™ 0.02CB, 96 mg L^{-1} of spinosad, in adults of *Ceratitis capitata* (Wiedemann) as a study model. The insect origin (laboratory population, LT_{50} = 11.16 h), time of toxic bait supply (4 h, LT_{50} = 16.85 h), food deprivation (12 h, LT_{50} = 15.55 h), and the adult age (5 days, LT_{50} = 35.31 h) of *C. capitata* showed higher susceptibility and a shorter LT_{50} lethal time to the Success™ 0.02CB toxic bait. However, the absence or presence of a food source ($LT_{50} \approx 31.17$ h) during the bioassay did not affect the susceptibility of *C. capitata*. Further, adults of *C. capitata* who were deprived and in the absence of a food source (artificial diet) had higher consumption of toxic baits (mg) during treatment exposure. The use of a 12-h deprivation period, toxic baits offered for 4 h, and absence of an artificial diet for adults of *C. capitata* at 5 days of age during the bioassay are considered adequate for evaluating formulations of toxic baits under laboratory conditions.

Introduction

Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is a polyphagous and cosmopolitan specie with a high capacity for infesting and damaging 361 host species belonging to 63 botanical families worldwide (McQuate & Liquido 2017). In Brazil, there are records of the occurrence of the pest in 93 host species belonging to 27 botanical families (Adaime *et al* 2017). Due to the high number of hosts and large dispersal capacity of *C. capitata*, especially in areas of commercial fruit production, the main management strategy is through chemical control with the use of insecticides in cover sprays and toxic baits (Morelli *et al* 2012, Botton *et al* 2016).

The application of toxic baits in aerial or terrestrial spray is a good alternative to fruit fly management (Raga & Sato 2005, Navarro-Llopis *et al* 2012, Raga & Galdino 2018). Because they are formulated from a food lure composed of proteins and carbohydrates associated with a lethal agent (Medina *et al* 2007), toxic baits act by attracting and intoxicating adults of the Mediterranean fruit fly who are searching for food sources for maintenance of energy reserves and maturation of the reproductive apparatus (Silva-Neto *et al* 2010, Raga & Sato 2016). In Brazil, sugarcane molasses (a byproduct of the sugar-making process containing reducing sugars and uncrystallized sucrose) and hydrolyzed proteins of plant origin (maize) are the main attractions used in the formulation of toxic baits for the management of *C. capitata* and *Anastrepha fraterculus* (Wiedemann) (Diptera:

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Tephritidae) (Raga & Sato 2005, Morelli *et al* 2012, Araújo *et al* 2013, Borges *et al* 2015, Härter et al. 2015). However, in addition to the toxic baits formulated in rural properties (Botton *et al* 2016), a ready-to-use toxic bait formulation (Success™ 0.02CB) has recently been introduced in the Brazilian market that has spinosad as a lethal agent. This agent, known internationally as GF-120™ NF (Prokopy *et al* 2004), is authorized for use in several crops in Brazil for the control of *A. fraterculus*, *Anastrepha obliqua* (Mcquart) (Diptera: Tephritidae), *Bactrocera carambolae* Drew and Hancock (Diptera: Tephritidae), and *C. capitata* (Agrofit 2018).

To develop, improve, and make available new formulations of toxic baits for the handling of fruit flies in the field, laboratory studies are important for the characterization of possible factors that may interfere in the toxicological characterization of the products, such as the type of attractant feeding time, exposure time, insect origin (field population or laboratory population without insecticide selection pressure), insect age, feed deprivation period, and mode of availability of the formulations (Stark *et al*. 2004, Medina *et al* 2007, Raga and Sato 2011, Gazit *et al* 2013). Analysis of these factors is fundamental for determining and establishing adequate bioassay methodologies in order to avoid misguided and erroneous conclusions about a certain toxic bait formulation and to affect management recommendations (Zwolfer 1983).

In testing and toxicological characterization of formulations of toxic baits on fruit fly species, there is no methodological standardization for evaluations and this may negatively affect results and compromise the reliability of the data (Humeres *et al* 1999, Robertson *et al* 2007, Gazit *et al* 2013, Paramasivam & Selvi 2017). To address these limitations, in this study, was to develop a bioassay methodology, considering factors such as age and origin of the population, time of food deprivation, and form and time of availability of the product to assess the effects on adults of *C. capitata* using as toxicological standard the Success™ 0.02CB commercial formulation (96 mg L^{-1} active ingredient spinosad).

Material and Methods

Insects

Adults of *C. capitata* were obtained from the Entomology Laboratory of Embrapa Grape and Wine, Bento Gonçalves, Rio Grande do Sul, Brazil, and were kept in air-conditioned rooms (temperature of 25 ± 2°C, relative air humidity of 70 ± 10%, and photoperiod of 12 h), rearing *C. capitata* adults with an artificial diet composed of a mixture of wheat germ, beer yeast, and brown sugar (3:1:1) offered in Petri dishes, and larvae fed with an artificial diet according to Nunes *et al* (2013).

Bioassays

All bioassays were performed in a climate room (temperature of 25 ± 2°C, relative air humidity of 70 ± 10%, and photoperiod of 12 h). The insects used in the different bioassays came from a susceptible population of *C. capitata*, which is kept in laboratory for 5 years, free of selection pressure by insecticides. For the bioassays, adults of *C. capitata* were packed inside plastic containers (300 mL) as proposed by Medina *et al* (2007) and Gazit *et al* (2013). The toxic bait used in the bioassays was the Success™ 0.02CB formulation (Dow Agrosciences, Santo Amaro, Sao Paulo, Brazil), diluted in proportion as one-part commercial product to 1.5 parts water, resulting in a concentration of 96 mg L^{-1} of spinosad (Barry *et al* 2006, Agrofit 2018). The bioassay method was based on the ingestion of a drop of 40 μL deposited on a 1-cm^2 polyethylene terephthalate (PET) plastic plate with the aid of graduated single-channel micropipette Gilson™ model Pipetman U76928A with 1-mL capacity and left for 2 h at room temperature (25°C) to allow drying and evaporation of water. For all bioassays, the toxic bait was provided for 4 h. The insects were fed an artificial diet composed of a mixture of wheat germ, beer yeast, and brown sugar (3:1:1) supplied inside acrylic lids (2-cm diameter) and distilled water in plastic caps (2-cm diameter) lined with hydrophilic cotton. Similarly, in all bioassays, adult mortality of *C. capitata* in treatments and for estimates of lethal times (LT_{50}) was evaluated at 1, 3, 6, 12, 24, 48, 72, and 96 h after exposure (HAE) of the toxic bait. Insects were considered dead when they did not exhibit reaction to the touch of a fine brush. Adult mortality of *C. capitata* was corrected by the formula of Henderson & Tilton (1955).

*Susceptibility of **C. capitata** populations as a function of origin*

Two populations of *C. capitata* were used: a population from the field (first generation) from insects collected from mango fruits (*Mangifera indica* L.) collected in the municipality of Casa Nova, Bahia, Brazil (9°1850679S; 40°5250000W), infested with *C. capitata* larvae, and a population established and maintained in the laboratory on an artificial diet as previously described. Adults that emerged from the two larval feeding substrates (mango fruits or artificial diet) were grown in semitransparent plastic cages (41.0 × 29.5 × 30.0 cm in length, width, and height, respectively) containing distilled water supplied via capillarity by means of a strip of vegetable sponge (Spontex™) and were fed an artificial diet composed of soybean extract, wheat germ, and brown sugar at the ratio of 3:1:1. The experimental design was completely randomized with 10 replicates (five couples per replicate) per treatment. Subsequently, adults of both populations (treatments) within 5 to 8 days old were packaged in plastic

containers (300 mL) and fed with a toxic bait (Success™ 0.02CB, one drop of 40 μL) for a period of 4 h. After this time, the insects were fed with an artificial diet. As a negative control, adults of *C. capitata* were fed only the artificial diet. The experimental design was completely randomized with 10 replicates (five couples) per treatment.

*Availability of toxic bait to **C. capitata** together with the supply of artificial food*

Adults of *C. capitata* (5 days old) obtained from the group maintained on the artificial diet were placed inside plastic containers (300 mL), as described in the previous bioassay. To verify the effects of the presence or absence of the artificial diet on adults of *C. capitata* during the period of exposure to the feeding of the toxic bait formulation, the treatments (T) were the following: (T1) adults of *C. capitata* exposed to the toxic bait Success™ 0.02CB (one drop of 40 μL) + distilled water provided as previously described, with the provision of artificial diet; (T2), adults of *C. capitata* were exposed only to toxic bait (Success™ 0.02CB, one drop of 40 μL) + distilled water as feed substrates, according to the methodology described above; and (T3), adults of *C. capitata* received only artificial diet + water, without the presence of toxic bait. The experimental design was completely randomized with 10 replicates (five couples per replicate).

*Exposure time of toxic bait to adults of **C. capitata***

To evaluate the exposure time of the toxic bait, adults of *C. capitata* (5 to 6 days old) obtained from the group maintained in the artificial diet were packed inside plastic containers (300 mL), as previously described. Subsequently, the insects were exposed to feed for 1, 2, 4, and 8 h on the toxic bait Success™ 0.02CB (one drop of 40 μL) without the presence of artificial diet. After the withdrawal of the toxic bait during the determined exposure periods, artificial diet was offered to the adults until the end of evaluation. The experimental design was completely randomized with 10 replicates (five couples per replicate).

*Toxicity of toxic bait on adults of **C. capitata** as a function of the age of the insects*

In this bioassay, adults of *C. capitata* from the rearing of maintenance with ages (treatments) of 1, 5, and 8 days old (after the emergence) were used. These are the ages at which females present a lower percentage of mature ovaries, developing ovaries, and high mature ovaries (Arita 1982). The insects were packed into plastic containers (300 mL) and then the toxic bait (Success™ 0.02CB, one drop of 40 μL) was provided for a 4-h period as described above. After this period, the toxic baits were removed, and the insects were fed with artificial diet and water. The experimental design was completely randomized with 10 replicates (five couples per replicate).

Period of food deprivation prior to toxic bait exposure

Adults of *C. capitata* (5 days old) from the group maintained on the artificial diet were submitted to periods (treatments) of 0, 12, and 24 h of food deprivation (artificial diet). After the deprivation periods of each treatment, the insects were transferred to cages made of plastic containers (300 mL), as described previously, where they were exposed to a drop of 40 μL of the Success™ 0.02CB toxic bait. As a negative control (control treatment), the insects received only artificial diet and water. The experimental design was completely randomized with 10 replicates (five couples per replicate).

Data analysis

The data on mortality and consumption of toxic bait were submitted to studentized residual analysis to confirm the assumption of normality using the Shapiro-Wilk test with the PROC UNIVARIATE procedure in SAS 9.1 (SAS Institute 2011). The resulting percentage data were submitted to arcsine square root transformation prior to analysis using the SAS function ARSIN (SQRT(x)). After arcsine square root transformation, the data met the assumption of normality required for ANOVA tests. Then, all data were subjected to analyses using the PROC GLM procedure in SAS 9.1. (SAS Institute 2011). Treatment differences were determined using least-square means statements (LSMEANS) at a $P = 0.05$ level of significance in SAS 9.1 (SAS Institute 2011). In the bioassay to evaluate the availability of toxic bait and populations, the data were submitted to the *t* test at 5% significance. For the evaluation of the effect of each treatment on insect survival, survival curves and respective lethal times (LT_{50}) were determined through the Kaplan-Meier analysis, comparing the survival curves by the log-rank test through the program SigmaPlot (v.12.5, Systat Software Inc., California, USA). Kaplan-Meier and log-rank analyzes were also used to compare the mortality of males and females from each treatment in all bioassays. The determination of the consumption of the toxic baits was carried out by weighing the blade in a precision scales Mettler Toledo model MS204S/A01, by subtracting the initial mass (IM) by the final mass (FM). The possible evaporation of the toxic bait was corrected by the evaporation of the toxic bait containing plates that remained in the same conditions of each experiment (negative control), but without being offered to the insects. The toxic bait consumption data were evaluated for normality by the Shapiro-Wilk test and homoscedasticity by Hartley and Bartlett and later submitted to analysis of

variance (ANOVA) using the *F* test ($P < 0.05$) (PROC GLM, SAS Institute 2011). Finding statistical significance, the averages were compared by the Tukey test at a 5% level of significance ($P < 0.05$) (SAS Institute 2011).

Results

Susceptibility of populations of ***C. capitata***

Adults of *C. capitata* that were laboratory bred on an artificial diet (laboratory population) presented a higher mortality rate ($F_{3,40} = 11.27$; $P < 0.001$) when exposed to the toxic bait formulation in the evaluations performed up to 96 HAE (97% mortality) when compared to the field population (72% mortality) (Table 1). The adult survival curves of *C. capitata* (males and females) showed that *C. capitata* adults from the laboratory population were more susceptible to toxic bait formulation in time, presenting the lowest values of LT_{50} (LT_{50} (CI 95%) = 11.16 (9.84–12.47) hours) when compared to insects from the field population (LT_{50} (CI 95%) = 18.11 (14.22–22.00) hours) (Table 1, Fig 1). Regarding the consumption of toxic baits, there were no significant differences ($F = 3.31$; $t > 0.05$) between the two populations (laboratory and field population) of *C. capitata* for both the laboratory population and the artificial diet (2.22 mg) and field population (1.75 mg) (Table 1).

Effect of availability of artificial diet

The presence or absence of artificial diet together with the toxic bait Success™ 0.02CB formulation + water did not affect the survival ($F_{2,20} = 8.11$; $P < 0.001$) of *C. capitata* adults (males and females) up to 96 HAE (100% mortality) (Table 1, Fig 2). Due to the overlapping of the confidence intervals, *C. capitata* adults showed the same susceptibility when the insects were exposed to the treatments containing toxic bait Success™ 0.02CB + water + artificial diet (LT_{50} (CI 95%) = 31.09 (27.37–34.80) hours) than insects exposed to the treatment containing toxic bait Success™ 0.02CB + water, without the presence of artificial diet (LT_{50} (CI 95%) = 31.17 (27.72–34.61) hours) (Fig 2). However, in the presence of an artificial diet together with toxic bait Success™ 0.02CB + water, adults of *C. capitata* showed a lower (6.87 mg) toxic bait consumption ($t = 5.71$; df = 39; $P = 0.028$) when compared with insects for which no artificial diet was available (9.33 mg) (Table 1).

Table 1 Average number of alive insects (N ± SE), percentage of mortality (%M), lethal time LT_{50} (hours), and confidence interval (CI) and toxic bait consumption (mg) of *Ceratitis capitata* adults on ingestion bioassay when submitted to the Success™ 0.02CB toxic bait formulation

Bioassay	96 HAE		LT_{50} (CI 95%) (hours)[3]	Toxic bait consumption (mg)[4]
	N ± SE[1]	% M[2]		
Susceptibility of *C. capitata* population				
Laboratory population	0.3 ± 0.30 a	97	11.16 (9.84–12.47)	2.22 ± 0.19 a
Wild population	2.7 ± 0.93 b	72	18.11(14.22–22.00)	1.75 ± 0.37 a
Availability of artificial diet with toxic bait				
Toxic bait + artificial diet	0.0 ± 0.00 a	100	31.09 (27.37–34.80)	6.87 ± 0.79 b
Toxic bait	0.0 ± 0.00 a	100	31.17 (27.72–34.61)	9.33 ± 0.66 a
Exposure time of toxic bait				
1 h	2.1 ± 0.71 b	76	23.90 (18.98–28.82)	2.28 ± 0.24 b
2 h	0.2 ± 0.13 a	98	24.01 (20.16–27.86)	2.90 ± 0.22 b
4 h	0.0 ± 0.00 a	100	16.85 (14.37–19.32)	4.45 ± 0.31 a
8 h	0.0 ± 0.00 a	100	14.89 (12.46–17.33)	4.65 ± 0.23 a
Influence of *C. capitata* age on toxicity of toxic bait				
1 day old	6.6 ± 0.83 c	33	15.51 (12.95–18.07)	0.93 ± 0.17 c
5 days old	1.0 ± 0.45 a	89	35.51 (31.14–38.88)	1.57 ± 0.20 b
8 days old	2.9 ± 0.81 b	67	45.56 (36.18–54.94)	2.00 ± 0.19 a
Time of food diet deprivation				
0 h deprivation	0.0 ± 0.00 a	100	24.72 (21.51–27.94)	2.28 ± 0.19 b
12 h deprivation	0.0 ± 0.00 a	100	15.55 (12.96–18.13)	4.64 ± 0.15 a
24 h deprivation	0.0 ± 0.00 a	100	9.24 (6.83–13.65)	5.33 ± 0.29 a

[1] Values represent means ± SE. A separate ANOVA (Tukey's test, $P < 0.05$) was conducted for treatments within each column (means followed by the same letter in column are not significantly different).

[2] Mortality calculated by the formula of Henderson and Tilton (1955).

[3] LT_{50} = time required to kill 50% of a tested population.

[4] Estimated value through of subtraction of the initial mass (IM) of the final mass (FM) in each blade.

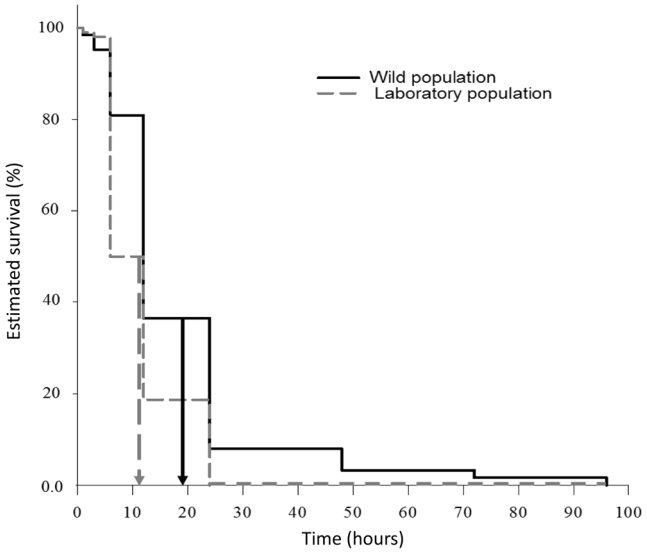

Fig 1 Survival of *Ceratitis capitata* adults of which larvae were reared in fruits of mango (*M. indica*) (wild population) and artificial diet (laboratory population) and exposed to the toxic bait Success™ 0.02CB, containing 96 mg L^{-1} of spinosad in laboratory. Arrows indicate the lethal time (LT_{50}) of the Success™ 0.02CB toxic bait in each parameter evaluated.

Exposure time of toxic bait to adults of ***C. capitata***

Insects that were exposed to toxic bait formulation for 2, 4, or 8 h presented 100% mortality and a higher susceptibility ($F_{3,38} = 21.14$; $P < 0.001$) to toxic bait when compared to exposed adults of *C. capitata* for 1 h (76% mortality) (Table 1). However, due to the overlap of confidence intervals estimated from survival curves, insects exposed for 8 h (LT_{50} (CI 95%) = 14.89 (12.46–17.33)) and 4 h (LT_{50} (CI 95%) = 16.85 (14.37–19.32)) to toxic baits presented the lowest values of LT_{50} (Table 1, Fig 3), differing from adults of *C. capitata* who were exposed to toxic baits for 1 h (LT_{50} (CI 95%) = 23.90 (18.98–28.82)) and 2 h (LT_{50} (CI 95%) = 24.01 (20.16–27.86)) (Table 1). Therefore, insects exposed to toxic bait feeding for 4 h (4.45 mg) and 8 h (4.65 mg) presented a higher consumption ($F_{3,38} = 20.59$; $P < 0.0001$) when compared to insects exposed for 1 h (2.28 mg) and 2 h (2.90 mg) (Table 1).

Influence of age on toxicity of ***C. capitata*** *to toxic bait*

In evaluations of up to 96 HAE, it was observed that adults of *C. capitata* that were 1 day old had the lowest susceptibility ($F_{3,39} = 14.08$; $P < 0.001$) to the formulation of the toxic bait (33% mortality) (Table 1). However, the highest adult mortality of *C. capitata* occurred with 5-day-old insects (89% mortality) and was statistically higher ($F_{3,39} = 14.08$; $P < 0.001$) than 8-day-old insects (67% mortality) (Table 1). When analyzing survival curves and estimates of lethal times, it was found that 8-day-old adults of *C. capitata* responded similarly (LT_{50} (CI 95%) = 15.51 (12.95–18.07)) to adults of *C. capitata* at 5 days old (LT_{50} (CI 95%) = 35.51 (31.14–38.88)), but less rapidly compared to 1-day-old insects (LT_{50} (CI 95%) = 45.56 (36.18–54.94)) (Table 1; Fig 4). However, 8-day-old insects had a higher consumption of toxic bait (2.00 mg), being significantly higher ($F_{2,28} = 8.34$, $P < 0.001$) when compared to 5-day-old insects (1.57 mg) and 1-day-old insects (0.93 mg) (Table 1).

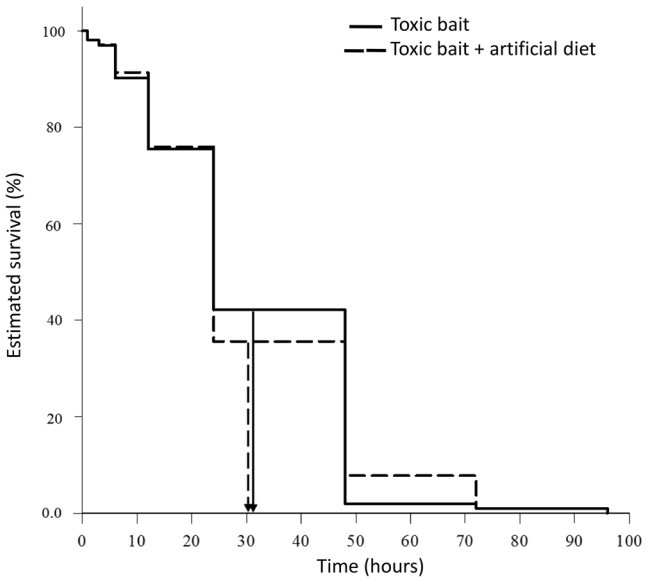

Fig 2 Survival of *Ceratitis capitata* adults after exposure to the toxic bait Success™ 0.02CB, containing 96 mg L^{-1} of spinosad alone or with artificial diet in laboratory. Arrows indicate the lethal time (LT_{50}) of the Success™ 0.02CB toxic bait in each parameter evaluated.

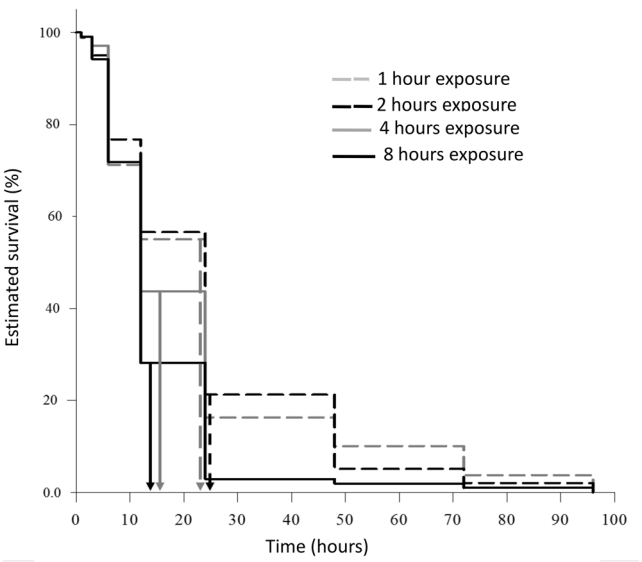

Fig 3 Survival of *Ceratitis capitata* adults after exposure to the toxic bait Success™ 0.02CB, containing 96 mg L^{-1} of spinosad offered for 1, 2, 4, and 8 h in laboratory. Arrows indicate the lethal time (LT_{50}) of the Success™ 0.02CB toxic bait in each parameter evaluated.

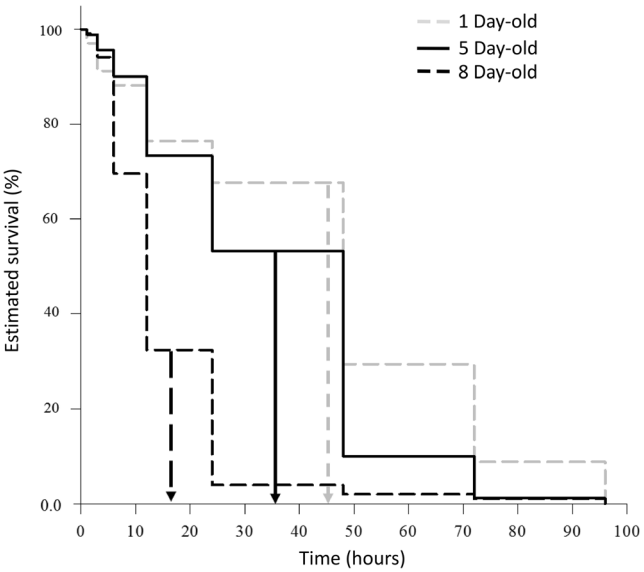

Fig 4 Survival of *Ceratitis capitata* adults exposed to the toxic bait Success™ 0.02CB, containing 96 mg L^{-1} of spinosad in laboratory. Arrows indicate the lethal time (LT_{50}) of the Success™ 0.02CB toxic bait in each parameter evaluated.

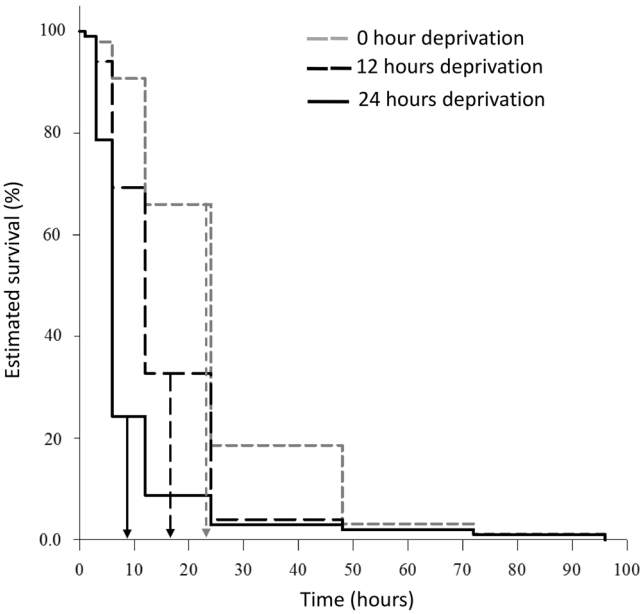

Fig 5 Survival of *Ceratitis capitata* adults submitted to 0-h, 12-h, and 24-h deprivation periods and exposed to the toxic bait Success™ 0.02CB, containing 96 mg L^{-1} of spinosad in laboratory. Arrows indicate the lethal time (LT_{50}) of the Success™ 0.02CB toxic bait in each parameter evaluated.

*Period of food deprivation before exposure **C. capitata** to toxic bait*

There were no significant differences ($F_{2,28} = 9.12$; $P > 0.05$) in mortalities of *C. capitata* adults in the presence or absence of food deprivation (artificial diet) up to 96 HAE (100% mortality) (Table 1). However, when analyzing survival curves and LT_{50} values, it was observed that insects that were deprived of food for 24 h showed a greater susceptibility to the toxic bait formulation in time (LT_{50} (CI 95%) = 9.24 (6.83–13.65) hours) when compared to insects that experienced 12 h (LT_{50} (CI 95%) = 15.55 (12.96–18.13) hours) and 0 h of food deprivation (LT_{50} (CI 95%) = 24.72 (21.51–27.94) hours) (Table 1; Fig 5). In relation to the consumption of toxic bait, insects that were deprived for 12 h (4.64 mg) and 24 h (5.33 mg) of the food source (artificial diet) had a higher ($F_{2,28} = 34.41$, $P < 0.0001$) toxic bait consumption when compared to insects that were not deprived (0-h deprivation) of the food source (2.28 mg) (Table 1).

Discussion

The development of an adequate bioassay methodology with toxic baits is fundamental to obtain satisfactory and reliable results for the design of management strategies. In the present study, when analyzing the parameters of origin of the insect population, period of food deprivation, time, and supply of the toxic bait together with another food source (artificial diet), a difference was found in the behavioral response of the individuals, due to the need to ingest nutritional foods for the maintenance of the energy reserves and maturation of the reproductive apparatus of the females (Bateman and Morton 1981, Raga and Sato 2016). In all bioassays performed, adults of *C. capitata* were fed the toxic bait drops (40 µL) of the Success™ 0.02CB formulation, containing 96 mg L^{-1} of spinosad. This aspect is very important for the evaluation of the toxicity of formulations of toxic baits, as one of the main factors influencing the efficiency of the formulations is the phage-stimulating effect of the supplied material, which provides a greater or lesser attractiveness (Nestel *et al* 2004).

Adults of *C. capitata* from larvae developed on artificial diet showed greater susceptibility to the Success™ 0.02CB toxic bait compared to insects from larvae developed in mango fruits, even though they had the same bait consumption. These factors may be related to the activation or inhibition of detoxifying enzymes induced by allelochemicals present in the food source (Fitt 1986). Thus, the insects that developed during the larval phase in mango fruits may have consumed chemical compounds that are present and that, in turn, provided less susceptibility in relation to those insects that developed in artificial diet. Although there are no records of populations of *C. capitata* adults resistant to spinosyn-based insecticides in Brazil, the fact of adults who emerged from field collected in mango orchards in the Casa Nova, BA municipality, larvae may be an indication of changes in the allelic frequency of resistance genes of the population (Couso-Ferrer *et al* 2011). The fact for this possibility is related to the high population level of *C. capitata* in the region and

the Success™ 0.02CB toxic bait is used in a large scale for *C. capitata* management (Baronio *et al* 2018).

The survival curves obtained for the treatments (with and without the presence of a food source along with the toxic bait) verified that the addition of a food source to the toxic bait exposure did not provide less susceptibility to the population. However, in the absence of the food source, the adults of *C. capitata* presented a higher intake of the toxic bait drop. For an ingestion experiment aimed at the evaluation of formulations of toxic baits, the longer the insect remains feeding and the greater the amount of food intake consumed, the greater the possibility of intoxication by the lethal agent and, consequently, the faster the effect of the product on the insect (Raga & Galdino 2018). Thus, the supply of another food source along with the toxic bait can reduce the consumption of the toxic bait and, consequently, the amount of lethal agent ingested by the insect (Nestel *et al* 2004, Raga & Galdino 2018). This fact may underestimate the lethal dose and the lethal time of the food attraction evaluated, especially if the insecticide does not present a knock-down effect, such as spinosad. However, previous studies have shown that supply of a food source to insects during the bioassay period is necessary, once adults are exposed to only toxic bait or water insect mortality can be high, leading to erroneous conclusions in relation to the toxicity of the toxic bait formulation (Medina *et al* 2007).

From 12 h of food deprivation, regardless of the supply period of the toxic bait, ranging from 1 to 8 h, there is a difference in the individuals' response to lethal time and adult survival. This fact may be associated with the fasting period of insects that induced *C. capitata* adults to ingest the toxic bait soon after the supply, resulting in a faster death. However, toxic baits offered during a longer time frame (more than 2 h) resulted in higher food consumption by the insects, and consequently, a greater intoxication. This fact demonstrates that consumption of greater than 2.90 mg of the toxic bait Success™ 0.02 CB was sufficient to cause significant mortality of the group composed of 10 adult insects of *C. capitata*.

The higher consumption of toxic bait by insects that were deprived of food for 12 and 24 h is explained by the compensation reaction caused by the time in which the insects remained without feeding, leading them to increase the consumption of the toxic bait after the fast (Raga & Sato 2005, Medina *et al* 2007). Females of *Ceratitis cosyra* (Walker) (Diptera: Tephritidae), fertilized and unfertilized, and deprived of food for 12 h, were more attracted by odors derived from foods such as chicken feces, beer yeast, guava juice, and cane molasses sugar when compared to flies that previously fed on a sugar and protein diet (Manrakhan & Lux 2008). However, prolonged periods of deprivation (greater than 12 h) increased the mortality of the insects of the control treatment, a fact observed in the present study, probably due to the reduction of the energetic reserves (Vargas *et al* 2002). Warburg & Yuval (1997) observed increased intake of sugars and proteins by *C. capitata* after periods of 24-h food deprivation. Likewise, Yee & Alston (2016) found that adults of *Rhagoletis indifferens* Curran (Diptera: Tephritidae) who received spinosad without food supplementation (sugar and yeast extract) showed a greater response to a toxic bait composed of sugar and spinosad, presenting faster mortality compared to those who had access to the diet.

Another factor that leads to a higher intake of toxic bait composed of a mixture of an alimentary attractant and a lethal agent is conditioned by the physiological stage of the adults, which is directly associated with the age of the insects (Kapsi *et al* 2002). These factors influence the search for food, copula, and oviposition sites in the field required for the reproductive success of the species (Rull & Prokopy 2000). In the case of *C. capitata*, the highest consumption of toxic bait occurred at the ages of 5 and 8 days after emergence, being associated with a greater need to obtain energy and nutrients for the development of the ovaries during the initial phase or for energy replacement (Silva-Neto *et al* 2010). In addition, the intake of higher amounts of protein during the adult phase of *C. capitata* prolongs its life cycle by 25 days, allowing an increase in the period in which adults can move to different areas, increasing the amount of damaged fruits (Kouloussis *et al* 2017).

Based on these results, it was evidenced that the origin of the population of *C. capitata* (wild or from laboratory), the form and the time of offer of the toxic bait Success™ 0.02CB, the age, and the period of food deprivation of adults of *C. capitata* directly influenced the toxicity of the toxic bait formulation. Thus, along with the evaluation of the parameters that may interfere negatively in the execution of the bioassays, the definition of a suitable methodology for the evaluation of toxic baits will aid in refining the data and designing bioassays to study new formulations. With adults of *C. capitata* as the experimental model, it was evidenced that adults 5 days old (after emergence), obtained from laboratory rearing on an artificial diet, deprived of food for 12 h, and exposed to toxic bait for 4 h, are suitable for the definition and installation of laboratory bioassays for the development of results that are reliable and replicable.

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