Original article

Effect of toxic baits used for fruit fly control against *Trichogramma pretiosum* and *Chrysoperla externa*

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Summary

Introduction – **Toxic baits are an important tool in fruit flies management; however, some beneficial insects such as natural enemies can be impaired by this technique. In this study, the toxicity of some selected baits on *Trichogramma pretiosum* and *Chrysoperla externa*, which are important as biological control agents of fruit pests, was evaluated.** *Materials and methods* – **Bioassays were conducted under laboratory conditions (temperature of 25±2 °C, relative humidity 70±10% and 14 h day length) and consisted on the exposure of adults to a fine film of bait (drops with ~4 mm of diameter). Based on either, reduction of the parasitism capacity for *T. pretiosum* or mortality for *C. externa,* the baits were grouped in the IOBC toxicity categories.** *Results and discussion* – **The malathion-based baits composed by the attractants sugarcane molasses (7%), Biofruit® (5%) and ANAMED® are harmful to both species based on reduction of >96% in the parasitism capacity of *T. pretiosum* and 100% mortality for *C. externa*. Toxic baits composed by the aforementioned attractants with the insecticide spinosad added, and the commercial bait Success® 0.02 CB are harmful to *T. pretiosum*, with reductions on parasitism capacity higher than 87%. However, these baits are relatively selective to *C. externa* adults, with mortality ranging from 23 to 60%.** *Conclusion* – **Based on the obtained results, toxic baits with spinosad are more selective to predators and considered a more ecofriendly option for integrated fruit flies management.**

Keywords
Brazil, fruit flies management, biological pest control, food attractants

Significance of this study

What is already known on this subject?

- The use of toxic baits is an efficient option for fruit flies management; however, non-target arthropods such as natural enemies can be negatively affected by this technique.

What are the new findings?

- The parasitism capacity of *Trichogramma pretiosum* is strongly reduced when in touch with toxic baits, regardless of insecticide or attractant used. Toxic baits with spinosad are relatively less toxic to the predator *Chrysoperla externa* compared with malathion toxic baits.

What is the expected impact on horticulture?

- The use of safer toxic bait formulations can help in preservation of these two natural enemies in orchards and consequently contribute for development of integrated pest management program.

Introduction

The fruit flies (Diptera: Tephritidae) are the main pests of fruit crops in Brazil. These insects reduce the quality of fruit by direct and indirect damages (Nascimento and Carvalho, 2000). In southern Brazil, the main fruit fly species that occurs in orchards is *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae) which can damage stone fruits, apples, grapevines, citrus and small fruits (Nava and Botton, 2010).

Due to the high damage potential of *A. fraterculus,* the use of broad spectrum insecticides to control this species is still a common practice among fruit growers in Brazil (Harter *et al*., 2010). However, a more ecofriendly alternative for management of this species is the use of toxic baits, where insecticides are mixed with food baits and applied in a lower volume in specific sites in the orchard, thereby minimizing negative impacts such as resurgence of pests, outbreak of secondary pests, mortality of beneficial arthropods, environmental contamination and presence of insecticide residues in the fruits (Harter *et al*., 2010, 2015).

Despite being considered an environmentally less intrusive option due to the lower volume of insecticide sprayed, the toxic baits can attract and negatively affect beneficial insects such as natural enemies, impairing the natural biological control of other pests species present in orchards (Botton *et al*., 2014). The egg parasitoid *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidae) exerts parasitism on lepidopteran pests that occur on fruit crops such as the apple leafroller *Bonagota salubricola* (Meyrick,

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1937) (Lepidoptera: Tortricidae) (Pastori *et al*., 2008), the oriental peach moth *Grapholita molesta* (Busck, 1916) (Lepidoptera: Tortricidae) (Rodrigues *et al*., 2011) and the citrus fruit borer *Gymnandrosoma aurantianum* Lima, 1927 (Lepidoptera: Tortricidae) (Molina and Parra, 2006). The predator *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) plays an important role in the control of the European red mite *Panonychus ulmi* (Koch, 1836) (Acari: Tetranychidae), the twospotted spider mite *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae), the white scale *Pseudaulacaspis pentagona* (Targioni-Tozzetti, 1885) (Hemiptera: Diaspididae), the aphid *Brachycaudus persicae* (Passerini, 1860) (Hemiptera: Aphididae), and small larvae and eggs of Lepidoptera (Freitas, 2002; Nava *et al*., 2014). These two species are important biological control agents in fruit crops and their preservation in agroecosystem should be encouraged. Furthermore, the easy mass rearing of these two species makes them good candidates to be used in applied biological control programs in fruit crops (Freitas, 2002; Parra, 1997).

Studies that evaluate the effect of toxic baits against natural enemies in laboratory are important to enable the compatibility of this technique with biological control in fruit fly management programs, since it can provide information to predict a possible negative impact on these beneficial arthropods in field conditions**.** These studies are targeted almost exclusively to tephritid fruit fly parasitoids (Ruiz *et al*., 2008; Wang *et al*., 2005; Zanardi, 2011), while other species of natural enemies are relegated. Thus, this research aimed to evaluate in laboratory the effect of toxic baits formulations used for fruit flies control against the egg parasitoid *T. pretiosum* and the predator *C. externa*.

Materials and methods

The insects used in the bioassays were obtained from colonies kept in laboratory under controlled conditions (temperature 25 ± 2 °C, relative humidity 70 ± 10% and 14 h day length). The parasitoid *T. pretiosum* was reared in eggs of the alternative host *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae), according to the methodology described by Parra (1997). The rearing of the predator *C. externa* followed the methodology proposed by Carvalho and Souza (2000). Eggs of the alternative prey *A. kuehniella* were offered for feeding of the larval stage while adults were fed with an artificial diet (Vogt *et al*., 2000).

The formulations of toxic baits evaluated were composed by the attractants sugar cane molasses (7%), hydrolyzed protein (Biofruit® 5%) and ANAMED® (emulsion of oils and waxes with proteins and fruit essences, preservatives and emulsifiers). Each bait was tested alone and in combination with the insecticides Malathion® CE 1000 (malathion at 2.0 g L^{-1} or kg^{-1}) and Tracer® 480 SC (spinosad, 0.096 g L^{-1} or kg^{-1}). The commercial formulation Success® 0.02 CB (hydrolyzed corn protein, invert sugar, oil, gum, potassium sorbate, ammonium acetate and the insecticide spinosad at 0.24 g L^{-1}), diluted in the ratio of 1 part of product to 1.5 parts of water (recommended label dilution rate) was also evaluated.

The toxic baits formulations were offered to adults of *T. pretiosum* and *C. externa* as droplets of approximately 4 mm in diameter (Borges *et al*., 2015), using a 5 mL plastic syringe. Ten droplets were deposited on a rectangular paper film (1 cm width and 6 cm length), which was inserted into exposure cages. The *C. externa* exposure cage was composed by a methacrylate ring with 10 cm in diameter and 3 cm high, closed by two square glass plates (12 × 12 cm) that served as bottom and cover. As for *T. pretiosum*, exposure cage was composed by a square aluminum frame (13 × 13 cm) with 1.5 cm high and 1 cm wide closed at the top and bottom with square glass plates (13 × 13 cm). After assembling the exposure cages containing the toxic baits, the natural enemies were released within them. Approximately 250 adults of *T. pretiosum* were released inside each cage, while for *C. externa* five couples were released per cage.

In the bioassays with *T. pretiosum*, cards with 1.5 cm width and 5 cm length, containing approximately 1,050 sterile eggs of *A. kuehniella* each, were offered to parasitism at 24 h (three cards), 48 h (two cards) and 96 h (one card) after exposure to the toxic baits, since the peak of parasitism occurs in the first 24 h and gradually decreases over the days (Bueno *et al*., 2010). Based on the sex ratio of 0.7 observed for *T. pretisum* on the bioassays, each exposure cage received about 175 females, resulting in approximately 36 available eggs per female. The average parasitism in the treatments (toxic baits) and control (diet consisting of 3 g gelatin, 100 mL water and 200 g honey) was determined by counting the total number of parasitized eggs and dividing it by the number of females per cage. The counting of parasitized eggs was performed using a stereomicroscope and a handheld counter, at 7 days after being offered to parasitoids, since they turn black and can be easily differentiated from non-parasitized eggs. The parasitism reduction caused by toxic baits was calculated, using the formula:

$$PR = (1 - Pt/Pc) \times 100$$

where PR is the parasitism reduction (in %), Pt is the average parasitism in toxic bait and Pc is the average parasitism in the control (Hassan and Abdelgader, 2001).

In the bioassays with *C. externa*, mortality (number of dead insects) was determined at 24 h, 48 h and 72 h after insects' exposure to toxic baits. The cumulative mortality (at 72 h) was calculated for each treatment and corrected according to the control (artificial diet) using Schneider-Orelli's formula (Püntener, 1981). The surviving adults from each treatment were collected and transferred to cages (15.5 cm height and 18.5 cm diameter) for checking possible deleterious effects on female fecundity and fertility. Four samples of eggs laid on a 24 h interval were collected. The number of eggs from each sample was measured and divided by the number of females in the cage in order to determine the fecundity (number of eggs $female^{-1}$ day^{-1}). Eggs were incubated until hatching for determining the fertility rate (percentage of hatched larvae).

Four replicates were used for each treatment. Each exposure cage was considered an experimental unit in a completely randomized design. The studies were divided in three and two bioassays for *T. pretiosum* and *C. externa*, respectively, according to the operational capacity of the laboratory and availability of insects.

The toxic bait formulations were classified for *T. pretiosum* based on parasitism reduction and for *C. externa* based on the mortality at 72 h, in the "International Organization for Biological and Integrated Control of Noxious Animals and Plants" (IOBC) toxicity categories: 1) harmless (< 30%); 2) slightly harmful (30–79%); 3) moderately harmful (80–99%), and 4) harmful (> 99%) (Hassan and Abdelgader, 2001). Additional statistical analyses were performed using the software Winstat 1.0 (Machado and Conceição, 2007). Data were subjected to analysis of variance and means were compared by Tukey test ($\alpha = 0.05$).

Results

Except for the treatment composed only by sugarcane molasses, the number of parasitized eggs per *T. pretiosum* female after exposure to toxic baits formulations was significantly lower than in their respective controls (Table 1). The toxic baits containing the insecticides malathion and spinosad (whether from Tracer® 480 SC incorporation or from the "ready to use" formulation Success® 0.02 CB) reduced in more than 87% the parasitism capacity of *T. pretiosum*. For each attractant, it wasn't observed significant difference in the number of parasitized eggs per female between the two insecticides used. The hydrolyzed protein (Biofruit®) in association with malathion and spinosad reduced in more than 96% the parasitism capacity of *T. pretiosum*, being moderately harmful to these parasitoids. The "ready to use" toxic bait formulation Success® 0.02 CB was moderately harmful

Table 1. Mean number (±SE) of eggs parasitized by *Trichogramma pretiosum*, parasitism reduction (%) and IOBC toxicity classification of different toxic bait formulations.

Treatment	Parasitized eggs/female[1]	PR[2]	C[3]
Bioassay I			
Control	22.5 ± 1.2 a	–	–
Biofruit® (5%)	7.1 ± 1.6 b	68.3	2
Biofruit® (5%) + spinosad (0.096 g L^{-1})	0.7 ± 3.9 c	98.2	3
Biofruit® (5%) + malathion (2.0 g L^{-1})	0.8 ± 1.5 c	96.4	3
Bioassay II			
Control	33.5 ± 1.0 a	–	–
Sugar cane molasses (7%)	35.3 ± 1.1 a	0.0	1
Sugar cane molasses (7%) + spinosad (0.096 g L^{-1})	0.0 ± 0.0 b	100.0	4
Sugar cane molasses (7%) + malathion (2.0 g L^{-1})	0.0 ± 0.0 b	100.0	4
Bioassay III			
Control	31.4 ± 1.2 a	–	–
ANAMED®	10.6 ± 1.0 b	66.4	2
ANAMED® + spinosad (0.096 g kg^{-1})	0.3 ± 0.2 d	99.2	4
ANAMED® + malathion (2.0 g kg^{-1})	0.3 ± 0.1 d	99.2	4
Success® 0.02 CB (spinosad 0.096 g L^{-1})	4.1 ± 0.9 c	87.1	3

[1]Means followed by the same letter in the column do not significantly differ by Tukey test ($p \leq 0.05$). Bioassay I: F = 63.79, df = 3, $p < 0.0001$; Bioassay II: F = 40.74, df = 3, $p < 0.0001$; Bioassay III: F = 53.01, df = 4, $p < 0.0001$.
[2]PR = Parasitism reduction compared to the control (%).
[3]C = Toxicity classes from IOBC: 1 = harmless (<30%), 2 = slightly harmful (30–79%), 3 = moderately harmful (80–99%), 4 = harmful (>99%).

Table 2. Cumulative mortality (no. ± S.E), final mortality (%) and IOBC classification when adults of *Chrysoperla externa* were exposed to toxic bait formulations.

Treatment	24 h[1]	48 h[1]	72 h[1]	M (%)[2]	C[3]
Bioassay I					
Control	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 d	–	–
Success® 0.02 CB (spinosad 0.096 g L^{-1})	0.5 ± 0.3 c	2.5 ± 0.3 b	6.0 ± 0.7 b	60.0	2
Biofruit® (5%)	0.3 ± 0.3 c	0.5 ± 0.3 c	5.0 ± 0.6 bc	50.0	2
Biofruit® (5%) + spinosad (0.096 g L^{-1})	1.5 ± 0.3 b	2.3 ± 0.6 b	3.8 ± 0.5 c	37.5	2
Biofruit® (5%) + malathion (2.0 g L^{-1})	10.0 ± 0.0 a	10.0 ± 0.0 a	10.0 ± 0.0 a	100.0	4
Bioassay II					
Control	0.0 ± 0.0 c	0.3 ± 0.3 c	0.3 ± 0.3 c	–	–
Sugar cane molasses	0.5 ± 0.3 bc	1.0 ± 0.4 c	1.8 ± 0.5 c	15.4	1
Sugar cane molasses (7%) + spinosad (0.096 g L^{-1})	1.0 ± 0.0 bc	1.8 ± 0.5 c	2.5 ± 1.0 bc	23.1	1
Sugar cane molasses (7%) + malathion (2.0 g L^{-1})	10.0 ± 0.0 a	10.0 ± 0.0 a	10.0 ± 0.0 a	100.0	4
ANAMED®	0.3 ± 0.3 c	0.5 ± 0.3 c	0.8 ± 0.5 c	5.1	1
ANAMED® + spinosad (0.096 g kg^{-1})	2.5 ± 1.0 b	4.0 ± 0.9 b	5.0 ± 0.9 b	48.7	2
ANAMED® + malathion (2.0 g kg^{-1})	9.8 ± 0.3 a	9.8 ± 0.3 a	10.0 ± 0.0 a	100.0	4

[1]Results from four replicates with five couples each. Means followed by the same letter in the columns do not differ significantly by Tukey test ($p < 0.05$). Bioassay I – 24 h: F = 395.73; df = 4; $p < 0.0001$; 48 h: F = 144.56; df = 4; $p < 0.0001$; 72 h: F = 61.82; df = 4; $p < 0.0001$. Bioassay II – 24 h: F = 100.89; df = 6; $p < 0.0001$; 48 h: F = 88.75; df = 6; $p < 0.0001$; 72 h: F = 53.54; df = 6; $p < 0.0001$.
[2]M (%) = Final mortality corrected by Schneider-Orelli.
[3]C = Toxicity classes from IOBC: 1 = harmless (<30%), 2 = slightly harmful (30–79%), 3 = moderately harmful (80–99%), 4 = harmful (>99%).

to the parasitoid likewise. With respect to the toxic baits composed by sugarcane molasses + malathion and spinosad, those combinations fully reduced the parasitism capacity, being considered harmful to *T. pretiosum*. The same effect was observed for the combinations of ANAMED® with both insecticides, with similar reduction in the parasitism capacity of *T. pretiosum*. Taking into account the attractants only, without mixing with insecticides, the sugarcane molasses diluted at 7%, was the only one harmless to the parasitoid *T. pretiosum*, allowing a parasitism rate equivalent to the control. The attractants Biofruit® and ANAMED® were slightly harmful to the parasitoid.

Toxic bait formulations containing malathion were harmful to adults of the predator *C. externa*, regardless of the attractant used, with no record of survivors 72 h after exposure (Table 2). Formulations combined with the insecticide spinosad were relatively less toxic to adults of *C. externa* when compared to malathion toxic baits. The toxic bait containing sugarcane molasses + spinosad was harmless, with less than 30% of mortality on adults, while Biofruit® + spinosad and ANAMED® + spinosad were slightly harmful. As for Success® 0.02 CB, that contains spinosad on its original formulation, it was also classified as slightly harmful with 60% of mortality. The treatments composed only by the attractant sugarcane molasses (7%) and ANAMED® showed no deleterious effect on the survival of *C. externa* adults and were considered harmless, while the attractant Biofruit® was slightly harmful since it caused 50% mortality.

In the reproductive performance assessment of *C. externa* survivor adults, it was verified detrimental effect only on the fecundity of females exposed to ANAMED® + spinosad, where the number of eggs female^{-1} day^{-1} was significantly lower than the control (Figure 1A). Fecundity was not negatively affected by the other toxic bait formulations evaluated. Fertility didn't differ significantly from the control in all formulations evaluated (Figure 1B).

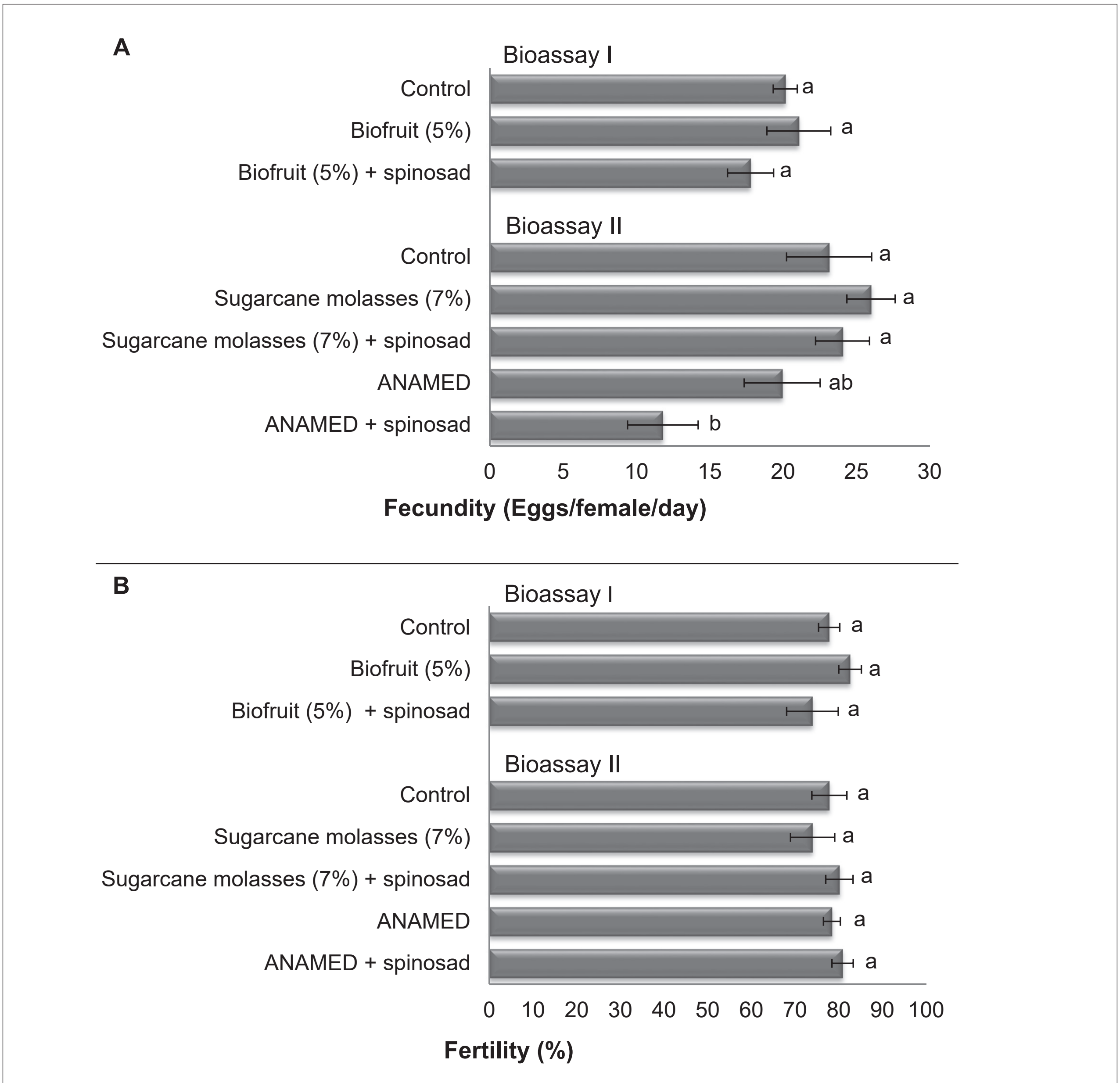

Figure 1. Fecundity (A) and fertility (B) of *Chrysoperla externa* after exposure to toxic baits in adult stage. Means followed by the same letter do not significantly differ by the Tukey test ($p < 0.05$). Fecundity – Bioassay I: F = 1.09; df = 2; $p = 0.3941$; Bioassay II: F = 5.47; df = 4; $p = 0.0072$. Fertility – Bioassay I: F = 1.17; df = 2; $p = 0.3717$; Bioassay II: F = 0.58; df = 4; $p = 0.6792$.

Discussion

Organophosphate insecticides are usually noxious to natural enemies and pollinators (Manrakhan *et al*., 2013). The high susceptibility of *T. pretiosum* and *C. externa* adults to organophosphate insecticide malathion was recorded in previous studies (Manzoni *et al*., 2006; Castilhos *et al*., 2011) and again in our study when used in toxic baits. Zanardi (2011) evaluated the effect of different toxic baits on the fruit fly parasitoid *Diachasmimorpha longicaudata* (Ashmead, 1905) (Hymenoptera: Braconidae) in laboratory and found high toxicity of sugarcane molasses associated with malathion. According to the refereed author, by being more attractive, sugarcane molasses + malathion was more harmful to the parasitoid when compared to Biofruit® + malathion, fact that resembles the observed for *T. pretiosum* in our study.

Sugarcane molasses 7% (without the addition of insecticide) is an energetic compound to insects due the high carbohydrate content, and didn't reduce *T. pretiosum* parasitism capacity. This attractant also did not cause high mortality and deleterious effects on fecundity and fertility of *C. externa*. Sugarcane molasses is a suitable food source for these natural enemies, which tend to be attracted and feed on toxic baits composed with this attractant. The toxicity of sugarcane molasses + malathion for *T. pretiosum* and *C. externa*, and sugarcane molasses + spinosad for *T. pretiosum* is associated with high intake of the insecticides by the insects as consequence of the high attractiveness of molasses. However, in some field conditions, factors such as the presence of other natural food sources in the orchard can reduce the propensity of these beneficial insects to feed on toxic baits containing this attractant, making them less vulnerable (Mahat, 2009).

Sugarcane molasses is still one of the attractants most used in toxic baits by fruit growers, however, due to its high attractiveness, the use of this substance poses a risk to natural enemies and pollinators, especially when used in combination with organophosphate insecticides (Botton *et al*., 2014). An alternative to be used as an attractant in toxic baits is hydrolyzed protein. In this context, Biofruit® was developed for specific management of fruit flies, and is largely used with toxic baits in Brazil due its lower attractiveness to beneficial entomofauna. In our study, when insects were exposed to Biofruit®, without the addition of insecticide, their nutritional and energetic requirements were not supplied by this attractant, consequently resulting in deleterious effects on *T. pretiosum* parasitism capacity and *C. externa* adults. According to Medina *et al.* (2007), attractants composed by hydrolyzed protein, despite the high protein content, cannot be used as food replacement by insects because they are deficient in carbohydrates.

The attractant plays an important role in the impact of toxic baits, but the insecticide incorporated is still the main factor responsible for natural enemies' mortality. The results obtained by Michaud (2003) suggest that GF-120 (spinosad), despite its greater palatability, is generally less toxic to natural enemies that occur in citrus, in comparison with Nu-Lure® (malathion), which was not very palatable to natural enemies, but caused high mortalities due to the higher toxicity of the insecticide incorporated. The referred author verified that Nu-Lure® (malathion) caused high mortality on the coccinellids *Curinus coeruleus* (Mulsant, 1850), *Cycloneda sanguinea* (Linnaeus, 1763), *Exochomus childreni* (Mulsant, 1850) and *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae); the syrphid *Pseudodorus clavatus* (Fabricius, 1794) (Diptera: Syrphidae); the green lacewing *Chrysoperla rufilabris* (Burmeister, 1839) (Neuroptera: Chrysopidae) and the parasitoids *Aphytis melinus* (DeBach, 1959) (Hymenoptera: Aphelinidae) and *Lysiphlebus testaceipes* (Cresson, 1880) (Hymenoptera: Aphidiidae), while GF-120 (spinosad) was only toxic to the two aforementioned parasitoid species, with low mortality to predators. Similarly, in this study, the toxic bait Success® 0.02 CB (equivalent to the commercial formulation GF-120) was less harmful to the predator *C. externa* in comparison with the parasitoid *T. pretiosum*.

The insecticide malathion has been used for decades in the control and eradication of fruit flies in different countries worldwide (Urbaneja *et al*., 2009). However, due to the toxicity of this insecticide to natural enemies and pollinators, in addition to the restriction of some importing countries to residues of this active ingredient in the fruit, its use tends to gradually decrease. Therefore, the search for an alternative to replace this active ingredient is necessary (Manrakhan *et al*., 2013). Within this context, the insecticide spinosad is a viable alternative to be used in toxic bait formulations for the control of the South American fruit fly *A. fraterculus* (Harter *et al*., 2015; Borges *et al*., 2015; Raga and Sato, 2005). Spinosad is considered one of the most promising insecticides for use in combination with hydrolyzed protein in the control of the fruit fly *Ceratitis capitata* (Wiedemann, 1824) and *Ceratitis rosa* (Karsch, 1887) (Diptera: Tephritidae) (Manrakhan *et al*., 2013).

Spinosad is classified as a biopesticide, formed by a mixture of spinosyns A and D, derived from the actinomycete *Saccharopolyspora spinosa*, and has the advantages of low toxicity to mammals, moderated residual effect, and relative selectivity to predators (Thompson *et al*., 2000). The selectivity of spinosad to predators was also observed in our study. The mortality induced by this insecticide mixed with the attractants sugar cane molasses, Biofruit® and ANAMED® has not exceeded 48.7%. Among the baits containing spinosad, higher mortality of *C. externa* adults was obtained for Success® 0.02 CB, however, this formulation was still grouped as slightly harmful. As described by Mangan *et al.* (2006), the formulation Success® 0.02 CB is composed by a spray-dried enzymatically hydrolyzed protein originated from industrial processing of corn, and other additives such as feeding stimulants, adjuvants, conditioners and attractants, that went through further refinements for effectiveness improvement. According to the aforementioned authors, Success® 0.02 CB has high efficacy and persistence against tephritid flies when used at the label concentration, with reports of effectiveness up to 20 days. These properties may explain the highest mortality of this formulation on *C. externa* in comparison to the other spinosad baits evaluated.

The toxicity of toxic baits containing spinosad was moderate compared to malathion, which caused 100% mortality regardless of attractive used. Similar results for lacewings were reported by Hernández-Fuentes *et al.* (2015), who found an adverse effect of malathion mixed with hydrolyzed protein on larvae and adults of *Ceraeochrysa valida* (Banks, 1895) (Neuroptera: Chrysopidae), while the spinosad-based bait GF-120 was less harmful, with 50% of mortality on adults of that species.

The toxic baits sugarcane molasses + spinosad and ANAMED® + spinosad were harmful to *T. pretiosum*, with mortality rates equivalent to those caused by malathion. The higher susceptibility of parasitoids to baits containing spinosad may be explained by its smaller size compared to *C. externa*, and also by factors such as possible greater ingestion and differences in metabolic detoxification system (Croft and Morse, 1979). Although spinosad is considered a low-risk pesticide, adverse effects on some non-target organisms, in particular hymenopteran parasitoids, are reported to baits containing this active ingredient (Michaud, 2003; Urbaneja *et al*., 2009), corroborating with the findings in our study.

The commercial toxic bait formulation Success® 0.02 CB was developed as a "ready to use" option for the control and eradication of fruit flies, being considered a more ecofriendly option of toxic bait. As described by Mangan and Moreno (2009), GF-120 (equivalent to Success® 0.02 CB) poses certain repellence to the honey bee *Apis mellifera* (Linnaeus, 1758) (Hymenoptera: Apidae), which is extremely relevant for preservation of this important pollinator of many fruit crops worldwide. In the same way, the stingless bees *Trigona fulviventris* (Guérin, 1853) and *Scaptotrigona mexicana* (Guérin-Meneville, 1845) (Hymenoptera: Apidae) are not attracted by GF-120; nonetheless, in specific field conditions, bees may feed on this formulation indirectly when mixed with food resources as nectar and pollen (Gómez-Escobar *et al*., 2014). Despite the selectivity to pollinators, this formulation can cause a negative effect on parasitoids. According to Wang *et al.* (2005) the fruit fly parasitoids *Fopius arisanus* (Sonan, 1932), *Diachasmimorpha tryoni* (Cameron, 1911) and *Pysttalia fletcheri* (Silvestri, 1916) (Hymenoptera: Braconidae), as well as the aphid parasitoid *Aphidius transcaspicus* (Telenga, 1958) (Hymenoptera: Aphidiidae) were susceptible to toxic bait formulation GF-120 in laboratory bioassays. Negative effects of GF-120 in the survival and reproduction of the parasitoid *D. longicaudata* were also observed by Ruiz *et al.* (2008) in laboratorial studies. Similarly, Success® 0.02 CB caused adverse effect on *T. pretiosum* in our study. Thus, the use of this formulation should be cautious in situations where parasitoids are released or occur naturally in commercial orchards.

The attractant ANAMED® was released in 2012 in the Brazilian market for South American fruit fly control and is based on the SPLAT® technology (Specialized Pheromone and Lure Application Technology) (Botton *et al*., 2014). Due to its pasty formulation, ANAMED® shows higher resistance to the rainfall and ultraviolet degradation, resulting in an increased useful time (Borges *et al*., 2015). The greater persistence of ANAMED® is useful for fruit fly management. However, the impact of these toxic baits on *T. pretiosum* and *C. externa* populations can be intensified, especially when combined with malathion. As stated by Harter *et al.* (2015), the persistence of toxic baits with malathion against the fruit fly *A. fraterculus* in field conditions can reach 10 days, but can be shortened with rain incidence. For this reason, the combination of this insecticide with the rainfall-resistant attractant ANAMED® can subject natural enemies' populations to a wide period of exposure.

The deleterious effect on fecundity of *C. externa* females caused by ANAMED® + spinosad can impair population growth of this predator and decrease its biological control effectiveness in orchards. Sublethal effects on fecundity, fertility, longevity, development, mobility, feeding and oviposition behavior must be taken into consideration as they may compromise an Integrated Pest Management Program (Desneux *et al*., 2007).

Toxic baits are sprayed usually in alternate rows inside and/or on the edge of the orchard, and in some cases in the adjacent native woods (Nava and Botton, 2010). In this case, susceptible natural enemies that migrate from external areas may be adversely affected when entering the orchard.

Because field tests are expensive and require more time and labor, the conduction of preliminary laboratorial tests to evaluate toxic baits toxicity on beneficial insects is highly recommended (Medina *et al*., 2007). The bioassays with both natural enemies in our study were carried using an adaptation of IOBC methodologies established for testing pesticides, which recommends laboratory, semi-field and field steps. Results obtained in laboratory should be extrapolated with caution to field, since factors as increased degradation, possibility of escape and alternative food sources for natural enemies can mitigate the effect of toxic baits in the orchards. Therefore, the conduction of field trials is necessary for more information about the impact of toxic baits on *T. pretiosum* and *C. externa*.

Conclusions

The results obtained in this study show that toxic baits with the attractants sugar cane molasses (7%), Biofruit® (5%), and ANAMED® containing the insecticide malathion are highly toxic to *T. pretiosum* and *C. externa*, while the same aforementioned attractants mixed with spinosad, and the formulation Success® 0.02 CB are relatively selective to *C. externa*. Thus, the use of toxic bait formulations containing spinosad is a more ecofriendly option for integrated fruit flies management.

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