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Lethal and sublethal effects of toxic bait formulations on *Doryctobracon areolatus* (Hymenoptera: Braconidae) and implications for integrated fruit fly management

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The use of toxic baits has become one of the main methods of management of fruit flies in Brazil. The application of toxic baits may cause side effects on the native parasitoid *Doryctobracon areolatus* (Hymenoptera: Braconidae). Based on the results, formulations made from the food attractants 3% Biofruit, 1.5% Ceratrap, 1.25% Flyral, 3% Isca Samaritá, 3% Isca Samaritá Tradicional, and 7% sugarcane molasses associated with the Malathion 1000 EC and the ready-to-use toxic bait Gelsura (containing the active ingredient alpha-cypermethrin) were classified as harmful (class 4) to *D. areolatus* (mortality > 85% at 96 HAE). In contrast, for toxic baits formulated with insecticide phosmet, the mortality ranged from 38% to 72%, classified as slightly harmful or moderately harmful. However, when phosmet was added to the 3% Samaritá Tradicional bait, the mortality was only 3.9% (class 1—harmless), similar to the toxicity observed for the Success 0.02 CB ready-to-use bait (0.24 g a.i. spinosad/l) (<5% mortality). Although toxic baits were formulated with spinosyn-based insecticides, all toxic bait formulations were classified as harmless or slightly harmful (<50% mortality) to *D. areolatus*, with the exception of 1.5% Ceratrap + spinetoram and 7% Sugarcane molasses + spinosad (≈ 60% mortality—moderately harmful). In addition, these formulations did not show sublethal effects in reducing the parasitism and emergence rate of the F_1 generation of *D. areolatus* in *A. fraterculus* larvae. The results serve as a basis for the correct use of toxic food baits without affecting the biological control.

Key words: selectivity, chemical control, fruit fly, native parasitoid

Introduction

In Brazil, the use of toxic food baits has become one of the main methods of controlling *Anastrepha fraterculus* (Wiedemann) and *Ceratitis capitata* (Wiedemann), (Diptera: Tephritidae), which are considered the main pests associated with local fruit production (Urbaneja et al. 2009, Nava and Botton 2010). Toxic bait a food attractant mixed a lethal agent (insecticide) (Botton et al. 2016, Baronio et al. 2019, Nunes et al. 2020).

Food attractants are usually composed of sugars or hydrolyzed protein since fruit fly adults, especially females, need to ingest protein and energy foods during the postemergence period to reach sexual maturation (Heath et al. 1994). In the field, toxic bait formulations are applied at specific locations within or outside orchards with the aim of forming a protective barrier between the crop and the surrounding landscape, where fruit fly infestations usually originate (Baronio et al. 2019).

In Brazil, organophosphate insecticides are predominantly used in the formulation of toxic baits (Harter et al. 2015, Raga and Galdino 2018). Oganophosphates have rapid action and high toxicity on fruit fly adults after ingestion (Casida and Quistad 1998, Vayssieres et al. 2009, Raga et al. 2018). However, in recent years, new insecticides (lethal agents), especially spinosyn-based insecticides (Schutze et al. 2018) and ready-to-use formulations of toxic baits, such as Gelsura and Success (Botton et al. 2016), have been made available.

Table 1. Food attractants used in Brazil for formulations of toxic baits to control fruit flies

Food attractant	Description	Tested concentration (%)[a]	Origin
Anamed	Contains fruit plant extracts and phagostimulants	SPLAT 40% + food attractant 24.2%; undiluted	Isca Tecnologias Ltda., Ijuí, RS, Brazil
Biofruit	Hydrolyzed corn protein	3	BioControle Métodos de Controle de Pragas Ltda., Indaiatuba, São Paulo, Brazil
CeraTrap	Enzymatic hydrolyzed protein of animal origin	1.5	BioIbérica S.A., Barcelona, Spain
Flyral	Enzymatic hydrolyzed protein of animal origin	1.25	BioIbérica S.A., Barcelona, Spain
Samaritá bait	Hydrolyzed corn protein	3	Samaritá Indústria e Comércio Ltda., Artur Nogueira, São Paulo, Brazil
Samaritá Tradicional bait	Vegetable protein, reducing sugars and preservatives	3	Samaritá Indústria e Comércio Ltda., Artur Nogueira, São Paulo, Brazil
Sugarcane molasses	By-product containing reducing sugars and uncrystallized sucrose	7	From the sugar manufacturing process by the sugarcane industries

[a]Concentration (mL) of food attractant used for the formulation of toxic baits.

Integrated pest management (IPM) is an important tool that helps to reduce fruit fly populations without requiring large-scale application of insecticides (Navarro-Llopis et al. 2012, Baronio et al. 2019, Nunes et al. 2020, Borges et al. 2021). However, it is important to verify the compatibility of the insecticide used to formulate toxic bait with other control strategies, such as biological control (Baldin et al. 2018, Bernardi et al. 2019, Farah et al. 2019, Cardoso et al. 2021). In Brazil, several species of fruit fly parasitoids have been reported to occur naturally, such as *Doryctobracon areolatus* (Szépligeti) (Hymenoptera: Braconidae), *D. brasiliensis* (Szépligeti) (Hymenoptera: Braconidae), *Opius bellus* (Gahan) (Hymenoptera: Braconidae), and *Aganaspis pelleranoi* (Brèthes) (Hymenoptera: Figitidae) (Ovruski et al. 2000, Marsaro Júnior et al. 2011, Nunes et al. 2012). However, the larval parasitoid *D. areolatus* has been shown to be the most promising natural enemy for the management of *A. fraterculus* and *C. capitata* (Marinho et al. 2009, Nunes et al. 2011, Garcia and Ricalde 2013, Murillo et al. 2015, Gonçalves et al. 2018). In addition to being a native parasitoid (Zucchi and Moraes 2008), rearing techniques for *D. areolatus* have already been developed for biological control programs (Gonçalves et al. 2018).

Despite the advantages and potential of the use of parasitoids in the management of fruit flies, few studies have been carried out to verify the selectivity of toxic baits on beneficial insects. These aspects are of paramount importance for IPM since programs of mass releases of *D. areolatus* in the field are being investigated. Therefore, the objective of this study was to determine the lethal and sublethal effects of formulations of toxic baits recommended for the management of fruit flies on the parasitoid *D. areolatus*.

Materials and Methods

Insects

Anastrepha fraterculus and *D. areolatus* adults were raised at Embrapa Clima Temperado, where they were kept in an acclimatized room (temperature: 25 ± 2°C, relative humidity: 70 ± 10%, and photophase: 12 h). *Anastrepha fraterculus* adults were raised in plastic cages (57 cm × 39 cm × 37 cm) and fed a solid diet based on sugar, wheat germ, and brewer's yeast in a proportion of 3:1:1 (Nunes et al. 2013). Eggs were collected daily and placed in aeration for 24 h (Kamiya 2010) and then placed on an artificial diet based on wheat germ. After completing the third instar, the diet was removed from the larvae, and they were deposited in cornmeal (substrate for pupation). The pupae were transferred to maintenance cages until adult emergence.

The raising of *D. areolatus* was carried out according to the methodology described by Gonçalves et al. (2018). The adults were kept in screen cages (40 cm × 27 cm × 23 cm) and fed honey. The multiplication of the parasitoids was accomplished through the daily provision of second instar larvae of *A. fraterculus* (6 days old) in an acrylic plate (4 cm in diameter × 0.2 cm in height) containing hydrated wheat fiber covered with voile tissue (1 mm grid). After 12 h of parasitism, the larvae were placed on an artificial diet based on wheat germ to complete development and then transferred to corn flour for pupation.

Toxic Bait Formulations

The food attractants evaluated were (i) Anamed, (ii) 3% Biofruit, (iii) 1.5% CeraTrap, (iv) 1.25% Flyral, (v) 3% Samaritá bait, and (vi) 3% Samaritá Tradicional bait with 7% sugarcane molasses (Table 1). For the formulation of toxic baits (treatments), the following insecticides were used: Malathion 1000 EC (malathion 1,000 g of active ingredient—g a.i./l; Cheminova Ltda., São Paulo, Brazil); Imidan 500 WP (phosmet 500 g a.i./kg; Syngenta Crop Protection, São Paulo, Brazil); Delegate 250 WG (spinetoram 250 g a.i./kg; Dow AgroSciences Industrial Ltda., São Paulo, Brazil); and Tracer 480 SC (spinosad 480 g a.i./L; Dow AgroSciences Industrial Ltda., São Paulo, Brazil) (Agrofit 2022).

The ready-to-use formulations used were (i) Success 0.02 CB (0.24 g a.i. spinosad/l; Dow AgroSciences Industrial Ltda., São Paulo, Brazil) diluted in water at a ratio of 1:1.5 volume/volume (v/v; 1 part of commercial product to 1.5 parts of water) and Gelsura (6.0 g a.i./l alpha-cypermethrin, a polymer matrix containing the active ingredient alpha-cypermethrin; BASF SA, São Paulo, Brazil) diluted 1:2 and 2:1 v/v (parts of the commercial product: parts of water). As a negative control for all toxic bait treatments, an 80% honey solution in water was used. For each treatment, blue liquid food coloring (Exberry, São Paulo, Brazil) (2 ml of dye: 100 ml of syrup) was added to confirm ingestion by visualizing the coloring of the insects' abdomen with an AxioCam MRc digital camera (both Zeiss, Sao Paulo, Brazil).

Bioassays

The bioassays were carried out at the Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas, Brazil. The lethal and sublethal toxicity in adults of *D. areolatus* was verified through tests of ingestion of toxic baits. The experimental design was completely randomized, with 10 replications per treatment. The treatments were food attractant + insecticide, only food attractant, and 80% honey solution. In each repetition, 10 pairs of *D. areolatus* 4–6 days old were used.

Lethal Effects

The experimental insects were placed inside plastic containers (500 ml) with the top cut and covered with voile fabric to allow aeration. They were deprived of food for 20 h. After this time, with the aid of a micropipette (100 µl), a drop (10 µl) of the respective treatment was offered on Parafilm paper (Bemis Company, Inc., USA). The treatments were available to the insects for ingestion for 24 h. After exposure, the treatments were removed, and an aqueous solution of 80% honey was offered. At 24, 48, 72, and 96 h after exposure (HAE), the number of live and dead insects was recorded. Insects that did not react to the touch of a fine-tipped brush were considered dead. At 24 HAE, the number of dye-labeled parasitoids (dead and alive) was counted to estimate treatment intake. The surviving adults were used to quantify potential sublethal effects.

To isolate the effect of each food attractant, the mortality caused by each formulation of toxic baits (food attractant + insecticide) was corrected with the respective food attractant using the formula of Henderson and Tilton (1955). Similarly, the mortality caused by the food attractant was corrected with the negative control (80% water and honey solution). Based on the mortality data (M) in the evaluation carried out at 96 HAE, the treatments were classified according to the criteria defined by the IOBC/WPRS: class 1 = harmless ($M < 25\%$), class 2 = slightly harmful ($25\% \leq M \leq 50\%$), class 3 = moderately harmful ($51\% \leq M \leq 75\%$), and class 4 = harmful ($M > 75\%$).

Sublethal Effects

To evaluate sublethal effects, treatments that presented mortality values lower than 50% (Classes 1 and 2) in the lethal toxicity bioassay were used. For this, adults were deprived of food for 20 h and placed inside cages (as described above). At 24 HAE, the surviving adults were fed 80% honey solution until the end of the bioassay. For 10 consecutive days, second instar larvae of *A. fraterculus* were offered (±10 larvae/female in a mixture with corn flour and wheat fiber diet) (Gonçalves et al. 2018). After 7 h of exposure, the larvae were removed and transferred to a cornmeal-based diet to complete larval development. After 4 days, the larvae were transferred to plastic containers (80 mL) containing cornmeal (1 cm) as a substrate for pupae formation. After approximately 4 days, the pupae were separated from the substrate until adult emergence in an air-conditioned room. When insects (*A. fraterculus* or *D. areolatus*) emerged, they were counted. The pupae that remained intact were dissected to check for the presence of flies or nonemerged parasitoids to determine the actual rate of parasitism.

The reduction in the capacity of parasitism (PR) and of emerged (ER) insects for each treatment was determined by comparison with the negative control (80% honey) and calculated using the formula PR or ER = $[(1 - T/C)^{*} 100]$, where T is the average parasitism or average emergence in the treatment (formulation of the toxic bait or only food attractant) and C is the average parasitism or emerged insects observed in the negative control. Based on the reductions in parasitism (%) and emergence (%) of *D. areolatus*, the treatments were classified according to the International Organization for the Integrated Biological Control of Noxious Animals and Plants IOBC (Hassan et al. 2000) as follows: (i) harmless (PR or ER < 30%); (ii) slightly harmful (30 ≤ PR or ER ≤ 79%); (iii) moderately harmful (80 ≤ PR or ER ≤ 99%), and (iv) harmful (PR or ER > 99%).

Statistical Analysis

The data were initially subjected to residual analysis to confirm the normality assumption by the Shapiro–Wilk test and homogeneity of variances of Bartlett using the PROC UNIVARIATE procedure in SAS 9.1 software (SAS Institute 2011). The survival rates of the *D. areolatus* adults that did not present a normal distribution were submitted to Box–Cox transformation before the analyses were carried out. Subsequently, all data were subjected to a 2-way analysis of variance with PROC GLM using the F test ($P < 0.05$) (SAS Institute 2011). The differences between the treatments were determined by the least squares means (PDIFF option in PROC GLM) using Tukey's adjustment at 5% significance in SAS 9.1 software. To evaluate the side effects on adults of *D. areolatus*, data on parasitism (%) and emergence (%) were evaluated for normality by the Shapiro–Wilk test and homoskedasticity by Hartley and Bartlett and then subjected to analysis of variance (ANOVA option in PROC GLM) using the F test ($P < 0.05$). When statistically significant, the means were compared by Tukey's test at the 5% significance level ($P < 0.05$) (SAS Institute 2011).

Results

Lethal effects

During the exposure time of the food attractants alone or associated with insecticides, it was verified that all the adults of *D. areolatus* fed. This fact was confirmed by the blue coloring of the abdomen of the insects after 96 HAE. Based on this result, it was evident that the food attractants Anamed, 3% Biofruit, 1.5% CeraTrap, 1.25% Flyral, and 7% sugarcane molasses showed low toxicity (mortality < 25%) to *D. areolatus* adults; these attractants were statistically similar to the control (negative control) after 96 h, being classified, according to the IOBC, as harmless (class 1) (Tables 2 and 3).

When the attractants were combined with the insecticide malathion (with the exception of Anamed + malathion), the mortality of *D. areolatus* adults was greater than 44% in the first 24, 48, and 72 HAE (Table 2). However, at 96 HAE, with the exception of the toxic bait Anamed + malathion 2.0 g a.i./l (M = 51.0%, moderately harmful), the other formulations resulted in a mortality of *D. areolatus* adults higher than 75%, classified as class 4 (harmful) (Table 2). In contrast, a lower toxicity on *D. areolatus* adults (mortality between 25% and 75%) was verified for the toxic baits formulated with the use of the insecticide phosmet (Table 2) at all evaluation times, classified according to the IOBC as slightly harmful or moderately harmful for *D. areolatus* adults (Table 2).

For the group of insecticides based on spinosyns, at 24, 48, 72, and 96 HAE, with the exception of 1.5% Ceratrap + spinetoram and 7% Sugarcane molasses + spinosad (mortality of approximately 60%), all toxic bait formulations were classified as harmless or slightly harmful over time for *D. areolatus* adults (Table 3). In addition, the attractants Anamed, 3% Biofruit, and 1.25% Flyral mixed with spinosad provided the lowest mortality rates (M < 25%), being considered harmless (class 1) to adults of *D. areolatus* at all evaluation times (Table 3). These results were similar to those for Anamed + spinetoram (M < 15%—classified as harmless) (Table 3). However, the other formulations containing 3% Biofruit + spinetoram, 1.25% Flyral + spinetoram, 3% Isca Samaritá + spinetoram, and 3% Isca Samarita Tradicional + spinetoram were considered slightly harmful (class 2) or moderately harmful (class 3), with a mortality between 25% and 60% (Table 3).

Table 2. Mean number of live insects ($N \pm$ SE) and corrected mortality (M%) of *D. areolatus* exposed to food baits with or without organophosphate insecticides and different food attractants

Treatments	24 h		48 h		72 h		96 h		IOBC/WPRS Class[c]
	N ± SE[a]	M%[b]	N ± SE	M%	N ± SE	M%	N ± SE	M%	
Anamed + malathion 2.0 g a.i./l	13.6 ± 0.47Aa*	17.6	8.0 ± 0.80Bb*	49.4	7.5 ± 0.77Bb*	51.9	7.5 ± 0.77Bb*	51.0	3
Anamed + fosmete 1.0 g a.i./kg	13.8 ± 0.41Aa*	16.7	9.3 ± 0.66Bb*	41.1	9.3 ± 0.66Bb*	40.4	9.3 ± 0.66Bb*	38.4	2
Anamed	16.5 ± 0.52Aa[ns]	14.1	15.8 ± 0.44Aa*	15.0	15.6 ± 0.54Aa*	15.2	15.1 ± 0.60Aa*	17.5	1
3% Biofruit + malathion 2.0 g a.i./l	9.1 ± 0.80Ba*	44.2	3.7 ± 0.65Cb*	75.8	3.6 ± 0.61Cb*	77.2	3.4 ± 0.60Cb*	78.5	4
3% Biofruit + phosmet 1.0 g a.i./kg	8.9 ± 0.62Ba*	45.4	8.1 ± 0.67Ba*	47.1	8.0 ± 0.63Ba*	49.4	7.8 ± 0.53Ba*	50.6	2
3% Biofruit	16.3 ± 0.55Aa[ns]	15.1	15.3 ± 0.66Aa*	17.7	15.8 ± 0.78Aa*	14.1	15.8 ± 0.78Aa*	13.7	1
1.5% CeraTrap + malathion 2.0 g a.i./l	1.8 ± 0.62Ca*	89.8	1.8 ± 0.62Ca*	88.9	1.8 ± 0.62Ca*	88.9	1.6 ± 0.49Ca*	90.1	4
1.5% CeraTrap + phosmet 1.0 g a.i./kg	7.0 ± 1.00Ba*	60.2	6.8 ± 1.04Ba*	58.0	6.6 ± 1.03Ba*	59.3	6.6 ± 1.03Ba*	59.3	3
1.5% CeraTrap	17.6 ± 0.45Aa[ns]	8.3	16.2 ± 0.86Aa[ns]	12.9	16.2 ± 0.86Aa*	12.0	16.2 ± 0.86Aa[ns]	11.5	1
1.25% Flyral + malathion 2.0 g a.i./l	2.2 ± 1.0Ca*	87.0	1.8 ± 1.05Ca*	88.6	1.6 ± 0.93Ca*	89.4	1.6 ± 0.93Ba*	89.4	4
1.25% Flyral + phosmet 1.0 g a.i./kg	9.6 ± 1.10Ba*	43.2	8.8 ± 1.08Ba*	44.3	8.8 ± 1.08Ba*	41.7	8.6 ± 0.94Ba*	43.0	2
1.25% Flyral	16.9 ± 0.84Aa[ns]	12.0	15.8 ± 1.05Aa*	15.0	15.1 ± 0.87Aa*	18.0	15.1 ± 0.87Aa*	17.5	1
3% Samaritá bait + malathion 2.0 g a.i./l	1.1 ± 0.23Ca*	93.0	0.9 ± 0.23Ca*	93.8	0.9 ± 0.23Ca*	93.7	0.9 ± 0.23Ca*	93.7	4
3% Samaritá bait + phosmet 1.0 g a.i./kg	5.4 ± 0.85Ba*	65.8	4.5 ± 0.76Ba*	69.2	4.2 ± 0.85Ba*	70.8	4.0 ± 0.93Ba*	72.0	3
3% Samaritá bait	15.8 ± 0.77Aa*	17.7	14.6 ± 0.81Aa*	21.5	14.4 ± 0.77Aa*	21.7	14.3 ± 0.80Aa*	21.9	1
3% Samaritá Tradicional bait + malathion 2.0 g a.i./l	6.7 ± 0.73Ba*	60.8	2.8 ± 0.59Bb*	82.0	2.8 ± 0.59Bb*	81.9	2.8 ± 0.59Bb*	81.9	4
3% Samaritá Tradicional bait + phosmet 1.0 g a.i./kg	15.9 ± 0.54Aa*	7.0	15.0 ± 0.44Aa*	3.8	15.0 ± 0.44Aa*	3.2	14.9 ± 0.50Aa*	3.9	1
3% Samaritá Tradicional bait	17.1 ± 0.67Aa[ns]	10.9	15.6 ± 0.87Aa*	16.1	15.5 ± 0.81Aa*	15.8	15.5 ± 0.81Aa*	15.3	1
7% Sugarcane molasses + malathion 2.0 g a.i./l	1.9 ± 0.54Ca*	87.7	1.5 ± 0.58Ca*	89.5	1.4 ± 1.4Ca*	90.2	1.1 ± 0.60Ca*	92.2	4
7% Sugarcane molasses + phosmet 1.0 g a.i./kg	6.8 ± 1.37Ba*	54.0	5.4 ± 1.40Ba*	62.2	5.4 ± 1.4Ba*	62.2	5.4 ± 1.40Ba*	61.7	3
7% Sugarcane molasses	14.8 + 0.57Aa*	22.3	14.3 ± 0.59Aa*	23.1	14.3 ± 0.59Aa*	22.3	14.1 ± 0.56Aa*	22.9	1
80% Honey–water solution (negative control)	19.2 ± 0.20 a[ns]	—	18.6 ± 0.33a[ns]	—	18.4 ± 0.30a[ns]	—	18.3 ± 0.33a[ns]	—	—

[ns]Not significant when compared to the negative control in the same column.
*Significant compared to the negative control in the same column.
[a]Average of live insects followed by the same capital letters in the column do not differ significantly from each other when compared to the toxic bait formulation with the respective food attractant and, lowercase letters in the row, do not differ significantly from each other over time by Tukey's test ($P < 0.05$).
[b]Corrected mortality of the toxic baits with the respective food attractant using the formula of Henderson and Tilton (1955).
[c]IOBC/WPRS class: Class 1 = harmless (M < 25%), Class 2 = slightly harmful (25% ≤ M ≤ 50%), Class 3 = moderately harmful (51% ≤ M ≤ 75%), and Class 4 = harmful (M > 75%) 96 h after ingestion.

Table 3. Mean number of live insects (N ± SE) and corrected mortality (M%) of *D. areolatus* exposed to food baits with or without spinosyn insecticides and different food attractants

Treatments	24 h		48 h		72 h		96 h		
	N ± SE[a]	M%[b]	N ± SE	M%	N ± SE	M%	N ± SE	M%	IOBC/WPRS Class[c]
Anamed + spinosad 0.096 g a.i./l	13.6 ± 0.77Aa[*]	18.1	12.2 ± 0.96Aa[*]	22.3	12.2 ± 0.96Ba[*]	21.8	11.7 ± 0.89Ba[*]	23.0	1
Anamed + spinetoram 0.075 g a.i./kg	14.7 ± 0.55Aa[*]	11.4	13.5 ± 0.61Aa[*]	14.6	13.2 ± 0.57ABa[*]	15.4	13.2 ± 0.57ABa[*]	13.2	1
Anamed	16.6 ± 0.52Aa[ns]	10.3	15.8 ± 0.48Aa[*]	12.2	15.6 ± 0.58Aa[ns]	8.2	15.2 ± 0.55Aa[ns]	9.5	1
3% Biofruit + spinosad 0.096 g a.i./l	14.6 ± 0.73Aa[*]	11.0	12.8 ± 0.80Aab[*]	17.4	12.2 ± 0.62ABb[*]	18.7	12.0 ± 0.66ABb[*]	20.0	1
3% Biofruit + spinetoram 0.075 g a.i./kg	13.3 ± 0.89Aa[*]	18.9	11.3 ± 1.09Aa[*]	27.1	11.0 ± 1.09Ba[*]	26.7	10.6 ± 1.06Ba[*]	29.3	2
3% Biofruit	14.4 ± 0.58Aa[*]	0.0	14.4 ± 0.71Aa[*]	11.1	14.4 ± 0.81Aa[*]	15.3	14.4 ± 0.81Aa[ns]	16.7	1
1.5% CeraTrap + spinosad 0.096 g a.i./l	10.8 ± 0.53Ba[*]	39.0	9.4 ± 0.6Ba[*]	42.7	9.4 ± 0.60Ba[*]	42.3	8.6 ± 0.66Ba[*]	47.2	2
1.5% CeraTrap + spinetoram 0.075 g a.i./kg	6.7 ± 1.00Ca[*]	62.1	6.0 ± 0.84Ca[*]	63.4	5.9 ± 0.82Ca[*]	63.8	5.8 ± 0.86Ca[*]	64.4	3
1.5% CeraTrap	17.7 ± 0.42Aa[ns]	4.3	16.4 ± 0.83Aa[ns]	8.9	16.3 ± 0.81Aa[ns]	4.1	16.3 ± 0.81Aa[ns]	3.0	1
1.25% Flyral + spinosad 0.096 g a.i./l	15.0 ± 0.80Aa[*]	12.3	14.2 ± 0.86ABa[*]	11.2	14.0 ± 0.89ABa[*]	7.9	14.0 ± 0.89ABa[ns]	7.9	1
1.25% Flyral + spinetoram 0.075 g a.i./kg	12.9 ± 1.40Ba[*]	24.6	11.2 ± 1.28Ba[*]	30.0	11.2 ± 1.28Ba[*]	26.3	11.2 ± 1.28Ba[*]	26.3	2
1.25% Flyral	17.1 ± 0.84Aa[ns]	7.6	16.0 ± 1.03Aab[ns]	11.1	15.2 ± 0.86Ab[ns]	10.6	15.2 ± 0.86Ab[ns]	9.5	1
3% Samaritá bait + spinosad 0.096 g a.i./l	10.3 ± 0.36Ba[*]	35.6	8.7 ± 0.42Bab[*]	42.0	8.4 ± 0.42Bb[*]	43.2	8.3 ± 0.42B[*]	43.1	2
3% Samaritá bait + spinetoram 0.075 g a.i./kg	8.8 ± 0.69Ba[*]	45.0	7.5 ± 0.65Ba[*]	50.0	7.4 ± 0.66Ba[*]	50.0	7.3 ± 0.68Ba[*]	50.0	2
3% Samaritá bait	16.0 ± 0.64Aa[ns]	13.5	15.0 ± 0.74Aa[*]	16.7	14.8 ± 0.71Aa[ns]	13.0	14.6 ± 0.73Aa[ns]	13.1	1
3% Samaritá Tradicional bait + spinosad 0.096 g a.i./l	14.3 ± 0.80Ba[*]	16.9	11.5 ± 1.07Bb[*]	27.7	11.4 ± 1.04Bb[*]	27.9	11.4 ± 1.04Bb[*]	27.8	2
3% Samaritá Tradicional bait + spinetoram 0.075 g a.i./kg	13.3 ± 0.74Ba[*]	22.7	11.9 ± 0.84Bab[*]	25.2	11.7 ± 0.70Bb[*]	25.9	11.4 ± 0.62Bb[*]	27.8	2
3% Samaritá Tradicional bait	17.2 ± 0.64Aa[ns]	7.0	15.9 ± 0.76Aa[*]	11.7	15.8 ± 0.71Aa[ns]	7.1	15.8 ± 0.71Aa[ns]	5.9	1
7% Sugarcane molasses + spinosad 0.096 g a.i./l	7.8 ± 0.62 Ca[*]	48.0	7.6 ± 0.93Ba[*]	47.9	7.0 ± 0.95Ba[*]	52.0	6.8 ± 0.85Ba[*]	52.8	3
7% Sugarcane molasses + spinetoram 0.075 g a.i./kg	9.8 ± 0.81Ba[*]	34.7	4.0 ± 0.57Cb[*]	72.6	3.7 ± 0.59Cb[*]	74.7	3.6 ± 0.61Cb[*]	75.0	3
7% Sugarcane molasses	15.0 ± 0.61Aa[*]	18.9	14.6 ± 0.61Aa[*]	18.9	14.6 ± 0.61Aa[ns]	14.1	14.4 ± 0.60Aa[ns]	14.3	1
80% Honey–water solution (negative control)	18.5 ± 0.40a[ns]	—	18.0 ± 0.47a[ns]	—	17.0 ± 0.55a[ns]	—	16.8 ± 0.51a[ns]	—	—

[ns]Not significant when compared to the negative control in the same column.
[*]Significant compared to the negative control in the same column.
[a]Average of live insects followed by the same capital letters in the column do not differ significantly from each other when compared to the toxic bait formulation with the respective food attractant and, lower case letters in the line, do not differ significantly from each other over time by the Tukey test ($P < 0.05$).
[b]Corrected mortality of the toxic baits with the respective food attractant and from this with the negative control (80% honey) using the formula of Henderson and Tilton (1955).
[c]IOBC/WPRS class: Class 1 = harmless (M < 25%), Class 2 = slightly harmful (25% < M < 50%), Class 3 = moderately harmful (51% < M < 75%), and Class 4 = harmful (M > 75%) 96 hours after ingestion.

The ready-to-use bait Success 0.02 CB caused low toxicity (<5% mortality at 24, 48, 72, and 96 HAE) to adults of *D. areolatus*, being classified as harmless (class 1), not differing statistically from the negative control with 80% honey ($P < 0.05$; Table 4). However, the parasitoids that were exposed to Gelsura's formulations (2.0 and 4.0 g a.i./l alpha-cypermethrin) experienced greater than 83% mortality in the first 24 HAE. Gelsura's formulations (2.0 and 4.0 g a.i./l alpha-cypermethrin) were classified as harmful to the parasitoids at all evaluation times (class 4).

Sublethal Toxicity

Among the toxic baits analyzed, 16 formulations caused adult mortality of less than 50% at 96 HAE. Thus, they were used for sublethal assessments. Based on this, it was observed that only the formulations Samaritá Tradicional + phosmet (38.5%) and Samaritá + spinosad (36.6%) showed direct negative effects in reducing the emergence of *D. areolatus*, which differed significantly from the negative control. Consequently, they were classified as slightly harmful (class 2). In contrast, the formulations Anamed + spinosad, Anamed + spinetoram, Biofruit + spinosad, Biofruit + spinetoram, Biofruit + phosmet, and Flyral + spinosad did not show negative effects on *D. areolatus* adults either in reducing parasitism or in reducing emergence (PR or ER < 30%; class 1) (Table 5). The insecticides spinosad, spinetoram, and phosmet associated with the Biofruit attractant showed the highest rates of parasitism (40.9%, 42.5%, and 47.8%, respectively) and emergence (22.9%, 25.4%, and 27.8%, respectively) of *D. areolatus* (Table 5). The ready-to-use formulation Success, despite showing the greatest reduction in parasitism (27.9%), was considered harmless according to the IOBC (PR or ER < 30%; class 1).

Discussion

In this study, the amount of food attractant ingested by the insects during the exposure time (24 h) was not measured. However, *D. areolatus* adults were attracted to and fed on all treatment baits offered. Based on the isolated effects of the food attractants, all attractants in the absence of insecticide caused low toxicity (M < 25%) and did not show sublethal effects (reduction of parasitism) on surviving insects after feeding on food attractants when compared to the insects fed the water and 80% honey solution, which was considered the standard for rearing and multiplication of the species in the laboratory (Gonçalves et al. 2018).

Based on our results, the highest mortality rates of adults of *D. areolatus* were recorded with formulations of toxic baits containing the insecticide malathion 1000 EC (2.0 g a.i./l) (harmful—Class 4). The toxicity of organophosphate insecticides has been reported for other braconid species, such as *Aphidius gifuensis* Ashmaed (Hymenoptera: Braconidae) and *Diachasmimorpha longicaudata* (Ashmaed) (Hymenoptera: Braconidae) (Ohta and Takeda 2015, Harbi et al. 2017, Bernardi et al. 2019). Due to their high toxicity to fruit fly species, organophosphate insecticides have commonly been used in the formulation of toxic baits (Harter et al. 2015, Nunes et al. 2020, Borges et al. 2021). In addition, the ease of acquisition and the low cost of the active ingredients constitute advantages to fruit growers that utilize toxic bait formulations (Botton et al. 2016). Although the repellency of *D. areolatus* adults was not evaluated in the present study, recent research has shown that in the field, some food attractants, when mixed with malathion, are repellent to beneficial insects, such as bees (Padilha et al. 2019). However, for *D. areolatus*, future studies should be evaluated in the field to verify this effect without harming the applied or natural biological control, since during the period of fruit maturation in the field, the highest population density of the parasitoid in the field occurs (Nunes et al. 2012). In contrast, phosmet-based toxic bait showed less lethal toxicity to *D. areolatus* compared to those containing malathion, despite belonging to the same chemical group (organophosphates).

Similar results were obtained for adults of *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) (Farah et al. 2019). According to Farah et al. (2019), the lower toxicity of the phosmet insecticide compared to malathiom occurs because the phosmet insecticide is a wettable powder (WP) formulation. This fact means that when the product is diluted in water or with the food attractant, it provides

Table 4. Mean number of live insects (*N* ± SE) and corrected mortality (M %) of *D. areolatus* subjected to treatment with ready-to-use toxic baits

Treatments	24 h		48 h		72 h		96 h		IOBC/WPRS Class[c]
	N ± SE[a]	M%[b]	*N* ± SE	M%	*N* ± SE	M%	*N* ± SE	M%	
Success 0.02CB (spinosad 0.133 g a.i./l)	16.8 ± 0.48Aa[ns]	0.0	16.1 ± 0.54Aa[ns]	2.4	15.9 ± 0.52Aa[ns]	3.05	15.8 ± 0.55Aa[ns]	3.7	1
Gelsura (alpha-cypermethrin 2.0 g a.i./l)	2.8 ± 0.41Ba[*]	83.3	2.2 ± 0.38Ba[*]	86.7	2.0 ± 0.39Ba[*]	87.8	1.8 ± 0.38Ba[*]	89.0	4
Gelsura (alpha-cypermethrin 4.0 g a.i./l)	2.3 ± 0.42Ba[*]	86.3	1.8 ± 0.41Bab[*]	89.1	1.6 ± 0.37Bab[*]	90.2	1.4 ± 0.30Bb[*]	91.5	4
80% Honey-water solution (negative control)	16.8 ± 0.29A[ns]	—	16.5 ± 0.37A[ns]	—	16.4 ± 0.42A[ns]	—	16.4 ± 0.42A[ns]	—	—

[ns]Not significant when compared to the negative control in the same column.
[*]Significant compared to the negative control in the same column.
[a]Average of live insects followed by the same capital letters in the column do not differ significantly from each other when compared to the toxic bait formulation with the respective food attractant and, lowercase letters in the row, do not differ significantly from each other over time by the Tukey test ($P < 0.05$).
[b]Corrected mortality using the formula Henderson and Tilton (1955).
[c]IOBC/WPRS class: Class 1 = harmless (M < 25%), Class 2 = slightly harmful (25% < M < 50%), Class 3 = moderately harmful (51% < M < 75%), and Class 4 = harmful (M > 75%) 96 hours after ingestion.

Table 5. Sublethal effect of food attractants and toxic baits on *D. areolatus* adults

Treatments	% Parasitism[a]	PR	IOBC/WPRS class	Emerged insects	ER	IOBC/WPRS class
Anamed + spinosad 0.096 g a.i./l	41.2 ± 5.71[ns]	—	1	16.1 ± 2.26[ns]	3.0	1
Anamed + spinetoram 0.075 g a.i. kg $^{-1}$	44.5 ± 5.45[ns]	—	1	20.8 ± 2.68[ns]	—	1
Anamed + phosmet 1.0 g a.i./kg	36.4 ± 4.69[ns]	9.1	1	14.9 ± 1.80[ns]	10.2	1
Anamed	39.0 ± 6.73[ns]	2.7	1	14.8 ± 2.34[ns]	10.2	1
3% Biofruit + spinosad 0.096 g a.i./l	40.9 ± 3.94[ns]	—	1	22.9 ± 2.15[*]	—	1
3% Biofruit + spinetoram 0.075 g a.i./kg	42.5 ± 6.19[ns]	—	1	25.4 ± 3.67[*]	—	1
3% Biofruit + phosmet 1.0 g a.i./kg	47.8 ± 7.20[ns]	—	1	27.8 ± 4.17[*]	—	1
3% Biofruit	44.5 ± 3.54[ns]	—	1	25.7 ± 2.25[*]	—	1
1.5% CeraTrap + spinosad 0.096 g a.i./l	38.6 ± 3.48[ns]	3.7	1	17.5 ± 1.50[ns]	—	1
1.5% CeraTrap	42.8 ± 3.26[ns]	—	1	20.5 ± 1.20[ns]	—	1
1.25 Flyral +spinosad 0.096 g a.i./l	42.5 ± 3.33[ns]	—	1	20.7 ± 2.26[ns]	—	1
1.25 Flyral + spinetoram 0.075 g a.i./kg	37.8 ± 5.80[ns]	5.7	1	18.2 ± 1.63[ns]	—	1
1.25 Flyral + phosmet 1.0 g a.i./kg	39.2 ± 2.12[ns]	2.2	1	23.8 ± 1.70[*]	—	1
1.25 Flyral	38.2 ± 2.99[ns]	4.7	1	18.2 ± 1.26[ns]	—	1
Samaritá bait 3% + spinosad 0.096 g a.i./l	37.6 ± 6.09[ns]	5.7	1	10.5 ± 2.26[*]	36.6	2
Samaritá bait 3% + spinetoram 0.075 g a.i./kg	38.4 ± 6.29[ns]	3.6	1	14.2 ± 2.00[ns]	14.4	1
Samaritá bait 3%	41.3 ± 2.03[ns]	—	1	16.0 ± 1.11[ns]	3.6	1
3% Samaritá Tradicional bait + spinosad 0.096 g i.a./l	39.3 ± 5.75[ns]	2.8	1	14.5 ± 2.29[ns]	12.6	1
3% Samaritá Tradicional bait + spinetoram 0.075 g i.a./kg	36.0 ± 3.06[ns]	10.2	1	12.3 ± 1.71[*]	25.9	1
3% Samaritá Tradicional bait + phosmet 1.0 g a.i./kg	37.3 ± 4.71[ns]	6.9	1	10.2 ± 2.32[*]	38.5	2
3% Samaritá Tradicional bait	39.9 ± 1.86[ns]	0.5	1	18.5 ± 1.68[ns]	—	1
Success 0,02CB (spinosad 0.133 g a.i./l)	28.9 ± 0.49[*]	27.9	1	14.8 ± 2.21[ns]	10.8	1
80% Honey–water solution (negative control)	40.1 ± 2.92[ns]	—	—	16.6 ± 1.29[ns]	—	—

PR = Reduction in parasitism and ER = Emergency reduction compared to the negative control.
ns = not significant in relation to the negative control and to each other according to Tukey's test ($P < 0.05$).
[*]Significantly different from the negative control and from each other according to Tukey's test ($P < 0.05$).
OBC/WPRS class: 1) harmless (PR or ER < 30%); 2) slightly harmful (30 ≤ PR or ER ≤ 79%); 3) moderately harmful (80 ≤ PR or ER ≤ 99%; and 4) harmful (PR or ER > 99%).
[a]Parasitism obtained from surviving insects (mortality less than 50%) in bioassays of ingestion of toxic baits after 96 HAE.

the formation of a suspension that is not as stable as that formed by the emulsifiable concentrate (EC) formulation of Malathion 1000 EC. Therefore, the formulation requires continuous agitation for the syrup to remain homogeneous. However, despite the lower lethal toxicity, practically all baits containing phosmet (except for 3% Biofruit + phosmet) had a sublethal effect on reducing parasitism and insect emergence. Similar results were obtained for the predator mite *Neoseilus californicus* (McGregor) (Acari: Phytoseiidae) and lacewing *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) (Monteiro 2001, Ferreira et al. 2006).

Similar values to those of toxic baits formulated with the malathion insecticide were obtained with the ready-to-use Gelsura (~ alpha-cypermethrin 2.0 and 4.0 g a.i./l). The toxic bait Gelsura is considered efficient in the management of *A. frateculus* and *C. capitata* in the field (Baronio et al. 2019, Nunes et al. 2020), and high toxicity (mortality > 85%) to *D. areolatus* adults was observed. This effect may be mainly associated with the knockdown effect caused by the pyrethroid insecticide (alpha-cypermethrin) present in bait formulations (Casida and Durkin 2013). This was evident due to the rapid mortality caused to *D. areolatus* adults after bait ingestion (up to 24 h after ingestion). Thus, the application of Gelsura and *D. areolatus* for the management of fruit flies for must be carried out considering the residual period of the bait (approximately 14 days) (Baronio et al. 2019) so that there are no negative effects on the parasitoid.

For toxic spinosyn-based baits, mortality varied according to insecticide and food attractant (M < 50%—slightly harmful—Class 2). However, there was a greater tendency toward lethal toxicity for treatments containing spinetoram, mainly when mixed with 1.5% CeraTrap and 7% sugarcane molasses (mortality between 51% and 75%—moderately harmful). Similar results were obtained for adults of *D. longicaudata* (Bernardi et al. 2019). Although the detailed composition of the sugarcane attractant is unavailable from the manufacturer, the vegetable protein obtained from sugarcane can likely undergo a fermentation process after the addition of water, resulting in the generation of byproducts that are toxic to *D. areolatus*, such as ethanol. Similar findings have been reported for *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Lynch et al. 2017). In addition, the greater toxicity of spinetoram compared to spinosad may be related to the fact that spinetoram is a semisynthetic molecule originating from the chemical modification of natural spinosyns. This provides a greater insecticidal action compared to spinosad, which in turn is derived from the biological fermentation of *Saccharopolyspora spinosa*, a naturally occurring organism in the soil (Sparks et al. 2001, Stark et al. 2004, Thomas and Mangan 2005, Miles 2006, Pedroso et al. 2011, Bernardi et al. 2019).

However, among the toxic bait formulations with insecticides based on spinosyns, the 1.25% Flyral + spinosad treatment presented the highest mean number of live insects, 96 HAE, which did not differ from the respective food attractant and negative control. Flyral is a food attractant of animal origin that was recently introduced in the Brazilian market, and it has a high degree of purity and percentage of hydrolyzed protein (36%) (Zucoloto 2000, Baronio et al. 2019). This finding might indicate that this attractant provides the nutrients (carbohydrates) necessary for the survival of individuals during the first days of life. According to previous studies, this period is considered crucial for the development and maturation of the ovaries, which is necessary for reproduction of the species of parasitoids (Sivinski et al. 2006, Benelli et al. 2017). However, one of the disadvantages of using toxic baits formulated with the Flyral food attractant is the low resistance in the presence of rain. This fact makes the floral food attractant less used by producers for the formulation of toxic baits (Ruiz et al. 2008, Harter et al. 2015, Harbi et al. 2017, Baronio et al. 2019). In contrast, Anamed + spinosad, in addition to providing high toxicity to *A. fraterculus* adults (Borges et al. 2021), was considered harmless to *D. areolatus* in both evaluations (lethal and sublethal). In addition, the Anamed + spinosad formulation presents high resistance to precipitation and degradation by sunlight (Borges et al. 2015, 2021, Harter et al. 2015, Baronio et al. 2019). This fact is considered beneficial for producers because it can shorten field application intervals (Borges et al. 2015). Similar results were found for the ready-to-use formulation Success 0.02 CB (spinosad), which caused low lethal toxicity (<5% mortality 96 HAE) to *D. areolatus* adults; however, it showed low resistance to rain when applied in the field (Baronio et al. 2019). In addition, unlike other bait formulations containing spinosyn-based insecticides, Success 0.02 CB caused a reduction in parasitism (27.9%).

Based on our combined results, the formulations of toxic baits with less lethal toxicity on *D. areolatus* were as follows: ready-to-use baits Success < spinosyn-based baits (spinosad and spinetoram) < baits based on organophosphates (phosmet and malathion) < ready-to-use bait Gelsura. For reduced sublethal effects, baits containing insecticides from the spinosyn group (spinosad and spinetoram) are preferable compared to phosmet or ready-to-use bait success. However, the results were obtained in a laboratory situation in which the insects were submitted to controlled and restricted feeding conditions. In the field, parasitoids can move around and use other food sources to survive, such as sugar substances present in the floral resources of adjacent plants (Lee et al. 2006). In addition, in the field, the presence of solar radiation or rain can accelerate the degradation of the active ingredient, causing less toxicity of the bait (Flores et al. 2011). Based on this situation, the release of the parasitoid can occur after the residual effect of the product used in the formulation of the toxic bait. Thus, fruit flies can be managed by combining chemical control through the application of toxic baits with biological control through the use of *D. areolatus* for the suppression of pest populations. However, new studies should be performed under semifield and field conditions for a better understanding of the effect of the tested formulations on the parasitoids.

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