DOI: 10.1590/1808-1657000682018

Attractive lures for fruit flies in an organic guava orchard

Iscas atrativas para a mosca-das-frutas em um pomar orgânico de goiaba

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ABSTRACT: Fruit flies are the biggest obstacle in guava cultivation, with the monitoring of population a fundamental aspect for their management. The objective of this study was to assess the effectiveness of attractive lures for fruit flies in guava trees, produced in an organic system. McPhail traps were installed with 400 mL of solution, in four repetitions, and distributed in randomized blocks in six treatments: Isca Mosca® (5%); Isca Samaritá Tradicional® (5%); Torula® yeast; Bio Anastrepha® (5%); Ceratrap® and guava juice (50%), with 3 weekly reviews. Data on capture were subjected to analysis of variance, and averages were compared (Tukey 5%). The values of fly trap per day (FTD) were computed. The correlation between flies and meteorological variables were evaluated, as well as the monthly cost of lures. A total of 37,917 individuals from the genus *Anastrepha* and 122 species of flies *Ceratitis capitata* were collected, being *Anastrepha* spp. the main genus of fruit fly in the region. The Ceratrap product proved to be superior, followed by Torula, Isca Mosca, and Bio Anastrepha; Isca Samaritá and guava juice presented the lowest results. The standard trapping of females was like the total. The uniformity of attraction presented a drop in all treatments, and Ceratrap was more constant after seven days. The correlations between meteorological factors and population variation were positive and significant only for precipitation. The most expensive treatments (Ceratrap and Torula) were also the most efficient and selective, and the Ceratrap product required less manpower.

KEYWORDS: *Psidium guajava*; *Anastrepha* spp.; population monitoring.

RESUMO: A presença de mosca-das-frutas é o maior obstáculo à produção de goiabas, sendo o monitoramento populacional de fundamental importância para seu manejo. O objetivo deste trabalho foi avaliar a eficácia de iscas atrativas para mosca-das-frutas em goiabeiras, produzidas em um sistema orgânico. Foram instaladas armadilhas McPhail contendo 400 mL de solução, em quatro repetições e distribuição em blocos randomizados, em seis tratamentos: isca Mosca® (5%); isca Samaritá Tradicional® (5%); levedura Torula®; Bio Anastrepha® (5%); Ceratrap® e suco de goiaba (50%), com 3 avaliações semanais. Os dados da captura foram submetidos à análise de variância e às médias comparadas (Tukey 5%). Os valores de Mosca por Armadilha por Dia (MAD) foram calculados. A correlação entre moscas e as variáveis meteorológicas foi avaliada e o custo mensal das iscas foi calculado. Foram coletados 37.917 indivíduos do gênero *Anastrepha* e 122 moscas da espécie *Ceratitis capitata*, sendo a *Anastrepha* spp. o principal gênero de mosca-das-frutas da região. O produto Ceratrap mostrou-se superior, seguido pelas iscas Torula, Isca Mosca e Bio Anastrepha; a Isca Samaritá e o suco de goiaba apresentaram os menores resultados de captura. O padrão de captura de fêmeas foi semelhante ao total. Verificou-se uma queda na uniformidade de atração de todos os tratamentos, sendo o Ceratrap o que apresentou maior constância após sete dias. As correlações entre os fatores meteorológicos e a flutuação populacional foram positivas e significativas apenas para a precipitação. Os tratamentos mais onerosos (Ceratrap e Torula) também foram os mais eficientes e seletivos, sendo o produto Ceratrap o que demandou menor mão de obra.

PALAVRAS-CHAVE: *Psidium guajava*; *Anastrepha* spp.; monitoramento populacional.

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Received on: 05/25/2018. Accepted on: 08/30/2019

INTRODUCTION

Brazil is one of the largest guava producers in the world. The fruit is appreciated for its aroma and flavor, as well as its high nutritional value. Despite its economic relevance in the world scenario, Brazilian's guava exportation *in natura* is insignificant. The presence of fruit flies (Diptera: Tephritidae) in Brazilian orchards is considered the biggest obstacle to market and export the Brazilian production (HERNANDES et al., 2013).

Fruit flies have great taxonomic diversity. They include over 5,000 species belonging to the family Tephritidae, distributed worldwide (MONTES et al., 2011). Within the family Tephritidae, the genus *Anatrepha* is the most economically important (NORRBOM et al., 1999). Damage occurs due to oviposition by females in developing fruits, causing depreciation of the product for consumption (NUNES et al., 2013). Indirectly, the hole made for laying and feeding the larvae facilitates contamination by microorganisms, thus rotting the fruits. When they fall from the tree, they also favor other phytosanitary problems (NASCIMENTO et al., 2000).

Many fruits of the Myrtaceae family are primary hosts of fruit flies (SILVA et al., 2010; SILVA et al., 2011; BIRK; ALUJA, 2011). In Brazil, more than 11 species of *Anastrepha* and *Ceratitis capitata* (Wied.) have been reported for infesting guava fruits (SOUZA FILHO et al., 2009; PEREIRA et al., 2010; JESUS-BARROS et al., 2012).

Fruit fly population fluctuates due to a succession of primary or alternative hosts, environmental complexity, and abiotic factors (MONTES et al., 2011). With a population monitoring study, it is possible to determine pest fluctuation in a specific area to detect exotic or quarantine species, enabling a more accurate characterization of the pest population in qualitative and quantitative terms (MACIEL et al., 2017). Information on fruit fly population fluctuation and its relationship with biotic and abiotic factors must be obtained and interpreted appropriately for regional control (ALUJA et al., 2012).

A key aspect of fruit fly management is population monitoring. This should give information that represents the behavior of the species population in the monitored area. The evaluation of effective and reliable food attractions should be performed on a permanent basis. The rational and efficient control of fruit flies has as a prerequisite the knowledge of the right moment for following control measures (KOVALESKI, 1997; NORA; SUGIURA, 2001). According to NASCIMENTO et al. (2000), among the several factors involved in the capture of fruit flies, the efficiency of the attractant used stands out

Available new attractions, such as hydrolyzed proteins [Bio Anatrepha® (Biocontrol - Pest Control Methods Ltda.) and Isca Mosca® (Isca Technologies Ltda.)], in addition to Torula® yeast, significantly improved pest monitoring, with more consistent results than those obtained with grape juice (SCOZ et al., 2006; MONTEIRO et al., 2007; ZUANAZZI, 2012; NUNES et al., 2013; BORTOLI, 2014). However, depending on the region, culture, and method of evaluation of the attractions, results are not repeated, generating doubts as to when producers should implement monitoring. For this reason, assessing effective and reliable food attractions should be permanently performed (MACHOTA JUNIOR, 2015).

In recent years, new long-lasting liquid attractants for use in mass capture traps have been developed (EPSKY et al., 2014). Among these new attractions is the hydrolyzed Ceratrap® protein (Bioibérica S.A., Barcelona, Spain), initially introduced and validated in Spain, to monitor and control *C. capitate* (SIERRAS et al., 2006). The product is formulated as a protein hydrolyzed, obtained from porcine intestinal mucosa through a cold enzymatic hydrolysis process (SANTOS-RAMOS et al., 2011; EPSKY et al., 2014; NAVARRO-LLOPIS; VACAS, 2014). The process reduces physical and chemical changes (EPSKY et al., 1993), with improvements in attractiveness. The product maintains the alkaline pH of solutions and conserves amino acids (EPSKY et al., 2014).

Monitoring results vary depending on the region of work conduction, culture (JAHNKE et al., 2014), and the presence of different fruit fly species belonging to the genus *Anastrepha*. Therefore, the choice and validation of the most effective attractants for pest monitoring is essential to improve species management, allowing to direct efforts in a specific way for each crop and orchard infestation hotspots (MENDONÇA et al., 2003). Seen that, the objective of the present study was to test the effectiveness of different attractive food baits for fruit flies in a guava crop, produced in an organic system, and to verify the influence of climatic factors on their population fluctuation.

MATERIAL AND METHODS

The study was carried out in the experimental area of APTA Regional Centro Norte, in Pindorama City, São Paulo state, Brazil, at 21º13'S, and 48º55'W, an altitude of 527 m, with an annual average temperature of 22.8°C, average precipitation 1,390.3 mm, and annual average relative humidity of 71.6%. According to Köppen's classification, the climate fits in the Aw type, defined as humid tropical, with a rainy season in summer and dry in winter.

The plants used in the experiment belongs to the Guava Germplasm Bank, grown in an organic system. McPhail traps were installed in guavas about 1.60 m high, inside the treetops and in the shade, on February 3rd, 2017, at the peak of fruit fly infestation in the study area. Each trap received 400 mL of an attractive solution. Each plot, represented by a trap, was repeated four times and distributed in blocks with randomized plots. Each treatment (trap) was installed 30 m away from the other, so as not to interfere with attractive solutions.

Six treatments were used: T1: 5% Isca Mosca®; T2: 5% Isca Samaritá Tradicional®; T3: Torula® yeast (3 tablets/trap); T4: 5% Bio Anastrepha®; T5: Ceratrap® (ready-to-use product); and T6: 50% sugary guava juice. Commercial products were used at the concentrations recommended by the manufacturer. Guava sugar juice was made following the method of GALLI et al. (2008),

by boiling the ripe guava pulp in water and sugar, in the ratio of 400 mL of water to 200 g of crystal sugar for each set of six large fruits. After boiling, the mixture was sieved and packaged in 500 mL plastic bottles for freezer storage. The concentration used was 50% of sugared juice per liter of water.

The evaluations were made on Mondays, Wednesdays, and Fridays, when the content retained in the attractive solutions of each trap were transferred, with the aid of a sieve, to plastic bottles with lid, with 70% alcohol, and taken to the laboratory for counting and separation by sex. After the insects were removed, the liquid was returned to its trap. Treatments T1, T2, T4, and T6 had the trap content replaced weekly on Fridays; treatment T3, fortnightly; and treatment T5 had no change, because it was a monthly replacement product.

The capture data observed in each plot were summed and transformed into the square root (x + 0.5) and submitted to analysis of variance. Means were compared with the Tukey test at a 5% probability. The analyses were performed with the aid of the AgroEstat statistical program.

The fly trap day (FTD) values obtained for each assessment were calculated based on the period in which the trap was exposed, and the immediately preceding assessment using the formula:

FTD: F / T x D
FTD: Fly / Trap / Day
F: number of flies caught in the period
T: number of orchard traps
D: number of exposure days

The association between the number of fruit flies of the genus *Anastrepha* spp. captured in traps and meteorological variables was assessed with Pearson's correlation coefficient ($p < 0.05$). Daily records for maximum and minimum temperatures (°C) and rainfall (mm) were obtained from the weather station located within the research unit, 400 m from the orchard. For temperature, daily values were used, with the averages recorded on the days the traps remained in the field. For precipitation, the accumulation in the period was used. The analyses were performed with the aid of the Statistica 7.0 statistical program. The correlation was not performed for *C. capitate* species due to the small number of captured individuals.

The cost of the food attractive used per monitoring point (trap) was calculated, as well as the monthly cost of maintaining the attractants, based on the prices of products.

RESULTS AND DISCUSSION

During the entire evaluation period, 37,917 *Anastrepha* individuals were collected, compared to 122 *Ceratitis capitata* flies, indicating that *Anastrepha* spp. was the main genus of fruit flies in the study region.

The *Anastrepha* genus predominance verified in the collections corroborates the results of several authors. MACIEL et al. (2017) found that all fruit flies collected in the traps belonged to the genus *Anastrepha* in a commercial guava orchard in São Luís City, Maranhão state, Brazil. CALORE et al. (2013) found that the genus *Anastrepha* corresponded to 97.02% of the total Tephritids sampled in an organic guava orchard, agreeing with results of ARAÚJO et al. (2005) and SILVA et al. (2007). DINIZ (2016) found that all captured *Anastrepha* flies were identified as *A. fraterculus* in an experiment conducted at the same place of the present study. According to ALUJA (1999), *A. fraterculus* is mainly associated with the Myrtaceae family.

Treatment 5 (Ceratrap) was superior in all evaluation times (Table 1), followed by treatment 3 (Torula), Isca Mosca, and Bio Anastrepha. In Fig. 1, data analyzed together (average of the eight evaluation dates) are presented, where the best attraction was Ceratrap (T5), without significantly differing from treatments T3 (Torula) and T1 (Isca Mosca). The treatments Bio Anastrepha, Isca Samaritá, and guava juice did not differ and presented the lowest capture results.

Similar results have been reported by several authors. LASA; CRUZ (2014) tested the effectiveness of attractive baits in a mango orchard in Mexico and found that Ceratrap was more efficient at capturing adults from *Anastrepha obliqua* than Torula baits. BORTOLI (2014) collected 703 individuals belonging to the species *A. fraterculus* in Ceratrap-baited traps in a citrus orchard in Serra Gaúcha region, Brazil, followed by Torula with 239, Bio Anastrepha, with 237, glucose, with 149, and grape juice with 82 individuals. respectively.

In Figure 2, data analyzed together (average of the eight evaluation dates), referring to *Ceratitis capitata* capture, are shown. The best attraction was Torula, without significantly differing from Ceratrap and Bio Anastrepha. Contrary to the results observed in Figure 1, the attractive Isca Mosca was in fourth place in the attractiveness of *C. capitata*, without differing from the attractive Isca Samaritá and guava juice, which were the least attractive.

SCOZ et al. (2006) suggest the use of Torula yeast as a reference for fruit fly detection or monitoring programs, because it presents a little variation in composition, it is more specific and attracts significantly more fruit flies when compared to grape juice at 25% and Bio Anastrepha hydrolyzed protein at 5%. In the present experiment, although not statistically different (Fig. 1), the greater attractiveness of the Torula before Bio Anastrepha was also verified. RAGA et al. (2006) demonstrated that the hydrolyzed proteins Bio Anastrepha and Isca Mosca, in an orange orchard, were efficient in attracting fruit flies without differing from each other, contrary to the data observed here, where even without a statistical difference, a higher efficiency of Isca Mosca before Bio Anastrepha was seen (Table 1 and Fig. 1).

Hydrolyzed proteins offer free amino acids for nutrition and reproduction, and contain stimulants, thus with high attractiveness to insects (VARGAS; PROKOPY, 2006). Protein-based feeding increases the likelihood of copulation among fruit flies (PEREIRA et al., 2013; TAYLOR et al., 2013), leading to greater attractiveness of sexually immature flies for hydrolyzed protein traps (BORTOLI, 2014; PEREA-CASTELLANOS et al., 2015).

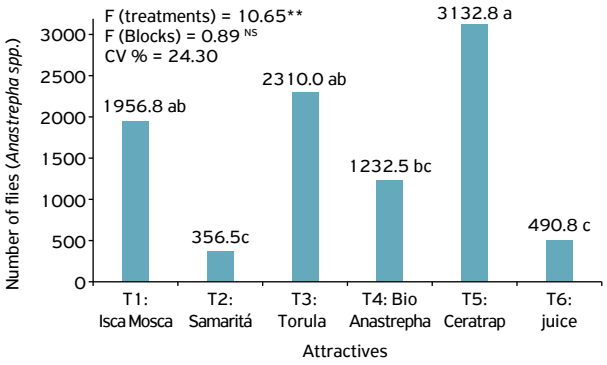

Figure 1. Comparison between attractive solutions in relation to the average number of fruit flies of the genus *Anastrepha* spp., captured during the experiment period. Bars followed by the same letter do not differ from each other. Tukey test at 5% probability.

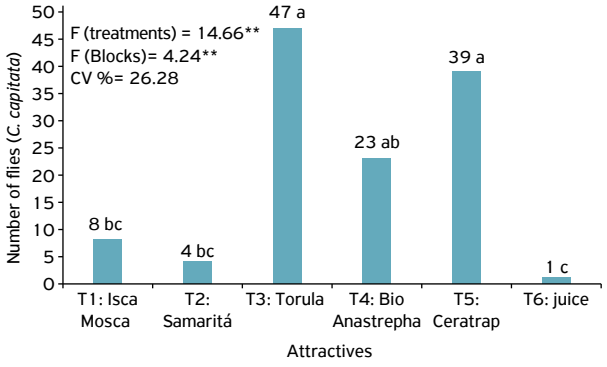

Figure 2. Comparison between attractive solutions in relation to the average number of fruit flies of *Ceratitis capitata* species, captured during the experiment period. Bars followed by the same letter do not differ from each other. Tukey test at 5% probability.

Despite the superiority of protein baits to the use of fruit juice, Isca Samaritá bait did not differ significantly from guava sugar juice in the present experiment, both presenting the smallest fly captures. According to RAGA; VIEIRA (2015), although hydrolyzed proteins are food attractants readily available in the market, many producers report variations in the attractiveness of these substances according to the manufacturing batch.

Regarding the uniformity of attraction, there was a decrease in the attractiveness of all treatments, according to the attraction exposure in the field (Table 2 and Fig. 3). Seen that Ceratrap

Table 2. Comparison of attractiveness of solutions for *Anastrepha* spp. in the 3rd, 5th, and 7th day after exposure.

Attractive	Days after exposure			
	3rd.	5rd.	7rd.	Average
Isca Mosca	386.8	226.3	108.8	240.6 bc
Isca Samaritá Tradicional	103.8	64.5	37.0	68.4 d
Torula	550.0	271.0	209.3	343.4 b
Bio Anastrepha	307.5	181.0	115.3	201.3 c
Ceratrap	561.8	589.5	502.0	551.1 a
Guava juice	168.3	57.0	22.5	82.6 d
Average	346.37 A	231.55 B	165.82 C	
F (treatments)	34.6833**			
F (days)	20.1389**			
F (treat. x days)	1.0377NS			
CV (%)	24.05			

Data transformed into root x (Table shows original data). Averages followed by the same lowercase letter in the column and uppercase in the row do not differ from each other. Tukey test at 5% probability. **significant at the 1% probability level ($p < .01$); NS: non-significant ($p > = .05$)

Table 1. Evaluation of the effectiveness of attractive baits for *Anastrepha* fruit flies.

Treatments	Valuation dates													
	02/06/17		02/08/17		02/10/17		02/13/17		02/15/17		02/17/17		02/20/17		02/22/17	
T1	386.8	ab	226.3	bc	108.8	bcd	461.5	ab	149.5	ab	116.5	abc	364.0	ab	143.5	ab
T2	103.8	b	64.5	c	37.0	cd	58.0	c	36.8	bc	20.3	bc	23.0	b	13.3	b
T3	550.0	a	271.0	ab	209.3	b	342.5	ab	127.8	ab	128.5	ab	461.5	a	219.5	a
T4	307.5	ab	181.0	bc	115.3	bc	276.8	abc	46.8	bc	47.0	abc	191.3	ab	67.0	ab
T5	561.8	a	589.5	a	502.0	a	593.3	a	283.5	a	205.8	a	240.8	ab	156.3	ab
T6	168.3	b	57.0	c	22.5	d	152.3	bc	14.5	c	8.8	c	46.0	b	21.5	b
F (treat.)	6.1463**		11.5591**		28.4587**		9.0915**		11.3443**		6.5345**		5.0727**		5.9200**	
F (Blocks)	1.3069NS		0.5351NS		0.3895NS		1.2826NS		0.5904NS		0.8628NS		0.0898NS		2.0355NS	
s.m.d. (bl)	7.25		5.98		4.01		6.71		4.72		5.60		9.69		5.97	
s.m.d. (treat)	9.98		8.24		5.52		9.24		6.51		7.71		13.35		8.22	
CV	24.82		25.94		21.30		24.48		30.72		41.94		45.48		41.02	

Treatments: T1: Isca Mosca; T2: Isca Samaritá; T3: Torula; T4: Bio Anastrepha; T5: Ceratrap; T6: Guava sugar juice. Data transformed into root x (table shows original data). Averages followed by the same lowercase letter in the column do not differ from each other. Tukey test at 5% probability. **significant at 1% probability level ($p < .01$); NS: non-significant ($p > = .05$)

product was not replaced, and Torula attractive replacement was performed after two weeks, the statistics were performed only during the first seven days of exposure. Treatment 5 (Ceratrap) presented greater constancy in the attraction of flies until the seventh day, differing significantly from the others; the attractive Isca Samaritá and guava juice were those that mostly lost their attraction after seven days in the field.

In the literature, many studies have demonstrated the durability and stability of Ceratrap formulation under field conditions and high attractiveness for species, such as *Anastrepha ludens* and *Ceratitis capitata* (SANTOS-RAMOS et al., 2011; EL ARABI et al., 2011; LASA et al., 2013; NAVARRO-LLODIS et al., 2014; HAFSI et al., 2015).

The main target of fruit fly monitoring is the capture of females, which represented 49.7% of the captured *Anastrepha* individuals. Among treatments, the capture pattern was similar to the total capture, and Ceratrap was the most efficient attraction to females, without differing significantly from Torula and Isca Mosca. Treatments with guava juice and Isca Samaritá did not differ and captured the smallest number of females in the experiment (Fig. 4).

Although differing from each other, all treatments presented high FTD indices (Table 3). In general, the FTD index equal to or greater than 0.5 is suggested as the moment to adopt *A. fraterculus* population control measures for all crops (CARVALHO, 2005). Thus, all the attractions used were efficient in meeting their goal, which was monitoring. It is noteworthy that because it is a guava orchard and being guava the preferential host of *A. fraterculus*, the capture of flies with traps reached very high numbers (37,917 adults captured in total). In other crops, such as peach, apple, and citrus, depending on the management (conventional or organic), and the time of trap installation, traps capture a smaller number of fruit flies, and that is why it is interesting to adopt a larger bait attractive power to avoid the risk of errors when adopting control measures.

Correlations between meteorological factors and fruit fluctuation were only positive and significant for rainfall (Table 4 and Fig. 5), and not significant for the temperature. Contrary to these results, CALORE et al. (2013), in a similar experiment, found no correlation with precipitation, but found significance for minimum, average and maximum temperatures,

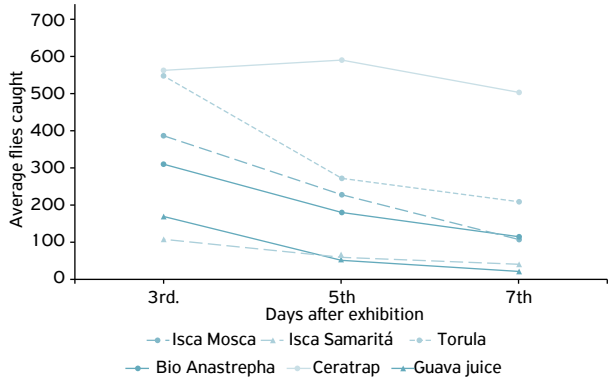

Figure 3. Comparison of the attractiveness of the tested solutions in the three periods after field traps.

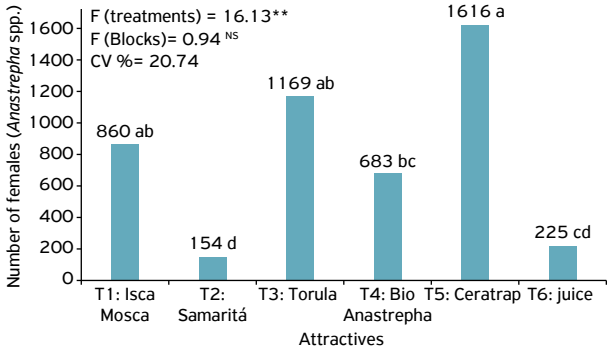

Figure 4. Comparison between attractive solutions to the average number of fruit flies females, captured during the experiment conduction period. Bars followed by the same letter do not differ from each other. Tukey test at 5% probability.

Table 4. Correlation coefficient between the total number of *Anastrepha* fruit flies caught in the traps, the number of females, the flies per trap per day index and the minimum temperature meteorological factors, maximum temperature and rainfall.

	Coefficient of linear correlation		
	TMIN (°C)	TMAX (°C)	RAIN (mm)
Anastrepha spp.	-0.2676^{NS}	-0.4475^{NS}	0.8170*
Females	-0.2841^{NS}	-0.4523^{NS}	0.8155*
FTD	0.0272^{NS}	-0.3353^{NS}	0.7503*

*Significant at 5% probability; ^{NS}: non-significant; TMIN: minimum temperature; TMAX: maximum temperature; RAIN: rainfall.

Table 3. Average number of *Anastrepha* flies per trap per day, captured on exposure dates to different food attractions.

Attractive	FTD							
	02/06/17	02/08/17	02/10/17	02/13/17	02/15/17	02/17/17	02/20/17	02/22/17
Isca Mosca	128.9	113.1	54.4	153.8	74.8	58.3	121.3	71.8
Isca Samaritá	34.6	32.3	18.5	19.3	18.4	10.1	7.7	6.6
Torula	183.3	135.5	104.6	114.2	63.9	64.3	153.8	109.8
Bio Anastrepha	102.5	90.5	57.6	92.3	23.4	23.5	63.8	33.5
Ceratrap	187.3	294.8	251.0	197.8	141.8	102.9	80.3	78.1
Guava juice	56.1	28.5	11.3	50.8	7.3	4.4	15.3	10.8

indicating that pest population growth is favored at higher temperatures. SANTOS et al. (2017) found that the population fluctuation of *Anastrepha fraterculus* in apple orchards did not correlate with precipitation.

Analyzing the financial viability of treatments (Table 5), it seems that the most expensive ones, Ceratrap and Torula, were also the most efficient in the capture of insects and were the most selective, and in the evaluations, almost all the captured individuals were fruit flies. In addition to efficiency, Ceratrap product demands less labor, because it does not have to be diluted and has monthly replacements. The attractive Torula, the second most expensive, has a fortnightly replacement, which facilitates before others. The sugary guava juice did not have the price calculated, because it is a product obtained in the property. Thus, it becomes practical due to its easy access by the producer, and fulfills its monitoring role, although it is laborious to use cooking in its preparation. Among hydrolysed proteins, all were low-cost, but the product Isca Mosca was the least selective, attracting all types of insects and with an extremely unpleasant odor, which made evaluations very difficult. It is up to farmers, with all these pieces of information, to decide what is best for their property, combining efficiency and practicality, as well as economy.

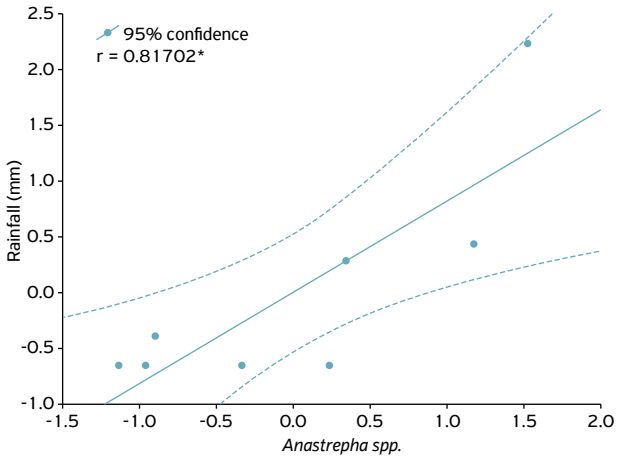

Figure 5. Correlation between fruit flies caught in the trap and rainfall recorded in the period.

ACKNOWLEDGMENT

We thank Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp) for financially funding the study (Process 2016/15647-8).

Table 5. Cost of food attractants used in the experiment.

Attractive	Comercial unit	Price Jan/2017	Dose/trap	Cost/trap	Duration	Monthly Cost/trap
Isca Mosca	500 mL	US$ 4.76	20 mL	US$ 0.19	1 week	US$ 0.76
Isca Samaritá	1 L	US$ 13.33	20 mL	US$ 0.27	1 week	US$ 1.07
Torula	100 tablets	US$ 66.67	3 tablets	US$ 2.00	2 week	US$ 4.00
Bio Anastrepha	500 mL	US$ 4.44	20 mL	US$ 0.18	1 week	US$ 0.71
Ceratrap	5 L	US$ 66.67	400 mL	US$ 5.33	4 week	US$ 5.33
Guava juice	-	-	200 mL	-	1 week	-

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