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CROP SCIENCE

# Search hours for food attractant by *Anastrepha fraterculus* and *Ceratitis capitata* (Diptera: Tephritidae) adults in guava orchards

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**Abstract:** *Anastrepha fraterculus* (Wiedemann, 1830) and *Ceratitis capitata* (Wiedemann, 1824) are considered the main pests of Brazilian fruit production. Understanding the behavior of species is of great importance for the success of management strategies. This study was to determine the period and search time by attractive food for *A. fraterculus* and *C. capitata* adults by using three commercial food attractants: BioAnastrepha™ 5%; Isca Samaritá Tradicional™ 5% and Ceratrap™ 1.5%. The largest catches of *A. fraterculus* and *C. capitata* adults in McPhail traps occurred during the day between 6:30 am and 6:30 pm for both species. The BioAnastrepha™ food attractant provided the largest catches compared to Isca Samaritá Tradicional™ and Ceratrap™. In addition, there was a higher prevalence of capturing females than males, for both flies' species. The period of largest search activity for food attractant was observed from 12h:31 pm to 04:30 pm for *A. fraterculus* and *C. capitata*, time of greatest temperature on the day. The definition of the period of largest activity of *A. fraterculus* and *C. capitata* adults in the field helps in the elaboration of management strategies to be adopted.

**Key words:** fruit flies, hydrolyzed protein, monitoring, feeding period.

## INTRODUCTION

Fruit flies are considered to be one of the main phytosanitary problems in fruit production areas worldwide, with approximately 5000 species described and the potential to infest more than 400 hosts (Norrbom & Korytkowski 2012). The main species of economic importance in Brazil are *Anastrepha fraterculus* (Wiedemann, 1830) and *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) (Botton et al. 2016, Bortoli et al. 2016, McQuate & Liquido 2017, Araujo et al. 2019a, b). The damage caused by these insects is carried out both by the females, who pierce the fruits when they oviposit and by the larvae which in addition to consuming the pulp, cause the induction of early maturation, and consequent premature fall of the fruits (Nava & Botton 2010). Approximately $ 1 billion is lost every year due to damage caused by fruit flies worldwide (Leonardo & Faria 2019).

To avoid economic losses, the management adopted by fruit growers is based on monitoring adult population levels in orchards (Nava & Botton 2010). The control is carried out mainly by use of synthetic insecticides (eg pyrethroids, spinosyns, and phosphorous), applied in total area (Botton et al. 2016). However, in recent years, studies have shown the use of toxic baits is a viable alternative with satisfactory results in the management of species (Baronio et al. 2019, Nunes et al. 2020).

The success of using chemical insecticides via spraying the entire area or application in toxic baits strategy depends on the activity of species

adult's movement inside the orchards (Navarro-Llopis et al. 2012, Da Rosa et al. 2017, Hafsi et al. 2019). Similarly, the presence of vegetal host's species in the surrounding vegetation (as well as the availability of fruits in these hosts and their ripening process) could favor the movement of Tephritidae flies towards the orchards (Galli et al. 2019). As well as, the availability and maturation of the fruits inside the orchard and the presence of vegetation with surrounding host species, the study area favors the locomotion of the pest in the orchard (Araujo et al. 2019a, b). In Brazil, the monitoring of *A. fraterculus* and *C. capitata* in agricultural orchards has been carried out using McPhail-type traps (Bortoli et al. 2016), baited with hydrolysed proteins of vegetal or animal origin (Raga et al. 2006, Bortoli et al. 2016). Although for *C. capitata* there is the option of using parapheromone (Nava & Botton 2010). The use of attractive protein food is associated with the physiological need of the adult fruit fly to ingest protein for maturation of the reproductive system (Lasa et al. 2014).

In view of this, using the food attractions BioAnastrepha® 5%, Traditional Samaritá Bait 5% (proteins of vegetable origin) and Ceratrap® 1.5% (protein of animal origin), the objective was to know the period and the search time for *A. fraterculus* and *C. capitata* adults to the food attractions inside the monitoring traps. In view of this, using the food attractants BioAnastrepha™ 5%, Isca Samaritá Tradicional™ 5% (proteins of vegetable origin) and Ceratrap™ 1.5% (protein of animal origin), the aim was to know the period and the search time for *A. fraterculus* and *C. capitata* adults to the food attractions inside the monitoring traps in guava orchard.

## MATERIALS AND METHODS

This study was carried out in two areas, during two different periods, March 26 to 30 and June 10 to 14 of 2019. Both areas were located in the South Region of Brazil. The first experimental site was a 0.5-ha guava orchard (*Psidium guajava* L., Myrtaceae), cultivar Paluma, with trees younger than 10 years, spaced 1.5 m × 4.5 m (between rows), and located in the Estação Experimental Cascata da Embrapa Clima Temperado, Rio Grande do Sul, Brazil (31º40'48.48"S, 52º26'42.71"W, 170 m altitude). At the edges of the orchard, the following fruits were grown: cherry guava (*Eugenia uniflora* Linnaeus), guabiju [*Myrcianthes pungens* (Berg) Legrand], and two vigorous loquat trees [*Eriobotrya japonica* (Thunb.) Lindley], all of them belong to the Rosaceae family. The second experimental site was a 0.7-ha guava orchard, cultivar Paluma, with trees younger than 8 years, spaced 1.8 m × 4.0 m (between and rows), and located in the Campus Visconde de Graça of the Instituto Federal de Educação, Ciência e Tecnologia Sul-Riograndense (IFSul) (31° 42' 52.762" S, 52° 18' 35.435" W, 15 m altitude). In the surroundings of this experimental area, the main vegetal host for Tephritidae flies was cherry guavas (*E. uniflora*). Both experimental sites were chosen due to the high incidence of tephritid flies and because pesticides and/or phytosanitary products are not used. In the first site, *A. fraterculus* predominates, meanwhile in the second experimental site the predominant species is *C. capitata*. During the period of study, both areas were in the growing cycle of *P. guajava* fruits.

The periods (day or night) and the time of largest search activity of *A. fraterculus* and *C. capitata* adults by the food attractant was evaluated for each area. For this, three food attractants were used: BioAnastrepha™ at 5% (BioControle – Métodos de Controle de Pragas Ltda., Indaiatuba, SP, Brazil); Isca Samaritá Tradicional™ at 5% (Samarita, Ltda, Barcelona, Spain), and Ceratrap™ 1.5% (Bioibérica S.A.,

Barcelona, Spain). McPhail-type monitoring traps were used, baited with 400 ml of food attractant solution (five traps per food lure). The traps were distributed under the canopy of the plants, 1.5 m above the ground, spaced equidistantly every 10 m (Nascimento et al. 2000).

To evaluate the search period, the evaluations were carried out during two different periods (day and night). The daytime period was between 6:30 am and 6:30 pm and night-time period between 6:30 pm and 6:30 am. By the end of each period, all adult fruit flies captured by trap were removed with the aid of a plastic sieve (2mm mesh), as proposed by Bortoli et al. (2016). The insects were stored in plastic containers (100 mL), containing 70% alcohol. In the laboratory, the specimens were separated by sex and identified with the aid of the dichotomous key (Zucchi 2000). The identification of the species was performed by observation of morphology features of the wing, thorax, and the ventral surface of the sting (Alberti et al. 2012).

To determine the search activity time of fruit flies' adults by the food attractant, every two hours, during the day, the number of insects captured in each trap was evaluated. The evaluation times were: 6h30 am to 8h30 am, 8h31 am to 10h30 am, 10h 31 am to 12h30 pm, 12h31 pm to 2h30 pm, 2h31 pm to 4h30 pm, and from 4h31 pm to 6h30min pm. For each evaluation period, the captured fruit fly adults were collected and stored in plastic containers (15 mL), containing 70% alcohol and transported to the laboratory for the quantification and identification of specimens, as described above. The evaluation periods were determined according to the sunrise time for Pelotas, Rio Grande do Sul, Brazil, obtained from the Observatório Nacional no Rio de Janeiro-RJ, Brazil (Moreira 2004).

The climate of the region is of the "Cfa" type, subtropical (without dry season and hot summer), with well-distributed rains and well-defined seasons, according to the Köppen-Geiger classification (Peel et al. 2007). The average environmental conditions (temperature) during the experiments were 23ºC (March) and 18ºC (June). The temperature during the period of the experiments was obtained from the meteorological stations of Embrapa Clima Temperado and Campus Visconde de Graça of the Federal Institute of Education, Science and Technology Sul-Riograndense.

### Statistical analysis

For both experiments (period and search activity for food attractant), the experimental design was completely randomized and carried out for five consecutive days. Generalized linear models (GLM) with a poisson distribution and a log link function were used to assess if the abundance of flies during the period of time (day or night) captured in each treatment and experiment carried out (Nelder & Wedderburn 1972). For both study areas, the first factor (A) was represented three food lures. The second factor (B) was represented by period (day or night) and third factor was represented by season (March or June). These three factors were considered fixed in the model. If significant differences were detected between treatments, the data were subjected to the Tukey test at the level of 5% significance. To determine the difference in catch between males and females of *A. fraterculus* or *C. capitata* within the same food attractant, the data were submitted to the t test at 5% significance. All analyzes were performed using the statistical software "R" version 2.15.1 (R Development Core Team 2012).

## RESULTS

For *A. fraterculus*, were captured 456 adults (235 females and 221 males) in the food attractant BioAnastrepha™; 72 adults (40 females and

32 males) in the Isca Samaritá Tradicional™ and 341 adults (225 females and 116 males) in Ceratrap™. For *C. capitata*, were captured 440 adults (225 females and 215 males) in the BioAnastrepha™, 75 adults (45 females and 30 males) in the Isca Samaritá Tradicional™ and 358 adults (228 females and 130 males) in the Ceratrap™. In relation to the feeding schedule, it was verified that adults of *A. fraterculus* (Fig. 1a) and *C. capitata* (Fig. 2) showed activity of feeding and searching for food attractants during the daytime. In the night-time only three *A. fraterculus* adults were captured in each trap baited with BioAnastrepha™ and Ceratrap™ (Fig. 1b), no individual of *C. capitata* was captured in this evaluation period. The food attractants BioAnastrepha™ and Ceratrap™ provided the largest number of *A. fraterculus* adults (F= 7.11; d.f.= 2, 14; *P*< 0.0001) (Fig. 1a) and *C. capitata* (F= 10.12; d.f.= 2, 14; *P*< 0.0001) (Fig. 2) captured. It was verified by the t test that the number of captured females was always higher than the number of males for both species during the day: *A. fraterculus* (BioAnastrepha™: t = 8.36, d.f. = 26.02; *P* < 0.0001, Isca Samaritá Tradicional™: t = 2.14, d.f. = 23.11; *P* < 0.0001, and Ceratrap™: t = 4.12, d.f. = 24.38; *P* < 0.0001) and *C. capitata* (BioAnastrepha™: t = 2.11, d.f. = 23.02; *P* < 0.0001, Isca Samaritá Tradicional™: t = 5.10, d.f. = 28.10; *P* < 0.0001, and Ceratrap™: t = 2.19, d.f. = 25.44 *P* < 0.0001). Regarding the time of activity of searching for the food attractant, it was observed that *A. fraterculus* (Fig. 3) and *C. capitata* (Fig. 4) adults searched for food in the morning from 10h30 am, with an increase until 2h30 pm. However, the greatest activity of searching for food attractant was observed from 12h:31 pm to 4h:30 pm, time of highest temperature on the day (variation between 22 to 26ºC) (Fig. 3 and 4). During this period (12h:31 pm to 4h:30 pm), were captured between 70 to 80% of the adults of *A. fraterculus* (Fig. 3) and 60% of the adults of *C. capitata* (Fig. 4).

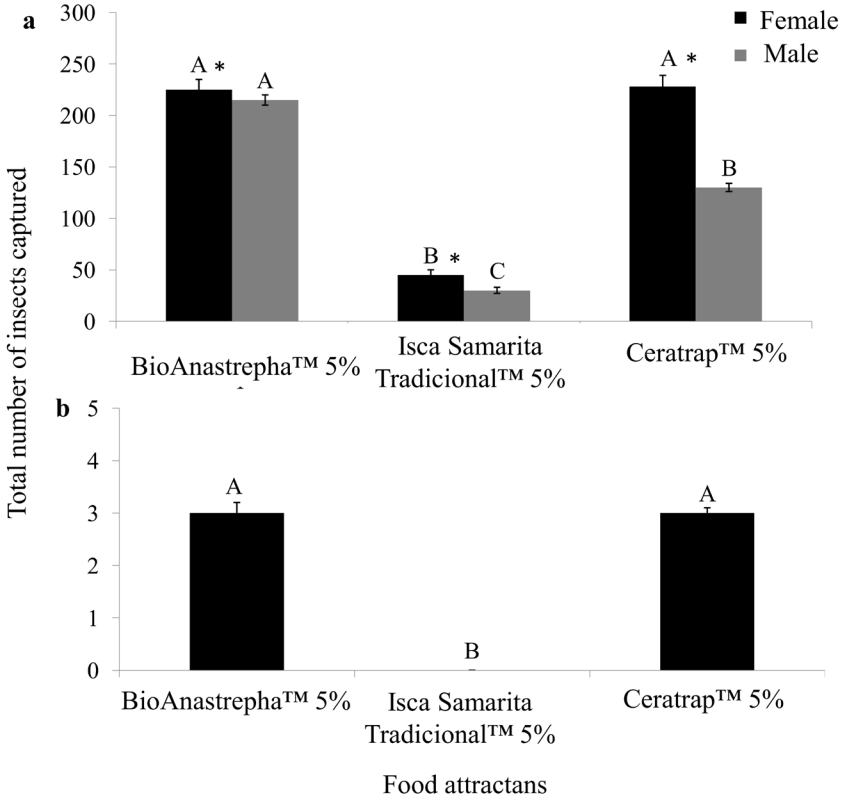


**Figure 1. Total number (± standard error) of males and females of *Anastrepha fraterculus* captured in McPhail traps baited with different food attractans during the day (6h31 am to 6h30 pm) (a) and nocturnal (6h30 pm to 6h31am) (b). Means (± SE) followed by the same capital letter in the same colored column do not differ significantly (Tukey, 0.05); Asterisks indicate significant differences between female and male within the same food attractant according to Student's t-test (*P* < 0.05).**

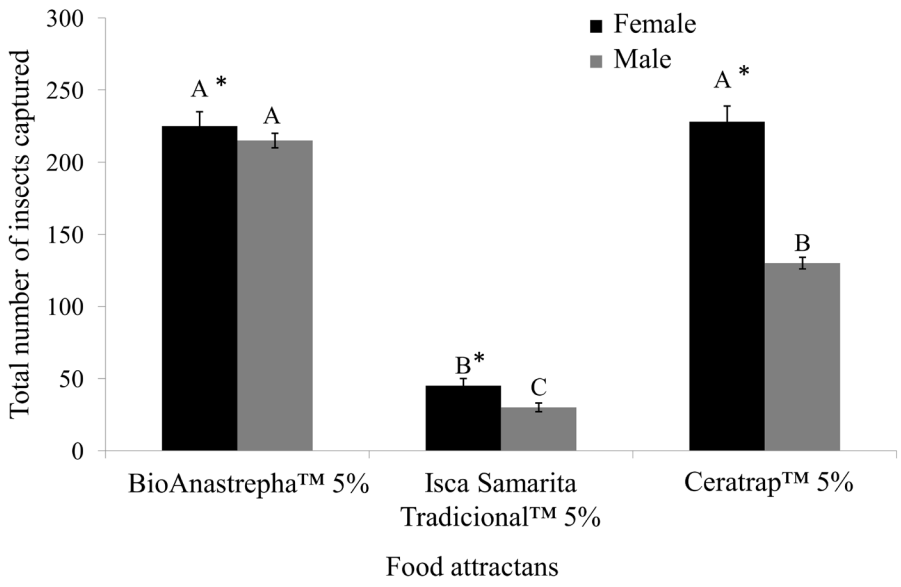


**Figure 2. Total number of males and females of *Ceratitis capitata* caught in McPhail traps baited with different food attractants during the day between 6h31am to 6h30 pm. Means (± SE) followed by the same capital letter in the same colored column do not differ significantly (Tukey, 0.05); Asterisks indicate significant differences between female and male within the same food attractant according to Student's t-test ($P < 0.05$).**

## DISCUSSION

The knowledge of the arrival behavior of fruit fly species inside the orchard can assist in the management of insects (Galli et al. 2019). In Brazil, the most used food attractants for the monitoring of *A. fraterculus* and *C. capitata* have been carried out using BioAnastrepha™ and Ceratrap™. Although Ceratrap™ is recommended for use for the mass capture strategy (Stupp et al. 2020). Using these two food attractants, the largest catches of *A. fraterculus* and *C. capitata* adults were observed during the daytime, between 06h:30 am and 6h:30 pm. According to Sugayama et al. (1997), the largest capture of adults during the day is associated with the behavior and bioecology of fruit flies, since during the night they are protected in the adjacent vegetation and during the day they migrate into the orchard, mainly in the first hours. During the night-time between 6h:30 pm and 6h:30 am, only three *A. fraterculus* adults were captured. However, the presence of these insects in the traps at night, probably occurred due to the capture of the insects in the first hours after the last evaluation carried out at 6h30 pm.

For all food attractants evaluated, the number of females was higher than the number of males this can be explained by the greater nutritional and physiological need of female fruit flies to consume protein components to occur sexual maturation and to generate offspring (Lasa et al. 2014, Bortoli et al. 2016). In addition, there was a numerical predominance of *A. fraterculus* adults captured in relation to *C. capitata* in McPhail traps in all evaluated attractions, as observed in other studies by Bortoli et al. (2016). This was due to *A. fraterculus* being the species with the highest occurrence in orchards in southern Brazil (Nunes et al. 2012, Bortoli et al. 2016, Araujo et al. 2019a) and because the species *C. capitata* is more concentrated in urban areas (Ricalde et al. 2012).

Regarding the activity search time for the food attractant, *A. fraterculus* adults looked for food in the morning from 10h30 am, with an increase until 2:30 pm. This information is extremely important for the management of the species in the orchard, since it helps in the definition of the best management strategies and the best time to meet the adults inside the crop (Sugayama et al. 1997). Probably the increase

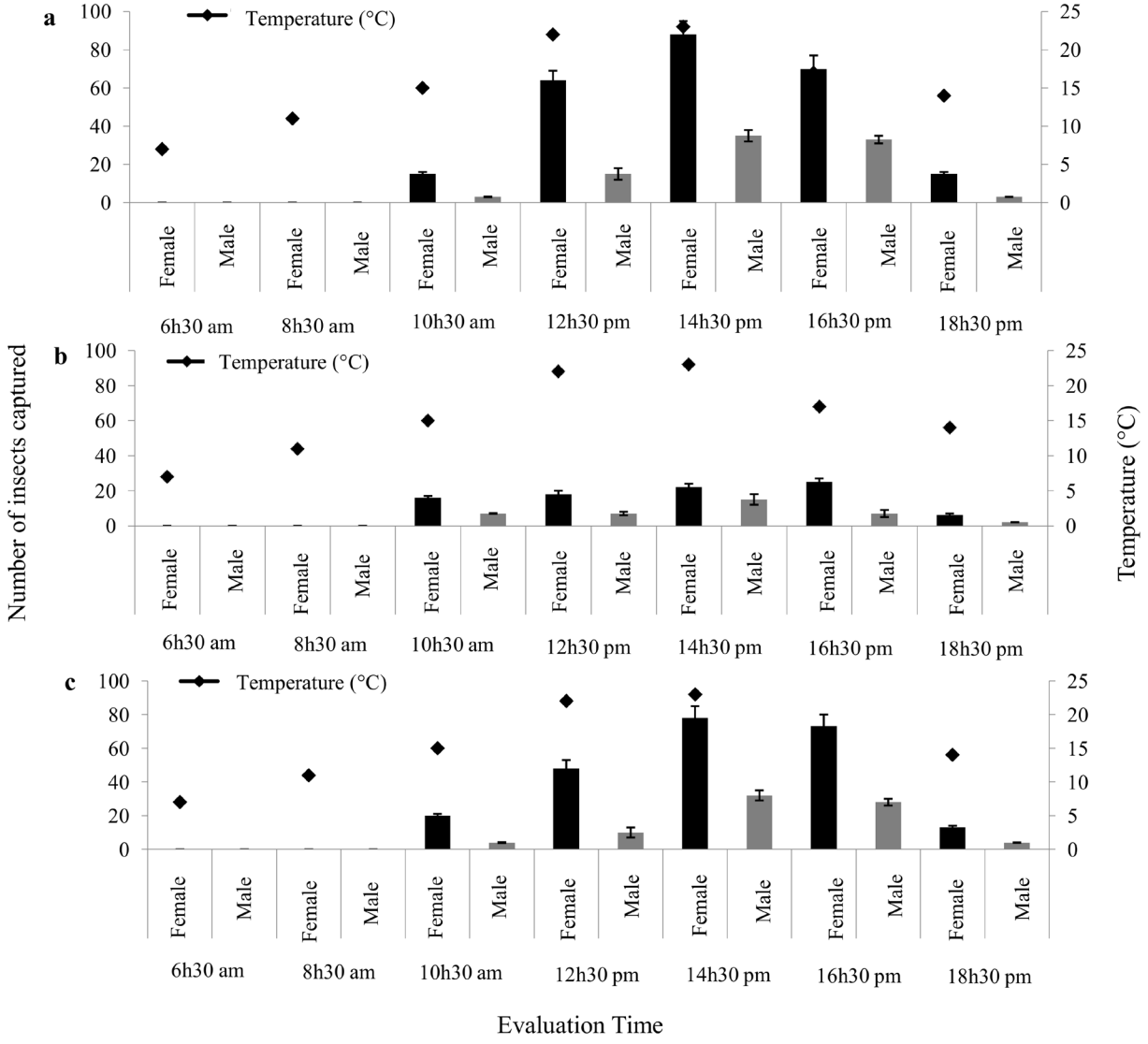


**Figure 3. Number of *Anastrepha fraterculus* adults captured every two hours during the day between 6h30 am to 6h30 pm in three food attractants. (a) BioAnastrepha™ 5%; (b) Traditional Samaritá™ bait 0.5% and (c) Ceratrap™ 1.5%.**

in temperature around 2h:30 pm provided a greater activity of searching for food in the orchards and, consequently, a greater capture in the monitoring traps. According to Lasa et al. (2014), higher temperatures can provide greater fermentation activity of the food attractants and, consequently, greater volatilization of the components of the attractant, favoring the search for odors released by fruit flies adults, which may have occurred in this study.

The main management strategies for *A. fraterculus* and *C. capitata* in Brazil, include the use of synthetic insecticides via total area application or applied in toxic bait form (Baronio et al. 2019, Nunes et al. 2020). In this way, the definition of the moment to find the adults of the pest inside the orchard can contribute to increase the efficiency of these strategies in the field (Lasa & Cruz 2015, Galli et al. 2019).

Good practices for the application of pesticides recommend doing it at the

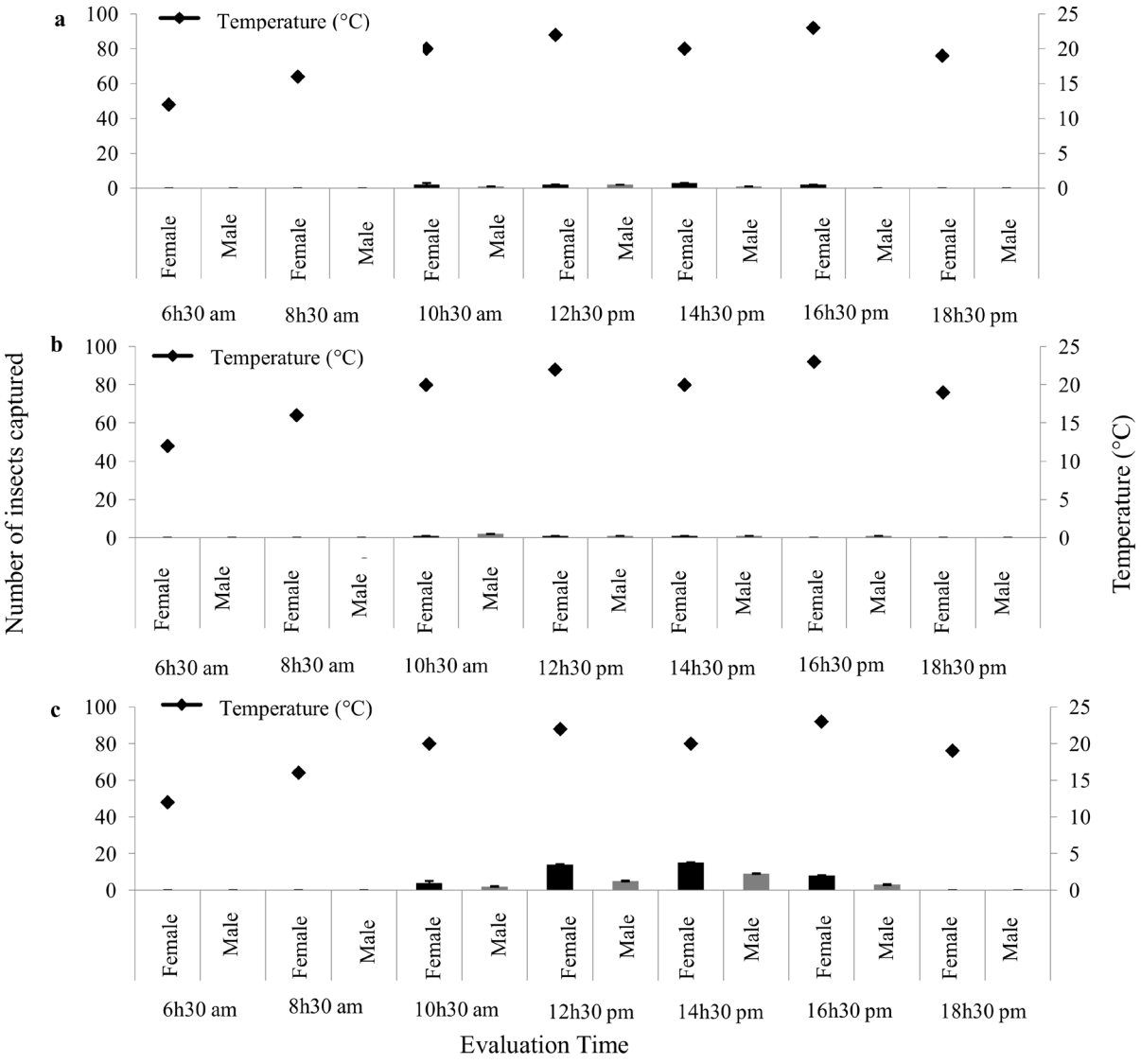


**Figure 4. Number of *Ceratitis capitata* adults captured every two hours during the day between 6h30 am to 6h30 pm in three food attractants. (a) BioAnastrepha™ 5%; (b) Traditional Samaritá™ bait 0.5% and (c) Ceratrap™ 1.5%.**

beginning or at the end of the morning when the temperature is milder and in this particular case for *A. fraterculus* and *C. capitata* it is recommended to apply around 4 pm when the insects are in the orchard and the temperature is already dropping. This situation occurs mainly for insecticides that have topical and contact action, as occurs with phosphorus and pyrethroid insecticides, chemical groups widely used in the management of fruit flies in Brazil (Botton et al. 2016).

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J. A. C. M.: investigation, methodology, data curation and writing—original draft; B. P.: investigation, methodology, and data curation; D. B.: writing—original draft and data analysis and conducting statistical analyses, review; D. E. N.: conceptualization, funding acquisition, project administration, supervision, writing—original draft, writing—review and editing. All authors have read and agreed to the published version of the manuscript.