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Infestation of guava by *Anastrepha fraterculus* (Diptera: Tephritidae): preferred ripening stages and influence of fruit peel coloration

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Abstract: The current study aimed to verify the preferred guava fruit ripening stage for egg laying by *Anastrepha fraterculus* (Diptera: Tephritidae) and to determine the influence of peel coloration of the fruits of 2 cultivars on infestation. The cultivars used were Paluma and Século XXI. The infestation level was evaluated in cages, considering 2 conditions: multiple-choice and nonchoice tests. The infestation levels of *A. fraterculus* differed between the green and the green-ripe stages in the multiple-choice test. In Paluma fruits with the nonchoice test, the infestation was highest during the ripe stage. There were no differences in infestation levels of *A. fraterculus* between the green and the green-ripe stages in Século XXI. In general, the green-ripe and the ripe stages were preferred for egg laying by *A. fraterculus*. In Paluma fruits there were significant correlations of infestation levels of *A. fraterculus* with luminosity (L) and chromaticity (C) in the nonchoice assay. In Século XXI fruits there was a significant correlation of infestation levels of *A. fraterculus* with color angle (*h*) in the nonchoice assay. No correlation was found between theses parameters in the multiple-choice test.

Key words: Fruit flies, Myrtaceae, preference-performance

1. Introduction

Over 2000 million hectares in Brazil are devoted to fruit, with a production of about 43 million tons/year, highlighting a regional specialization that is mainly influenced by weather. The regions closer to the equator produce tropical fruits such as guava (*Psidium guajava* (Myrtaceae)), banana (*Musa* spp. (Musaceae)), mango (*Mangifera indica* (Anacardiaceae)), melon (*Cucumis* spp. (Cucurbitaceae)), and cocoa (*Theobroma* spp. (Malvaceae)). In the southern regions there are temperate fruit trees such as peach (*Prunus persica* (Rosaceae)), apple (*Malus domestica* (Rosaceae)), and grape (*Vitis vinifera* (Vitaceae)) (Ibraf, 2010; Fachinello et al., 2011).

Fruit flies are major pests that decrease the production of fruits in Brazil and reduce its exports due to quarantine regulations imposed by importing countries (Duarte and Malavasi, 2000; Klassen and Curtis, 2005; Oliveira et al., 2014). The damage caused by these flies is reflected in the domestic market with the loss of fruit caused by larval feeding and by adults, specifically females, who make holes in the peel of fruits for egg laying, which in turn allows the entry of fungi, causing the fruits to rot. According to the FAO (United Nations Food and Agriculture), fruit flies cause damage amounting to $1,7 billion worldwide, 10% of which is Brazil's share (Ibraf, 2010).

Anastrepha fraterculus (Wiedemann, 1830) (Diptera: Tephritidae) is considered a major pest of guava (Malavasi and Morgante, 1981) and it has also been found on a wide number of other hosts, including commercial crops (White et al., 1994). A direct relationship between the infestation of fruit flies and fruit rottenness incidence in some crops, such as apples, can be frequently seen (Santos et al., 2008). The different behavior preference or nonpreference of *A. fraterculus* for egg laying in cultivars may be related to the nutritional quality of the fruits as well as their chemical characteristics and visual appearance (Zucoloto, 1993). During site selection for oviposition, aspects like plant size, color, odor, taste, ripening stage, and the presence of other eggs are evaluated by the tephritid females (Slansky, 1982). Chemical stimuli, nutrients, volatile substances, and inhibitors for feeds and feeding stimulation also have an effect on resource localization (McInnis, 1989). The determination of the preferred ripening stage for egg laying is of great importance for the cultural management

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of fruit flies. This preference can be dependent on the cultivar. Therefore, this study aimed to determine the preferred ripening stage of guava fruits for egg laying by *A. fraterculus* and its relation to physiochemical parameters of the Paluma and Século XXI fruit cultivars.

2. Materials and methods

The research was conducted in the Laboratory of Plant Resistance to Insects, Department of Plant Protection, Faculty of Agriculture and Veterinary Sciences (FCAV), UNESP – Jaboticabal, São Paulo, Brazil. The test of maturity stages of guava fruits preferred for egg laying of *A. fraterculus* was conducted with fruits of the cultivars Paluma and Século XXI. The fruits were obtained from the VAL Fruit Company, located in Vista Alegre do Alto, 35 km from Jaboticabal - SP. These cultivars were chosen based on their frequent cultivation in Brazil (El-Buluk, 1995; Anuário Brasileiro da Fruticultura, 2009). There is a difference in susceptibility between these 2 cultivars (Oliveira et al., 2014), Século XXI is more susceptible than Paluma to infestation by *A. fraterculus*, but these results are only applicable to the green-yellow fruits of guava.

Guava fruits were assayed during 3 stages of physiological maturity: the green, the green-yellow ripe, and the fully ripe stages. The stages of maturity were assayed according to Cavalini et al. (2006). Fruits were cleaned with sodium hypochlorite (1%), water-rinsed, and dried at room temperature on a paper towel before being placed in the test cages. Tested specimens of *A. fraterculus* were taken from mass rearing on an artificial diet (Salles, 1992), held at the Laboratory of Radioentomologia in the Centre for Nuclear Energy in Agriculture - CENA/USP, located in Piracicaba - SP. Attractiveness and infestation levels were determined in bioassays for each cultivar separately, considering 2 situations: multiple-choice test and nonchoice test. Both bioassays were conducted in a room at an average temperature of 25 ± 1 °C, relative humidity of 60 ± 10%, and photophase of 12 h.

Attractiveness and infestation levels were determined in both tests for each cultivar and expressed as number of visits (female/fruit) and number of offspring (larvae/female/fruit), respectively. The number of females attracted by the different treatments was counted at 1 min, 3 min, 5 min, 10 min, 20 min, 30 min, 60 min, 120 min, 180 min, 360 min, and 1440 min. The number of insects found on each fruit at each time increment was considered as the number of visits. After 10 days the fruits were dissected and the number of *A. fraterculus* larvae in each fruit was counted and recorded according to the procedure adopted by Oliveira et al. (2012).

2.1. Multiple-choice test

Three fruits (1 from each stage) of each cultivar were placed equidistant from each other inside a plastic cage (30-cm long, 30-cm wide, and 40-cm high). Ten such cages were set up and each cultivar was evaluated separately. Each cage was a replicate and corresponded to a block, allowing a randomized block design to be used. Fertile females (10 days after emergence) of *A. fraterculus* were used, with 3 being released per cage.

2.2. Nonchoice test

One fruit was placed on a cylindrical plastic cage (15 cm in diameter and 10-cm high). One fertile female (10 days after emergence) of *A. fraterculus* was released per cage. Ten cages for each stage/cultivar were arranged; each cage was a replicate and a fully randomized design was set. For the evaluations, the procedures utilized were the same as for the multiple-choice test.

2.3. Peel coloration analysis

Fruits of the 2 cultivars were harvested at the following stages: green, green-yellow, and fully ripe stages. The fruits were analyzed at the Laboratory of Technology of Agricultural Products, Department of Technology, Faculty of Agricultural Sciences and Veterinary (FCAV) - UNESP - Jaboticabal. We used 4 fruits for stage/cultivar, with each fruit considered a replicate.

The peel coloration of the fruits was inspected by a Minolta colorimeter, model CR-300 (configuration: LC h color system). Two readings were taken per fruit on the opposite sides of its equatorial region (McGuire, 1992). The parameter h (color angle) can range from 0° to 360°, where 0° corresponds to the color red, 90° to yellow, 180° to green, and 270° to blue. C is chromaticity, which sets the color intensity and assumes values close to 0 for neutral colors (gray) and around 60 for vivid colors (McGuire, 1992). L is luminosity of the peel; this parameter represents the brightness of the surface and follows a scale ranging from 0 (dark colors and opaque) to 100 (white color or maximum brightness).

2.4. Data analysis

The data regarding the presence of females for each treatment as a function of time were subjected to logistic regression analysis, fitting the data to binomial distribution, using the procedure GENMOD (SAS, 2006). The probability (P) of individuals occurring in the fruit was estimated using the model:

$$P = \exp(P_0 + P_1 x + P_2 x^2 + P_3 x^3)/1 + (\exp(P_0 + P_1 x + P_2 x^2 + P_3 x^3),$$

where exp is the exponential function, x is time and P_0, P_1, P_2, and P_3 are the logistic regression parameters associated with the slope of the curve. The infestations were compared by Student–Newman–Keuls (SNK) test ($P < 0.05$). We tested the normality (Shapiro–Wilk and Kolmogorov D) and homogeneity of variances (Bartlett's test). For physicochemical analyses, the data were subjected to ANOVA and the means were compared by SNK test. All figures were plotted by Sigma Plot software (version 11.0). Pearson's correlation analyses were used to study relationships between infestation levels and physicochemical parameters of fruits using the procedure CORR (SAS Institute, 2002).

3. Results

3.1. Multiple-choice test

The results in this assay showed that the interaction between ripening stage and cultivar was not significant ($F_{2,5}$ = 1.15, P > 0.05). Therefore, the data of infestation were summed and analyzed together. Fruit ripening stages significantly affected the infestation rate of *A. fraterculus*. The green-yellow stage was found to have the highest level of infestation (5.44 larvae/female/fruit), followed by the fully ripe stage (4.25 larvae/female/fruit), and the green stage (1.77 larvae/female/fruit) (Figure 1).

The curves of the visits proportion as a function of time were generated using the following model: $\exp(P_0 + P_1 x + P_2 x^2)/1 + (\exp(P_0 + P_1 x + P_2 x^2)$ (Figures 2 and 3). The quadratic coefficient of regression (P_2) was positive for

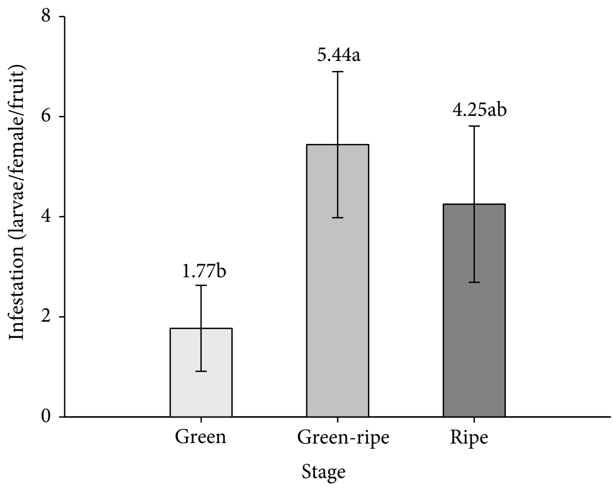

Figure 1. Multiple choice test: Mean (± SE) of the number of larvae of *Anastrepha fraterculus* per female per guava of the Paluma and Século XXI cultivars, at the green, green-ripe, and ripe stages. Means followed by the same letters do not differ by SNK test (P = 0.05).

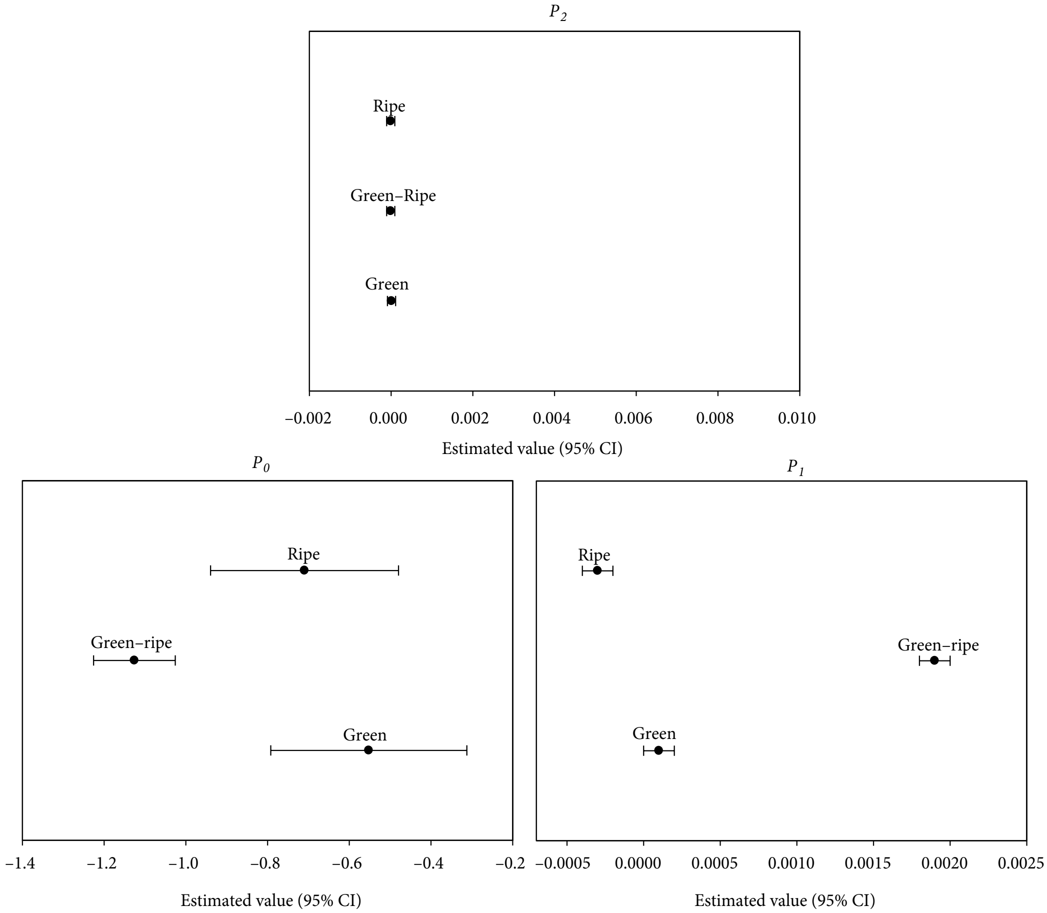

Figure 2. Multiple choice test: Estimates of logistic regression parameters and confidence intervals (95% CI) of visits proportion of *Anastrepha fraterculus* female/fruit of the Paluma cultivar. P_0, P_1, P_2 are the logistic regression parameters associated with the slope of the curve.

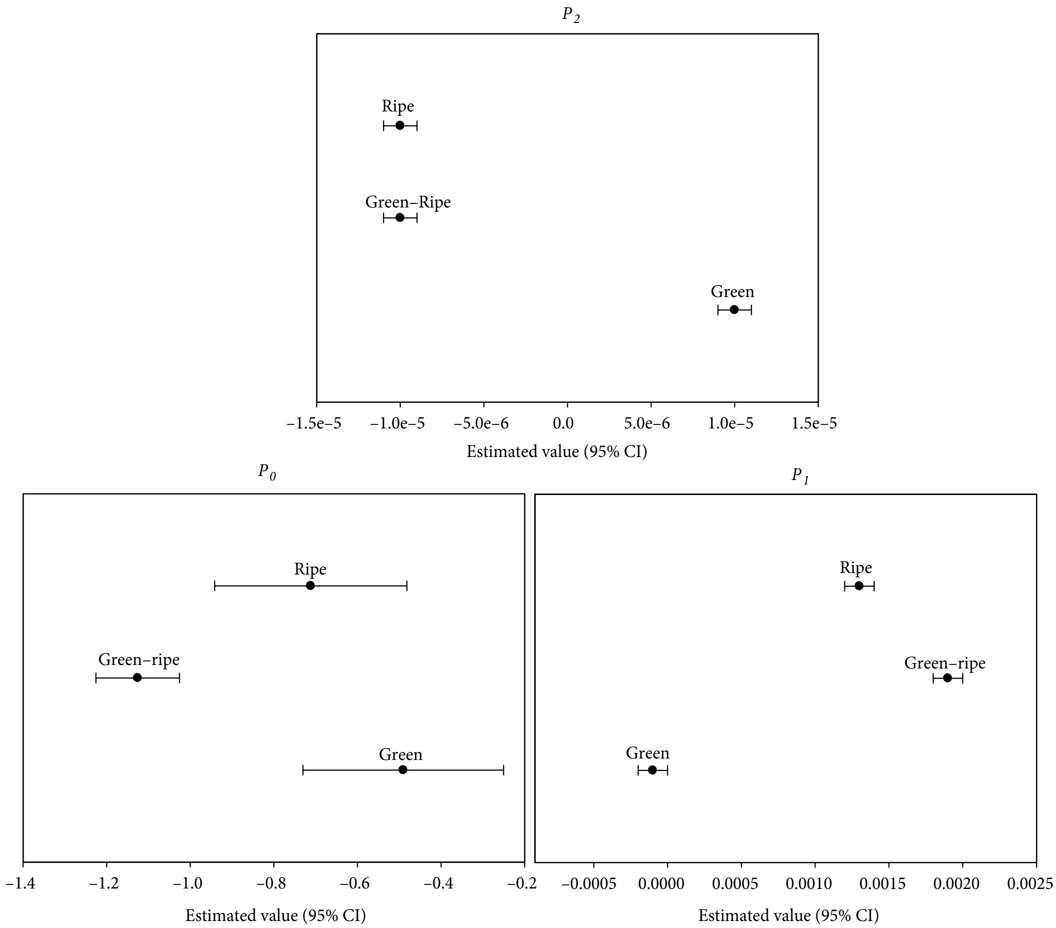

Figure 3. Multiple choice test: Estimates of logistic regression parameters and confidence intervals (95% CI) of visits proportion of *Anastrepha fraterculus* female/fruit of the Século XXI cultivar. P_0, P_1, P_2 are the logistic regression parameters associated with the slope of the curve.

the green stage in both cultivars. At the green and green-ripe stages, this coefficient was negative in Paluma and Século XXI cultivars (Figure 2). In the Século XXI fruits the visits proportions were significantly higher at the green stage than at the green-ripe and the ripe stages (Figure 3). However, in the Paluma fruits the visits proportions were significantly higher at the green-ripe stage than at the green and the ripe stages. The proportion of individuals per fruit increased at the green stage and significantly decreased at the other stages (green-ripe and ripe) 120 min after the release of *A. fraterculus* (Figure 4).

3.2. Nonchoice test

In the nonchoice test the interaction between stage of ripening and cultivar was significant ($F_{2,5} = 3.21$, $P < 0.0001$). The *A. fraterculus* infestation in the Paluma fruits differed among the ripening stages, but did not significantly differ in the Século XXI fruits. In the Paluma fruits the fully ripe stage had the highest level of infestation (8.75 larvae/female/fruit), followed by the green stage (3.16 larvae/female/fruit) and the green-yellow stage (2.62 larvae/female/fruit). For the Século XXI cultivar, it was observed that the ripening stage with the highest rate of infestation was the green-yellow stage (7.10 larvae/female/fruit), but it was not significantly different from the green (6.10 larvae/female/fruit) and the ripe stages (3.10 larvae/female/fruit).

Regarding the comparison of *A. fraterculus* infestation levels between the 2 cultivars within the same stage, it was observed that only the infestation at the green stage did not show a significant difference. At the green-ripe stage Século XXI fruits had significantly higher infestation levels (6.10 larvae/female/fruit) than the Paluma fruits (2.62 larvae/female/fruit), while at the fully ripe stage the Paluma fruits were significantly more infested (8.75 larvae/female/fruit) than the Século XXI fruits (3.10 larvae/female/fruit) (Figure 5).

The curves of visits proportions as a function of time were generated using the model: exp $(P_0 + P_1 x)/ 1 +$ (exp

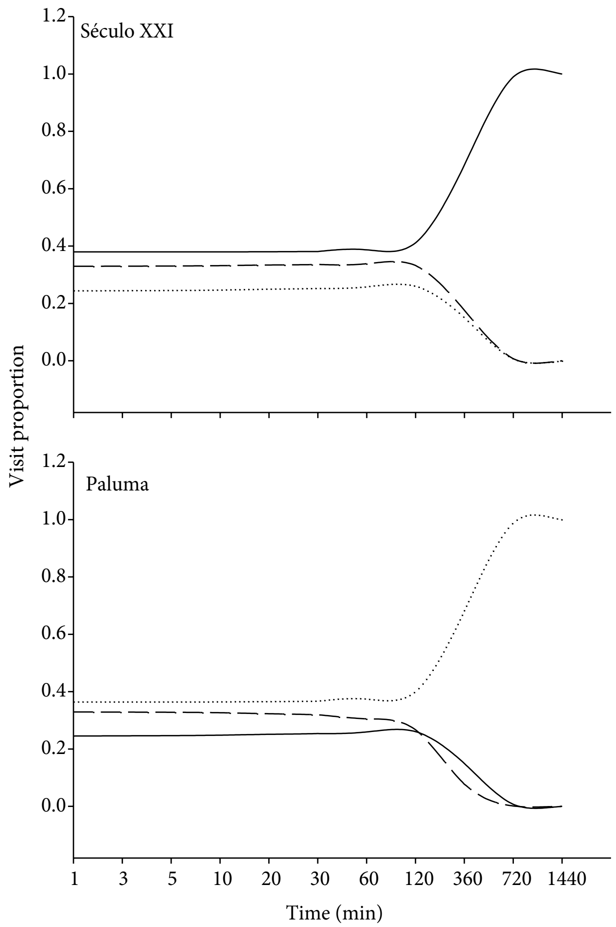

Figure 4. Multiple choice test: Logistic regression of visits proportion of *Anastrepha fraterculus* female/fruit of the guava cultivars of Paluma and Século XXI at the following stages: green (———), green-ripe (············), and ripe (------).

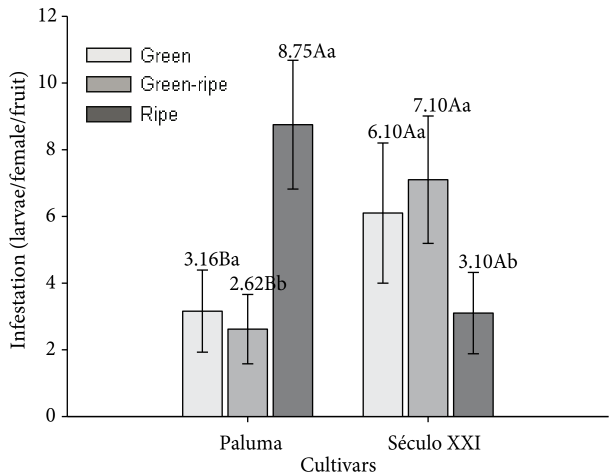

Figure 5. Nonchoice test: Mean (±SE) of the number of larvae of *Anastrepha fraterculus* per female per guava of the Paluma and Século XXI cultivars, at the green, green-ripe, and ripe stages. Means followed by the same capital letters (within same cultivar) and lower letters (within same stage) do not differ by SNK test (P = 0.05).

(P_0 + P_1 x). In the nonchoice test the linear regression coefficients (P_1) were positive for the green-ripe and fully ripe stages of the Paluma (Figure 6), and for the green stage of the Século XXI (Figure 7); they were negative for the remaining conditions(Figures 6 and 7). In the Paluma fruits, for the majority of the time, the visits proportions were significantly higher at the green stage than at the green-ripe and ripe stages (Figure 8). The visits proportion increased significantly in the green-ripe stage fruits of Paluma after 720 min. In the Século XXI cultivar, however,

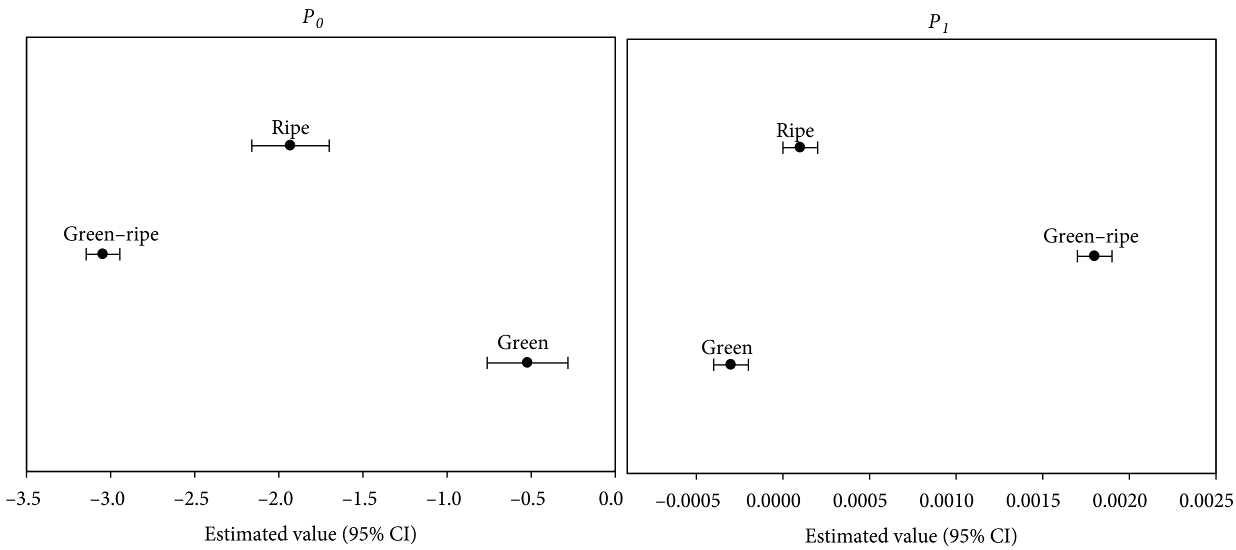

Figure 6. Nonchoice test: Estimates of logistic regression parameters and confidence intervals (95% CI) of visits proportion of *Anastrepha fraterculus* female/fruit of the Paluma cultivar. P_0, P_1 are the logistic regression parameters associated with the slope of the curve.

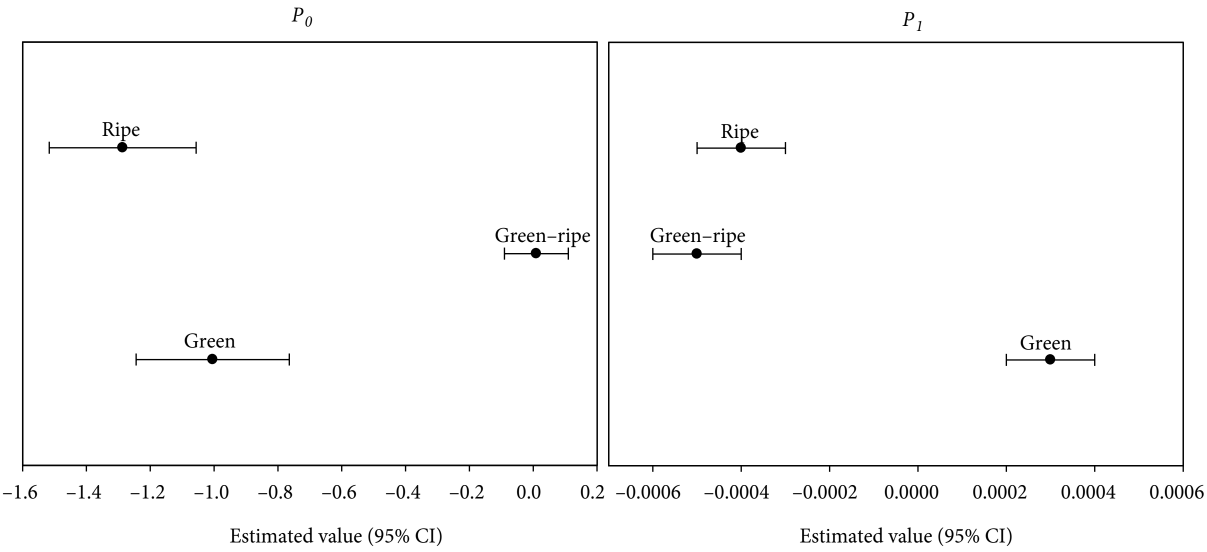

Figure 7. Nonchoice test: Estimates of logistic regression parameters and confidence intervals (95% CI) of visits proportion of *Anastrepha fraterculus* female/fruit of the Século XXI cultivar. P_0, P_1 are the logistic regression parameters associated with the slope of the curve.

the green-ripe stage fruits had the highest visits proportion between 1 min and 720 min. The visits proportion of *A. fraterculus* was practically constant for 30 min following the release of females on the green stage fruits of the Século XXI cultivar; after 120 min a significant increase in this proportion was found. A decrease in the visits proportions at the green-ripe and the ripe stages was observed 120 min following the release of the insects (Figure 8).

3.3. Peel coloration and relation with infestation level of *A. fraterculus*

There was a significant difference between the ripe and the green stages in the color parameters luminosity (L) and color angle (h) in the Paluma fruits. With regards to the chromaticity (C) parameter in the Paluma fruits, the stage that provided the highest value was the green-ripe stage (48.43), followed by the green (45.97) and the ripe (40.45) stages. There was no difference among the green, the green-ripe, and the ripe stages in the luminosity and chromaticity color parameters. A significant difference was found in the color angle (h) of Século XXI fruits, with the lower h parameter being observed at the green-ripe stage; there was no difference between the green and the ripe values of h (Table 1).

Correlations were observed between infestation levels of *A. fraterculus* and luminosity (L) and chromaticity (C) of the Paluma fruits in the nonchoice assay. There was a significant correlation between the infestation levels of *A. fraterculus* and the color angle (h) of Século XXI fruits in the nonchoice assay. No correlation was found between these parameters in the multiple-choice test (Table 2).

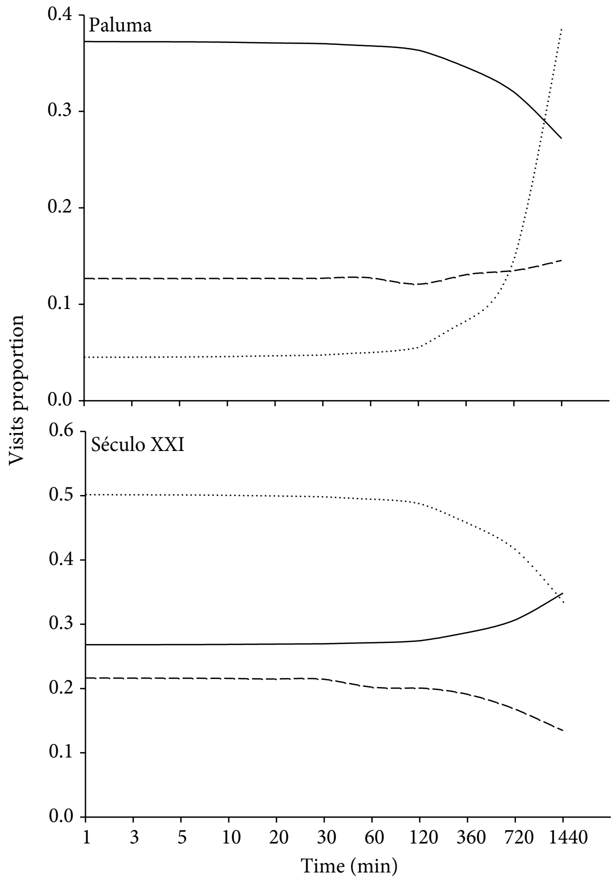

Figure 8. Nonchoice test: Logistic regression of visits proportion of *Anastrepha fraterculus* female/fruit of the guava cultivars Paluma and Século XXI at the following stages: green (———), green-ripe (············), and ripe (------).

Table 1. Peel coloration of the 3 stages of fruit ripening of the Paluma and Século XXI cultivars.

Stage	Cultivars					
	Paluma			Século XXI		
	L	C	H	L	C	h
Green	63.90 ± 1.23 b	45.97 ± 0.89 b	106.87 ± 5.06 a	64.12 ± 3.08 a	48.73 ± 2.03 a	108.40 ± 2.54 a
Green-ripe	74.18 ± 2.09 ab	48.43 ± 1.08 a	99.95 ± 2.34 ab	69.74 ± 4.55 a	51.78 ± 4.57 a	97.47 ± 1.06 b
Ripe	80.15 ± 1.25 a	40.45 ± 0.97 c	86.59 ± 1.08 b	64.57 ± 1.36 a	49.44 ± 1.29 a	104.60 ± 2.67 a

L = luminosity; C = chromaticity; h = color angle. Means followed by the same letter do not differ by SNK test at 5% probability.

Table 2. Values of Pearson's correlation coefficients[1] and probabilities[2] between infestation level of *Anastrepha fraterculus* and fruits' physicochemical characteristics of the Paluma and Século XXI cultivars.

Cultivar/Stage	Parameter		
	L	C	h
Paluma			
Multiple choice	r = 0.5145; P = 0.2667	r = 0.1710; P = 0.8906	r = –0.7300; P = 0.4667
Nonchoice	r = –0.7281; P = 0.0480	r = 0.9100; P ≤ 0.0001	r = –0.5010; P = 0.6670
Século XXI			
Multiple choice	r = 0.6396; P = 0.5582	r = 0.5100; P = 0.6700	r = –0.4047; P = 0.7347
Nonchoice	r = 0.6299; P = 0.5672	r = 0.5157; P = 0.6550	r = –0.9799; P ≤ 0.0001

L = luminosity; C = chromaticity; h = color angle. [1]r = Pearson's correlation coefficients. [2]P = Probability > r.

4. Discussion

The results of the present study showed that differences exist in the infestation levels of *A. fraterculus* among the stages studied. Moreover, the cultivar differences depend on the bioassay (multiple or nonchoice test). In the multiple-choice test, lower infestation was found in the green stage in comparison to the green-ripe stage. In the nonchoice test significant differences were detected between the cultivars and among the ripening stages. In the nonchoice test the susceptibility of guava cultivars depended on the maturity stage. In fact, in Paluma fruits the highest infestation level was observed in the ripe fruits. However, in the ripe fruits of the Século XXI cultivar a lower infestation was observed in comparison with other stages of this cultivar and with Paluma ripe fruits (Figure 2). Similar results were found in others studies (Uchôa-Fernandes et al., 2002). Therefore, the maturity stage of the hosts can be a decisive factor for the attractiveness and level of infestation of *A. fraterculus*, considering that this factor is directly related to the peel color of the hosts and the levels of nutrients. The fruit flies of tropical and subtropical regions are transient and tend to settle in areas where fruits undergo a ripening process (Selivon, 2000). Senescent fruits are more susceptible to attacks by fruit flies than fruits early in the season (Greany, 1989). The susceptibility data are of great importance for the design of management strategies and the development and assessment of plant-based synthetic odor baits (Nyasembe et al., 2014). According to Oliveira et al. (2014), in both tests (multiple and nonchoice) the rate of fruit infestation by *A. fraterculus* did not differ among the Pedro Sato, Paluma, and Cascão cultivars, whereas the fruits of the Século XXI cultivar were more infested. In that study only the green-ripe fruits were evaluated. In *B. dorsalis*, a greater preference was found for green-ripe or ripe mangoes due to the softness of the pericarp; the chemical differences of fruits in different ripening stages are seen as responsible for fruit fly attacks (Reissig, 1979; Bower, 1997). *Anastrepha obliqua* (Diptera: Tephritidae) were more attracted to lime-green, orange, and yellow spheres than to red, black, or white spheres (Lopéz-Guillén et al., 2009).

Flies were more attracted to lime-green spheres baited with mombin fruit odor, *Spondias mombin* L., than to spheres offering either visual or chemical cues alone (Lopéz-Guillén et al., 2009). In the present study it was observed that the data of infestation levels of *A. fraterculus* did not follow predicted patterns of infestation: the visits proportions in the Século XXI fruits were significantly higher in the green stage than in the green-ripe and ripe stages (Figure 3). However, in the Paluma fruits the visits proportions were significantly higher in the green-ripe stage than in the green and ripe stages. The difference between infestation data and attractiveness may be due to various reasons for visiting the fruits, such as rest or refuge, rather than solely for egg laying. Furthermore, the action of inserting the ovipositor depends on factors such as type of host (primary or secondary) and physicochemical fruit aspects, and takes into account the degree of fruit maturation and the presence of marking pheromones and previously laid eggs (Aluja et al., 2000).

Fruit flies from larger fruits with better nutritional composition are expected to show better performance, especially regarding reproductive success, as this is directly related to the intake during the larval stage of 2 primary nutrients, proteins and carbohydrates. In the immature stage, the quantity and quality of nutrient intake affect weight, development time, the chemical composition of the body, adult size, and egg production, as the reproductive success of fruit flies depends on the ability of the female to find a host that provides all the nutrients for their young (Zucoloto, 2000). The coloration of fruits and the odors released by the fruits during the ripening stages are also related to their attractiveness as hosts for fruit flies (Vargas, 1991; Messing and Jang, 1992; Bautista and Harris, 1996). In some cases, the preference of a fruit fly host is due to the richness of sugars. However, other factors such as color, texture, and volatiles emitted by plants also influence the choice of the fruit fly for egg laying (Cruz, 2000). The pH indexes did not interfere with the infestation of *A. fraterculus*, whereas a high rate of soluble solids and low color angle appear to be crucial for discriminating the fruits of the most susceptible cultivars. Infestation rates of Século XXI fruits displayed significant correlations with °Brix and color angle (h) of guava fruits under the multiple-choice conditions (Oliveira et al., 2014). The results of the present study showed that the highest value of the chromaticity (C) parameter was detected in the green-ripe stage of Paluma fruits. With respect to the hue angle (h), the stages of the studied cultivars showed a yellow color with values of around 100, assuming that 90° corresponds to yellow (Table 1). In Século XXI fruits a lower value of the h parameter was observed in the green-yellow stage. In addition, the relation between peel and infestation level depends on specific parameters within each cultivar. In Paluma fruits, there were significant correlations between infestation levels of *A. fraterculus* and luminosity (L) and chromaticity (C) in the nonchoice assay. In Século XXI fruits, there was a significant correlation between infestation levels of *A. fraterculus* and color angle (h) in the nonchoice assay.

A high flexibility of behaviors displayed by a species makes it more likely to adapt to new conditions, as stated in Sugayama and Malavasi (2000). Thus, a polyphagous insect with high behavioral flexibility may, for example, maintain a large number of hosts, which can be related to the present study, where all stages of ripening of the varieties studied were, to some degree, infested and attractive to *A. fraterculus*. In the multiple-choice test the stage preferred by *A. fraterculus* infestation was green-yellow; in the nonchoice test, *A. fraterculus* preferred to lay eggs in ripe fruits in the Paluma cultivar. In Paluma fruits, there were significant correlations between infestation levels of *A. fraterculus* with luminosity (L) and chromaticity (C) in the nonchoice condition. In Século XXI fruits, there was a significant correlation between infestation levels of *A. fraterculus* with color angle (h) in the nonchoice condition. These results are of great importance in the cultural control of *A. fraterculus*, as the adoption of criteria on plants baits and the development of baits with synthetic odor depend on the ripening stages of the cultivar. In particular, tephritid studies about genotypes or ripening stages in terms of low susceptibility to fruit flies may contribute to the IPM programs for these pests, especially for management of insect resistance, because it is possible to use the most susceptible cultivar as a refuge in areas with insect resistance genotypes. However, the results of laboratory tests with picked fruits need to be interpreted with caution because fruit conditions and chemical profiles of the fruits can change radically after they are removed from the tree (Turgeon et al., 1998), thereby affecting the adult preference of a fruit and the offspring performance of a species (Lloyd et al., 2013).

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