

## Research Article

## Performance of *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) rearing on fruits of two guava varieties under forced infestation

Rendimiento de *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) en frutas de dos cultivares de guayaba bajo infestación forzada

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**Abstract.** *Anastrepha fraterculus* (Wiedemann) is dominant in the main guava's crops. Guavas are an important host of *A. fraterculus* for providing a good performance for the immature and high reproductive capacity. We evaluated the performance of *A. fraterculus* in two guava varieties related to the fruit physicochemical composition under laboratory conditions. Mature-green guavas of "Tailandesa" (red pulp) and "Kumagai" (white pulp) varieties were exposed to forced infestation by *A. fraterculus* in laboratory. An additional sample of each variety was submitted to physicochemical analysis. Similar quantities of pupae per fruit (128.5 – 156.0) and pupal viability (85.9% and 87.5%) were obtained per variety, but "Kumagai" produced significantly more pupae of *A. fraterculus* per fruit mass (1000.6 pupae/kg). Pupal weight was higher in "Tailandesa" (115.3 mg/10 pupae) than "Kumagai" (82.6 mg/10 pupae). Significant positive correlations were obtained between pupae per fruit or kg versus adults per kg for both varieties. A positive correlation was observed between pupae per fruit and pupae per kg of "Kumagai" guavas. Peel and pulp firmness were inversely correlated with pupae per fruit and pupae per kg. Weight of 10 pupae of *A. fraterculus* was correlated with fruit weight of "Tailandesa" guavas. The multivariate analysis PCA showed a relationship between pupae per kg and peel firmness, pulp firmness, Ratio, soluble solids, and titratable acidity in "Kumagai" guava. This explains the higher infestation index pupae per kg and better reproductive performance of *A. fraterculus* in "Kumagai" guava.

**Key words:** Infestation indices, Insecta, physicochemical parameters, *Psidium guajava*.

**Resumen.** *Anastrepha fraterculus* (Wiedemann) es dominante en los principales cultivos de guayaba. Las guayabas son un hospedador importante de *A. fraterculus* por brindar un buen desempeño a los inmaduros y con alta capacidad reproductiva. Evaluamos el comportamiento de *A. fraterculus* en dos variedades de guayaba relacionados con la composición fisicoquímica del fruto en condiciones de laboratorio. Las guayabas maduras de color verde de los cultivares "Tailandesa" (pulpa roja) y "Kumagai" (pulpa blanca) fueron expuestas a la infestación forzada por *A. fraterculus* en el laboratorio. Una muestra adicional de cada variedad se sometió a análisis fisicoquímico. Se obtuvieron cantidades

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similares de pupas por fruto (128,5 - 156,0) y viabilidad de pupas (85,9% y 87,5%) por cultivar, pero "Kumagai" produjo significativamente más pupas de *A. fraterculus* por masa de fruto (1000,6 pupas / kg). El peso de las pupas fue mayor en "Tailandesa" (115,3 mg / 10 pupas) que en "Kumagai" (82,6 mg / 10 pupas). Se obtuvieron correlaciones positivas significativas entre pupas por fruto o kg versus adultos por kg para ambos cultivares. Se observó una correlación positiva entre pupas por fruto y pupas por kg de guayabas "Kumagai". La firmeza de la cascara y la pulpa se correlacionó inversamente con las pupas por fruto y las pupas por kg. El peso de 10 pupas de *A. fraterculus* se correlacionó con el peso del fruto de las guayabas "Tailandesa". El análisis multivariado PCA mostró una relación entre pupas por kg y firmeza de la cascara, firmeza de la pulpa, relación sólidos solubles y acidez titulable en la guayaba "Kumagai". Esto explica el mayor índice de infestación de pupas por kg y el mejor desempeño reproductivo de *A. fraterculus* en la guayaba 'Kumagai'.

**Palabras clave:** Índices de infestación, Insecta, parámetros fisicoquímicos, *Psidium guajava*.

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## Introduction

In fruit flies (Diptera: Tephritidae), host-marking generally involves dragging the aculeus inside the fruit and depositing a host-marking pheromone on it. Females should be selective — choosing to place eggs on hosts that tend to be associated with relatively high juvenile growth and survival (Diaz-Fleischer *et al.* 2000). The knowledge of larval performance under different fruit compositions should therefore shed light on the determinants of insect host ranges (Hafsi *et al.* 2016).

The perfect correlation between oviposition preference and performance indicate that *Bactrocera invadens* (Drew, Tsuruta & White) females maximize offspring survival (Akol *et al.* 2013). Females of *Anastrepha ludens* (Loew) not only produce eggs continuously to quickly respond to egg-laying opportunities, but the eggs they produce are largely fertile (Aluja *et al.* 2011).

Fruit flies are persistent pests in guava (*Psidium guajava* L.) crops (Gould & Raga 2002). *Anastrepha fraterculus* (Wied.) has been observed in 116 fruit hosts (Zucchi & Moraes 2008) and is found in wild and commercial guavas in Brazil (Raga *et al.* 2005, 2006). Fruit fly infestations begin when guavas reach 2 cm (Souza-Filho *et al.* 2009) and remain until harvesting.

Guava is one of the most widely grown fruits in tropical and sub-tropical regions of the world, with established markets in more than 60 countries (Mitra & Irenaeus 2018). The production area of guava in Brazil was estimated to be 21,500 ha in 2018 (IBGE 2019). Under pruning and irrigation systems, the growing region of Campinas (São Paulo) produces table guavas of both red and white pulps year-round.

Orchards have higher uniformity because trees of the same cultivar are clones produced via vegetative propagation and grafted on to rootstocks that are now commonly produced vegetatively as well (Kogan & Hilton 2009). The continuous and high-level of damage inflicted by fruit flies on guava crops is caused by both susceptible variety and continuous fructification (Raga *et al.* 2006). The susceptibility of commercial guava varieties likely is related to fruit volatiles for female oviposition and favourable physicochemical characteristics of the fruit for larval development.

Guava exhibit thick pulp, few seeds and stone cells, and the desirable fruit characteristics for table purposes are high sugar concentrations and typical aroma (Singh 2011). Collecting fruits from guava germplasm field, Raga *et al.* (2006) obtained a maximum of 49 pupae of fruit fly per fruit, where *A. fraterculus* was dominant. The larval density of tephritids varied according to the guava growing region and respective temporal sampling (Raga *et al.* 2005); however, little is known about the level of larvae support each fruit provides and the

consequent larval performance in different varieties. Here, we associate the performance of *A. fraterculus* rearing in two guava varieties with the physicochemical composition of the fruit under laboratory conditions.

## Material and Methods

### *Infestation Bioassay*

Mature green guavas from cultivars "Tailandesa" (red pulp) and "Kumagai" (white pulp) were collected from a commercial orchard located in Campinas, State of São Paulo, Brazil. The orchard conducts fruit bagging without application of synthetic insecticide. Fruit were immediately brought to the laboratory and washed with a neutral detergent to remove any residue. The mean fresh fruit weight (g) was measured immediately prior to infestation.

*Anastrepha fraterculus* specimens tested here were sourced from colonies that have been maintained in the Instituto Biológico (Campinas) since 1993 (Raga & Sato 2011). Forty-two fruits of each variety were exposed to mature, 17-day-old females of *A. fraterculus* for 24 hours, with 10 females being exposed per fruit. The total number of guavas per variety were separated equally into two laboratory cages (1 × 1 × 1 m) during the infestation, under 14:10 L:D photoperiod.

After infestation, the guavas were separated and kept in circular plastic containers, 15 cm in diameter (1 litre), containing approximately 1.5 cm of vermiculite substrate. The containers were capped with voile and bound with an elastic. We evaluated the recovered pupae and adults approximately 22 and 38 days after infestation, respectively. Ten pupae per fruit were weighted (mg). Experiments were undertaken in a room at 21.4-26.0 °C and 46-68% RH.

### *Fruit physicochemical analysis*

Ten additional fruits of each variety were collected on the same date of the infestation assay for physicochemical analysis. The following fruit parameters were analysed in the Laboratory of Physiology and Postharvest of the Instituto Agrônômico.

**Colour:** determined using the Minolta BC 10 colourimeter and expressed according to the system proposed by the Commission Internationale de L'Eclairage (CIE) in  $L^* a^* b^*$  (two readings per peel and pulp colour). The values were expressed in  $a^*$  (from green to red),  $b^*$  (from blue to yellow), luminosity (L), hue angle, which indicates the location of the colour in a diagram and is calculated using the formula:  $\text{Hue} = \text{tg}^{-1} (b / a)$ , and chromaticity, which indicates the intensity of colour and is calculated using the formula:  $C = (a^2 + b^2)^{1/2}$ ;

**Firmness:** determined with a manual penetrometer with a tip diameter of 8 mm and a penetration of 6 mm. Two readings per fruit in the middle region (before and after peel removal) were taken. The results were expressed in Newtons (N);

**Titrateable acidity (TA):** determined using 10 g of pulp ground in a blender and homogenized with 90 mL distilled water. The sample was titrated using a pH-meter with a sodium hydroxide solution (0.1N) until a pH of 8.1 was reached. Results were expressed in grams of citric acid per 100 g of sample;

**pH:** measured using a digital pH-meter with the electrode placed directly into the blended pulp;

**Soluble solids (SS):** estimated using a bench refractometer with a  $\pm 0.0003$  precision by placing a small sample of blended pulp on the reading prism. Results were expressed as percentages;

**Ratio:** the ratio between SS/TA.

### Statistical analysis

We considered each fruit as one replication for the fruit fly infestation assay. The level of infestation for the two species and for the various bag colours was measured by the number of pupae and adults produced per fruit and was compared using a one-way analysis of variance (Sisvar, version 5.6) at  $P < 0.05$  (Ferreira 2019). Pearson's correlation (p-value  $< 0.05$ ) using SAS University Edition software (Version 3.8) (SAS Enterprise Miner 13.1. SAS Institute Inc., Cary, NC.) was used to determine the interaction between the two factors. Principal component analysis (PCA) was conducted using R statistical software, version 4.0.1 (R Core Team 2019), and applied to all analytical data collected for the guavas.

## Results

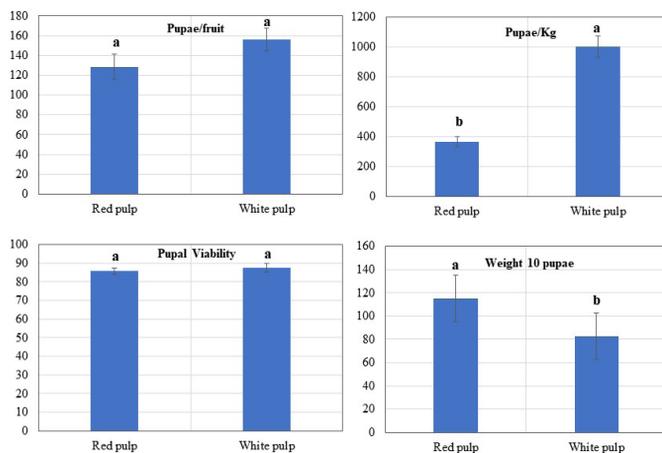
"Tailandesa" guavas produced similar quantities of *A. fraterculus* compared to "Kumagai" guavas, corresponding to 128.5 (range 0 – 382) and 156.0 (range 3 – 304) pupae per fruit (Student's test,  $P < 0.05$ ), respectively (Figs. 1, 2). However, significantly more pupae of *A. fraterculus* per kg of fruit were recovered from "Kumagai" guavas than from "Tailandesa" guavas, corresponding to 1000.6 (range 21.1 – 1788.2) and 365.3 (range 0 – 855.5) pupae, respectively (Figs. 1, 2). No statistical differences were detected between guava varieties in terms of pupal viability (85.9% and 87.5%) (Figs. 1, 2). Pupal weight was higher in "Tailandesa" (115.3 mg/10 pupae) than in "Kumagai" (82.6 mg/10 pupae) (Figs. 1, 3).

The values of physicochemical characteristics from the two guava varieties are shown in Table 1. "Tailandesa" guavas exhibited a significantly higher fruit weight, a, hue and chroma of pulp (Student's test,  $P < 0.05$ ). L,  $a^*$ ,  $b^*$ , hue and chroma of skin, pH, titratable acidity (TA) and ratio (SS/TA) were statistically similar between the varieties. "Kumagai" (white pulp) fruit exhibited higher values of pulp L and b and peel and pulp firmness than "Tailandesa" fruit (red pulp).

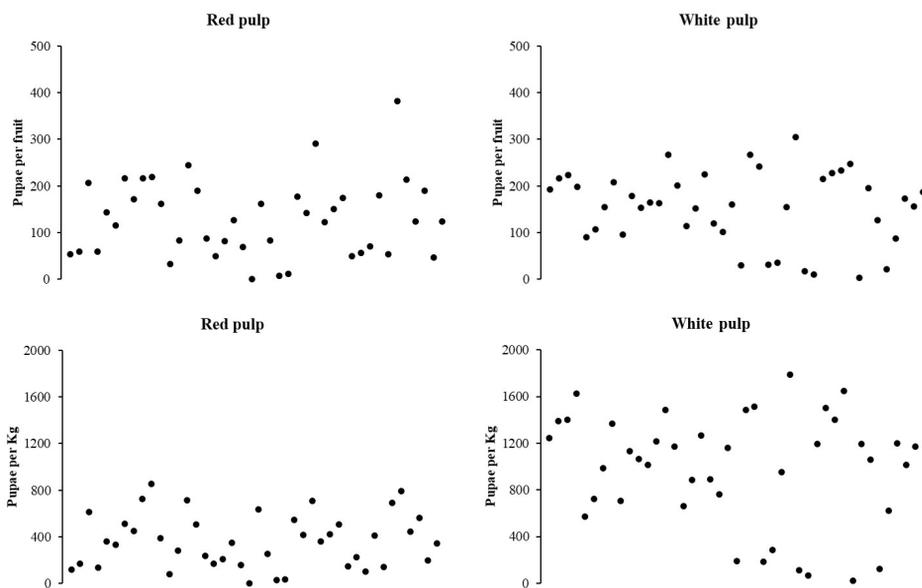
We obtained a significant positive correlation between the number of pupae per fruit or kg versus the number of adults per kg for "Tailandesa" ( $r = 0.94$ ,  $P < 0.0001$ ;  $r = 0.99$ ,  $P < 0.0001$ ) and "Kumagai" ( $r = 0.95$ ,  $P < 0.0001$ ;  $r = 0.94$ ,  $P < 0.0001$ ), respectively. No significant correlation was detected between pupal viability and infestation indices for "Tailandesa" guavas (Fig. 3); however, a positive correlation was observed between pupal viability with pupae per fruit ( $r = 0.85$ ,  $P < 0.001$ ) and pupae per kg ( $r = 0.80$ ,  $P < 0.001$ ) of "Kumagai" guavas (Fig. 3). Peel ( $r = -0.59$ ,  $P = 0.0741$ ;  $r = -0.68$ ,  $P = 0.0308$ ) and pulp ( $r = -0.71$ ,  $P = 0.0225$ ;  $r = -0.72$ ,  $P = 0.0193$ ) firmness were inversely correlated with pupae per fruit and pupae per kg, respectively. The weight of 10 pupae of *A. fraterculus* was correlated with the fruit weight of "Tailandesa" guavas (Fig. 3). No correlations were found between pupal infestation and the remaining physicochemical parameters.

Principal components analysis (PCA) was performed on the dataset of the results for the two guava varieties and 20 traits assessed. The total variability was explained by one principal component (PC). Of these, the first (PC1) accounted for 100% of the total variation (Fig. 4). PC1 was effective in separating the two guava varieties. The examination of the PC1 loadings suggests that this separation is due to pupae per fruit, pupae per kg, pupal viability, peel and pulp luminosity, peel and pulp  $b^*$ , peel hue angle, peel chromaticity, peel and pulp firmness, titratable acidity, soluble solids and SS/TA ratio. The PC1 scores and

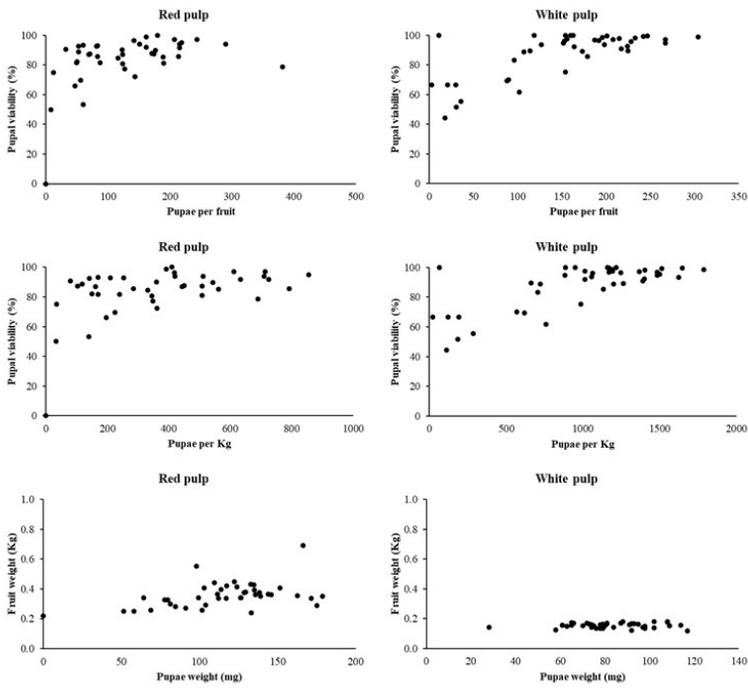
loadings suggest that the concentrations or values of these compounds were highest for white pulp and lowest for red pulp, which were located on the negative side of the PC1 axis. The opposite was observed for pH, pulp chromaticity, peel  $a^*$ , pulp  $a^*$ , pulp hue angle and pupal weight, which also contributed to the separation of the guava varieties in PC1, although with negative loadings. The PC1 scores and loadings suggest that pupal viability was present at a distant level in the red and white guavas, which were centred on the PC1 axis.



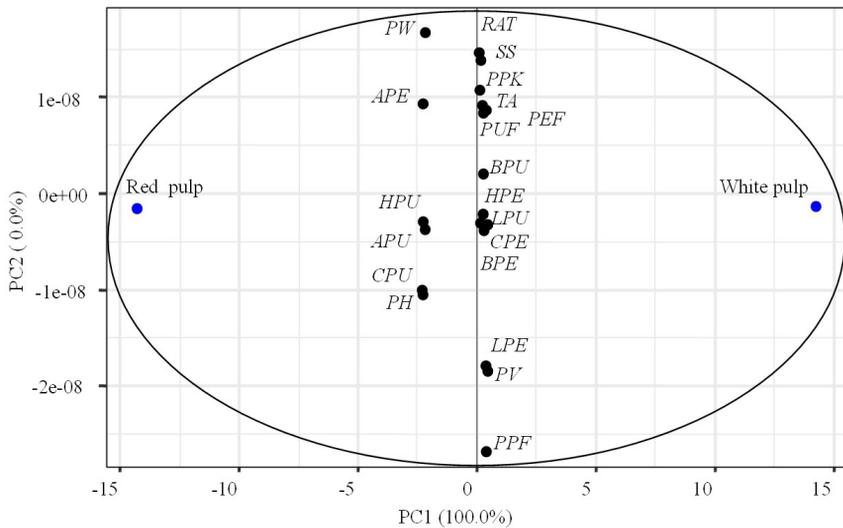
**Figure 1.** Infestation parameters of *Anastrepha fraterculus* in guavas “Tailandesa” (red pulp) and “Kumagai” (white pulp). Bars with different letters indicate significant differences by Student’s t-test ( $P < 0.05$ ).



**Figure 2.** Pupal infestation pattern of *Anastrepha fraterculus* in guavas “Tailandesa” (red pulp) and “Kumagai” (white pulp) (n=42).



**Figure 3.** Pupae of *Anastrepha fraterculus* per fruit or kg as function of pupal viability and, pupal weight versus guava weight of “Tailandesa” (red pulp) and “Kumagai” (white pulp) varieties (n=42).



**Figure 4.** Plots of the principal component analysis of physical and physicochemical profile data for two guavas. PC1/PC2 scores (●) and loadings plot (●) accounted for 100.00% of the total variation. Samples: Red pulp (“Tailandesa” guava), White pulp (“Kumagai” guava). Trait abbreviations: pupae per fruit [PPF], pupal weight [PW], pupae per kg [PPK], pupal viability [PV], luminosity peel [LPE], a\* peel [APE], b\* peel [BPE], angle Hue peel [HPE], chromaticity peel [CPE], luminosity pulp [LPU], a\* pulp [APU], b\* pulp [BPU], angle Hue pulp [HPU], chromaticity pulp [CPU], peel firmness [PEF], pulp firmness [PUF], pH [PH], titratable acidity [TA], soluble solids [SS], ratio [RAT].

**Table 1.** Physicochemical parameters of mature fruits of “Tailandesa” (red pulp) and “Kumagai” (white pulp) guava varieties (n = 10, except fruit weight n = 42).

Parameter	Red pulp	White pulp
Fruit weight	0.357a	0.155b
L skin	65.29a	65.93a
a skin	-10.97a	-12.40a
b skin	45.29a	42.71a
Hue skin	104.55a	106.28a
Chroma skin	44.04a	44.53a
L pulp	69.33b	83.76a
a pulp	20.61a	-3.01b
b pulp	20.87b	24.27a
Hue pulp	225.83a	96.69b
Chroma pulp	29.80a	24.50b
Peel firmness	60.07b	85.16a
Pulp firmness	55.22b	74.19a
pH	3.94a	3.89a
titratable acidity (TA) (g 100g <sup>-1</sup> )	0.58a	0.62a
soluble solid content (SS)	7.72b	9.06a
SS/TA (ratio)	13.39a	14.80a

Significant differences within a row are indicated by different letters Student's t-test ( $P < 0.05$ ).

## Discussion

When searching for comparative trends in the evolution of oviposition behaviour, one point of focus for any phytophagous insect is a possible linkage between the preference for host oviposition sites and the performance of larvae in the host (Diaz-Fleischer *et al.* 2000). Guava volatiles positively affect male sexual performance of *A. fraterculus* (Bachmann *et al.* 2015) and stimulate oviposition. In the present study, the forced *A. fraterculus* infestation provided similar quantities of pupae per fruit, showing adequate substrate (pulp) for larval development.

Adult emergence above 85% supports the close relationship between *A. fraterculus* and fruits of the Myrtaceae family, especially guavas (Sugayama *et al.* 1998). The correlation between infestation indices and pupal viability possibly is related to the available endosymbionts during the larval development of *A. fraterculus* (Selivon *et al.* 1996, 2002; Noman *et al.* 2020) and their beneficial nutritional function for immatures.

Fruit weight had an incremental effect on tephritid infestation rates of *Rhagoletis completa* Cresson (Tephritidae) in walnuts (Guillén *et al.* 2011) and *Bactrocera oleae* (Gmelin) in olives (Garantonakis *et al.* 2016). In our study, infestation differences between guava varieties when considering weight was due to “Kumagai” guava being less heavy than “Tailandesa” guava. “Kumagai” guavas produced on average 174% more pupae per kg. Higher pupal

weights of *A. fraterculus* were obtained from higher fruit weights (“Tailandesa”), although the fruit mass did not affect the pupal viability.

Rattanapun *et al.* (2009) found that ripe and fully-ripe mangoes were more suitable for larval development, with higher larval survival and shorter larval development times for *Bactrocera dorsalis* (Hendel). In our case, “Kumagai” mature guava produced on average approximately 1 pupa of *A. fraterculus* per gram of fruit, although both varieties stimulated a similar oviposition rate per fruit.

The performance of fruit species depends on the nutritional content of the host, but many tephritids prefer ripe and fully-ripe fruit for oviposition because they have adequate components for larval development and exhibit low content of secondary metabolites (Birke & Aluja 2018). In an earlier study, Raga *et al.* (2020) obtained up to 202 pupae of *A. fraterculus* per unbagged, ripe “Tailandesa” guava in the laboratory. No other fruit host species of *A. fraterculus* has shown such a high level of infestation under the same conditions.

The dimension of guava infestation range (both indices) shows the potential impacts in terms of yield losses, the spread of immature stages during the commercialisation process and quarantine risks during domestic and international trade. Added to this are the qualitative losses during pre- and post-harvesting processes (Louzeiro *et al.* 2020). The potential infestation in guavas and pupal viability of *A. fraterculus* are indicator variables of the risk of possible establishment and dispersal of pests (Heather & Halmann 2008).

An investigator might determine which of the same range of hosts supported larval development in those species (Diaz-Fleischer *et al.* 2000). Fifteen species of *Anastrepha* plus *Ceratitis capitata* (Wied.) infest *P. guajava* in Brazil (Zucchi & Moraes 2008). However, in the state of São Paulo, *A. fraterculus* is dominant (Raga *et al.* 2005), although co-infestation may occur in guavas (Raga *et al.* 2006).

Multivariate analysis of our results showed that pulp and peel firmness, soluble solids, titratable acidity, and SS/TA ratio are associated with a higher number of pupae of *A. fraterculus* per mass of “Kumagai” guavas. Oliveira *et al.* (2014) found that the highest rates of infestation of *A. fraterculus* in some guava varieties were correlated with the highest values of soluble solids and observed that the pH index had no direct relation to *A. fraterculus*'s attraction to and infestation of the guava fruits. Firmness is an important factor for *A. ludens* (Diaz-Fleischer & Aluja 2003). Mature guava is the best medium for larval development and therefore performance testing (Cunningham *et al.* 2016). This is because immediately after harvesting, the peel and pulp maintain their firmness, thus stimulating fruit fly oviposition. The same was observed for papaya infested by *C. capitata* (Joachim-Bravo *et al.* 2001) and mangoes infested by *B. dorsalis* (Rattanapun *et al.* 2009).

No significant effects on egg and pupal performance of *C. capitata* were obtained from insects reared in different citrus varieties; however, the fruits did affect larvae and pupal weights (Papachristos *et al.* 2008). The insect performance varied according to the suitable host, the region and climatic conditions (Medeiros *et al.* 2007; Bonebrake *et al.* 2010). We consider *P. guajava* as the primary host of *A. fraterculus* in southeastern Brazil, from which large populations spread to other fruit crops, such as citrus and stone fruits (Raga *et al.* 2002, 2017). Under field conditions, guavas were susceptible to *A. fraterculus* attack from when the fruits were undeveloped to when they began to ripen (Birke *et al.* 2015). Future field studies should be developed to determine the guava fruit stage preference and the per cent degree of symptomatic and asymptomatic ripe fruits during the harvesting process to guarantee fruit health and quarantine safety.

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