

Damage and Development of *Anastrepha fraterculus* (Diptera: Tephritidae) in Fruits of Two Pear Cultivars

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Summary

Anastrepha fraterculus is the main horticultural pest for food crops in southern Brazil. This study aimed to identify the damage caused by this species, evaluate its development, and correlate its infestation rate with physical and chemical characteristics of Packhams and Williams pear fruit cultivars at five different stages of development. In the field, cages were installed on branches of the pear plants in which two couples of *A. fraterculus* were released for a period of 48 hours. The damage resulting from oviposition was evaluated at fifteen-day intervals from the day the insects were released until harvest. The evaluation of damage consisted of visual observation of decayed and deformed fruits and the presence of larvae. In the laboratory, two couples were individualized with one fruit in a 750 mL pot for 48 hours. The evaluations consisted of counting the number of living third-instar larvae, pupae and adults. The physical and chemical analyses consisted of the determination of fruit peel and pulp texture, color, soluble solid content and transversal diameter. The incidence of the fruit fly on Packhams and Williams fruits occurred when fruits measured 54.9 and 52.8 mm respectively. The development of *A. fraterculus* in pear fruits of both cultivars is related mainly to fruit peel and pulp hardness.

Keywords: fruit fly, integrated pest management, physicochemical characteristics, peel hardness

Resumen

Daños y desarrollo de *Anastrepha fraterculus* (Diptera: Tephritidae) en frutos de dos cultivares de pera

Anastrepha fraterculus es la principal plaga de la producción de frutas en el sur de Brasil. El objetivo de este estudio fue identificar los daños causados por esta especie, evaluar su desarrollo y correlacionar el índice de infestación con las características físicas y químicas de los cultivares de pera Williams y Packhams en cinco distintas etapas de desarrollo. En el campo, se instalaron jaulas en las ramas de los perales para aislar frutas en las que se colocó dos parejas de *A. fraterculus* durante 48 horas. Se evaluó, en intervalos de 15 días, el daño provocado por la oviposición a través del recuento de los frutos caídos y deformes y de la presencia de larvas en su interior desde el inicio del experimento y hasta el momento de la cosecha. La evaluación de los daños consistió en la observación visual de frutas podridas y deformes y la presencia de larvas. En el laboratorio, se separó cada uno de los frutos de pera en potes de 750 mL y se los dejó con dos parejas de moscas durante 48 horas. La evaluación consistió en contar el número de larvas de tercer instar vivas, pupas y adultos emergentes. El análisis físicoquímico de las frutas determinó la textura de su piel y pulpa, el color, los sólidos solubles totales y su diámetro transversal. Se observó la incidencia de moscas en los cultivares Packhams y Williams cuando las frutas midieron 54,9 y 52,86 mm respectivamente. El desarrollo de *A. fraterculus* en frutos de pera de ambos cultivares está relacionado principalmente con la textura de la piel y pulpa de las frutas.

Palabras clave: mosca de la fruta, manejo integrado de plagas, características físicoquímicas, resistencia de la piel

Introduction

The pear tree (*Pyrus communis* L.) is cultivated in many countries, and pear fruits have great economic importance on national and international markets (Fioravanço, 2007).

In Brazil, the pear fruit is the third most consumed fruit, second only to apples and peaches (Botrel *et al.*, 2010). As the areas cultivated with this crop are not enough to meet the consumption demand this fruit has to be imported, which makes Brazil the second largest importer of pear fruit in the world (210.000 tons) (FAO, 2013). Growing pear trees is promising in southern Brazil, especially in the Highlands of Santa Catarina because of its suitable climatic conditions and the logistical structure already installed for apple crops, which can be used for pears.

The fruit flies that belong to the Tephritidae family are the main pests of world horticulture (Uchôa, 2012). The South American fruit fly, *Anastrepha fraterculus* (Wiedemann), is a polyphagous species that is found from southern Texas, in the United States to Argentina (Norrbon *et al.*, 1999). In the south region of Brazil, the South American fruit fly is the dominant species, and it is the most commonly found species in monitoring traps (Garcia y Corseuil, 1998; Garcia *et al.*, 2003; Husch *et al.*, 2012; Nunes *et al.*, 2013). It also bears the main responsibility for production losses of temperate fruits (Zucchi, 2008). Fruit losses occur by the larval development on the fruit pulp, which induces its rot, and by the insertion of the ovipositor, which induces the death of the cells that are adjacent to the puncture site, causing fruit deformation and inducing premature fruit decay (Aguiar-Menezes *et al.*, 2004).

The stage of maturity of the fruits modifies their physical and chemical characteristics such as color, firmness, aroma, starch proportion and free sugars (Yashoda *et al.*, 2007; Prasanna *et al.*, 2007) and influences oviposition by fruit flies (Salles, 1999). For example, damage to peach fruits begins in the period of swelling (Salles, 1994); in apples, damage occurs when fruit diameter is larger than 20 mm (Sugayama *et al.*, 1997); in plums, the puncture is perceived in fruits whose diameter ranges from 22 to 28 mm (Salles, 1999). In addition to the plant species, the cultivar can influence oviposition as well. In kiwi fruit, cultivar Bruno is immune to *A. fraterculus*; however, for cultivar MG06, oviposition is achieved mainly at the beginning of fruiting (Lorscheiter *et al.*, 2012). In vines, Zart *et al.* (2011) studied larval development in Cabernet Sauvignon, Moscato Embrapa, and Isabel cultivars and found that oviposition occurred only in the Moscato Embrapa cultivar.

There is scarce information about the moment when the South American fruit fly uses the pear fruit as an oviposition site, and about the influence of the physical and chemical properties of the fruit on larval development. Knowledge of the interactions between *A. fraterculus* and its host is very important for the adoption of control strategies; therefore, this study aimed to characterize the damage caused by *A. fraterculus* as well as to correlate the number of larvae found at the different stages of fruit ripeness with the physical and chemical characteristics of pear fruits of the two cultivars.

Material and Methods

The study was set up in a pear orchard located in the municipality of São Joaquim, Santa Catarina state (28°16'33" S, 49°56'12" O and 1,406m high), Brazil. The orchard consists of a collection of pear cultivars with an area of 0.5 ha. Ten 29-year-old plants of the cultivars Packhams and Williams trained to the central leader system, spaced 4 m between plants and 6 m between rows, were used for this study. Cropping practices were performed as usual during the period of the experiment except for insecticide application, which was not performed. A sample of 500 fruits per cultivar were enclosed with polypropylene bags (21 x 25 cm) when fruits had two centimeters of transversal diameter and were on the «J» stage of development (growth of fruit) according to the phenological scale proposed by Minost (2013). The fruits that were protected by polypropylene bags were used in infestations tests both in the field and at the laboratory, and for physical and chemical determinations.

Artificially reared eight-generation adult fruit flies, maintained at the Laboratory of Entomology, Agroveterinary Center, State University of Santa Catarina, were used in the infestation tests. The substrate used for oviposition in the artificial fruit fly rearing was papaya. The diet used for adults consisted of wheat germ, refined sugar and yeast on a 3:1:1 ratio, and water *ad libitum*. A sample of 100 insects was collected from the artificial rearing and identified according to dichotomous keys of the genus *Anastrepha* (Steyskal, 1977). All insects were classified as *A. fraterculus*. After identification, the insects were sent to Instituto Biológico, São Paulo-Brazil, where a fruit fly specialist confirmed the species as *A. fraterculus*.

Field evaluation of damage caused by *A. fraterculus*

Five artificial infestation trials were performed in pear fruits with adults of *A. fraterculus* aged between 14 and 17 days

on the five following dates: 11/23/11; 12/14/11; 12/28/11; 01/11/12 and 01/25/12. On each date, 30 fruits per plant were selected; 15 of them were infested with adult fruit fly, and 15 fruits that were not infested remained as control. Before the release of adults of *A. fraterculus*, the polypropylene bags that had been protecting the fruits were replaced by cylindrical cages measuring 40 cm length and 25 cm in diameter, closed at both extremities with wires. Each cage received two couples of *A. fraterculus*, in reproductive age (14 to 17 days), for a period of 48 hours with a honey solution (10 %) that was provided in caps containing hydrophilic cotton. After this period, the fruit flies were removed from the cages and the fruits remained protected until the harvest season. Every 15 days until harvest (01/15/12), the evaluations were carried out to determine the presence of deformed fruits or the occurrence of premature fruit decay. The fruits were dissected every time that a decayed fruit was found and at the time of fruit harvest with the objective of evaluating the presence of galleries and/or larvae. Data on fruit decay for Packhams and Williams cultivars were compared, in each period, with the respective controls by using an independent t-test. Data on the percentage of malformed fruits, percentage of fruits with the presence of galleries and percentage of fruits with living larvae in each cultivar were subjected to One-Way ANOVA, and the means were compared with Tukey's test at 5 % of significance in each infestation period.

Larval development of *A. fraterculus* at the laboratory and evaluation of physical and chemical parameters of pear fruits

At the same time as field tests, 40 fruits of each cultivar were collected in each period; 20 of them were used in the laboratory infestation tests, and 20 served as control (infestation-free) in a total of five infestations.

For larval development tests, the fruits were placed individually in plastic pots (750 mL) with two adult couples of *A. fraterculus* aged 14 to 17 days. Food consisted of a honey solution at 10 % provided in hydrophilic cotton. The insects remained inside the cages in a temperature-controlled room with temperature at 25 ± 2 °C, relative humidity at 60 % and a photo phase of 14 hours, for a period of 48 hours. After this period, the flies were removed and the fruits remained in the room until the larvae completed their development. After that, the fruits were dissected to quantify the number of living larvae, which were transferred to plastic pots coated with vermiculite and maintained in a temperature-controlled room for the count of pupae and adults.

Physical and chemical analysis

Physical and chemical analyses were performed at the same time as field and laboratory infestations. Thirty fruits were collected and taken to the laboratory for analysis of equatorial diameter, peel and pulp textures, soluble solid content and peel color. Diameter was measured with a digital caliper. Peel and pulp textures were evaluated at two points in the equatorial region of the fruits with a TAXT-Plus™ electronic texturometer (Stable Micro Systems Ltda., United Kingdom). To quantify the force required to break epidermis and penetrate into the fruit pulp, a 2-mm PS2 tip was introduced at 8 mm depth into the fruit pulp with pre-test, test and post-test of 10, 1 and 10 mm s⁻¹, respectively. Soluble solid content was determined through extraction of the pear juice, which was measured by a digital refractometer and expressed in °Brix. Color was determined by a Minolta CR 400 colorimeter, positioned on the opposite sides of the pear fruit, and luminosity (L), chroma (C) and hue angle (h°) values were measured. The luminosity parameter can range from 0 (dark and opaque) to 100 (white or maximum brightness). Chroma is related to color intensity and assumes values close to 0 for neutral colors (gray) and around 60 for vivid colors (McGuire, 1992). Hue angle can range from 0° to 360°, where 0° corresponds to the color red, 90° to yellow, 180° to green, and 270° to blue.

Data on the number of larvae found per fruit were transformed in $\sqrt{x+0.5}$ and submitted to analysis of variance and to Tukey's mean comparison test at 5 % significance. Pearson's correlation was performed between the mean of larvae per fruit and the mean value of the physical and chemical properties on the different infestation dates.

Results and Discussion

Fruit decay in the infested Packhams cultivar varied between seven and 13 % on the different dates of infestation during cultivation (Table 1); however, there were no significant differences when compared to control. On 28/12/2011, when fruits had 46.2 mm in diameter, 35 % of the decayed fruits had galleries and living larvae on their pulp, indicating initial larval development of *A. fraterculus*. At this stage, the pulp of the damaged fruits had brown spots and small galleries.

Natural fruit decay was higher in cv. Williams in comparison with Packhams (Table 1). Moreover, fruit fly infestation may have contributed to decay in fruits whose diameter was equal to or above 52.8 mm. Galleries occurred for the infestation performed on 12/28/2011; however larval develop-

Table 1. Percentage of fruit drop, percentage of damage (deformation and galleries) and percentage of fruits with larvae in fruits of Packhams and Williams pear cultivars after infestation with adults of *Anastrepha fraterculus* in field conditions. Crop 2011/12. São Joaquim, SC.

| Cultivar | Date of infestation | Fruit diameter (mm) | Fruit drop | | Type of damage found | | |
|----------|---------------------|---------------------|----------------------------------|---------|----------------------|-----------|---------|
| | | | Release of <i>A. fraterculus</i> | Control | Deformation | Galleries | Larvae |
| Packhams | 11/23/2011 | 46.1 | 13.0A | 20.0A | 7.0 a | 0.0 b | 0.0 b |
| | 12/14/2011 | 48.2 | 7.0A | 13.0A | 7.0 a | 0.0 b | 0.0 b |
| | 12/28/2011 | 54.9 | 13.0A | 7.0A | 0.0 a | 35.0 ab | 35.0 ab |
| | 11/1/2012 | 70.3 | 13.0A | 7.0A | 0.0 a | 40.0 ab | 40.0 ab |
| | 1/25/2012 | 78.4 | 7.0A | 13.0A | 0.0 a | 53.0 ab | 53.0 a |
| Williams | 11/23/2011 | 38.8 | 26.0A | 33.0A | 0.0 a | 0.0 c | 0.0 b |
| | 12/14/2011 | 43.9 | 20.0A | 27.0A | 7.0 a | 0.0 c | 0.0 b |
| | 12/28/2011 | 52.8 | 47.0A | 13.0 B | 0.0 a | 43.0 b | 0.0 b |
| | 1/11/2012 | 63.6 | 80.0A | 13.0 B | 0.0 a | 67.0 a | 60.0 a |
| | 1/25/2012 | 77.1 | 73.0A | 20.0 B | 20.0 a | 67.0 a | 60.0 a |

Means followed by the upper case letters within a line and lower case letters within the columns are not different by the Tukey test ($P < 0.05$).

ment occurred only on fruits that were infested on 01/11/2012 and 01/25/2012.

The analysis of variance between the two cultivars over time in the field infestations revealed significant differences in the percentage of decayed fruits ($F_4 = 2.735$, $P = 0.036$), fruits with galleries ($F_4 = 11.577$, $P < 0.001$) and fruit with larvae ($F_4 = 15.750$, $P < 0.001$); therefore, it can be inferred that the Williams cultivar fruits are more prone to oviposition by *A. fraterculus* compared with the Packhams cultivar. Larvae present in the fruit pulp damage the internal tissues of the fruit, and such damage may increase the release of ethylene, which accelerates the fruit abscission process. Moreover, premature fruit decay is also related to the occurrence of enterobacteria in the gut of adult flies, which are transferred to the fruit during oviposition. These bacteria establish and proliferate in the fruit pulp and, together with the activity of the larvae, accelerate early fruit decay (Behar *et al.*, 2008). Deformations from fly oviposition were not observed in Packhams and Williams fruits. This may be due to the difficulty in distinguishing the lesion caused by *A. fraterculus* from the damage caused by a hailstorm that occurred at the beginning of fruit development and damaged fruit peel of both cultivars.

Laboratory results (Table 2) corroborated those found in the field. For the Packhams cultivar, a small number of larvae were observed in fruits with 54.9 mm in diameter; how-

ever, these larvae did not complete their development. There was a small number of larvae in fruits with 70.3 mm in diameter; however they could reach full development. Fruits with 78.4 mm diameter showed a significant increase in the number of larvae. For the Williams cultivar, larval development occurred only in fruits with 52.8 mm in diameter and increased in the subsequent infestations, when fruits measured 63.6 and 77.1 mm. This increase in the number of living larvae may be due to sugar content in the fruits, which is an oviposition stimulant for fruit flies (Rattanapun *et al.*, 2009) and produces an increase in larval performance (Lee *et al.*, 2011). This is opposed to the results found in other temperate fruit trees such as apple (Sugayama *et al.*, 1997), pome (Salles, 1999), and kiwi fruit (Lorscheiter *et al.*, 2012) where the authors found that the damage caused by *A. fraterculus* occurred at the early developmental stage. Damage to the pear cultivars in this study occurred when fruits reached 70 % of their final size. According to Diaz-Fleisher y Aluja (2003), this discrepancy in host utilization is due to the natural variation in fruit physicochemical characteristics, which lead the insects to exploit the fruits or not. The variance on the development between native and cultivated fruits in an ecosystem provides a constant offer of hosts to the South American fruit fly, ensuring population maintenance all over the year and its status as a crop pest. The number of pupae obtained in this study differed from the number found by

Table 2. Mean number \pm (SE) of larvae, pupae and adults of *Anastrepha fraterculus* obtained from pear fruits of Packhams and Williams cultivars infested from 11/23/11 to 1/25/12 in laboratory.

| Cultivar | Date | Fruit diameter (mm) | Larvae | Pupae | Adults |
|----------|------------|---------------------|------------------|------------------|-----------------|
| Packhams | 11/23/2011 | 46.1 | 0.0 b | 0.0 b | 0.0 b |
| | 12/14/2011 | 48.2 | 0.0 b | 0.0 b | 0.0 b |
| | 12/28/2011 | 54.9 | 0.1 \pm 0.1 b | 0.0 b | 0.0 b |
| | 1/11/2012 | 70.3 | 0.7 \pm 0.4 b | 0.3 \pm 0.2 b | 0.1 \pm 0.0 b |
| | 1/25/2012 | 78.4 | 8.9 \pm 1.4 a | 3.9 \pm 0.9 a | 2.6 \pm 0.8 a |
| Williams | 11/23/2011 | 38.8 | 0.0 c | 0.0 c | 0.0 b |
| | 12/14/2011 | 43.9 | 0.0 c | 0.0 c | 0.0 b |
| | 12/28/2011 | 52.8 | 3.3 \pm 1.2 bc | 1.2 \pm 0.5 bc | 0.7 \pm 0.3 b |
| | 1/11/2012 | 63.6 | 14.0 \pm 2.4 a | 7.7 \pm 1.4 a | 4.0 \pm 0.9 a |
| | 1/25/2012 | 77.1 | 7.6 \pm 1.9 | 3.6 \pm 1.0 b | 1.6 \pm 0.7 b |

Means followed by the same letter within a column are not significantly different by the Tukey test ($P < 0.05$).

García y Norrbom (2011). The authors found an average of 0.52 larvae per fruit of pear (*Pyrus communis*) collected at the ripe stage under natural conditions. This difference may be due to several factors that are not possible to determine in natural conditions, such as size of fruit fly population, age, nutritional and weather conditions and the chance that the flies had to oviposit at random, which did not occur at the laboratory where we defined the age of flies, diet, temperature, humidity and after all, restricted oviposition of two couples to a single fruit.

The linear model was the most appropriate to evaluate the relationship between fruit diameter and larval infestation. The equation obtained for Packhams cultivar was: $y = -3.61 + 1.85x$ ($R^2 = 0.56$) and for Williams was: $y = -3.78 + 2.92x$ ($R^2 = 0.61$). These data demonstrate that fruit size is not the only factor responsible for the increase in the number of larvae in pear fruits. Studies conducted by López-Guillén *et al.* (2009) revealed that adults of *Anastrepha obliqua* (Mcquart) are more attracted to spheres with 8, 10 and 12 cm in diameter to those measuring 4 or 6 cm. However, according to Gregorio *et al.* (2010), the colors of the oviposition substrate did not affect the fecundity of *A. fraterculus*.

In both cultivars, fruit color ranged between green and yellow-green. Color attributes also showed a moderate negative correlation with the number of larvae per fruit in both cultivars (Table 3).

Visual cues have a great importance for Tephritidae for the location of hosts and in search of mating partners (Papa-

dopoulos *et al.*, 2006). As far as fruit location is concerned, fruit color is one of the factors responsible for location detection by fruit flies (Aluja y Mangan, 2008; García, 2009). According to studies made by Cytrynowicz *et al.* (1982), adults of both *A. fraterculus* and *Ceratitidis capitata* (Wiedemann) were more attracted to the yellow rectangles than to the orange, green and red ones in field experiments. Despite the importance of color for host location, Gregorio *et al.* (2010) observed that the color of the oviposition substrate did not affect the fecundity of *A. fraterculus*.

Nonetheless, a negative correlation between number of larvae and texture of fruit peel was observed for Packhams and Williams. Pulp texture and solid soluble content were only correlated with the number of larvae in the Packhams cultivar.

Data on peel and pulp texture showed that these factors are determinants to infestation of pear fruits by *Anastrepha fraterculus*, indicating that females respond positively to ripening fruits. Peel and pulp hardness of a fruit that is at the beginning of development may have worked as a barrier to the insertion of the ovipositor and/or to larval development. Balagawi *et al.* (2005) studied the relationship between peel hardness of tomato fruits and their susceptibility to infestation by *Bactrocera tryoni* and found that fruits of the cultivar «Cherry», whose peel is tougher than that of cultivars «Grosse Lisse» and «Rome», was less frequently attacked. Rattanapun *et al.* (2009) also verified that ripe mango fruits are more adequate to larval development and granted a bigger survivorship rate and a shorter period of development compared

Table 3. Physicochemical characteristics of Packhams and Williams cultivars and Pearson's correlation coefficients and probabilities between the number of larvae and attributes of color (L, C and h), texture (fruit peel and pulp) and content of soluble solids.

| Cultivar | Date | Larvae | Color | | | Texture | | °Brix |
|----------|------------|----------------|----------------|----------------|----------------|---------|-------|-------|
| | | | L ¹ | C ² | h ³ | Peel | Pulp | |
| Packhams | 11/23/2011 | 0 | 52.76 | 30.4 | 112.9 | 21.24 | 14.59 | 7.4 |
| | 12/14/2011 | 0 | 53.36 | 36.9 | 112.2 | 20.22 | 13.62 | 8 |
| | 12/28/2011 | 0.35 | 57.61 | 37.8 | 110 | 17.49 | 11.41 | 9 |
| | 1/11/2012 | 0.4 | 57.59 | 37.8 | 109.1 | 15.97 | 9.1 | 9.1 |
| | 1/25/2012 | 0.53 | 60.98 | 36.5 | 97.7 | 14.01 | 7.02 | 11.2 |
| | | R ² | 0.98 | 0.58 | -0.80 | -0.98 | -0.96 | 0.91 |
| | | p | 0.001 | 0.150 | 0.050 | 0.002 | 0.005 | 0.015 |
| Williams | 11/23/2011 | 0 | 48.74 | 25.87 | 113.6 | 23.76 | 17.2 | 8.00 |
| | 12/14/2011 | 0 | 48.76 | 30.36 | 105.9 | 23.89 | 17.3 | 9.00 |
| | 12/28/2011 | 0 | 52.83 | 31.54 | 90.9 | 22.01 | 17.13 | 10.00 |
| | 1/11/2012 | 0.6 | 54.51 | 34.11 | 92.9 | 20.21 | 15.37 | 9.50 |
| | 25/01/2012 | 0.6 | 59.27 | 36.48 | 81.3 | 14.63 | 7.21 | 10.00 |
| | | R ² | 0.84 | 0.82 | -0.74 | -0.83 | -0.75 | 0.49 |
| | | p | 0.036 | 0.043 | 0.049 | 0.040 | 0.073 | 0.200 |

¹Brightness.²Chroma.³Hue angle.

with unripe fruits. The development of pectinase during the ripening process reduces cell wall hardness, thus encouraging the insertion of the ovipositor.

As observed for the Packhams cultivar, oviposition rate and larval development have been positively related with the increase of soluble solid content, which is a result of the conversion of free acids and starch into sugars during ripening process. According to studies carried out by Lorscheiter *et al.* (2012) on the development of *A. fraterculus* in kiwi fruits, sugar content in a fruit seems to be a decisive factor for larval development since larvae were detected when the amount of soluble solids doubled to 6.4 % and 7 % in MG06 and Bruno cultivars, respectively. Lee *et al.* (2011) verified that the increment of the °Brix of blueberry, cherry and mulberry were correlated with an increase in the number of postures and developed eggs. The °Brix value was also responsible for a higher survivorship of *Bactrocera dorsalis* in mango fruits (Rattanapun *et al.*, 2009).

Conclusions

Both Packhams and Williams cultivars are hosts of the South American fruit fly, but larval development only occurs when fruits reach a size bigger than 54.9 and 63.6 millimeters in diameter, respectively. However, fruit decay was influenced by fruit fly damage only in the Williams cultivar, which increased when the diameter of the fruits was larger than 52.8 mm. Fruit parameters other than size, such as color, peel and pulp hardness, and sugar content, are strongly correlated with the number of larvae found in pear fruits, since they enable oviposition by adult females and larval development.

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