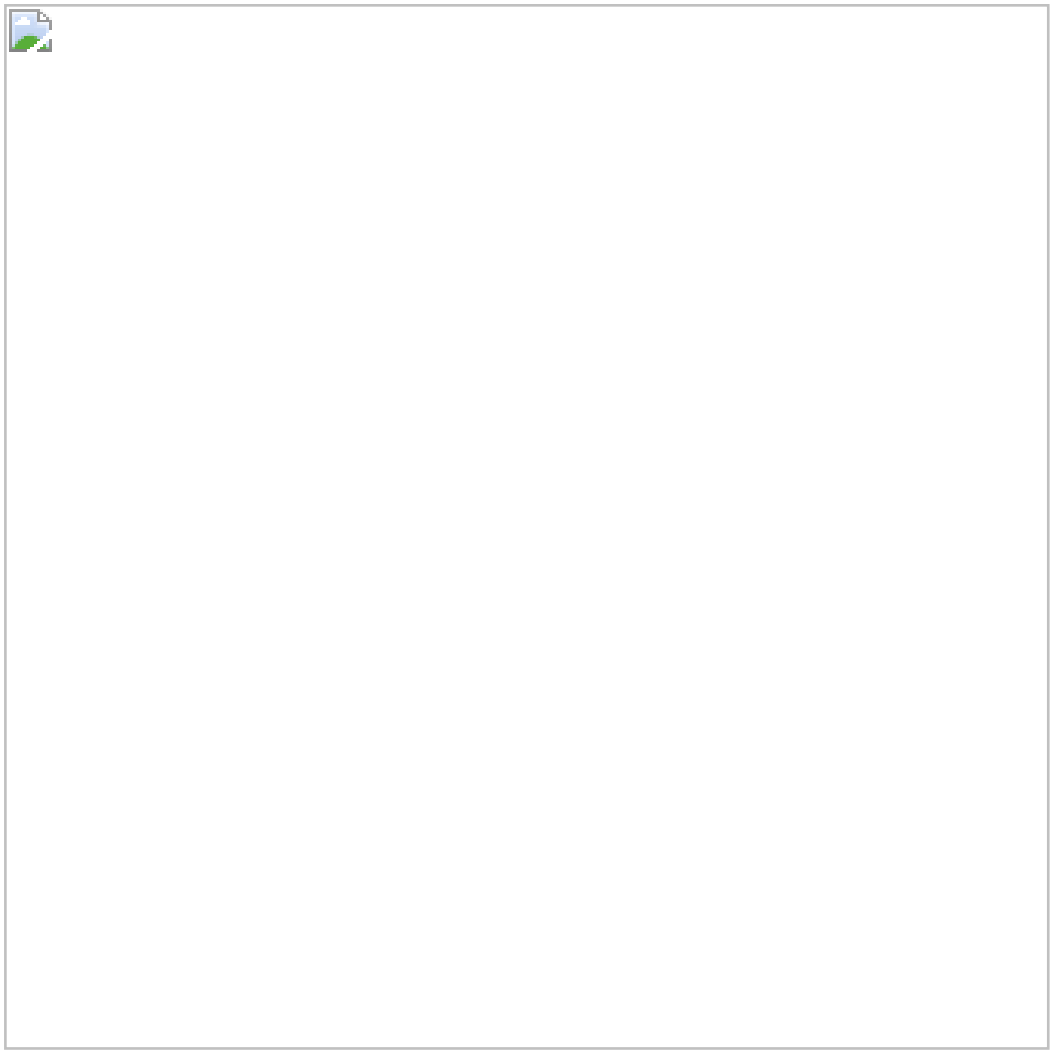

Nonhost Status of Commercial Sweet Granadilla (Passiflora ligularis) in Peru to Ceratitis capitata (Diptera: Tephritidae) and Anastrepha fraterculus

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Research

Nonhost Status of Commercial Sweet Granadilla (*Passiflora ligularis*) in Peru to *Ceratitis capitata* (Diptera: Tephritidae) and *Anastrepha fraterculus*

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Abstract

The host status of sweet granadilla (*Passifflora ligularis* Juss.) to *Ceratitis capitata* (Wiedemann) and *Anastrepha fraterculus* (Wiedemann) in Peru was determined. Experiments were conducted in Pasco (Peru) in four different orchards, over 2 yr (2016 and 2017), two orchards per year. Choice (granadilla plus natural host) and no-choice foraging behavior trials were conducted using sleeves under field conditions, and forced infestation was examined in laboratory cages, with five females per fruit. The development time of *C. capitata* was determined, and the oviposition behavior of *C. capitata* and *A. fraterculus* was examined. Three fruit maturity stages of intact (n = 1,320) and punctured (n = 1,320) granadilla fruits were examined. Adult *C. capitata* (n = 4,418) and *A. fraterculus* (n = 2,484) were trapped in the orchards, and commercial granadilla fruits (n = 1,940) sampled and dissected. Fruit fly infestation was not found in any intact granadilla fruits. Larvae and pupae were found inside punctured granadilla only in fruits broken after 20 d, and adults only emerged when those pupae were removed from the fruit. *Ceratitis capitata* development time was longer in punctured granadilla than that in host fruit. In the oviposition test, *A. fraterculus* and *C. capitata* did not lay eggs in intact granadilla, and *C. capitata* laid eggs in punctured fruits but larvae were not found. Because of the resistance mechanisms of the pericarp, commercial fruits of *Passiflora ligularis* are not a natural host of *C. capitata* and *A. fraterculus* in Peru.

Key words: Granadilla, *Anastrepha*, *Ceratitis*, Tephritidae, host status

Passiflora ligularis Juss. is commonly known as sweet granadilla or yellow passion fruit, and is native to Peru (Chóez et al. 2015). *Passiflora* is the genus of greatest economic importance in the family Passifloraceae with approximately 530 species distributed in the new world (Ulmer and MacDougal 2004). The main passion fruit producers are found in South America, primarily in Brazil, Colombia, Peru, and Ecuador. Commercial passion fruit crops are also found in Australia, Hawaii (United States), India, New Guinea, Kenya, South Africa, Sri Lanka, and Costa Rica. In Peru, sweet granadilla is cultivated primarily in highland valleys and jungles, such as those found in the Pasco, Cajamarca, La Libertad, and Piura departments, whose commercial areas are considered the largest in the country.

Korytkowski (2001) in a review of the genus *Anastrepha* in Peru and linked some of the recorded species with passion fruit by noting that *A. chiclayae* Greene had been bred from *Passiflora*. Furthermore, *A. curitis* Stone, is mentioned in connection with *Passiflora quadrangularis* L. and the fruits of *Passiflora* sp. Similarly, the author states that *Passiflora quadrangularis* is a host of *A. pseudoparalella* Loew.

Castillo and Ortiz (2011) sampled fruit in Oxapampa (Pasco, Peru), and infestations of *Ceratitis capitata* (Wiedemann) and *Anastrepha fraterculus* (Wiedemann) were not found in sweet granadilla fruits, but fruits were infested by *Dasiops* sp. and *Neosilva* sp. (both in the family Lonchaeidae). However, information on the condition of the fruits (i.e., maturity stage, presence of damages, whether from a plant or fallen on the ground) was not provided. In addition, these authors related the infestation to the larvae found but not to emerged adults. They also noted that flies of the genus *Dasiops* oviposit on and infest flowers and fruits of sweet granadilla *Passiflora ligularis*; however, larvae remained inside fruits and only left them after fruits fell to the ground. This behavior suggests that Lonchaeidae larvae are not capable of leaving the fruits for pupation and have to wait for the fruit pericarp to break naturally to exit, which demonstrates that the pericarp is the fruit cover that provides mechanical resistance to the fruit and thereby prevents larvae from leaving.

In Hawaii, Liquido et al. (1990) collected *Passiflora ligularis* fruits from the plant and those fallen on ground and found infestation

by *C. capitata*, but the maturity level and fruit conditions were not indicated.

In Venezuela, Carrero et al. (2000) reported *A. pallidipennis* Greene from damaged fruits of 'parchita maracuya' (*Passiflora edulis* f. sp. *flavicarpa* Sims) for the first time. In the laboratory, adults were obtained from naturally infested fruits. When fruits that remained attached to plants were collected and moved to laboratory, the examination revealed larvae with typical dipteran characteristics, and when they completed their development to adult fruit flies, the genus *Anastrepha* was identified.

In Colombia, Rengifo et al. (2011) conducted a host status study for the fruit fly *C. capitata* in purple passion fruit *P. edulis* f. *edulis*. The authors did not find larvae or emerged adults from 976 fruits manually harvested or from 623 fruits fallen on the ground. Under laboratory conditions, forced infestation experiments with punctured and intact fruits were conducted with *C. capitata* to determine the acceptability of different maturity stages and the physiological suitability of different stages of fruit development. For intact fruits, *C. capitata* females oviposited exclusively in fruits with a zero level of maturation (Maturity index 18.4; ICONTEC 1997), with 41.67% of fruits accepted for oviposition at a rate of 183.1 ± 33.8 eggs per fruit. However, oviposition was not reported for fruits with maturation levels of 2 and 4 (maturity index 26.0 and 32; ICONTEC 1997). In punctured fruits, *C. capitata* laid 84,410 and 84,250 eggs inside fruits with maturation levels of 0 and 2, respectively, but the emergence of *C. capitata* adults did not occur at any level of maturity. Based on these laboratory tests, the authors concluded that purple passion fruit was not a host to *C. capitata*.

In Colombia, Wyckhuys et al. (2012) determined the composition and seasonal dynamics of the Diptera species complex associated with three passion fruit crops (*P. edulis* f. *edulis*, *P. edulis* f. *flavicarpa*, and *P. ligularis*). The research was conducted from 2008 to 2010 by trapping in passion fruit commercial orchards. Fifty-five flies of the Tephritidae were found, with 37 individuals obtained from *P. ligularis* fruits and 18 from those of *P. edulis* f. *edulis*. Among the tephritid flies in *P. ligularis* orchards, 86% were *A. fraterculus*, 3% were *A. obliqua* Macquart, 5% were *A. striata*, and 5% were *A. grandis* Macquart. The tephritid flies captured in *P. edulis* f. *edulis* orchards included *A. fraterculus* (95%) and *A. striata* (5%).

Cowley et al. (1992) adopted the fruit fly host definition for quarantine situations: 'Any fruit or vegetable in which fruit fly oviposit under field conditions, the eggs hatch into larvae, and the larvae acquire sufficient sustenance to form viable pupae from which adults emerge and are capable of reproduction'. These authors also expressed that adult fruit flies should be obtained by rearing from infested, unsprayed hosts (i.e., wild rather than laboratory-bred flies should be used). Because flies derived from wild populations often fail to mate or oviposit, a laboratory-breeding colony can be used. However, a breeding colony should be supplemented with the addition of wild flies every 2 yr to maintain genetic similarity between the laboratory colony and the wild population. With the supplementation of laboratory colonies with wild flies, situations are avoided in which laboratory flies develop different ovipositional preferences.

Canteri et al. (2010) examined the chemical structure and composition of the pericarp of granadilla fruit sand note that the most abundant component of pericarp and its fractions (epicarp or exocarp, mesocarp, and endocarp) is total dietary fiber (48–65%), with 61, 66, 48, and 65% fiber for the exocarp, mesocarp, endocarp, and pericarp, respectively. In addition, the xylose content was relatively high at 133.4, 31.8, and 15.9 mg g^{-1} for the exocarp, mesocarp, and endocarp, respectively. Other monosaccharides and polysaccharides were also found. The authors also stated that the highest content of pectin isolates (13.6%) of high esterification (79%) with the highest viscosity (3.41 dl g^{-1}) was found in the mesocarp fraction. The moisture contents were also low at 4.5, 6.1, 6.0, and 4.3% for the exocarp, mesocarp, endocarp, and pericarp, respectively.

Chóez et al. (2015) complemented the information on chemical composition and found 22 chemical compounds associated with essential oils in the shell of granadilla *P. ligularis* in Ecuador. Approximately 34% of the chemical compounds corresponded to squalene, which according to Ahmed et al. (2018), has toxic and repellent effects against phytophagous insects and mites. This result suggests that squalene prevents females from laying eggs, in addition to the hardness and thickness of the pericarp. Therefore, squalene could be the first instance of chemical resistance identified in granadilla fruits against tephritid fruit flies.

Because this background information does not conclusively state the host status of commercial granadilla fruits to tephritid fruit flies in Peru, a demonstration of the nonhost status of commercial sweet granadilla *P. ligularis* fruits to *C. capitata* and *A. fraterculus* under the specific conditions of sweet granadilla production areas, as well as identifying the resistance mechanisms that might be involved, is important.

Materials and Methods

The information on materials and methods applied and/or used in this study was obtained from Cowley et al. (1992), Aluja et al. (2004), Aluja and Mangan (2007), North American Plant Protection Organization NAPPO (2005), and International Standard for Phytosanitary Measures-ISPM standard #37. Although the preferred name for *P. ligularis* is sweet granadilla (CABI 2018), only granadilla is used this research paper.

Experimental Locations

The research area was in Oxapampa (409 km from Lima City), which is one of the provinces of the Pasco department that is geographically limited by Huanuco (north), Lima (west), Junin (south), and Ucayali (east). Oxapampa is the highest granadilla production area in Peru with approximately 2,000 ha. The predominant production scale is small-farmer based (based on area in accordance with references provided by the Food and Agriculture Organization of the United Nations-FAO 2017).

The climate in Oxapampa is characterized by medium rainfall levels (1,000–1,500 mm), temperature ranging from 18 to 25°C, and Relative Humidity between 70 and 90%. Weather data were obtained from the Oxapampa Meteorological Station that belongs to Servicio Nacional de Meteorologia e Hidrologia del Peru - SENAMHI (meteorological national authority).

Sweet passion fruit plants in Oxapampa study orchards were 3 yr old and grafted on passion fruit rootstock. Said orchards are surrounded by coffee, guava, citrus, among other crops. Four experimental orchards were used: two in Huancabamba sector, Laturachi subsector (Boronda and Ortiz orchards), and two in Chorobamba sector, San Martin subsector (Espinoza and Asconoa orchards). The Ortiz (10°22′34.25″ S, 75°34′33.89″ W; 2093 m a. s. l.) and Espinoza (10°35′46.62″ S, 75°29′3.64″ W; 1933 m a. s. l.) orchards were used in 2016; whereas the Boronda (10°22′12.44″ S, 75°35′10.45″ W; 2227 m a. s. l.) and Asconoa (10°36′4.47″ S, 75°29′18.51″ W; 1897 m a. s. l.) orchards were used in 2017.

Importantly, during the execution of the field experiments and at least 1 mo before they began, insecticides (including oils) were not sprayed over the lots under study. Therefore, control measures other than pesticides were implemented to solve specific pest problems.

Species of Tephritidae and Sources of Insects

Adults of *C. capitata* and *A. fraterculus* of wild origin (few generations under captivity and host fruits as the larval diet) were used in this study. The colony was collected from the study area, both species of fruit flies were reared for at least fifth filial generation [ISPM 37 (2017)]. The adult fruit flies were recovered from field-collected host fruits, such as mango *Mangifera indica* L. (Anacardiaceae) and guava *Psidium guajaba* L. (Myrtaceae) for *A. fraterculus* and peach *Prunus persica* L. (Rosaceae) and coffee *Coffea arabica* L. (Rubiaceae) for *C. capitata*. These fruits were maintained at 26 ± 2°C and 65 ± 5% RH in boxes with pupation media (we used sand instead of vermiculite) according to the procedure described by Aluja (2004). The emerged adults were placed in 40 × 40 cm Plexiglas cage with water and food (3:1 mixture of sugar and hydrolyzed protein). The adults were mass-reared in room conditions at 26 ± 2°C and 65 ± 5% RH, with a 12:12 (L:D) h photoperiod.

Trapping *C. capitata* and *Anastrepha* spp. Adults in Experimental Granadilla Orchards

To corroborate the occurrence of tephritid species, a trapping system was implemented in the experimental granadilla orchards. The system was set up according to the SENASA Phytosanitary Programs and Fruit Fly Section (SMFPF 2006). McPhail traps were baited with Torula yeast pellets (Coltec Comercializadora Agrotecnologica S.A., Guatemala; Lot Number P155-715), 4 of which were dissolved in 250 ml of water with 5% of Borax as a lure for capturing adult *Anastrepha* spp. and *C. capitata*. The traps were rebaited on a weekly basis. The McPhail traps were hung from granadilla plant branches, and all flies captured were put in 50 cm^3 vials with 70% alcohol.

In Huancabamba sector, 7 km from Oxapampa, 3 and 11 McPhail traps were installed in San Martin and La Florida subsectors, respectively. In Chorobamba sector, 24 km from Oxapampa, 7, 10, and 6 McPhail traps were installed in Ancahuachanan, Lanturachi, and Mallapampa subsectors, respectively.

The traps were placed inside and around the experimental orchards at the rate of 1 trap per each 20 ha, which is equivalent to 5 traps per km^2 (=100 ha). The following parameters were recorded: 1) total number of hectares under trapping; 2) total number of traps; and 3) FTD (fruit flies/trap/day). All traps were georeferenced using GPS (Global Positioning System) and were checked by SENASA personnel specialized in fruit fly trapping management, following procedures in the National fruit fly detection system manual (SENASA 2010).

The information accumulated during 1 yr and 8 mo was used to determine the fruit fly population levels during this study.

Infestation-Level Assessment for *A. fraterculus* and *C. capitata* in Natural Hosts Located in Perimeter and Interior Portions of Experimental Orchards

To define the potential sources of fruit fly infestation that reached the experimental orchards, the fruits of all species of plant hosts and weeds with fleshy fruits that were growing either inside or outside of the experimental orchard perimeters, plus those found in surrounding native and wild vegetation, were sampled during the 2-yr study. Granadilla fruits that fell to the ground from each orchard were also included as noncommercial fruit, and any damage either mechanical or that produced by external feeders was recorded.

The sampled fruits were labeled and taken to the laboratory. The samples were weighed and placed in maturation boxes, with bottoms containing sand to facilitate pupation. After 10 d, all fruits that were not rotten were dissected to remove immature stages (larvae or pupae) that had not left the fruits.

Once removed from fruit, the pupae were placed in small plastic containers to recover adults. After emergence and once their wings reached complete coloration (2–3 d), the recovered adults were placed in small plastic containers containing 70% alcohol and identified to species.

Granadilla Fruit Sampling at the Experimental Orchards

To determine whether granadilla fruits could be infested by natural populations of fruit flies in each experimental orchard during harvest (Within April and November), 50 commercial fruits were randomly harvested every 15 d and transported to the laboratory using Tecnopor boxes, which were labeled by orchard name, sampling date, trap code, number of fruits, and name of collector. At the laboratory, 50% of the fruits were cut immediately and searched for eggs and larvae, whereas the other 50% were placed individually in maturation boxes and subsequently dissected after 15 d to search for fruit fly larvae and pupae. A 15-d period before dissecting the fruits was considered an appropriate length of time for fly development in fruits that had been exposed to naturally occurring fruit flies. The sampled and dissected fruits and recovered adults were recorded.

Granadilla Fruit Maturity Stages

To determine the fruit maturity stage, the ICONTEC (1997) Norma Tecnica Colombiana 4101 Fruta fresca. Granadilla. Specifications were used as a reference, and only the three maturity stages of green (Fig. 1A), green-yellow (Fig. 1B), and yellow (Fig. 1C) were considered in the study. The physical attribute (color) was the primary attribute, but chemical characteristics such as total soluble solids (TSS, °Brix), acidity (A, %), and maturity index (MI) were also evaluated. The fruit maturity index (MI), according to ICONTEC (1997), is a measure of the balance between sweetness and acidity, and the following formula was applied: MI = TSS/Acidity.

Forced Infestation Trials Using Sleeves Under Field Conditions

This research was conducted in two experimental orchards during the 2-yr study period. Organza-made sleeves (0.5 m in diameter and 1 m in length) were used to enclose granadilla fruit-bearing branches (Fig. 1D). In this experiment, the three maturity stages (green, green-yellow, and yellow) were tested (Every sleeve contained only one maturity stage). Each sleeve-enclosed branch contained five fruits. The age of the flies varied between 7 and 12 d (*C. capitata*) and 12 and 17 d (*A. fraterculus*). In each sleeve, sexually mature male and female fruit flies were released at a rate of five couples per fruit (Fig. 1E). These flies remained in the sleeve for four consecutive days with water and food (standard protein-sugar mixture in a solid state).

The fruit flies were released under two experimental fruit conditions: Choice (CH) and No-Choice (NCH). The treatments were intact (CH-intact and NCH-intact) and punctured (CH-puncture and NCH-puncture) fruits. The NCH experiment used five granadilla fruits naturally hanging from the plant. The CH experiment used five granadilla fruits naturally hanging and five natural host fruits (peach for *C. capitata* and mango for *A. fraterculus*), artificially hung from the plant with a 5 cm distance between a granadilla fruit and a host fruit. In addition, five natural host fruits alone artificially hung within a sleeve and were used as the control.

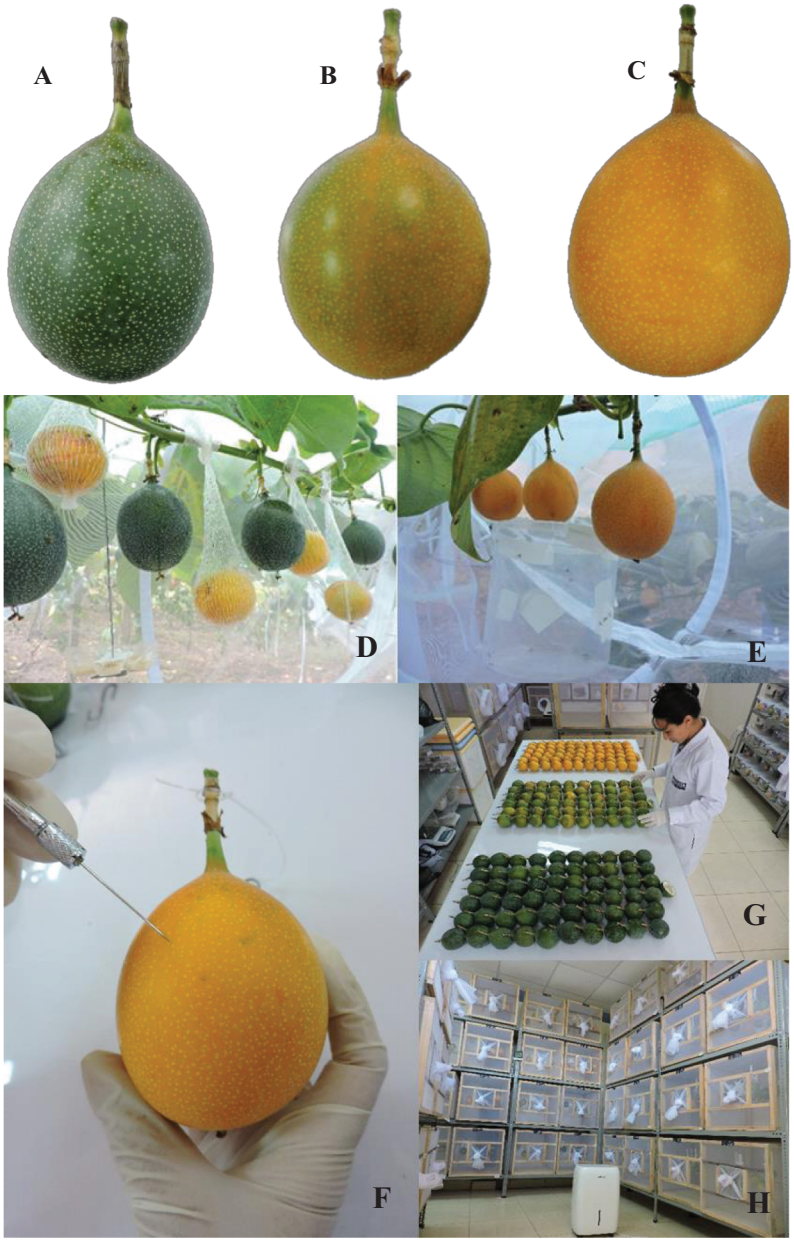

Fig. 1. Maturity stages of granadilla fruits: (A) Green stage; (B) Green-yellow stage; and (C) Yellow stage. Sleeves enclosing branches of green granadilla fruits with host fruits for the Choice test (D) and yellow (mature) granadilla fruits for the No-choice test (E); granadilla fruit being punctured for experimental test (F); Three maturity stages of granadilla fruits (G); and cages under laboratory conditions (H).

To ensure that fruit flies/fruit proportion did not change during the experiment, the sleeves were checked every morning, and another fly of the same species, age, and sex replaced each dead fly.

A total of 375 sleeves were used in the entire study. For tests with granadilla in 2016, 60 sleeves for *C. capitata* were set up in the Huancabamba sector. In 2017, 120 sleeves were installed for *A. fraterculus* in the Huancabamba sector, 60 sleeves for *C. capitata* in the Chorobamba sector, and 60 sleeves for *C. capitata* in the Huancabamba sector. Additionally, 75 sleeves (15 sleeves for each fruit fly species, experimental year, and place) were established for natural host fruits.

For the punctured CH and NCH tests, each granadilla fruit was punctured the day the trial began. The surface of each fruit was punctured 10 times by inserting a needle (Steel-made, 0.5-mm bore) (Fig. 1F) and as deep as 10 mm. To determine whether host fruits had been previously infested, 40 fruits per lot and per fruit species were placed into plastic bins or boxes (60 cm length × 50 cm width × 10 cm depth) to allow the eventual development of fruit fly larvae.

All tests were performed with only wild fruit fly females. After a 4-d exposure period, all fruits were removed, taken to laboratory, and individually weighed. The conditions were recorded when an infestation occurred.

After some time, when fruits started to rot, all larvae possible were expected to leave the fruits. Therefore, host fruits were dissected on day 10 after exposure. Because no larvae left granadilla fruits 20 d after exposure, fruits were also dissected on day 20 to observe their inner parts.

Because breaking the pericarp (shell) to open the fruits was an artificial intervention, the statistical analyses were conducted and the results discussed for two different scenarios: one scenario was for the data gathered until day 20 without opening the fruits, and the other scenario was for data gathered after opening the fruit on day 20.

Following Calzada (1982), the statistical analysis was based on a completely randomized block design (CRBD) with the data square-root transformed for normality required by ANOVA, which was followed by Student's *t*-tests and Tukey's tests. The following variables were analyzed: infestation percentage, number of larvae and pupae, number of adults emerged, and percentage of adult emergence with respect to larvae and pupae.

Granadilla Fruits Exposed to Female Fruit Flies Under Laboratory Conditions

This research was conducted in the laboratory during the 2-yr study period. Cages (0.5 m in diameter and 1 m in length) were used for this experiment in which granadilla and host fruits (Cherimoya in 2016 and peach in 2017 for *C. capitata*, and mango for *A. fraterculus*), were placed. The three maturity stages (green, green-yellow, and yellow) were used (Fig. 1G), and five fruits were placed per box in which sexually mature male and female fruit flies were released at a rate of five couples per fruit. The flies remained in these boxes (Fig. 1H) for four consecutive days with water and food. The granadilla fruits were harvested the day that the test began.

To ensure that the fruit flies/fruit proportion did not change during the experiment, the boxes were checked every morning, and another fly of the same species, age, and sex replaced each dead fly encountered.

The fruit flies were released under 2 experimental fruit conditions: Choice (CH) and No-Choice (NCH). The treatments were intact (CH-intact and NCH-intact) and punctured (CH-puncture and NCH-puncture) fruits. The NCH experiment used five granadilla fruits hung inside cages. The CH experiment used five granadilla fruits and five natural host fruits hung inside a cage with a 5 cm distance between a granadilla fruit and a host fruit. The fruit fly rate was five couples per granadilla or host fruit.

A total of 180 cages were used. For *C. capitata*, 36 cages were used in 2016 and again in 2017. Two experiments were conducted in 2017 with *A. fraterculus*, with 36 cages used for each experiment. In addition, 36 cages were used for natural host fruits (nine cages for each fruit fly species and experimental year).

At 8:00 a.m., 10- to 20-day-old *C. capitata* and 18- to 20-day-old *A. fraterculus* sexually mature adults were released inside cages according to treatments. The laboratory conditions were 26 ± 2°C and 65 ± 5% RH, with a 12-h photoperiod; these conditions remained throughout the assay.

The granadilla and natural host fruits were removed after 96 h of exposure and weighed individually on a digital balance (0.001 g precision), and the weights were recorded and each fruit was encoded.

Following Calzada (1982), the statistical analysis was based on a CRBD with the data square-root transformed for ANOVA, which was followed by Student's *t*-tests and Tukey's tests. The following variables were analyzed: infestation percentage, number of larvae and pupae, number of adults emerged, and percentage of adult emergence with respect to larvae and pupae.

Additional Experiments for Fruit Fly Species That Achieved Infestation on Punctured Granadilla

The granadilla fruits were punctured to facilitate infestation by breaking through the shell of fruits. These punctured fruits helped to demonstrate the role of the shell (pericarp) as a barrier resistance mechanism against fruit flies. Therefore, the following two experiments were conducted with fruit fly species that achieved infestation on punctured granadilla fruits.

Fruit Fly Developmental Period in Two Different Maturity Stages of Punctured Granadilla Fruit

The purpose of this test was to compare the development time for the fruit fly species that achieved infestation on two different maturity stages (yellow-green and yellow) of punctured fruit with that of flies on natural hosts.

To facilitate infestation, recently harvested granadilla fruits were taken to laboratory and punctured (10 holes per fruit) as deep as 10 mm. The fruits were then placed individually in cages and exposed for 2 h to 5- to 10-d-old *C. capitata* gravid females. The rate of 10 female fruit flies per fruit was selected to obtain approximately 100 eggs per fruit. The same procedure was performed to obtain infestation in the natural host fruit (peach).

At the end of the exposure, the fruits were weighed and placed in maturation boxes. Then, after 0, 12, 24, 36, 48, 60, 72, 96, 120, 144, 192, 240, 288, 336, 384, 480, 576, 720, 864, and 1,008 h, a group of fruits was dissected. The number of eggs, larvae (first, second, and third instars), and pupae were counted.

The eggs recovered from the punctured granadilla and natural host fruits during the evaluation were placed in hatching dishes (10-mm diameter, plastic Petri dishes containing absorbent wet cloth and two pieces of black cardboard on which the eggs were placed). The dishes were kept in a clean place for 4 d. Then, the number of emerged larvae was counted. This evaluation was used to determine the hatching percentage of eggs recovered from granadilla and natural host fruits. Larvae, pupae, and adults were also counted; larval instars were identified as first, second, and/or third.

According to Calzada (1982), statistical analyses of untransformed data and data transformed by natural logarithm were performed to calculate the mean development times for egg, larva (first, second, and third instars), and pupa stages. With oviposition the beginning, each developmental stage received a 'number of days' with a 50% value to calculate the cumulative percentage. The weight mean of the developmental time was calculated, and the developmental rate for each immature stage was estimated following a quadratic regression. The following data were obtained for each fruit, which were recorded and statistically processed: 1) number of recovered eggs, 2) number of live larvae, 3) number of pupae recovered, and 4) number of adults.

Oviposition Behavior of *C. capitata* and *A. fraterculus* in Field Sleeves

To determine the preference level for granadilla and natural host fruits exposed to 5- to 10-d-old *C. capitata* and 12- to 16-d-old *A. fraterculus* gravid females, an oviposition behavior test using sleeves was conducted under field conditions between July and August 2017. Three granadilla fruit maturity stages were used (green, green-yellow, and yellow), and the fruits were enclosed inside organza sleeves (1 and 0.5 m in length and width, respectively).

Observations were recorded during the hours of highest activity, from 9:00 a.m. to 12:00 p.m. for *C. capitata* and from 10:00 a.m. to 3:00 p.m. for *A. fraterculus*; therefore, the 1-day evaluation corresponded to 3 and 5 h for *C. capitata* and *A. fraterculus*, respectively. An evaluator was responsible for observing and recording the following behavioral parameters for one field sleeve: 1) number of visits to fruits; 2) oviposition attempts: ovipositor insertion without

aculeus dragging; and 3) successful oviposition: ovipositor insertion and subsequent aculeus dragging.

Each sleeve enclosed five fruits and included water and food. Five granadilla fruits and five natural host fruits were placed separately for each fruit fly species for the NCH test, and five granadilla fruits and five natural host fruits were placed together for each fruit fly species for the CH test. At the beginning of the test, sexually mature females were released at a 1:1 ratio (one fruit fly female per fruit). The fruit flies remained in the sleeve during the evaluation, and a 5 cm distance separated a granadilla fruit and a host fruit. A total of 90 sleeves were used in the entire study. For the tests involving granadilla, 36 sleeves were set up for *C. capitata* and 36 for *A. fraterculus*. In addition, 18 sleeves (nine per each fruit fly species) were established for the natural host fruits.

For the punctured treatments within CH or NCH experiments, each granadilla fruit was punctured on the day the test began. Ten punctures were randomly distributed on the fruit surface and were made by inserting a needle as deep as 10 mm.

To determine that the natural host fruits had not been infested previously, 10% of these fruits were placed in maturation boxes (60 × 50 × 10 cm in length, width, and depth, respectively). When fruit fly adults were not recovered from the fruits in these boxes, the absence of previous infestation was confirmed.

All tests used wild females. After completion of the test, all fruits were removed and taken to laboratory to be analyzed as previously explained.

Statistical descriptive and inferential evaluations were performed (Calzada 1982). The experiments were executed with type of fruit, field sleeve, fruit fly species, granadilla, and host fruit (peach and mango) for the 2017 harvest season. Three response variables were analyzed: NUMVISIT (number of visits), NUMINTOVI (number of oviposition attempts), and OVIPOEXI (number of successful ovipositions). The descriptive evaluation used previously gathered data regarding behavioral parameter per fruit fly species.

For the inferential evaluation, three maturity types of granadilla fruits were used for both fruit fly species: green, green-yellow, and yellow. Following Calzada (1982), statistical analysis was based on a CRBD with data square-root transformed for ANOVA, which was followed by Student's *t*-tests and Tukey's tests. Before using CRBD, the data were grouped by fruit fly species, experimental period, and type of fruit maturity. The following variables were compared: 1) number of visits to fruits; 2) number of oviposition attempts; 3) number of successful ovipositions; 4) number of recovered larvae; 5) number of recovered pupae; and 6) number of emerged adults.

Recovery of Fruit Fly Adults

The granadilla fruits from forced infestation experiments in both the field and laboratory were individually placed in 1.25-liter small plastic containers, with the bottom containing a sand layer as the pupation medium. The containers were tightly covered with a mesh lid (organza cloth) to avoid the escape of fruit fly larvae, to prevent the possible entrance of other insects such as *Drosophila* flies that could contaminate the sample, and to permit gas exchange. The containers were placed on wood/metal shelves (at various levels) in a room at 26 ± 1°C and 70 ± 10% RH.

After 20 d for *C. capitata* and 25 d for *A. fraterculus*, the containers were inspected for larvae and pupae. All fruits were dissected to remove any larvae that had not left the fruit for pupation, and the sand was checked for pupae. When present, larvae and pupae were counted and maintained in sand inside plastic containers (250 ml) to recover adults. After 15 d, the plastic containers were inspected daily to count and determine the sex of emerged adults. After this period, any pupae that remained without adult emergence were maintained for an additional month.

All recovered adults from granadilla fruits were put into bins or vials containing 70% alcohol. The adults were identified by properly trained SENASA personnel from Oxapampa. The experiments included host fruits that represented the controls, and these fruits were taken to laboratory and kept in the same facilities in which granadilla fruits were maintained, including applying control measures to prevent cross-contamination by *Drosophila* flies. The fruit flies from the colonies used in the assays, as well as the emerged flies of infested fruits, were stored in small plastic containers with 70% alcohol to maintain voucher specimens, which could eventually be required to corroborate the fruit fly taxon (family, genera, and/or species) or to provide other types of information.

Confidence Levels

According to Follet and Henneessey (2007), sample size and confidence levels would be significantly higher if the number of eggs laid by adult flies is estimated and used in calculations. To apply this statement, we included theoretical calculations of Confidence Levels using the formula suggested: $C = 1 - (1 - pu)n$; where C is the level of confidence, pu is the acceptable level of survivorship and n is the number of test insects, eggs in this case.

The confidence level was calculated for each fruit fly species by using the number of female fruit flies (12,480 of *C. capitata* and 2,490 of *A. fraterculus*), which were used during the study periods and the number of eggs that such females may lay in optimal conditions (this is understood as if the target fruit would not have resistance and as if puncturing would not have made). To do so, it was used the average number of eggs laid by females per day that corresponds to 25.2 for *A. fraterculus* (Malavasi and Zucchi, 2000) and 20 for *C. capitata* (De Graaf 2009).

Results

Records of Meteorological Parameters in the Experimental Zones During the 2016 and 2017 Seasons

During the 2016–2017 seasons, the mean ambient temperature in the granadilla production zones (Oxapampa, Pasco, Peru) varied from 17 to 20°C, the relative humidity varied from 80 to 94%, and the rainfall varied from 20 to 400 mm.

Fruit Fly Population Densities in the Experimental Orchards and their Surroundings for the 2016 and 2017 Seasons Based on a Trapping System

As shown in Fig. 2, fruit fly numbers fluctuated and peaks occurred from May to October, which is the fruiting season of many fruit species, providing good conditions for fruit fly reproduction. During this period, the fly density at population peaks varied between 0.2 and 1.6 flies per trap per day (FTD). The peaks with the highest FTD values occurred when rainfall and relative humidity were relatively low, and the lowest FTD values always coincided with the rainy season (January through March).

The trapped fruit fly adults confirmed the natural occurrence of *A. fraterculus* and *C. capitata* in Chorobamba and Huancabamba sectors and their corresponding subsectors. The total numbers of trapped *A. fraterculus* and *C. capitata* adults were 2,484 (1,306 males and 1,178 females) and 4,418 (1,142 males and 3,276 females), respectively.

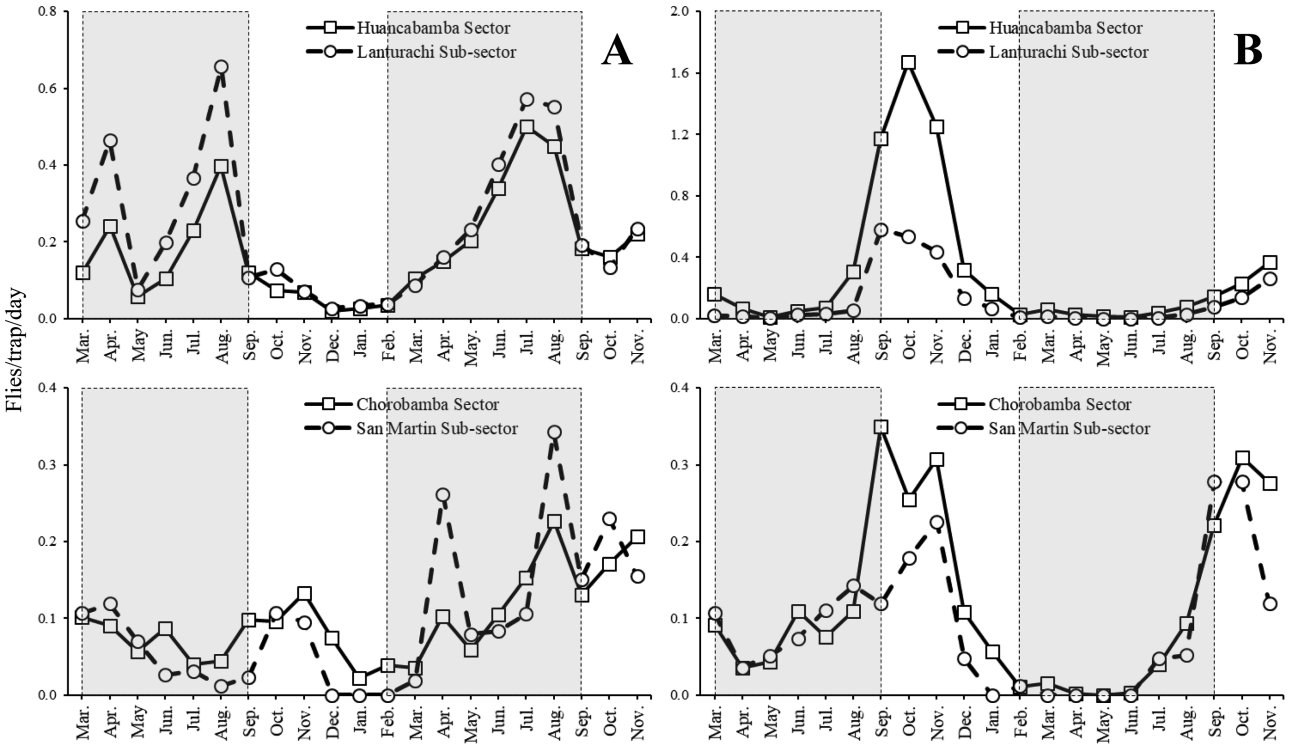

Fig. 2. Monthly values of flies per trap per day (FTD) for adult *A. fraterculus* (A) and adult *C. capitata* (B) captured in McPhail traps in granadilla production sectors and subsectors in 2016 and 2017. Oxapampa, Pasco, Peru.

Infestation-Level Assessment for *A. fraterculus* and *C. capitata* in Natural Hosts in Perimeter and Interior Portions of Experimental Orchards

To comply with the study protocol, the natural infestation of host fruit was required to demonstrate the natural occurrence of fruit flies. These data for potential host fruits are shown in Table 1.

A total of 8,490 fruits representing the fruits of 30 species were collected by SENASA fruit fly inspectors, which were evaluated in the SENASA laboratory in Oxapampa (Pasco). One thousand three hundred nine granadilla fruits were collected that could not be used commercially because the fruits were damaged and showed cracking and/or sunburn. These fruits were picked either from a plant or from the ground (fallen). Although these noncommercial granadilla fruits were vulnerable because of injuries, naturally occurring fruit flies did not infest them. By contrast, under the same natural soil and environmental conditions, host fruits generated fruit fly adults. In addition, *Anastrepha* spp. and *C. capitata* infested 16 natural host fruit species in the granadilla production areas.

Granadilla Fruit Sampling at the Experimental Orchards

A total of 1,940 commercial granadilla fruits were collected, 450 fruits in 2016 and 1,490 fruits in 2017. To determine the MI, 190 fruits were evaluated. Based on the dissection of 970 fruits (50% of the total) on the day of sampling and the other 970 fruits after 20 d in suitable containers, fruit fly eggs, larvae, or pupae were not found. These results demonstrated that naturally occurring fruit flies could not infest the granadilla fruits and are shown in Table 2.

According to the ICONTEC (1997), the MI should vary between 19.9 and 35.4. The range of MI values for Peruvian granadilla was higher (34–71, and most values were between 50 and 62) than that of previous publications, as shown in Table 3. The high levels of MI were explained by the high Brix values and low acidity levels for fruits with the surface mostly yellow.

The fruits were harvested with quantitative attributes also determined, such as the weight (121.21 ± 0.31 gr.) and maturity index (56.01 ± 0.8%), with levels/values for both parameters. In spite of the commercial attributes found for granadilla fruits, fruit fly immature stages (eggs, larvae, or pupae) were not found.

Choice and No-choice Foraging Behaviors Using Sleeves Under Field Conditions

First Evaluation: The Granadilla Epicarp Remained Unbroken (Intact) Until Day 20

In all field sleeve tests involving intact fruit, both *A. fraterculus* and *C. capitata* were unable to oviposit in the fruits through the epicarp, based on testing of all fruit maturity stages (green, green-yellow, and yellow). The infestations in host fruit species (control) confirmed that the female fruit flies used in the experiments were gravid and in an optimal condition for effective oviposition behavior. The resistance of the epicarp is fully discussed in the corresponding section of this article (Fig. 3).

Vargas et al. (2000) notes that *C. capitata* eggs hatch within 2–4 d and that larvae feed for another 6–11 d (at 13–35°C); therefore, third instar larvae take 6.3–10.2 d to leave the fruits. Similarly, Malavasi and Zucchi (2000) determined that *A. fraterculus* eggs hatch in 2.3–10.3 d at 15–30°C, and the larvae development take 11–34.5 d at 15–30°C; therefore, third instar larvae take 13.6–17.2 d to leave the fruits. The statistical comparisons between the treatments are shown in Tables 3 and 4.

Table 1. Infestation ratios for *Anastrepha* spp. and *C. capitata* in natural host fruits from granadilla production areas in Chorobamba and Huancabamba sectors in 2016 and 2017; Oxapampa, Pasco, Peru

Scientific name	Common name	Botanical family	Weight (Sum kg ± SEM)	Fruit fly Species	No of emerged individual
Annona cherimola Mill.	Cherimoya	Annonaceae	1.29 ± 0.11		
Capsicum frutescens L.	Pepper	Solanaceae	2.59 ± 0.01		
Capsicum pubescens Ruiz & Pav	Rocoto pepper	Solanaceae	5.28 ± 0.01		
Capsicum sp.	Paprika pepper	Solanaceae	0.02 ± 0.00		
Carica papaya L.	Papaya	Caricaceae	7.87 ± 0.09		
Citrus aurantium L.	Bitter orange	Rutaceae	2.87 ± 0.04		
Citrus jambhiri Lush.	Rough lemon	Rutaceae	12.15 ± 0.02	*A. fraterculus*	83
				C. capitata	10
Citrus limetta Risso	Sweet lemon	Rutaceae	1.46 ± 0.02	*A. fraterculus*	8
Citrus limettioides Tanaka	Sweet lime	Rutaceae	10.22 ± 0.02	*A. fraterculus*	89
Citrus limonia Risso	Rangpur	Rutaceae	58.4 ± 0.02	*A. fraterculus*	151
				C. capitata	51
Citrus reticulata Blanco	Mandarin orange	Rutaceae	1.51 ± 0.01	*A. fraterculus*	66
Citrus sinensis (L.) Osbeck	Orange	Rutaceae	34.92 ± 0.02	*A. fraterculus*	276
				C. capitata	54
Citrus X paradisi Macfad	Grapefruit	Rutaceae	3.59 ± 0.02	*A. fraterculus*	4
Citrus X tangelo J. W. Ingram & H. E. Moore	Tangelo	Rutaceae	0.70 ± 0.06	*A. fraterculus*	9
Coffea arabica L.	Coffee	Rubiaceae	12.39 ± 0.02	*C. capitata*	616
Cucurbita máxima Duchesne ex Lam.	Squash	Cucurbitaceae	10.49 ± 0.29	*A. grandis*	41
Cucurbita pepo L.	Pumpkin	Cucurbitaceae	2.80 ± 0.12		
Cyclantera pedata (L.) Shrader	Stuffing cucumber	Cucurbitaceae	17.16 ± 0.01		
Eriobotrya japónica (Thunb.) Lindl.	Loquat	Rosaceae	1.05 ± 0.02	*A. fraterculus*	13
Eugenia uniflora L.	Surinam cherry	Myrtaceae	1.92 ± 0.01	*A. fraterculus*	60
Inga sp.	Inga	Fabaceae	13.30 ± 0.01	*A. distincta*	68
Juglans regia L.	Walnut	Juglandaceae	10.91 ± 0.02	*A. schultzi*	298
Lucuma obovata	Lucuma	Sapotaceae	1.50 ± 0.02		
Passiflora ligularis Juss.	Sweet granadilla	Passifloraceae	115.64 ± 0.01		
Persea americana Mill.	Avocado	Lauraceae	7.02 ± 0.05		
Physalis peruviana L.	Goldenberry	Solanaceae	1.18 ± 0.00		
Prunus pérsica (L.) Batsch	Peach	Rosaceae	5.02 ± 0.01	*C. capitata*	503
Psidium guajaba L.	Guava	Myrtaceae	46.96 ± 0.00	*A. fraterculus*	63
				A. ornata	206
				A. striata	1,415
Solanum betaceum Cav.	Tamarillo	Solanaceae	1.86 ± 0.03		
Solanum quitoense Lam	Naranjillo	Solanaceae	1.34 ± 0.07		

Based on this information, without opening the fruit or breaking the epicarp, during the 20 d after intact and punctured granadilla were exposed to fruit flies, no larvae emerged from and no pupae were found outside these fruits. By contrast, larvae and pupae were found outside host fruits in high numbers. The host fruits started to decompose much earlier than the granadilla fruits because of the high infestation and therefore were opened 10 d after exposure.

Second Evaluation: The Granadilla Epicarp was Broken on Day 20

Results From Intact Granadilla Fruits.

In all field sleeve tests involving intact fruit, both *A. fraterculus* and *C. capitata* were unable to oviposit through the epicarp of all fruit maturity stages (green, green-yellow, and yellow). The infestation in host fruit species (control) confirmed that the female fruit flies were gravid and in optimal condition for effective oviposition behavior. To determine whether fruit flies completed the cycle inside of fruits, granadilla fruits were opened 20 d after exposure to female fruit flies, and no larvae and pupae were found inside or outside intact granadilla fruits.

In 2017, two trials were conducted for *A. fraterculus*. A total of 300 intact granadilla fruits (100 fruits per maturity stage) were exposed to 1,500 gravid females. After 20 d, granadilla fruits were dissected, and no infestation of intact granadilla fruits was observed. For *C. capitata*, three trials were conducted between 2016 and 2017, and 150 green, 150 green-yellow, and 150 yellow granadilla fruit were exposed to 2,250 gravid females. Fruit fly infestation was not observed in any maturity stage.

The natural host fruits used as controls were successfully infested. Mango was used for *A. fraterculus*. In the choice test, 150 fruits were exposed, and 81 (54%) were infested, from which 888 adults emerged (76% emergence) from 1,098 larvae and pupae. In the no-choice test, of the 150 fruits exposed, 79 (52%) fruits were infested, from which 555 adults emerged (79% emergence) from 699 larvae and pupae. Peach was the host fruit exposed to *C. capitata*. In the choice test, 225 fruits were exposed, and 165 (73%) were infested, from which 4,098 adults emerged (62%) from 6,599 larvae and pupae. In the no-choice test, 158 (70%) fruits were exposed and infested, from which 4,883 (62%) adults emerged from 7,934 larvae and pupae.

Results From Punctured Granadilla Fruits.

In punctured fruits, the results were different when the granadilla fruits were opened 20 d after exposure to fruit fly females. Some larvae and pupae were found inside the punctured granadilla.

Table 2. Granadilla fruits sampled from the production in Chorobamba and Huancabamba sectors in 2016 and 2017

Sample year	No. of fruits collected	Weight (g) (X±SEM)	Fruit maturity evaluation (X ± SEM)				No. of fruit fly immature stages
			N	% TSS	% Acidity	MI	
Sampling during 2016							
Chorobamba Sector	250	116.65 ± 1.00	20	12.35 ± 0.46	0.34 ± 0.04	43.47 ± 3.31	0
Huancabamba Sector	200	114.52 ± 0.89	20	13.35 ± 0.26	0.26 ± 0.01	52.00 ± 1.57	0
Sampling during 2017							
Chorobamba Sector	700	123.79 ± 0.50	70	14.67 ± 0.19	0.25 ± 0.00	59.38 ± 1.15	0
Huancabamba Sector	790	122.07 ± 0.46	80	15.34 ± 0.16	0.28 ± 0.01	57.20 ± 1.05	0
TOTAL:	1,940	121.21 ± 0.31	190	14.57 ± 0.13	0.27 ± 0.01	56.01 ± 0.8	0

Oxapampa, Pasco, Peru. TSS = total soluble solids and MI = maturity index.

Anastrepha fraterculus did not infest the 100 green fruits that were exposed. For the green-yellow fruits, five fruits were infested of the 100 (5% infestation) that were exposed, with 32 larvae and pupae recovered from which 12 adults emerged (38% emergence). For the yellow fruits, 34 fruits were infested of the 100 (34% infestation) that were exposed, with 309 larvae and pupae recovered from which 89 adults emerged (29% emergence). The performance of *C. capitata* was different. This fruit fly species was able to oviposit in the punctures of 52 green fruits of the 150 exposed (35%), with 527 larvae and pupae recovered from which 76 adults emerged (14% emergence). For the green-yellow fruits, 114 fruits were infested of the 150 exposed (75%), with 1,762 larvae and pupae recovered from which 274 adults emerged (16% emergence). For the yellow fruits, 100 fruits were infested of the 150 exposed (67%), with 1,456 larvae and pupae recovered from which 342 adults emerged (81% emergence). See Table 5.

A greater proportion of natural host fruits were infested than that of granadilla. For *A. fraterculus* in the choice test, 73 mango fruits were infested of the 150 (49% infestation), with 907 larvae and pupae recovered from which 673 adults emerged (74% emergence). For *C. capitata*, 138 peach fruits were infested of the 225 fruits exposed (61% infestation), with 3,256 larvae and pupae recovered from which 2,500 adults emerged (77% emergence).

Granadilla Fruits Artificially Exposed to Female Fruit Flies Under Laboratory Cage Conditions

This experiment was also conducted using the two evaluations, the first one simulated natural conditions before cutting the fruit after 20 d of having been exposed to fruit flies, verifying the presence of larvae and pupae outside fruits and/or in the sand. The second one was performed after cutting such fruits after 20 d of having been exposed to fruit flies, verifying the presence of larvae and pupae inside fruits.

First Evaluation: Granadilla Epicarp Remained Unbroken Until Day 20

Based on the life cycle information provided by Vargas et al. (2000) and Malavasi and Zucchi (2000) for both *C. capitata* and *A. fraterculus*, fruits were not opened for 20 d after intact and punctured granadilla were exposed to fruit flies. No larvae emerged and no pupae were found outside the fruits, which was in contrast to high-intensity infestation in host fruits.

In all laboratory cage tests involving intact fruit, both *A. fraterculus* and *C. capitata* were unable to oviposit in these fruits through the epicarp, at any of the fruit maturity stages (green, green-yellow, and yellow) that were subjected to forced infestation in experimental cages in the laboratory. The infestation in host fruit species (control) confirmed that the female fruit flies used in these experiments were gravid and in optimal condition for effective oviposition behavior.

Second Evaluation for Intact Granadilla Epicarp was Broken on Day 20 (Cage-Lab)

Two trials were conducted in 2017, and a total of 900 gravid females of *A. fraterculus* were exposed to 90 intact granadilla fruits (30 fruits per maturity stage). After the dissection of fruits, no infestation of intact granadilla fruits was observed in any maturity stage. Two trials were conducted for *C. capitata* between 2016 and 2017. A total of 900 gravid females were exposed to 60 green, 60 green-yellow, and 60 yellow granadilla fruits, and infestation was not observed at any maturity stage.

Table 3. Results from ANOVA (DF-treatment, DF-Replicate, *F*-value, *P*-value) and Tukey's comparison of means for *C. capitata* larvae and pupae observed outside fruits (granadilla and host) 20 d after exposure to fruit flies without opening granadilla fruits

A) *C. capitata* in Chorobamba sector-2016						
Variable	Yellow		Green-Yellow		Green	
DF-Treatment	6		6		6	
DF-Replicate	4		4		4	
F-value	8.61		7.52		6.71	
P-value	0		0		0	
Treatments	Mean	Group	Mean	Group	Mean	Group
NCh-host	1.6833	A	1.17	AB	1.529	A
Ch-pun-host	1.4367	AB	1.144	AB	0.669	AB
Ch-intac-host	2.5046	A	2.073	A	1.223	A
NCh-pun-gran	0	B	0	B	0	B
Ch-pun-gran	0	B	0	B	0	B
NCh-intac-gran	0	B	0	B	0	B
Ch-intac-gran	0	B	0	B	0	B
B) *C. capitata* in Chorobamba sector-2017						
Variable	Yellow		Green-Yellow		Green	
DF-Treatment	6		6		6	
DF-Replicate	4		4		4	
F-value	184.99		106.21		68.83	
P-value	0		0		0	
Treatments	Mean	Group	Mean	Group	Mean	Group
NCh-host	7.0127	A	4.642	A	6.517	A
Ch-pun-host	4.39262	B	2.327	B	4.019	B
Ch-intac-host	6.85385	A	4.74	A	4.556	B
NCh-pun-gran	0	C	0	C	0	C
Ch-pun-gran	0	C	0	C	0	C
NCh-intac-gran	0	C	0	C	0	C
Ch-intac-gran	0	C	0	C	0	C
C) *C. capitata* in Huacabamba sector-2017						
Variable	Yellow		Green-Yellow		Green	
DF-Treatment	6		6		6	
DF-Replicate	4		4		4	
F-value	149.78		50.78		17.53	
P-value	0		0		0	
Treatments	Mean	Group	Mean	Group	Mean	Group
NCh-host	5.18333	B	4.564	A	3.711	A
Ch-pun-host	4.76478	B	3.062	B	2.966	A
Ch-intac-host	6.95943	A	4.429	A	2.812	A
NCh-pun-gran	0	C	0	C	0	B
Ch-pun-gran	0	C	0	C	0	B
NCh-intac-gran	0	C	0	C	0	B
Ch-intac-gran	0	C	0	C	0	B

Results correspond with the field sleeve test with three maturity stages of granadilla in Oxapampa, Pasco, Peru. DF = degrees of freedom. Names of treatments are as follows: Ch-intac-gran = Choice-intact granadilla; Ch-intac-host = Choice-intact host; Ch-pun-gran = Choice-punctured granadilla; Ch-pun-host = Choice-punctured host; NCh-host = No-Choice host; NCh-intac-gran = No-Choice intact granadilla; and NCh-pun-gran = No-Choice punctured granadilla. Oxapampa, Pasco, Peru, 2017.

By contrast, the natural host fruits used as a control were successfully infested. Mango was used as the natural host for *A. fraterculus*. In the choice test, 90 fruits were exposed, and 57 were infested, with the recovery of 1,098 larvae and pupae from which 888 adults emerged. In the no-choice test, 30 fruits were exposed, and 27 were infested, with the recovery of 581 larvae and pupae from which 428 adults emerged.

The host fruits exposed to *C. capitata* were cherimoya in the 2016 trial and peach in the 2017 trial. In the choice experiment, 90 fruits were exposed, and 87 were infested, with the recovery of 8,181 larvae and pupae from which 5,997 adults emerged. In the no-choice test, 30 fruits were exposed and infested, and 3,902 larvae and pupae were recovered from which 2,453 adults emerged.

Second Evaluation for Punctured Granadilla Epicarp was Broken on Day 20 (Cage-Lab)

Anastrepha fraterculus infested 10 (17%) green fruits of the 60 exposed, and 64 larvae and pupae were recovered from which 12 adults emerged. For green-yellow fruits, 11 (18%) were infested of the 60 exposed, with the recovery of 658 larvae and pupae from

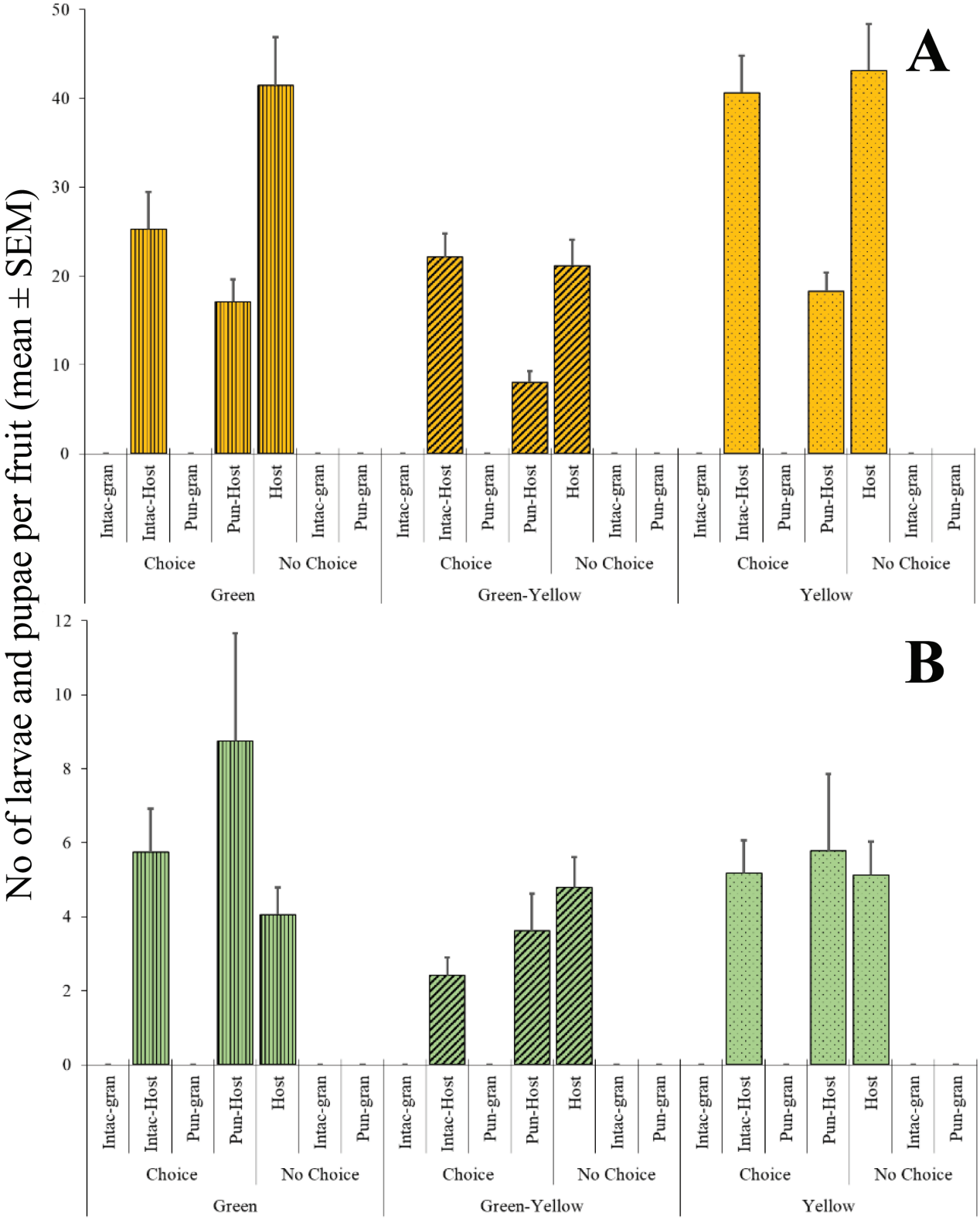

Fig. 3. Average number of larvae and pupae of *C. capitata* (A) and *A. fraterculus* (B) recovered after 20 d *without opening granadilla fruits* from sleeve field tests in 2016 and 2017. Treatments are as follows: Intac-gran = Intact granadilla; Intac-host = Intact host; Pun-gran = Punctured granadilla; Pun-host = Punctured host; Host = only host. Oxapampa, Pasco, Peru.

which 20 adults emerged. For yellow fruits, 28 (47%) were infested of the 60 exposed, and 360 larvae and pupae were recovered from which 143 adults emerged.

For *C. capitata*, 26 (43%) of 60 green fruits were infested, with the recovery of 153 larvae and pupae from which 21 adults emerged. In the green-yellow maturity category, 39 fruits (65%) of the 60

Table 4. Results from ANOVA (DF- degrees of freedom for treatments, DF-degrees of freedom for replicates, *F*-value, *P*-value) and Tukey's comparison of means for *A. fraterculus* larvae and pupae observed outside fruits (granadilla and host) 20 d after exposure to fruit flies without opening granadilla fruits

A) *A. fraterculus* in Huancabamba sector-2017-1

Variable	Yellow		Green-Yellow		Green	
DF-Treatment	6		6		6	
DF-Replicate	4		4		4	
F-value	15.63		16.85		12.96	
P-value	0.000		0.000		0.000	
Treatments	Mean	Group	Mean	Group	Mean	Group
NCh-host	1.89942	A	1.811	A	1.349	A
Ch-pun-host	1.83213	A	1.333	A	2.271	A
Ch-intac-host	1.61808	A	1.056	A	1.789	A
NCh-pun-gran	0	B	0	B	0	B
Ch-pun-gran	0	B	0	B	0	B
NCh-intac-gran	0	B	0	B	0	B
Ch-intac-gran	0	B	0	B	0	B

B) *A. fraterculus* in Huancabamba sector-2017-2

Variable	Yellow		Green-Yellow		Green	
DF-Treatment	6		6		6	
DF-Replicate	4		4		4	
F-value	15.06		8.77		13.57	
P-value	0.000		0.000		0.000	
Treatments	Mean	Group	Mean	Group	Mean	Group
NCh-host	1.27365	A	1.247	A	1.421	A
Ch-pun-host	1.10776	A	0.944	A	1.507	A
Ch-intac-host	1.80393	A	0.951	A	1.482	A
NCh-pun-gran	0	B	0	B	0	B
Ch-pun-gran	0	B	0	B	0	B
NCh-intac-gran	0	B	0	B	0	B
Ch-intac-gran	0	B	0	B	0	B

Results correspond with the field sleeve test with three maturity stages of granadilla in Oxapampa, Pasco, Peru. DF = degrees of freedom. Names of treatments are as follows: Ch-intac-gran = Choice-intact granadilla; Ch-intac-host = Choice-intact host; Ch-pun-gran = Choice-punctured granadilla; Ch-pun-host = Choice-punctured host; NCh-host = No-Choice host; NCh-intac-gran = No-Choice intact granadilla; and NCh-pun-gran = No-Choice punctured granadilla. Oxapampa, Pasco, Peru, 2017.

fruits exposed were infested, with the recovery of 1,093 larvae and pupae from which 149 adults emerged. In the yellow maturity category, 54 (90%) of the 60 fruits exposed were infested, with the recovery of 1,650 larvae and pupae from which 146 adults emerged.

The natural host fruits used for choice tests involving *A. fraterculus* were infested at a greater proportion than that of granadilla fruits. A total of 69 (77%) of the 90 mango fruits were infested, with the recovery of 1,459 larvae and pupae from which 1,186 adults emerged (81% emergence). For the host fruit of *C. capitata*, of the 90 fruits exposed, 89 (99%) were infested, and 6,317 larvae and pupae were recovered, from which 4,523 adults emerged (72% emergence).

Fruit Fly Developmental Period Test in Two Stages of Granadilla Fruit Maturity

The results obtained for green-yellow (Fig. 4A) and yellow (Fig. 4B) granadilla fruits were similar. Fifty percent of the eggs remained until 1.5 d after being placed on the granadilla fruit, which was a result similar to that of the host fruit. For the first-instar larvae, 50% appeared after 5 d, which was 1 d later than the appearance in the host fruit. For the second larval instar, 50% appeared after 12 d, which was 2 d later than the appearance in the host fruit (Fig. 4C). In addition, this larval stage was observed over 24 d.

For third-instar larvae, 50% entered this larval instar after 20 d, which was a couple of days later than the third instars in the host fruit. In addition, this larval instar was observed for over 36 d. The development of pupae inside green-yellow granadilla was longer than that in the host fruit, with 50% of pupae appearing after 36 d, which was 15 d later than the appearance in the host. In addition, pupae were recovered for over 42 d. Adult emergence was not observed from yellow and green-yellow granadilla fruits, in contrast to host fruits, which showed adult emergence.

The resistance of granadilla fruit against the fruit fly *C. capitata* was demonstrated again, because adults did not emerge from punctured granadilla fruits that were infested, at either green-yellow or yellow maturity levels. As previously observed, the ready-to-pupate larvae could not leave the fruits and were forced to pupate inside. These pupae eventually died inside.

The development times for the eggs, larvae, and pupae of *C. capitata* were similar between green-yellow and yellow granadilla fruits. However, compared with the host fruit (peach), eggs, larvae, and pupae needed longer to develop in granadilla fruits. Based on the increase in development time of larval instars I, II, and III and the absence of recovered adults, punctured granadilla was not a good host for this fruit fly species.

Table 5. Number of fruits infested, adults emerged per fruit, and number of larvae and pupae recovered when granadilla fruits were dissected 20 d after exposure to fruit fly females of *A. fraterculus* and *C. capitata* in field sleeve tests in 2016 and 2017; Oxapampa, Pasco, Peru

Treatments	*A. fraterculus*			*C. capitata*		
	No. of fruits infested	Larvae and pupae (mean ± SEM)	Adults emerged (mean ± SEM)	No. of fruits infested	Larvae and pupae (mean ± SEM)	Adults emerged (mean ± SEM)
Yellow						
Ch-intac-gran	0	0	0	0	0	0
Ch-intac-host	31	8.35 ± 1.08	6.32 ± 0.83	63	48.32 ± 4.4	24.59 ± 2.46
Ch-pun-gran	23	7.00 ± 0.95	5.13 ± 0.99	53	13.25 ± 3.20	12.19 ± 3.22
Ch-pun-host	24	12.04 ± 4.00	10.71 ± 3.88	53	25.92 ± 2.16	19.92 ± 1.67
NCh-host	27	9.48 ± 1.16	8.50 ± 1.15	55	58.85 ± 5.77	27.53 ± 2.73
NCh-intac-gran	0	0	0	0	0	0
NCh-pun-gran	11	13.45 ± 5.23	9.60 ± 4.81	47	16.04 ± 2.54	6.39 ± 1.02
Green-Yellow						
Ch-intac-gran	0	0	0	0	0	0
Ch-intac-host	23	5.26 ± 0.66	4.00 ± 0.5	59	28.12 ± 2.88	22.12 ± 2.74
Ch-pun-gran	0	0	0	65	15.98 ± 1.79	5.85 ± 1.01
Ch-pun-host	22	8.23 ± 1.90	5.19 ± 0.62	41	14.66 ± 1.68	12.85 ± 1.43
NCh-host	26	9.23 ± 0.92	8.25 ± 0.85	52	30.54 ± 3.45	24.69 ± 3.05
NCh-intac-gran	0	0	0	0	0	0
NCh-pun-gran	5	6.40 ± 3.11	4.00 ± 1.18	49	14.76 ± 2.29	3.68 ± 0.69
Green						
Ch-intac-gran	0	0	0	0	0	0
Ch-intac-host	27	10.63 ± 1.67	8.54 ± 1.45	43	44.09 ± 5.77	30.14 ± 3.61
Ch-pun-gran	0	0	0	33	13.42 ± 2.89	6.17 ± 1.69
Ch-pun-host	27	16.19 ± 4.99	13.04 ± 4.93	44	29.11 ± 3.34	23.10 ± 2.40
NCh-host	26	7.81 ± 0.93	6.12 ± 0.82	51	60.96 ± 6.43	40.88 ± 5.00
NCh-intac-gran	0	0	0	0	0	0
NCh-pun-gran	0	0	0	19	4.42 ± 0.86	1.00 ± 0.00

Names of treatments are as follows: NCh-host: No-Choice host; Ch-pun-host: Choice-punctured host; Ch-intac-host: Choice-intact host; NCh-pun-gran: No-Choice punctured granadilla; Ch-pun-gran: Choice-punctured granadilla; NCh-intac-gran: No-Choice intact granadilla; and Ch-intac-gran: Choice-intact granadilla. Maturity stages: Yellow = fully ripened; Green-Yellow = in the process of ripening; Green = unripe but ready to start color change.

According to Fig. 4A, when the development in yellow granadilla fruit and the host fruit (peach) was compared, the egg period in yellow granadilla fruits was similar to that in the host, with 50% of the eggs remaining until 1.5 d after being placed on the fruits. For larval instar I, the development in yellow granadilla fruits was longer than that in the host fruit, with 50% of the larvae appearing after day 5, which was 1 d later than the appearance in host fruit. In addition, the larvae at stage I were observed until day 12. For larval instar II, the development in yellow granadilla fruits was also longer than that in the host fruit, with 50% of the larvae appearing after day 12, which was 6 d later than the appearance in host fruit. In addition, the larvae at stage II were observed until day 16. For larval instar III, the development in yellow granadilla fruits continued to be longer than that in the host fruit, with 50% of the larvae appearing after day 20, which was 10 d later than the appearance in host fruit. In addition, the larvae at stage III were observed until day 30. For pupae, the development in yellow granadilla fruits was longer than that in the host fruit, with 50% of the pupae appearing after day 35, which was 20 d later than the appearance in the host fruit. In addition, pupae were observed up to day 42. For adults, no emergence was observed from granadilla fruits, because the hardness of the pericarp prevented larvae from leaving for pupation.

According to Fig. 4B, when the development in green-yellow granadilla fruit and the host fruit (peach) was compared, the egg period in the green-yellow granadilla fruits was similar to that of the yellow granadilla and to that of the host fruit, with 50% of the eggs remaining until 1.5 d after being placed on the fruits. The development of larvae I in green-yellow granadilla fruits was longer than that in the host fruit, with 50% of the larvae appearing after day 5, which was 1 d later than the appearance in the host fruit. In addition, instar I larvae were observed until day 14. The development of larvae II in green-yellow granadilla was also longer than that in the host fruit, with 50% of the larvae appearing after day 10, which was 2 d later than the appearance in the host fruit. In addition, instar II larvae were observed until day 24. The development of larvae III in green-yellow granadilla fruits continued to be longer than that in the host fruit, with 50% of the larvae appearing after day 14, which was 2 d later than the appearance in the host fruit. In addition, instar III larvae were observed until day 36. The development of pupae in green-yellow granadilla fruits was longer than that in the host fruit, with 50% of the pupae appearing after day 35, which was 15 d later than the appearance in the host fruit. In addition, pupae were observed up to day 42. For the green-yellow granadilla fruits, no adult emergence was observed, because the hardness of the pericarp did not allow larvae to leave for pupation.

Oviposition Behavior of *C. capitata* and *A. fraterculus* Under Field Conditions Using Sleeves

Results corresponding to *A. fraterculus* can be found in Fig. 5A (Choice) and 5B (No-Choice), and for *C. capitata* in Fig. 5C (Choice) and 5D (No-Choice). In all cases, intact and punctured green, green-yellow, and yellow granadilla fruits are included.

Anastrepha fraterculus females could not lay eggs in intact or punctured green, green-yellow, and yellow granadilla fruits, in both choice and no-choice tests. Although *C. capitata* could not lay eggs in intact

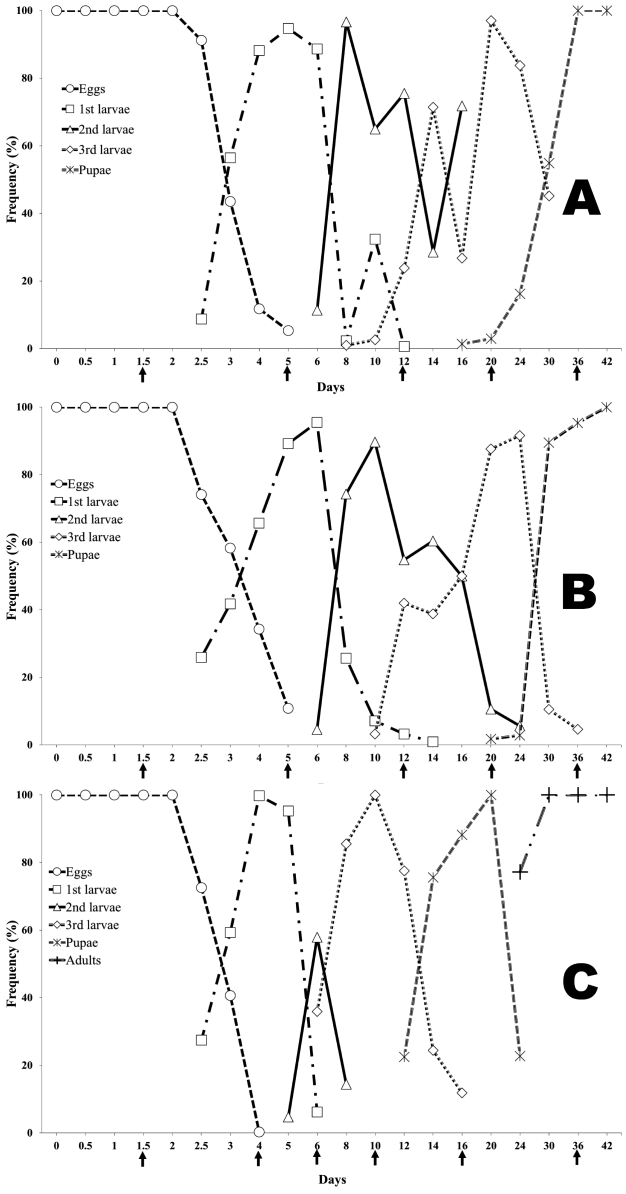

Fig. 4. Development period of *C. capitata* immature stages in yellow (A) and green-yellow (B) granadilla fruits, as well as in host fruit (C) in 2017.The arrows indicate the accumulated probability for each fruit fly instar (first-, second-, and third-instar larvae) and stage (egg, larva, and pupa). Oxapampa, Pasco, Peru.

granadilla fruits, eggs were successfully laid in a few punctured green-yellow (Choice and No Choice) and yellow granadillas; however, emerging larvae were not observed. By contrast, oviposition was successful in host fruits (control) and resulted in larvae, pupae, and adults. These results confirmed that the hardness of the epicarp is a resistance mechanism against fruit flies in intact granadilla fruits, regardless of the maturity stage. Corresponding ANOVA is shown in Table 6.

Discussion

The ranges of temperature and relative humidity recorded in the experimental zones during 2016 and 2017 were optimal for the granadilla crop and for fruit fly development. The discussion on rainfall (mm) is focused on the rainfall that occurred in December–March and the 'dry' season that occurred in April–November. The 'dry' season was when rainfall occurred but at reduced volumes. In general, rainfall can affect insect populations when drops of water make direct contact with insects and create good conditions for the progress of entomopathogens; however, some insects have evolved strategies to survive rainfall, including initiating activities following rainfall during the day, which is observed in the diurnal behavior of fruit flies. Variation was observed in the weather, particularly in rainfall, and the variation was a good indicator of the experimental replicate years 2016 and 2017. These differences in meteorological conditions between years helped to explain the overall results.

It is worth mentioning that *C. capitata* had a higher usage of punctured fruits since it laid eggs in 53.7% of such punctured fruits, comparing to *A. fraterculus* that laid eggs in just 15.4% of those punctured fruits. In contrast, usage of intact fruit was similar between these two fruit fly species since none of them could lay eggs on the hard skin of granadilla fruits.

Based on the trapping system and the fluctuations in fruit fly populations, the high capture of fruit fly males in McPhail traps was most likely explained by the feeding lure Torula and the pheromone produced by captured females before being trapped. The total number of trapped, naturally occurring *C. capitata* adults was almost double the total number of *A. fraterculus* adults in the Oxapampa experimental area. Moreover, the male:female ratios were 1:0.9 and 1:2.8 for *A. fraterculus* and *C. capitata*, respectively. The predominance of the naturally occurring Mediterranean fruit fly *C. capitata* may be of value for the aims of the study because of the high importance for quarantine.

The availability of host fruit was an important factor affecting fruit fly population fluctuations throughout the experimental periods. Similar to the months of the year when harvestable or commercial host fruit is available, most of the host fruit species had fruit available from May to October, when the fruit fly (*A. fraterculus* and *C. capitata*) populations peaked (from trapping system) during both 2016 and 2017 seasons. The highest FTD values were in 2016 for *C. capitata* (above 1.6), and this level could have been related to an increase in the number of host fruits available because of the rainfall amounts during January–March 2016, which were the highest during the entire experimental period from 2016 to 2017.

For clarity, the sleeve and cage experiments were interpreted using the two evaluations. In the first evaluation, intact and punctured granadilla fruits were not opened for 20 d after exposure to fruits flies. Larvae and pupae were not found outside either intact or punctured granadilla, whereas many larvae and pupae were recorded outside other host fruits. The punctured fruits had larvae and pupae inside, because the third-instar larvae could not leave the fruits for pupation as they did on host fruit. However, the second evaluation also suggests that when the epicarp is broken, artificial infestation can occur. Notably, fruits with a broken epicarp do not have commercial value and therefore are not exported, preventing the potential passive dispersion of fruit flies. After 20 d, larvae and pupae were not found inside or outside intact granadilla fruits, but some larvae and pupae were found remaining inside punctured granadilla fruits. Thus, both evaluations demonstrated the epicarp of granadilla fruit is an effective barrier in preventing fruit fly infestation and movement.

The larvae that developed from artificial oviposition performed by female fruit flies through the needle holes could not leave punctured granadilla fruits, and therefore, unusual pupation occurred inside those fruits. This internal pupation occurred only in some of the punctured granadilla fruits that were exposed to fruit fly females. The intact granadilla fruits remained completely free from fruit fly eggs and, therefore, also from larvae and pupae. This result suggests that the pericarp is a resistance mechanism, which is discussed in more detail as follows.

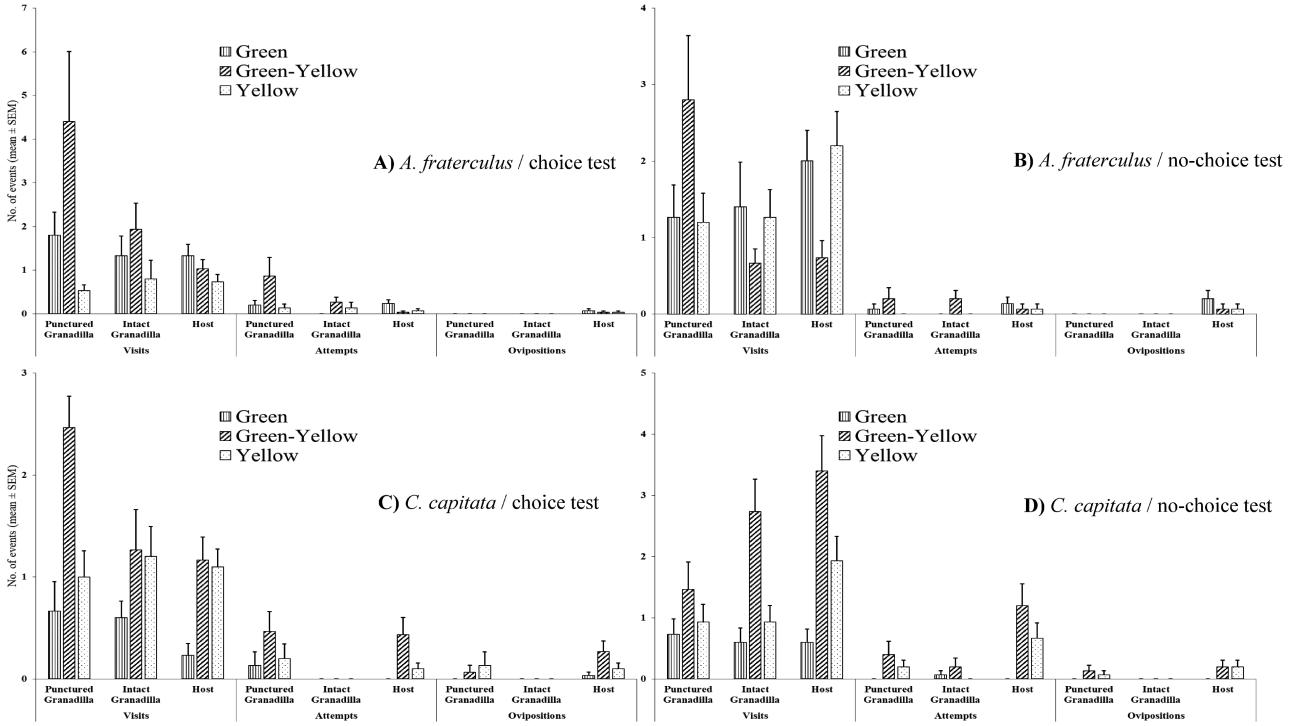

Fig. 5. Average visits, oviposition attempts, and successful ovipositions of *A. fraterculus* (A and B) and *C. capitata* (C and D) females for three maturity stages of granadilla fruits (green, green-yellow, and yellow) under Choice (A and C) and No-Choice (B and D) conditions involving intact and punctured granadilla fruits and natural host fruits.

Liquido et al. (1990) report 1.84 *C. capitata* flies/kilo of sampled granadilla fruits in Hawaii; however, said authors do not mention the fruit condition nor the maturity degree. In addition, according to their methodology, fruits were collected from trees and those fallen on the ground. In contrast, we did not report *C. capitata* infestation from the granadilla fruits collected from trees and from those fallen on the ground.

When fruits were punctured, juice emerged from some of the punctures and may explain why some eggs deposited in the needles holes hatched and some larvae developed and pupated inside fruits. The juice from the puncture would have provided moisture for the eggs and first instar larvae. The punctures without juice would have resulted in dry conditions for eggs and therefore affected their incubation period. The eggs surrounded by dry tissue of the mesocarp may have died or the incubation period may have been extended. If extended, the larvae that hatched may have been weakened or with poor growth, which may have complemented the subsequent and direct mechanical resistance on larvae and pupae. The slower egg development in granadilla than that in host fruit could be explained by the stress of a dry environment for eggs.

The failure of larvae to leave and the forced pupation inside punctured granadilla fruits indicated the barrier also worked from the inside. The ready-to-pupate third-instar larvae were not been able to perforate the endocarp, which is a paper-like, thick, hard, and dry tissue that the mouthparts of larvae cannot break, and were forced to pupate inside the fruits. Thus, when pupae were found in the pulp, the conclusion was that larvae could not perforate the endocarp.

However, other pupae were found within the mesocarp and endocarp, which indicated that the related larvae could perforate the endocarp but never the mesocarp and the epicarp. The inability to break through was likely related to the absence of free water in these layers, which increased the difficulties for larval movements.

Moreover, in addition to the difficulty caused by the paper-like and dry texture and the absence of free water in these layers, the hardness, explained by the very low water content and the high level of fiber and pectin, might also hinder the movement of hatched larvae (first instar). The paper-like and dry texture should be considered another type of mechanical resistance against first and second instar larvae and most likely caused abrasions to the relatively thin cuticle of the larvae. Some of these larvae were found dead within mesocarp and endocarp layers.

Anastrepha fraterculus used the punctures to lay eggs but only in green-yellow and yellow fruits, indicating that green fruits were not preferred even with holes available. The holes alone did not guarantee a completely successful *A. fraterculus* infestation because of the low percentage of larvae turning into pupae and pupae turning into adults. Because fruit flies are not usually attracted to green fruits, this non-preference for green fruits would not be considered as a form of resistance; however, the slowed development in green-yellow and yellow fruits may involve the resistance mechanisms previously addressed.

Similarly, in the experiment examining the development of *C. capitata*, the development time for eggs, larvae, and pupae was longer in granadilla fruit than that in host fruit. Although the MI of granadilla fruits was relatively high, the MI depends solely upon Brix degree and acidity; therefore, other factors may have also been important. Generally, development time in insects is expanded when conditions in a given fruit are not optimal, which can include certain chemical compounds that may affect the regular development. To complete the larval stage, the larvae must have eaten the arils of the pulp, which may have slowed development compared with that in the host fruit. In addition, because pupae are directly formed from larvae, they also showed slower development. Therefore, pupal development time depended not only on the pupal environment but

Table 6. ANOVA (df = degrees of freedom) and Tukey's comparison of means for oviposition behavior of *A. fraterculus* and *C. capitata* involving fruits of granadilla at different stages of maturity (yellow, green-yellow, and green); Oxapampa, Pasco, Perú

Anastrepha fraterculus

	Yellow						Green-Yellow						Green					
Variable	NUMVISIT		NUMINTOVI		OVIPOEXI		NUMVISIT		NUMINTOVI		OVIPOEXI		NUMVISIT		NUMINTOVI		OVIPOEXI	
Gl-Treatment	6		6		6		6		6		6		6		6		6	
Gl-Replicate	2		2		2		2		2		2		2		2		2	
F-value	2.460		0.540		0.820		2.500		2.050		0.850		0.730		1.320		1.890	
P-value	0.029		0.773		0.554		0.027		0.066		0.534		0.672		0.256		0.090	
Treatments	Mean	Group	Mean	Group	Mean	Group	Mean	Group	Mean	Group	Mean	Group	Mean	Group	Mean	Group	Mean	Group
NCh-host	1.279	A	0.067	A	0.067	A	0.610	A	0.067	AB	0.067	A	1.221	A	0.133	A	0.200	A
Ch-pun-host	0.683		0.067	A	0.000	A	0.777	A	0.067	AB	0.067	A	0.893	A	0.200	A	0.067	A
Ch-intac-host	0.494	B	0.067	A	0.067	A	0.714	A	0.000	B	0.000	A	0.913	A	0.228	A	0.067	A
NCh-pun-gran	0.843	AB	0.000	A	0.000	A	1.273	A	0.161	AB	0.000	A	0.828	A	0.067	A	0.000	A
Ch-pun-gran	0.533	AB	0.133	A	0.000	A	1.480	A	0.492	A	0.000	A	1.120	A	0.200	A	0.000	A
NCh-intac-gran	0.808	AB	0.000	A	0.000	A	0.589	A	0.200	AB	0.000	A	0.793	A	0.000	A	0.000	A
Ch-intac-gran	0.479	B	0.094	A	0.000	A	0.973	A	0.267	AB	0.000	A	0.810	A	0.000	A	0.000	A

Ceratitis capitata

Variable	Yellow						Green-Yellow						Green					
	NUMVISIT		NUMINTOVI		OVIPOEXI		NUMVISIT		NUMINTOVI		OVIPOEXI		NUMVISIT		NUMINTOVI		OVIPOEXI	
Gl-Treatment	6		6		6		6		6		6		6		6		6	
Gl-Replicate	2		2		2		2		2		2		2		2		2	
F-value	1.270		3.360		0.940		6.050		3.910		1.340		1.240		0.830		1.000	
P-value	0.277		0.005		0.469		0.000		0.002		0.247		0.293		0.546		0.430	
Treatments	Mean	Group	Mean	Group	Mean	Group	Mean	Group	Mean	Group	Mean	Group	Mean	Group	Mean	Group	Mean	Group
NCh-host	1.203	A	0.504	A	0.200	A	1.714	A	0.780	A	0.200	A	0.483	A	0.000	A	0.000	A
Ch-pun-host	0.948	A	0.133	AB	0.133	A	0.876	BCD	0.067	B	0.200	A	0.249	A	0.000	A	0.000	A
Ch-intac-host	0.732	A	0.067	B	0.067	A	0.780		0.550	AB	0.255	A	0.133	A	0.000	A	0.067	A
NCh-pun-gran	0.686	A	0.200	AB	0.067	A	0.934	BC	0.315	AB	0.133	A	0.571	A	0.000	A	0.000	A
Ch-pun-gran	0.759	A	0.161	AB	0.094	A	1.488	ABC	0.389	AB	0.067	A	0.459	A	0.094	A	0.000	A
NCh-intac-gran	0.725	A	0.000	B	0.000	A	1.522	AB	0.161	B	0.000	A	0.476	A	0.067	A	0.000	A
Ch-intac-gran	0.868	A	0.000	B	0.000	A	0.828	CD	0.000	B	0.000	A	0.561	A	0.000	A	0.000	A

Maturity stages: Yellow: fully ripened; Green-Yellow = in the process of ripening; and Green = immature but ready to initiate color changes. Oviposition behavior: NUMVIST: number of visits; NUMINTOVI: number of oviposition attempts; and OVIPOEXI: successful ovipositions. The names of the treatments are as follows: NCh-host: No-Choice host; Ch-pun-host: Choice-punctured host; Ch-intac-host: Choice-intact host; NCh-pun-gran: No-Choice punctured granadilla; Ch-pun-gran: Choice-punctured granadilla; NCh-intac-gran: No-Choice intact granadilla; and Ch-intac-gran: Choice-intact granadilla.

also on the food quality and medium that were associated with the larvae. To explain the slowed development, granadilla pulp aril may also have resistance mechanisms against fruit flies; e.g., the pulp aril has a glue-like or mucilage-like substance that could cover the spiracles, affecting respiration in larvae and pupae, or render movement of larvae difficult in the pulp. Such a combined effect would slow fruit fly development compared with that in host fruits, such as mango or peach. In addition, dead larvae and pupae were observed inside the pulp of granadilla fruits.

Regarding nutritional aspects, according to USDA's Food Data Central (2019), fruit fly host species such as peach, mango, and citrus contain an average of 86% of water content while granadilla fruits have 73% water content. This difference may suggest that the lower water concentration may have affected the larval development and pupa emergence.

The results from the development time studies also suggest that resistance mechanisms in yellow and green-yellow granadilla slowed the development of *C. capitata* eggs, larvae, and pupae compared with that in host fruits. Insects may extend their life cycle when some adverse conditions occur in the food; e.g., those affecting respiration, feeding, digestion, and displacement, among others.

To summarize the resistance mechanisms, fruit fly adults could not lay eggs in intact granadilla fruits because of the hardness and thickness of the pericarp and the likely repellent effect of squalene essential oil. In relation to the punctured granadilla fruits, fruit fly larvae usually make space while feeding to obtain oxygen from the entrance hole (oviposition hole), or when larger, larvae can make a hole in good host fruit species (far from the oviposition hole) to obtain the oxygen to continue development. However, the pericarp of granadilla fruits did not allow larvae to leave the fruits or to make respiratory holes through the pericarp, which may have affected their breathing. In addition, because arils provide a dense pulp texture in granadilla fruits, the glue-like or sticky medium could have increased the difficulty for fruit fly larvae to move and search for oxygen. The chemical resistance offered by squalene could have had negative effects during incubation and affected larvae as they attempted to move through the pericarp. Live or dead fruit fly adults were not observed inside the punctured fruits because of pupal death inside, but if adults did eventually emerge from pupae inside fruits, these adults would also die because they do not have the mouthparts suitable to perforate and emerge through the pericarp layers.

Finally, the results regarding the Confidence Levels have shown a 99.99% of confidence for both fruit fly species (*C. capitata* and *A. fraterculus*), which means a high level to ensure that granadilla fruits will not be infested by such fruit fly species.

Based on these results, the commercial fruits of *Passiflora ligularis* (Passifloraceae), well known as granadilla in Latin-America, must not be considered as a natural host of *C. capitata* and *A. fraterculus* in Peru. Therefore, these fruit fly species must not be regulated for granadilla because these pests do not follow the pathway when this fruit is imported by another country. We recommend that future research should determine the host status for other commercial species in the Passifloraceae.

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