QUARANTINE SECURITY: ASSESSMENT AND MITIGATION OF THE RISK OF *ANASTREPHA STRIATA*, *ANASTREPHA FRATERCULUS*, AND *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE) IN 'HASS' AVOCADO, *PERSEA AMERICANA*

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Experiments were conducted in Peru to determine the host status of mature green 'Hass' avocado, *Persea americana* Miller, to guava fruit fly, *Anastrepha striata* Schiner; South American fruit fly, *Anastrepha fraterculus* (Wiedemann); and, Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Fruit fly population densities in test orchard and surrounding areas were estimated by trapping and collecting natural host fruits. Commercial grade, mature green 'Hass' avocados were collected weekly from trees in the test orchard during the entire harvest season. Fruit fly infestation level was assessed through microscopic examination of peeled exocarp and thinly sliced mesocarp, and by holding fruits individually in rearing containers. Fruit fly infestation in fruits on the ground and culled fruits from a commercial packinghouse was also determined. Choice and no-choice forced oviposition tests using intact and punctured avocados were conducted in 4-m high by 4.5-m diameter field cages covering an entire tree. No-choice forced oviposition tests were also conducted using 1.5-m long by 1-m diameter sleeve cages enclosing an entire branch. We are reporting results of studies conducted in the Department of Piura, where natural populations of *A. striata, A. fraterculus*, and *C. capitata* occur.

Data collected from field studies showed that commercial grade, mature green Peruvian 'Hass' avocado is not a suitable field host of *A. striata, A. fraterculus*, and *C. capitata*. Based on these results, USDA published two final rules concluding that (1) Peruvian 'Hass' avocado is not a pathway for *A. striata*, or *A. striata* is not a pest of 'Hass' avocado in Perú; and,(2) mature green 'Hass' avocado is a conditional non-host of *A. fraterculus* and *C. capitata*. These rules signify that with appropriate safeguards to minimize the risks associated with the importation of 'Hass' avocados into the United States, conventional quarantine treatments specified in the USDA-PPQ Treatment Manual are no longer required.

Key Words: quarantine security, host determination, host suitability, fruit flies, 'Hass' avocado

SEGURIDAD CUARENTENARIA: EVALUACION Y MITIGACION DEL RIESGO DE *ANASTREPHA STRIATA*, *ANASTREPHA FRATERCULUS* Y *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE) EN PALTA 'HASS', *PERSEA AMERICANA*

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Experimentos fueron conducidos en Perú para determinar estatus de hospedante de palta 'Hass', *Persea americana* Miller, en madurez comercial a mosca de guayaba, *Anastrepha striata* Schiner; mosca sudamericana, *Anastrepha fraterculus* (Wiedemann); y mosca del mediterráneo, *Ceratitis capitata*. Las densidades poblacionales de moscas de la fruta en huertos experimentales y áreas circundantes fueron estimadas por trampeo y colecta de frutos hospedantes naturales. Frutos de palta 'Hass' comercial fueron colectados semanalmente de árboles del fundo experimental durante la estación completa de cosecha. El nivel de infestación de moscas de la fruta fue medido a través de examen microscópico del exocarpo pelado y en mesocarpo finamente cortado en tajadas y manteniendo frutos individualmente en cajas de crianza. La infestación de moscas de la fruta en frutos del suelo y frutos de descarte de una empacadora comercial también fue determinada. Pruebas de oviposición forzada de elección y no-elección usando paltas intactas y punzadas fueron conducidas en jaulas de campo de 4-m alto por 4.5-m diámetro cubriendo un árbol entero. Pruebas de oviposición forzada de no elección fueron también conducidos usando mangas de1.5-m largo y 1-m de diámetro encerrando una rama entera. Estamos reportando resultados de estudios conducidos en el Departamento de Piura, donde ocurren las poblaciones naturales de *A. striata, A. fraterculus*, y *C. capitata*.

Los datos colectados de estudios de campo mostraron que la palta 'Hass' con madurez comercial de Perú no es hospedante adecuado de *A. striata*, *A. fraterculus*, y *C. capitata*. En base a estos resultados, el USDA publicó dos normas finales concluyendo que (1) la palta 'Hass' peruana no es una via de ingreso para *A. striata*, o *A. striata* no es una plaga de palta 'Hass' en Perú; y (2) la palta 'Hass' con madurez comercial es un no-hospedante condicional de *A. fraterculus* y *C. capitata* y. Estas normas significan que con resguardos apropiados para minimizar los riesgos asociados con la importación de palta 'Hass' en los Estados Unidos, tratamientos cuarentenarios convencionales especificados en el Manual de Tratamientos USDA-PPQ ya no son requeridos.

Palabras Claves: seguridad cuarentenaria, determinación de hospedante, susceptibilidad de hospedante, moscas de la fruta, palta 'Hass'

INTRODUCTION

Host suitability is a critical parameter in developing risk mitigation measures and establishing the intensity of applicable commodity quarantine treatments. Assessing the risk of fruit fly pests associated with the movement of various economically important commodities is paramount in facilitating global trade. The risk assessment conducted in 2006 by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Center for Plant Health Science and Technology (CPHST) concludes that there are three fruit fly pests associated with the importation of 'Hass' avocado, *Persea americana* Miller, from Peru into the continental United States (USDA 2006). These regulated, high risk tephritid pests are guava fruit fly, *Anastrepha striata* Schiner; South American fruit fly, *Anastrepha fraterculus* (Wiedemann); and, Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). The Peruvian Ministry of Agriculture, National Agrarian Health Service (SENASA) responded to APHIS by proposing a mitigation procedure based on non-host status, and, subsequently, submitting a research protocol aimed to establish the non-host status of 'Hass' avocados to infestation by *A. striata, A. fraterculus*, and *C. capitata* (SENASA 2009). APHIS recommended complying with the standard stipulated in North American Plant Protection RSPM 30: "Guidelines for the Determination and Designation of Host Status of a Fruit or Vegetable for Fruit Flies (Diptera: Tephritidae)" (NAPPO 2008).

Based on APHIS recommendation, collaborative research among SENASA, the Peruvian Hass Avocado Growers Association (ProHass) and APHIS was conducted in the Departments of Lima, Ancash, and Piura. Following the guidelines of NAPPO RSPM 30, the research objective was to determine the host suitability of commercial grade, mature green 'Hass' avocados to *A. striata, A. fraterculus*, and *C. capitata*. Because of the required brevity for this presentation, we solely report on the results of studies gathered in Piura, the only study site where natural populations of *A. striata, A. fraterculus*, and *C. capitata* occur. We present data supporting the recent regulatory decisions published by USDA concluding that *A. striata* is not a pest of 'Hass' avocado; and, commercial grade mature green 'Hass' avocado is a conditional non-host of *A. fraterculus*, and *C. capitata*.

MATERIALS AND METHODS

Study Site

Studies were conducted in avocado fields of Agricola Saturno, SA located in the Department of Piura, District of Chuculanas, Sector Yapatera, Subsector La Villa Sol Sol-Paccha. Department is the primary level of geopolitical division in Peru, followed by District, Sector and Subsector. The daily mean ambient temperature (°C), relative humidity (%) and cumulative precipitation (cm) were recorded using a HOBO® weather station (ISETEK S.A., Lima, Peru) maintained at Agricola Saturno. All avocado production lots used in the studies reported here had no pesticide applications.

Test Insects

Laboratory Stock. Laboratory stock colonies were originally established from field-collected host fruits: guava (*Psidium guajaba* L.), peach (*Prunus persica* (L.) Batsch), loquat (*Eriobotrya japonica* [Thunb.] Lindl.), and mango (*Mangifera indica* L.) for *A. fraterculus* and *C. capitata*; and, guava alone for *A. striata*. They were mass-reared at SENASA facilities in Piura and Lima, following the guidelines and standard procedures of FAO, IAEA, and USDA to maintain the quality of mass reared insects (FAO/IAEA/USDA 2003). Larvae of *A. fraterculus* and *C. capitata* were reared using artificial diet, while those of *A. striata* were reared in guava, as a suitable rearing diet for this species has not been developed. To "rejuvenate" the laboratory colonies, adults from field collected natural host fruits were introduced into the mating and egging cages periodically. Stock rejuvenation was done at least once before attaining the 10th filial generation.

Wild stock. Wild stock colonies were established from the same natural host fruits mentioned above, and reared following the same standard guidelines and procedures as the laboratory stock colonies. However, wild stock colonies of each of the three species were offered natural host fruits for oviposition; thus, the ensuing larvae were reared in their respective preferred host fruits. Wild stock colonies of *A. striata*, *A. fraterculus* and *C. capitata* were refreshed by adding wild adults to the colony at or before the fifth filial generation.

Relative Densities of *Anastrepha* spp. and *C. capitata* in Traps and in Natural Host Fruits

Density of Adults Captured in Traps. Relative densities of adults of *A. striata, A. fraterculus*, and *C. capitata* inside and in areas surrounding the orchard were estimated using lure and protein bait traps placed along the trapping grid of SENASA's Fruit Fly and Phytosanitary Programs (SMFPF). McPhail traps with hydrolyzed protein bait (250 ml Buminal® [Bayer CropScience Tecniagro, S.L.], 5 g borax, and 235 ml water) were used for capturing adults of *A. striata, A. fraterculus*, and *C. capitata*; protein bait in each trap was replaced weekly. Males of *C. capitata* were also monitored using sticky Jackson traps baited with 2 g Trimedlure plugs; sticky inserts were replaced biweekly, while TML plugs were changed every three weeks.

Trap density was five McPhail traps and five Jackson traps per km^2, or 5 of each trap per 100 ha. Sector Yapatera, approximately 3,280 ha, had 151 McPhail and 152 Jackson traps. Subsector La Villa Sol Sol-Pacchas, approximately 1,218 ha, had 26 McPhail and 24 Jackson traps. The 'Hass' avocado fields of Agricola Saturno measured 153 ha and had 16 McPhail and 16 Jackson traps. The location of each trap was defined by its geographic information coordinates. Trapped adults were collected weekly and brought to the laboratory for identification and counting. Data were entered into SENASA's Integrated Fruit Fly Information System (SIIMF) database.

Infestation Rates in Natural Host Fruits. Fleshy fruits of all plant species growing inside and along the perimeter of Agricola Saturno orchard were collected from January to December 2009. Only ripe fruits on the ground were collected, as they had the higher likelihood of being infested. A total of 5,191 fruit samples belonging to 20 plant species were collected. Fruits were brought to SENASA's laboratory facility in Morropon, Piura for processing. They were weighed, placed in rearing boxes measuring 21 cm high x 38 cm long x 27.7 cm wide, and covered with a fine mesh cloth. Each rearing box contained either fine sand or sawdust at the bottom, as a larval pupation medium; fruits were separated from the sand or sawdust by a grill metal screen placed inside the box at about 10 cm high. After 15 days, fruits were dissected to recover larvae remaining in the fruit. Pupae in sand or

sawdust were recovered by sieving or picking individually with fine forceps. All pupae were placed in 7 cm diameter x 9 cm high plastic cups covered with a fine mesh cloth for adult emergence. Within 2-5 days after emergence, adults were placed in vials containing 70% alcohol and sent to SENASA's taxonomist for identification.

Assessment of Infestation Rates of *A. striata*, *A. fraterculus* and *C. capitata* in 'Hass' Avocados

Infestation Rate in 'Hass' Fruits on Trees in Experimental Orchard. Sampling was conducted weekly during the 2009 harvest season, starting on 20 March and ending on 29 April 2009. Sampling commenced when the fruit average dry matter content reached 21.5%, based on weekly field fruit monitoring conducted by SENASA and staff of Agricola Saturno orchard. Fruits with $\geq$ 21.5% dry matter content were acceptable for export. Avocado fields were divided into four-hectare quadrants; each quadrant was divided equally into four subquadrants, each containing approximately 100 trees. During each sampling occasion, quadrants were randomly chosen; five trees for fruit sampling were selected per quadrant as follows: one tree from each subquadrant and one tree from the midpoint of the diagonal of the quadrant. The canopy of each sample tree was divided into halves, the lower half and the upper half. Five fruit samples, one from each of the cardinal points and one from the interior, were collected from each half of the tree canopy. Fruits exhibiting any physical damage, diseases, and malformation described in Norma Técnica Peruana (2005) were excluded from sampling.

Each fruit was weighed, and the diameter measured using a digital caliper (CIMATEC SAC, Lima, Peru) at two points: above the peduncle and at the equatorial area. To determine the presence of oviposition punctures and/or fruit fly eggs, half of fruit samples were peeled using a potato peeler. Exocarp with discoloration or dark spots was examined under the dissecting microscope to search for eggs or any evidence of oviposition puncture. In addition, fruit pulp or mesocarp was sliced thinly to determine presence of eggs or larvae following the method described by Gould (1995). The remainder of fruit samples were held individually in rearing containers and processed to determine fruit fly infestation. Fruits were individually placed in 1-liter plastic container covered with a fine mesh cloth and containing a thin layer of sawdust as the pupation medium. The fruit holding room was maintained at 26±1ºC and 70±10% RH. Each fruit holding container was examined at least weekly for presence of adults. After 30 days, each fruit was dissected and sawdust was carefully inspected and sieved. To sum up, a total of 1,300 'Hass' avocados was collected; among these samples, 65 were used to estimate the percentage dry matter content, 650 avocados were peeled and sliced individually to determine presence of fruit fly eggs and/or larvae.

Determining Percentage Dry Matter Content. The dry matter content was estimated from 10%, or 65, of fruit samples that were peeled for presence of eggs or larvae. Using 10 g mesocarp, the percentage dry matter content was determined following a modified gravimetric method presented in Liquido et al. (1995). The formula for calculating percentage dry matter content was: %DMC = DW/FW x 100, where DMC, DW, and FW denote dry matter content, dry weight, and fresh weight, respectively. The percentage dry matter content is used as the index of commercial harvest maturity. Only fruits with at least 21.5% dry matter content were acceptable for export.

Infestation Rate in 'Hass' Avocados Collected from a Packinghouse. At the Sunshine packinghouse, the sole packinghouse for Agricola Saturno, two sets of samples were randomly collected: first, culled fruits from the culling bins; second, brushed and washed exportable fruits gathered directly from the conveyor belts prior to waxing and packing. Weekly sampling was conducted from 20 March to 15 April 2009. During the first week, 25 discarded and 50 export quality fruits were sampled; 100 culled and 100 exportable fruits were sampled weekly during the succeeding weeks. A total of 875 fruits were sampled, 425 culled and 450 exportable fruits. Procedures for estimating percentage dry matter content and determining fruit fly infestation were as described above.

Infestation Rate in Commercial Grade 'Hass' Avocados Left on the Ground. To determine whether commercial grade 'Hass' avocados on the ground were susceptible to infestation by *A. striata*, *A. fraterculus* and *C. capitata*, 600 newly harvested export quality mature green fruits were placed on the ground underneath the tree canopy; this was done by dropping each fruit from one meter height to the ground. After a one-day exposure on the ground in the field, two hundred fruits were collected; another 200 were collected after three days; and, the remaining 200 were collected

after being left on the ground for 7 days. Immediately after collection, fruits were taken to the laboratory, then held individually in 1-liter rearing containers and processed as described above to determine the rate of fruit fly infestation.

Forced Oviposition of *A. striata, A. fraterculus*, and *C. capitata* in 'Hass' Avocados

Oviposition behavior of gravid females of *A. striata*, *A. fraterculus* and *C. capitata* was conducted, separately by species, from April to August 2009 in 'Hass' avocado fields with six-year old, 3-4 m high trees. Tests were initiated when the percentage dry matter content of 'Hass' avocados reached 21.5%. Both choice and no-choice tests were conducted on trees inside large field cages; test trees were pruned and individually enclosed inside 8-sided cages measuring 4 m diameter and 4.5 m height (Figure 1). No-choice tests were also conducted on fruits on branches inside sleeve cages; branches were enclosed in 1 m diameter x 1.5 m long sleeve cages made of fine mesh fabric supported by cylindrical wires (Figure 2). While conducting each test, temperature (^{o}C) and relative humidity (%) inside each cage were measured using a hygrothermograph (CIMATEC SAC, Lima, Peru). Details of each test are presented below.

Choice Tests in Field Cages. Test insects were given a choice to oviposit between two test fruits: 'Hass' and natural host fruits; each test was designed as paired choice test. There were 15 'Hass' avocados hanging from tree branches and 15 natural host fruits artificially suspended from the tree using plastic netting tubes (Figure 1). 'Hass' and natural host fruits dangled at no more than 30 cm away from each other. The natural host fruits used for the tests were guavas for *A. striata*, mangoes for *A. fraterculus* and sweet oranges for *C. capitata*. Few days before conducting each test, all 'Hass' fruits on test trees inside the cages were covered with white paper bags; afterwards, the entire tree was thoroughly examined, searching and removing any flying insects and predatory spiders. On the day of each test, white bags covering 15 randomly selected 'Hass' test fruits were removed. Thus, only 15 'Hass' and 15 natural host fruits were exposed to the test insects, consisting of 15 gravid females that were 13-29 days old for *A. striata* and *A. fraterculus*, and 10-28 days old for *C. capitata*. All gravid females used in choice tests were from wild stock colonies. Choice tests for *A. fraterculus* and *C. capitata* were replicated 16 times; for *A. striata*, four times.

Figure 1. 'Hass' avocado tree inside a field cage measuring 4 m diameter x 4.5 m high for choice oviposition studies (left); 'Hass' avocado and orange as choice oviposition fruits for gravid *C. capitata* females (top right); detail observation of the oviposition behavior of fruit fly females on intact 'Hass' avocados during a forced, no-choice cage test (bottom right).

Gravid females' oviposition behavior was observed continuously, commencing immediately after releasing females at 9:00 in the morning and ending at 5:00 in the afternoon. Field technicians took turns in observing and recording test females' behavior inside the cage, which included: visiting the fruit, i.e., females alighted on the fruit without attempting to oviposit; attempting to oviposit, whereby a female attempted to insert the ovipositor into the fruit; and, ovipositing successfully, whereby egg deposition occurred. An oviposition attempt was recorded every time a female fly landed on a fruit, followed by repeated dabbing of labellum and probing of the ovipositor on the fruit surface. Insertion of the ovipositor into the fruit followed by egg deposition and aculeus dragging was recorded as successful oviposition. Accuracy of the categorical data on successful egg deposition was determined during processing of test fruit for presence of eggs and/or larvae.

At the end of each test, 'Hass' avocados observed to have been oviposited were labeled accordingly using white liquid paper, and then rebagged; the fruit sample identification label was also written on the white paper bag. Marked, bagged fruits were left attached onto the tree until they naturally detached from the peduncle.

Test fruits were brought to the laboratory and individually placed in one liter holding containers and processed for infestation as previously described. Control or natural host fruits which had been oviposited were immediately brought back to the laboratory after each test terminated. 'Hass' avocado test fruits were brought to the laboratory for fruit holding on the day they naturally detached or were harvested from the tree.

No-Choice Tests in Field Cages. The treatments for the no choice cage tests were intact 'Hass', punctured 'Hass', and natural host fruits. 'Hass' fruits were punctured using a tack pin with a maximum depth of 5 mm; each test fruit was punctured 10 times, with punctures distributed randomly. Similar to choice tests, the natural host fruits were guavas, mangoes, and oranges for *A. striata*, *A. fraterculus*, and *C. capitata*, respectively. Each cage had one type of treatment fruit and offered to test females separately by species. Fifteen gravid females were released inside the cage. Forced, no choice oviposition tests by treatment and by species had variable number of replicates, w/c for brevity are shown and explained in Table 3. The duration and procedures for observing and recording the behavior of gravid females were similar to those for the choice tests. Likewise, the procedures for labeling and holding fruits were the same as those for the choice tests.

No-Choice Tests in Sleeve Cages. Tests commenced once fruits on test trees attained $\geq$ 21.5% dry mater content. As in cage oviposition studies, no-choice forced oviposition tests in sleeve cages (Figure 2) were conducted separately among *A. striata, A. fraterculus*, and *C. capitata*. However, the experimental design included separate testing of wild and laboratory stock colonies. Each sleeve cage had either 5 'Hass' or natural host fruits for tests using with wild stock flies, and either 10 'Hass' or natural host fruits for tests using laboratory stock flies. The natural host fruits were guavas, mangoes, and oranges for *A. striata*, *A. fraterculus*, and *C. capitata*, respectively.

Figure 2. A branch of 'Hass' avocado being placed inside a cylindrical sleeve cage measuring 1 m diameter x 1.5 m long (left); a sleeve cage enclosing a branch bearing 'Hass' avocado fruits for no-choice oviposition tests (right).

By 9:00 in the morning, sexually-mature adults aged 13-29 days for *A. striata* and *A fraterculus*, and 10-28 days for *C. capitata* were released inside the sleeves at a density rate of 25 pairs for wild and 50 pairs for laboratory stock colonies. These test flies were kept in the sleeve for four days, with ample supply of water and food (mixture of sugar and hydrolyzed protein (Marca A-1, Lima, Peru). During the 4-day test duration, each sleeve was inspected daily and any dead fly was removed and replaced with robust flies of same species, age, and sex.

Tests were designed as paired no-choice tests; each 'Hass' avocado treatment cage was paired with a control cage containing the natural host fruit. Both wild and laboratory-stock colonies of *A fraterculus* and *C capitata* were used in the tests, while only wild stock colonies of *A. striata* were used. Forced, no choice oviposition tests by treatment and by species had variable number of replicates, as explained in Table 4.The gravidity of test females, based on presence of eggs in their ovaries, was reconfirmed by dissecting 3 laboratory stock and 10 wild stock females per sleeve cage. After the 4-day exposure period, 'Hass' and natural host fruits were brought to the laboratory and individually placed in one liter holding containers and processed for fruit fly infestation as previously described.

RESULTS

Climatic Conditions During the Study

Agricola Saturno orchard was very arid, with zero precipitation most of the year; the highest cumulative rainfall of approximately 11 cm was observed in March 2009; the rest of the year mostly had no precipitation. The recorded temperature was highest in March and April; while the highest ambient relative humidity occurred in February and March.

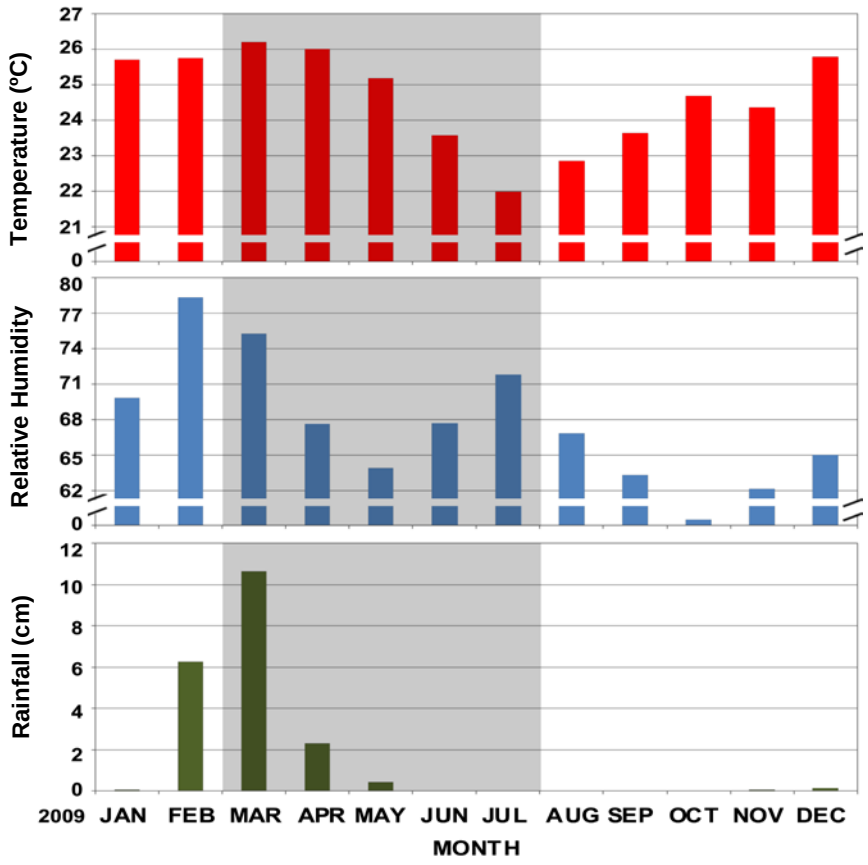

Figure 3. Mean monthly temperature (°C), mean relative humidity (%) and cumulative precipitation (cm) recorded inside 'Hass' avocado fields in Agricola Saturno orchard; Piura, 2009.

Relative Densities of *A. striata*, *A. fraterculus*, and *C. capitata* in Traps

The consistently low densities of *A. striata, A. fraterculus*, and *C. capitata* in McPhail and Jackson traps in Agricola Saturno orchard and surrounding areas are shown in Fig 3. *A. striata* was not detected, i.e., zero density, in McPhail traps inside avocado fields of Agricola Saturno. Similarly, *A. striata* adults were not detected in Subsector La Villa Sol Sol-Paccha, except on weeks 20 (11-17 May) and 21 (18-24 May). The highest density of 0.0165 *A. striata* adult/trap/day was observed on week 21, which coincided with peak guava fruiting.

A. fraterculus adults were detected in Agricola Saturno during weeks 11-18 (9-15 March to 27 April -3 May). The highest observed density of 0.225 *A. fraterculus* adult/trap/day was observed on week 17 (20-26 April). The highest density of *A. fraterculus* observed in Sector Yapatera was 0.59 adult/trap/day on week 12 (16-22 March). Agricola Saturno and Sector Yapatera had mango, guava, and citrus orchards harboring a low density population of *A. fraterculus*.

During 'Hass' avocado harvest season, *C. capitata* adults were not detected in Agricola Saturno and Subsector La Villa, except on weeks 23 to 24 (1-14 June). Adults of *C. capitata* were consistently very low in Sector Yapatera. The peak densities of *C. capitata*, both observed in Agricola Saturno, were 0.114 male/trap/day in Jackson traps on week 23 and 0.107 adult/trap/day in McPhail traps on week 24. The peak fruiting season of oranges, tangerines, lemons occurred in May and June.

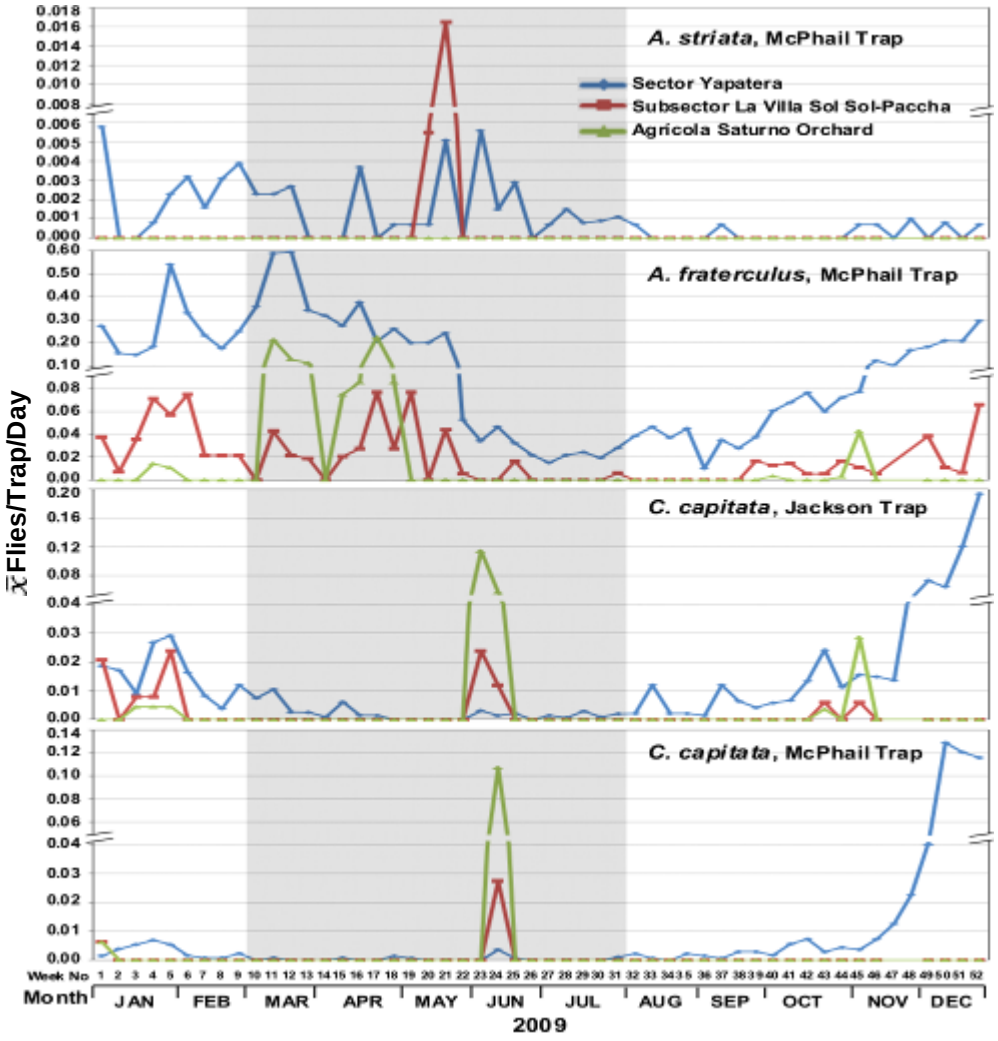

Figure 4. Relative population densities of *A. striata, A. fraterculus*, and *C. capitata* in Sector Yapatera, Subsector La Villa Sol Sol-Paccha, and Agricola Saturno orchard. Grey-shaded area indicates the 'Hass' avocado harvesting and packing season.

Infestation Rates of *Anastrepha* spp. and *C. capitata* in Natural Host Fruits

The low infestation rates of *Anastrepha* spp. and *C. capitata* in fruits of their natural host plants growing inside and along the perimeter of Agricola Saturno were evident (Table 1). Only one of 715 rough lemons was infested, with two adults of *C. capitata* emerging. Two of 204 grapefruits were infested with *C. capitata*, with 20 adults emerged. Four of 250 mandarin oranges were infested, with 15 *C. capitata* adults emerging. Seven of 160 mangoes were infested with with *A. fraterculus,* producing 45 adults. Fourteen of 120 guavas were infested, from which 16 *A. fraterculus* and 21 *A. striata* adults emerged.

Table 1. Infestation rates of *Anastrepha* spp and *C. capitata* in fruits of natural host plants growing inside and along the perimeter of Agricola Saturno orchard from January to December 2009. Samples were ripe fruits collected from the ground.

Scientific Name	Common Name	No. Fruit Collected	No. Fruit Infested	No. Emerged Adults[11]				
				Cc	***Af***	***As***	***Ad***	***Ao***
Annona muricata L.[1]	Soursop	15	0	0	0	0	0	0
Capsicum annuum L.[2]	Pepper	222	0	0	0	0	0	0
Citrus aurantiifolia (Christm.) Swingle[3]	Lime	120	0	0	0	0	0	0
Citrus jambhiri Lush.[3]	Rough lemon	715	1	2	0	0	0	0
Citrus X paradisi Mcfady[3]	Grapefruit	204	2	20	0	0	0	8
Citrus reticulata Blanco[3]	Mandarin orange	250	4	15	0	0	0	0
Citrus X tangelo J. W. Ingram & H. E. Moore[3]	Tangelo	70	0	0	0	0	0	0
Citrus sinensis (L.) Osbeck[3]	Sweet orange	100	0	0	0	0	0	0
Cucumis dipsaceus Ehrenb. ex Spach[4]	Wild cucumber	55	0	0	0	0	0	0
Inga feuillei DC[5]	Inga	136	59	0	0	0	314	0
Mangifera indica L.[6]	Mango	160	7	0	45	0	0	0
Momordica charantia L.[4]	Bitter gourd	295	0	0	0	0	0	0
Morinda citrifolia L.[7]	Indian mulberry	120	0	0	0	0	0	0
Passiflora foetida L.[8]	Fetid passionfruit	175	0	0	0	0	0	0
Persea americana Mill.[9]	Avocado	104	0	0	0	0	0	0
Psidium guajava L.[10]	Guava	120	14	0	16	21	0	0
Solanum nigrum L.[2]	Black nightshade	1260	0	0	0	0	0	0
Solanum pimpinellifolium Jusl.[2]	Currant tomato	630	0	0	0	0	0	0
Spondias cytherea Sonn.[6]	Otaheite apple	140	0	0	0	0	0	0
Spondias purpurea L.[6]	Spanish plum	300	176	0	0	0	0	268

[1]Family Annonaceae; [2]Solanaceae; [3]Rutaceae; [4]Cucurbitaceae; [5]Fabaceae; [6]Anacardiaceae; [7]Rubiaceae; [8]Passifloraceae; [9]Lauraceae; [10]Myrtaceae. [11]*Cc*, *Ceratitis capitata*; *Af*, *Anastrepha fraterculus*; As, *Anastrepha striata*; *Ad*, *Anastrepha distincta*; *Ao*, *Anastrepha obliqua*.

Assessment of Infestation Rates of *A. striata*, *A. fraterculus*, and *C.* capitata in 'Hass' Avocados on Trees, from a Pacckinghouse, and on the Ground

All exportable mature green 'Hass' avocados collected from trees inside Agricola Saturno orchard had no fruit fly infestation. Exportable and culled avocados sampled from Sunshine packinghouse also were not infested (Table 2). Similarly, all 600 commercial grade fruits left on the ground under 'Hass' avocado trees and recovered after one, three, and seven days of exposure were not infested either by *A. striata*, *A. fraterculus,* or *C. capitata.*

Table 2 . Absence of infestation by *Anastrepha* spp and *C. capitata* in 'Hass' avocados collected from Agricola Saturno orchard and Sunshine packinghouse; Piura, Peru, 2009.

Sampling Date	No. fruit Collected	Weight (g) ($\bar{x}$ ±SEM)	Diameter (cm, $\bar{x}$ ±SEM)		% Dry Matter Content		No. Eggs	No. Larvae + Pupae
			Minimum	Maximum	*n*	($\bar{x}$ ±SEM)		
Fruits on Trees, Agricola Saturno Orchard								
3/20/2009	100	154.85 ± 3.29	3.94 ± 0.04	5.74 ± 0.05	5	22.16 ± 0.40	0	0
3/25/2009	200	173.09 ± 2.91	4.62 ± 0.04	6.33 ± 0.04	10	22.89 ± 0.25	0	0
4/01/2009	200	150.61 ± 2.33	3.66 ± 0.03	6.02 ± 0.04	10	25.13 ± 0.41	0	0
4/08/2009	200	127.74 ± 2.41	3.93 ± 0.04	5.68 ± 0.04	10	22.92 ± 0.32	0	0
4/15/2009	200	127.36 ± 3.30	4.10 ± 0.03	5.64 ± 0.05	10	27.00 ± 0.45	0	0
4/22/2009	200	123.29 ± 2.91	3.87 ± 0.03	5.59 ± 0.05	10	26.47 ± 0.41	0	0
4/29/2009	200	106.82 ± 2.32	3.93 ± 0.03	5.32 ± 0.04	10	28.39 ± 0.21	0	0
Exportable Fruits, Sunshine Packinghouse								
3/20/2009	50	204.21 ± 4.88	4.41 ± 0.06	6.35 ± 0.06	3	22.50 ± 0.34	0	0
3/25/2009	100	200.12 ± 2.95	4.41 ± 0.05	6.60 ± 0.03	5	23.00 ± 0.26	0	0
4/01/2009	100	172.72 ± 4.87	4.30 ± 0.05	6.17 ± 0.06	5	23.20 ± 0.20	0	0
4/08/2009	100	209.99 ± 3.14	4.41 ± 0.06	6.78 ± 0.04	5	23.50 ± 0.54	0	0
4/15/2009	100	218.23 ± 1.40	4.84 ± 0.04	6.81 ± 0.02	5	26.80 ± 0.47	0	0
Culled Fruits, Sunshine Packinghouse								
3/20/2009	25	173.22 ± 5.68	3.86 ± 0.06	5.94 ± 0.13	2	20.70 ± 0.34	0	0
3/25/2009	100	177.48 ± 3.27	4.70 ± 0.05	6.32 ± 0.05	5	20.80 ± 0.39	0	0
4/01/2009	100	167.09 ± 2.87	3.78 ± 0.03	6.19 ± 0.04	5	24.50 ± 0.40	0	0
4/08/2009	100	176.51 ± 3.93	4.62 ± 0.04	6.28 ± 0.05	5	22.60 ± 0.27	0	0
4/15/2009	100	174.55 ± 4.08	4.68 ± 0.04	6.27 ± 0.06	5	27.20 ± 0.44	0	0

Forced Oviposition of *A. striata, A. fraterculus*, and *C. capitata* in 'Hass' Fruits

Choice and No-Choice Field Cage Tests. Results of all caged tests were combined and summarized in Table 3. In both choice and no choice field cage tests, gravid females of *A. striata* did not even attempt to oviposit on intact and punctured 'Hass' avocados. Gravid females of *A. fraterculus* attempted but failed to successfully oviposit in intact and punctured 'Hass' avocados in both choice and no choice tests. *C. capitata* females successfully oviposited in four of 240 intact 'Hass' avocados in choice tests. Five of 480 intact and 23 of 240 punctured avocados were successfully oviposited by *C. capitata* during no choice tests. However, all eggs oviposited by *C. capitata* were observed to be encapsulated by callous tissues, and eventually died (Figure 5). No successful infestation of intact and punctured 'Hass' avocados by *C. capitata* were observed. Control fruits, i.e., guavas, mangoes,and tangelo oranges were successfully infested by *A. striata, A. fraterculus*, and *C. capitata*, respectively.

No-Choice Sleeve Cage Tests. Both wild and laboratory colonies of *A. striata, A. fraterculus*, and *C. capitata* failed to infest 'Hass' avocado fruits in no-choice forced oviposition studies in sleeve cages (Table 4). These fruit flies successfully infested their respective control test fruits.

Table 3. Oviposition behavior and absence of infestation by *A. striata, A. fraterculus*, and *C. capitata* in 'Hass' avocados under choice and forced, no-choice conditions in large field cages subject to the natural field environment in Agricola Saturno orchard; Piura, 2009 and 2010.

	Tests[1]	Total No. Test Fruit	Total No. Test Female	Total No. Female Visit	Total No. Oviposition Attempt	Total No. Successful Oviposition	Total No. Fruit Oviposited	Total No. Fruit with Larvae and Pupae	Total No. Larvae and Pupae	Total No. Adults Emerged
***A. striata*,2009**										
Choice Test:	Hass	60	60	13	0	0	0	0	0	0
	Mango	60		70	40	28	28	24	103	31
No-Choice Test:	Hass Without Puncture	120	120	25	0	0	0	0	0	0
	Hass With Punctures	60	60	5	0	0	0	0	0	0
	Mango	60	60	79	54	15	15	12	49	17
***A. fraterculus*, 2009**										
Choice Test:	Hass	240	240	45	9	0	0	0	0	0
	Mango	240		186	53	20	20	14	59	40
No-Choice Test:	Hass Without Puncture	480	480	168	33	0	0	0	0	0
	Hass With Punctures	240	240	71	15	0	0	0	0	0
	Mango	240	240	137	33	12	11	3	14	6
***C. capitata*, 2009**										
Choice Test:	Hass	240	240	96	25	4	4	0	0	0
	Tangelo	240		399	240	34	26	0	0	0
No-Choice Test:	Hass Without Puncture	480	480	541	232	5	5	0	0	0
	Hass With Punctures	240	240	339	182	23	12	0	0	0
	Tangelo	240	240	352	233	47	29	0	0	0
***C. capitata*, 2010**										
Choice Test:	Hass	240	240	190	7	0	0	0	0	0
	Naranja	240		721	128	18	12	11	123	112
No-Choice Test:	Hass Without Puncture	480	480	544	56	0	0	0	0	0
	Hass With Punctures	240	240	359	93	10	6	0	0	0
	Sweet Orange	240	240	590	140	35	31	25	254	240

[1]Each replicate for choice test had 15 'Hass' and 15 host fruits; each replicate for a no-choice test either had 15 intact 'Hass', 15 punctured 'Hass', or 15 host fruits. Each replicate for both choice and no-choice tests had 15 gravid test females released in a cage; thus, 60, 240, and 480 test females signify 4, 16, and 32 replications.

Figure 5. A female *C. capitata* ovipositing on an intact, without puncture, mature green 'Hass' avocado (top, left); callused tissue resulting from insertion of the ovipositor into the fruit (top, right); dead *C. capitata* eggs with the encapsulating callused tissue removed (middle, right); tube-shaped callused tissue protruding internally, formed around the wound caused by pin punctures (bottom, left); dead *C. capitata* eggs inside the tube-shaped callused tissue.

Table 4. Absence of infestation by *A. striata, A. fraterculus*, and *C. capitata* in 'Hass' avocados in forced, no-choice oviposition studies conducted under natural field conditions in sleeve cages in Agricola Saturno orchard; Piura, 2009 and 2010.

Species	Hass					Host [1]				
	No. Test Fruit[2]	No. Test Female[2]	No. Infested Fruit	No. Larvae and Pupae	No. Emerged Adults	No. Test Fruit[2]	No. Test Female[2]	No. Infested Fruit	No. Larvae and Pupae	No. Emerged Adults
***A. striata*, 2009**										
Wild Adults	20	100	0	0	0	20	100	4	10	7
***A. fraterculus*, 2009**										
Wild Adults	5	25	0	0	0	10	50	2	38	26
Laboratory Adults	30	150	0	0	0	20	100	8	155	114
***C. capitata*, 2009**										
Wild Adults	10	50	0	0	0	10	50	0	0	0
Laboratory Adults	20	100	0	0	0	20	100	0	0	0
***C. capitata*, 2010**										
Wild Adults	10	50	0	0	0	10	50	5	18	17
Laboratory Adults	20	100	0	0	0	20	100	7	19	17

[1] Guava for *A. striata*, mango for *A. fraterculus*, and sweet orange for *C. capitata*.

[2] For both 'Hass' and host fruits, each replicate in a sleeve cage no-choice test using wild adults (by species) had 5 test fruits and 25 pairs of fruit fly adults; thus, 25, 50, and 100 test females signify one, two, and four replications, respectively. For tests using adults from laboratory colonies, each replicate had 10 test fruits and 50 pairs of fruit fly adults (by species); thus, 100 and 150 test females signify two and three replications, respectively.

DISCUSSION

USDA-APHIS, on 7 January 2009, published a proposed rule recommending the quarantine measures to mitigate the risks of tephritid pests associated with the importation of 'Hass' avocados into the continental United States (USDA 2009). These quarantine security measures include (1) establishment of an area free of *A. fraterculus* and *A. striata*, or trapping to demonstrate that places of production have a low prevalence of *A. fraterculus* and *A. striata*; (2) establishment of an area free of *C. capitata*; trapping to demonstrate that places of production are free of *C. capitata*; and, (3) application of appropriate commodity quarantine treatment approved by APHIS-PPQ (http://manuals.cphst.org/TIndex/treatmentSearch.cfm; https://epermits.aphis.usda.gov/manual/index.cfm). SENASA, as mentioned earlier, instead proposed extensive field and laboratory experiments aimed to establish that 'Hass' avocados grown in Perú are either a natural non-host or conditional non-host of *A. striata*, *A. fraterculus*, and *C. capitata*. Experiments were conducted and data gathered in collaboration with APHIS in 2009 and 2010 in the Departments of Lima, Ancash and Piura (SENASA 2010). Data collected in Piura in 2009, including data for forced oviposition tests in 2010 for *C. capitata*, are presented in this report.

Results presented here, and those conducted in Mexico (Aluja et al. 2004), showed that 'Hass' avocados are not natural host fruits of *A. striata*. Our detailed observations of the gravid females' foraging behavior revealed that females of *A. striata* do not even attempt to oviposit in 'Hass' avocados, even under forced, no-choice conditions. Based on the corroborating data obtained from studies conducted in Perú and Mexico, USDA-APHIS in a final ruling, published on 4 January 2010, removed *A. striata* from the list of pests of 'Hass' avocados of Perú, which translates to a conclusion that *A. striata* is not a pest of 'Hass' avocado in Perú (USDA 2010).

This paper is the first report on the status of 'Hass' avocado as a conditional non-host of *A. fraterculus*. A fruit commodity may be designated as a conditional non-host status under approved regulatory safeguards and defined limiting conditions, such as stage of maturity, other physiological conditions, and phenological asynchrony of the pest and the host commodity. In Perú, the percentage fruit dry matter content ($\geq$ 21.5%) is used as the index of exportable mature green fruits. Under forced, no-choice infestation conditions in large field and sleeve cages, *A. fraterculus* did not successfully infest both intact (without holes) and punctured mature green 'Hass' avocados. The inability of *A. fraterculus* to infest 'Hass' avocados under forced conditions, and the verifiable records that *A. fraterculus* has never been intercepted in avocados (USDA 2011a) suggest that *A. fraterculus* is either not a pest of avocado, or not a pest of mature green or harvest mature 'Hass' avocados. On 22 July 2011, USDA-APHIS published a rule concluding that commercial grade, mature green 'Hass' avocado is a conditional non-host of *A. fraterculus* (USDA 2011b).

Data presented here corroborate the findings of Willink and Villagran (2007) and De Graaf (2009) on the status of harvest mature 'Hass' avocado as a conditional non-host of *C. capitata*. Willink and Villagran (2007) evaluated the ability of *C. capitata* to infest 'Hass' avocados in Argentina. They conducted forced oviposition experiments in the laboratory and field, surveyed fruits extensively by collecting samples from production orchards and commercial packinghouses, and observed total absence of infestation of *C. capitata* in 'Hass' avocado. De Graaf (2009) conducted research on the susceptibility of Hass avocados to three *Ceratitis* species in South Africa: *C. capitata*, *Ceratitis rosa* Karsch and *Ceratitis cosyra* (Walker); his approach was similar to the methods presented here and by Aluja et al. (2004). Based on expansive fruit sampling in production orchards and packinghouses and forced oviposition studies in the laboratory and field, De Graaf (2009) concluded that mature green 'Hass' avocado is a conditional non-host of *C. capitata*, but a poor host of *C. rosa* and *C. cosyra.* USDA in a final rule concluded that commercial grade mature green 'Hass' avocado is a conditional non-host of *C. capitata* (USDA 2011b)

Results of studies conducted in Perú, and those of fruit fly workers in Mexico, Argentina and South Africa collectively support the regulatory decisions of APHIS: commercial grade, mature green 'Hass' avocado is not a host of *A. striata,* and is a conditional non-host of *C. capitata* and *A. fraterculus*, and, thus, conventional quarantine treatments specified in the USDA-PPQ Treatment Manual are no longer required. Therefore, with appropriate safeguards before and during harvest, during packing, and during shipment and arrival at port of entry, mature green 'Hass' avocados from Perú do not pose risks of introducing *A. striata*, *A. fraterculus*, and *C. capitata* into the United States.

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