Export of Commercial Hass Avocados From Argentina Poses Negligible Risk of *Ceratitis capitata* (Diptera: Tephritidae) Infestation

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ABSTRACT Argentina has to meet quarantine restrictions because of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), to export 'Hass' avocados, *Persea americana* Miller, to certain countries. Hass avocado at the hard, mature green stage is potentially a conditional nonhost for *C. capitata* and could open export markets without the need for a quarantine treatment. Trapping data from 1998 to 2006 showed that *C. capitata* was present in avocado orchards, particularly early in the harvest season. The host status of hard, mature green Hass avocado to *C. capitata* was evaluated using laboratory and field cage tests under no-choice conditions and by assessing natural levels of infestation in commercially harvested fruit from the main avocado production area. In total, 2,250 hard, mature green avocado fruit were exposed to 11,250 gravid females for 24 or 48 h after harvest in laboratory or field cages, and no infestations were found. During 11 seasons, 5,949 fruit in total were sampled from the trees and 992 fruit were collected from the ground, and in none of them were any live or dead fruit fly larvae found. Inspection of >198,000 commercial fruit at the packinghouse from 1998 to 2011 showed no symptoms of fruit fly infestation. These data exceed the published standards for determination of nonhost status, as well as the Probit 9 standard for development of quarantine treatments. Hass avocado harvested at the hard, mature green stage was not infested by *C. capitata* and seems to pose a negligible quarantine risk. As a consequence, no postharvest treatment or other quarantine actions should be required by importing countries.

KEY WORDS Mediterranean fruit fly, avocado, host plant resistance, quarantine security, risk analysis

Avocado, *Persea americana* Miller, is generically listed as a host for Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Liquido et al. 1991). Consequently, any country where *C. capitata* is established must control this quarantine pest in avocados before export to a country where *C. capitata* does not occur. Avocado is generally regarded as a nonpreferred or poor host for tephritid fruit flies (Miller et al. 1995), but susceptibility is specific to the species of fruit fly and is influenced by the cultivar and maturity level (Armstrong et al. 1983, Armstrong 1991, Aluja et al. 2004, Follett 2009, De Graaf 2009). Avocados are typically harvested at the hard, mature green stage and ripened at room temperature until they soften before consumption. Avocado becomes an increasingly favorable fruit fly host as it ripens and softens after harvest (Armstrong 1991, Oi and Mau 1989, Follett 2009). Determination and designation of host status helps regulatory authorities assign and manage risk in traded commodities. A conditional host is a commodity that is a host or nonhost under defined permissive or restrictive conditions, respectively (e.g., stage of maturity; Anonymous 2008). Avocado may be a nonhost for certain fruit flies at the hard, mature green stage (Armstrong et al. 1983, Armstrong 1991, Aluja et al. 2004, Follett 2009, De Graaf 2009). If a specific cultivar and maturity stage of avocado can be shown to be a conditional nonhost for a species of fruit fly, quarantine mitigation measures would not be needed.

Host status determination studies frequently follow the methods proposed by Cowley et al. (1992) to demonstrate nonhost status to fruit flies for particular commodities (e.g., Aluja et al. 2004, Mexican 'Hass' avocados). Cowley et al. (1992) proposed a three-tiered testing protocol and decision tree involving laboratory cage tests with punctured fruit, laboratory cage tests with unpunctured fruit, and field cage tests with unpunctured fruit attached to the tree. The laboratory cage trial with punctured fruit involves exposing 500 g of fruit to several gravid females to ensure that 250–500 eggs are laid, replicated five times. In assessing the results, if adults are reared from a single control replicate of a known host fruit exposed to gravid females and no adults are reared from the five replicates of trial fruit, then the trial fruit is declared a nonhost and further testing is unnecessary (Cowley

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et al. 1992). This protocol means nonhost status could be demonstrated by testing as few as 1,250–2,500 eggs and <100 fruit. This protocol has been adopted by New Zealand Ministry of Agriculture and Forestry and the Asia and Pacific Plant Protection Commission (Anonymous 2005).

More recently, Follett and Hennessey (2007) proposed that studies to demonstrate nonhost status should meet the same stringent standards applied to the development of postharvest quarantine treatments. This concept was incorporated into guidelines for the determination and designation of host status of a commodity for tephritid fruit flies published by the North American Plant Protection Organization (Anonymous 2008). For some countries, commodities, and pests, Probit 9 treatments have traditionally been required to provide an acceptable level of quarantine security during quarantine treatment development (Liquido et al. 1995, Follett and Neven 2006). A response at the Probit 9 level results in 99.9968% efficacy, an efficacy that can be proved at the 95% confidence level by treatment of 93,613 individuals with no survivors (Couey and Chew 1986). The U.S. Department of Agriculture (USDA) has used 99.9968% efficacy as the basis for approving many treatments as meeting quarantine security requirements for high-risk pests, particularly for tephritid fruit flies. Other countries require 99.99% efficacy (Australia, Japan, and New Zealand) that can be demonstrated by treatment of 29,956 individuals (Follett and Neven 2006). The required response may be mortality, sterility, or prevention of maturity. However, it may be not possible to provide adequate confidence levels for treatment efficacy in cases where the commodity is a poor host, given that oviposition may be reduced or absent and the required number of insects cannot be reared in the specific host. Landolt et al. (1994) proposed that in such cases a less severe postharvest treatment might still provide quarantine security. More recently, Follett and McQuate (2001) suggested that fewer insects can be used to develop quarantine treatments for poor hosts. With nonhost testing, the number of test subjects used to determine efficacy could be the number of fruit flies used in laboratory and field cage tests, the number of fruit exposed to fruit flies in cage tests, the number of fruit commercially harvested and inspected for evidence of infestation, or any combination of these options (Follett and Hennessey 2007, Aluja and Mangan 2008). Yet, confidence levels have not been reported consistently along with efficacy in nonhost status testing. As a result, it has been difficult to demonstrate equivalency between a postharvest quarantine treatment and nonhost status as alternative measures even when in both cases, the goal is to ensure quarantine security at a specified level of precision.

Hass avocado is potentially a conditional nonhost for *C. capitata* at the hard, mature green stage. In South Africa, De Graaf (2009) exposed freshly harvested unpunctured Hass fruit in no-choice tests to *C. capitata* in laboratory cages for 24 h and found no infestation. Likewise, caged fruit on the tree exposed for 48 h were not infested, and inspections for internal pests of packinghouse fruit and fruit exported to Europe found no *C. capitata* infestation. South African Hass avocados were infested at low levels by two other fruit flies, *Ceratitis rosa* (Karsh) and *Ceratitis cosyra* (Walker) (De Graaf 2009). As such, the quarantine risk of *C. capitata* being introduced as a result of Hass avocado trade is probably overstated, possibly due to insufficient information.

In Argentina, avocado is mainly produced in the northwestern provinces of Tucumán (1,500 ha), Jujuy (500 ha), and Salta (200 ha). Approximately 2,722 tons of Hass avocados were exported from Argentina in 2009. Export destinations are primarily Europe (Spain, France, Great Britain, and The Netherlands) and Chile. Quarantine restrictions because of *C. capitata* prevent Argentina from exporting avocados to Japan and the United States. If commercial quality Hass avocado could be shown to be a nonhost for *C. capitata*, these markets could be opened without the need for a quarantine treatment. The aim of this study was to determine the host status of hard, mature green Hass avocado to *C. capitata* by using laboratory and field cage tests under no-choice condition and to investigate natural levels of infestation in commercially harvested fruit from the main avocado production area in Tucumán, Argentina.

Materials and Methods

Trapping

Fruit fly trapping was done to evaluate *C. capitata* population levels and seasonality in a commercial orchard in the locality of Sauce Huacho, Famaillá department, province of Tucumán, Argentina (550 m above sea level; 26° 56′24.76″ S and 65° 28′14.72″ W) from 1998 to 2006. This orchard is located in the main avocado production area, at the foothills of Sierra del Aconquija. The climate is subtropical, with rainfall concentrated during the summer and dry winters. Sauce Huacho has ≈70 ha of cultivated avocado, 36 ha of which are Hass planted from 1968 to 1985 at a density of 65 trees per ha Hass avocado was introduced to La Cruz, Chile, from California in 1944 and from La Cruz to Tucumán, Argentina, in 1968. The plantation is surrounded by lemon [*Citrus limon* (L.), Burm.] orchards and natural rain forest.

Adult fly trapping began in April, 1 mo before the beginning of the harvest period for exportation, and continued until late August when the exportation was complete. A network of 32 Jackson traps (standard sticky traps baited with trimedlure plugs, Süsbin, Mendoza, Argentina) and 32 McPhail traps (baited with a food attractant: yeast plus borax, Süsbin, Mendoza, Argentina) was established in 10 orchards blocks within the 36-ha orchard growing fruit for export following Argentinean standards. The blocks had different sizes ranging from <1 ha to ≈5 ha and were surrounded by blocks with 'Torres' avocados and areas with natural vegetation. Dirt roads connect the blocks. The two trap types were deployed in pairs, ≈20–30 m apart. Trap density and network layout was deter-

mined with phytosanitary authorities in Argentina. An additional 44 Jackson traps and 44 McPhail traps baited with the same lures were placed in pairs and spaced as before around the Hass avocado blocks, in blocks with other avocado varieties, and around the perimeter of the orchard. Traps were monitored weekly and captured flies were sent to the laboratory of the Zoology Section of the Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Tucumán, Argentina, for counting. Trap captures were averaged to express the number of *C. capitata* captured per trap per day (FTD) within each plot, and grand means were plotted to track population trends. Although sexes were identified during counting, for the purpose of this study total *C. capitata* numbers are reported.

Forced Artificial Infestation Tests

Between 1998 and 2003, 15 infestation trials were performed whereby Hass avocados were exposed to *C. capitata* in 1) laboratory cage no-choice tests by using harvested fruit for 24 h or 48 h after harvest and 2) field cage tests of fruit naturally attached to the tree.

Insects. *C. capitata* used in the infestation trials were obtained from a colony reared at the EEAOC laboratories. This colony was initiated with wild material collected at different localities in northwestern Argentina and held under artificial rearing conditions by using the standard adult diet (sugar and yeast hydrolysate, 3:1 ratio, MP Biomedicals, Solon, OH) and the larval diet of Boller (1985). Laboratory insects were used instead of wild insects because wild insects are not readily available during the time of the year the tests were performed. To avoid any detrimental effect of laboratory inbreeding or adaptation, every year before the trials began, wild-type individuals were collected from infested fruit in citrus plantations and incorporated into the colony through four successive crosses of laboratory females with wild males, resulting in an injection of 93.75% of wild genetic background. Quality control of the laboratory strain was determined following standard procedures (Orozco et al. 1983, FAO–IAEA–USDA 2003). Egg hatch averaged 94.2%; average pupal weight, as measured 4 d after pupation, was 9.9 mg; adult emergence averaged 90.3%; flight ability averaged 90.1%; adult longevity averaged 37 d; and the male:female ratio was between 0.9 and 1.0. Before each trial, 10–17-d-old females were taken from the rearing cages and held in plastic containers with adult food at a 3:1 ratio of sugar and hydrolyzed yeast (MP Biomedicals) and water. This age ensured that females were sexually mature and fertile at the time experiments were conducted. To confirm this condition, before using a particular cage the occurrence of mating couples was checked visually.

Fruit. The fruit used was in all trials was Hass avocado that had reached the degree of harvest maturity required for export to Chile; i.e., the fruit was hard (>13 kg/cm^2 measured with a penetrometer [FT 327, McCormick, Facchini, Alfonsine, Italy] with a 10-mm-diameter cylindrical probe) and the exocarp (skin) was green. Dry matter content was $\geq$23%, the minimum acceptable value for commercial Hass avocados as set by the Argentine Secretary of Agriculture (resolution no. 756/97). Percentage dry matter content was determined at the Chemistry Laboratory of Agroindustrial Products at the EEAOC following the methodology of Liquido et al. (1995), but using a microwave oven to dry the fruit instead of a food dehydrator.

Laboratory Cage Tests With Harvested Fruit. Hass avocados harvested from the field were exposed to *C. capitata* in cages in the laboratory for 24 h or 48 h after harvest under no-choice conditions. Two to 3 h after harvest, avocados with stems attached were placed in infestation cages with five gravid females per fruit (totaling 50 fruit and 250 females) during 24 or 48 h. Cages consisted of voile cloth cylindrical sleeves (60 cm in length, 40 cm in diameter) supported with a wire frame, similar to those described in Aluja et al. (2004). After the exposure period, each fruit was placed in 0.5-liter plastic containers with a thin layer of sawdust to allow pupation. The container was covered with a voile cloth fastened by a rubber band and incubated at 25 $\pm$ 2°C and $>$70% RH. Every 3 d, the containers were checked for pupae. After 10 d of incubation, 50% of the fruit (the most mature fruit) were dissected to determine the presence of larvae and whether they were alive or dead. The remaining fruit were held for an additional 20 d, at which time they were dissected to determine the presence of larvae. In this case, fruit was cut with a sharp knife into small pieces that were inspected. With this procedure the presence of any larvae can be detected readily, even when in those few cases in which the fruit was mummified. In addition, during these 20 d, the sand was revised every 3 d in search of pupae.

Field Cage Tests With Fruit Attached to Tree. Field infestation tests were performed at five localities within Tucuman province, including Sauce Huacho (550 m; 26° 56′24.76″ S and 65° 28′14.7″ W), Taficillo (800 m; 26° 41′22.3″ S and 65° 16′57.4″ W), Alpachiri (500 m; 27° 20′04.7″ S and 65° 16′14.7″ W), Yerba Buena (500 m; 26° 48′26.1″ S and 65° 19′41.0″ W), and Timbó Nuevo (600 m; 26° 39′20.3″ S and 65° 03′57.2″ W). The Sauce Huacho locality corresponds to the orchard where the trapping network was set up.

At each locality, on each testing date, 50 fruit were evaluated. Groups of three to five Hass avocado fruit were enclosed in the same infestation cages used in the laboratory tests with five gravid females per fruit (15–25 flies per cage) under no-choice conditions; resulting in 10–17 cages per locality per date. Flies in sleeve cages were left in the field with the same adult food described above, and water and fruit attached to the tree for 48 h. Afterward, the fruit were harvested and taken to the laboratory. When cages were removed from the trees, live females were counted and taken to the laboratory for dissection to check whether they were sexually mature and inseminated. If $>$10% of the females were not sexually mature, virgin or dead, the cage was discarded and the fruit were not processed. In the laboratory, each fruit from

successful fruit fly exposures was placed in 0.5-liter plastic containers with a thin layer of sawdust to allow pupation. The container was covered with a voile cloth fastened by a rubber band and incubated at 25 ± 2°C with >70% RH. Every 3 d, the containers were checked for pupae. After 10 d of incubation, 50% of the fruit (the most mature fruit) were dissected to determine the presence of larvae and whether they were alive or dead. The remaining fruit were held for an additional 20 d for possible fly emergence. At this time, fruit also were dissected to check for the occurrence of larval development.

Female Reproductive Potential. To demonstrate the competence of gravid female flies and estimate their reproductive potential, an artificial substrate consisting of a 5-cm-diameter agar sphere completely wrapped with Parafilm (Boller 1968) was used as a permissive artificial oviposition substrate. Females were chosen randomly from the same set of females for the infestation trials and consequently were 10–17 d old. Four agar spheres were used for each trial, and five females were exposed to each agar sphere under laboratory cage conditions. After 48 h, agar spheres were dissected and the number of eggs laid was counted to estimate the average number of eggs laid per female for each test. Egg fertility was estimated from the quality control evaluation of the rearing cage from which the females were taken and consequently represented also a random sample of females from the same set of females used for the infestation trials.

Fruit Sampling for Natural Infestation

Harvest mature fruit were sampled from the field and from one packing house. Fruit sampling in the field was done from 1998 to 2011 at the Sauce Huacho, Famaillá, orchard during the harvest season for fruit exported to Chile (May–August). Fruit were taken every 3 wk from randomly selected sites throughout the orchard. A random sample of 10 fruit was cut from each tree, and whenever possible a second sample of equal size was collected from the ground. The fruit were taken to the laboratory and processed as described for the cage tests to determine infestation.

Fruit from the packinghouse placed at the orchard in Sauce Huacho, Famaillá, Tucuman, were examined for any internal pests by trained inspectors from Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA). This procedure is part of the export agreement between Chile and Argentina, and this packinghouse is the only authorized to export Hass avocados to Chile. The methodology consisted in a visual inspection of 2% of all fruit for symptoms, and fruit dissection by multiple cuttings for a thorough check of at least 1% of the fruit. Inspections were performed from 1998 to 2011 except for 2007 and 2008, when there were no exports (see Table 2).

Confidence Levels

In laboratory and field cage host status experiments, a known number of gravid adult female flies are exposed to the fruit, and typically many fruit flies are exposed to a smaller number of fruit. To determine the natural infestation rate, fruit are collected from the field and either dissected to count eggs and larvae, or they were held for adult fruit fly emergence. The numbers of adult fruit flies present in the orchard and the number of fruit that are visited by gravid flies during a defined period or phenological fruit stage is usually unknown because there are no visible signs of infestation or visitation. Therefore, the sampled number of fruit is used to determine the level of confidence. If the natural infestation rate is low, a large number of fruit samples will be required to determine nonhost efficacy with 95% confidence (e.g., Hennessey et al. 1992). Hence, confidence levels for fruit flies and fruit can be calculated for laboratory, field cage, and natural infestation experiments and used together to assess host status.

The level of confidence associated with treating a number of insects with zero survivors is given by the equation

$$C = 1 - (1 - p_u)^n$$

where p_u is the acceptable level of survivorship (as a proportion), and n is the number of test insects or fruits (Couey and Chew 1986). Confidence levels were calculated for the number of flies used in laboratory and field cage tests, for the number of fruit used in laboratory and field cage tests, for the fruit inspected at the packinghouse, and for the total number of flies and fruits used in the study (Follett and Hennessey 2007). Confidence levels were calculated assuming the required efficacy ($[1 - p_u] \times 100$) as 99.99 and 99.9968%.

Results

***C. capitata* Presence in Field.** Trapping data from 1998 to 2006 showed that *C. capitata* was present in avocado orchards, particularly early in the harvest season. Trap captures were highest for Jackson and McPhail traps in April. From May to August, captures never exceeded 0.01 flies per trap per d (Fig. 1). In particular, no *C. capitata* were captured during June–August in McPhail traps, and Jackson trap captures were low and sporadic during this period. This is in agreement with the fact that Jackson traps baited with the long-range attractant trimedlure generally captured more flies than McPhail traps baited with a food attractant.

Forced Artificial Infestation Tests. In total, 2,250 hard, mature green avocado fruit with dry matter content ranging from 23 to 32% were exposed to 11,250 fully mature gravid *C. capitata* females for 24 or 48 h after harvest in laboratory cages, or in field cages, on each of 15 trial dates performed from April to October during five different harvest seasons. No damage to the fruit surface was noticed, no live or dead *C. capitata* larvae were found in the dissected fruit, and no pupae were recovered from the fruit held for fruit fly development under the forced, artificial conditions of the laboratory or field cage tests (Table 1). Although egg

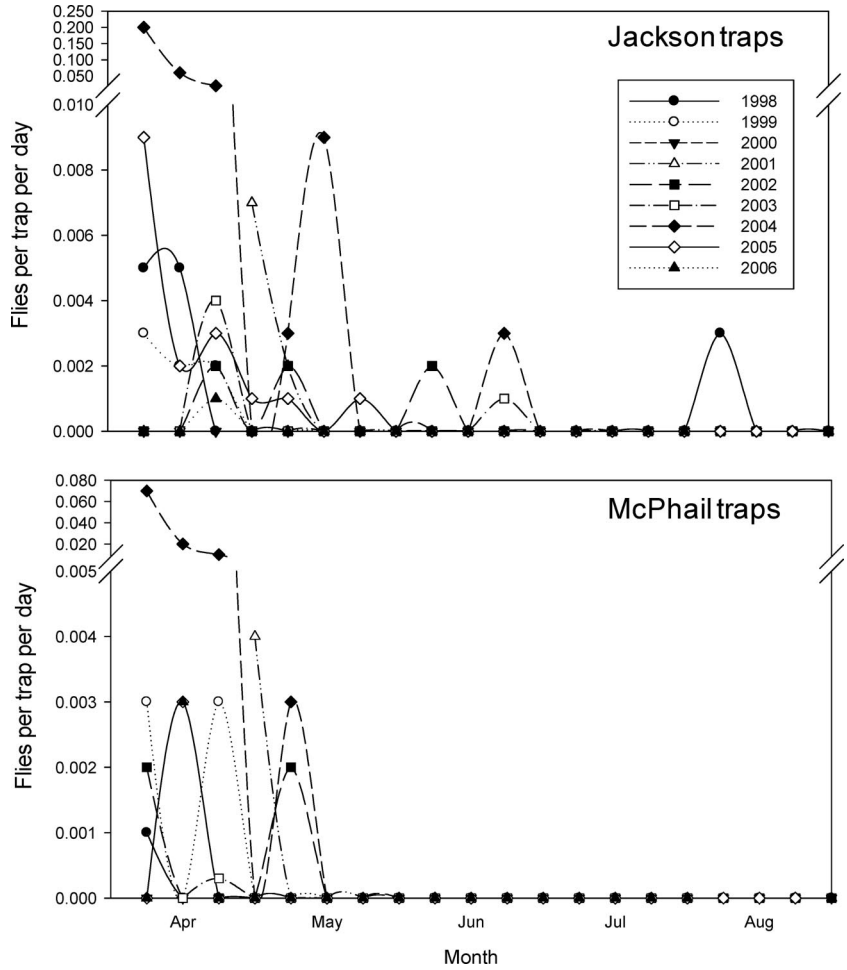

Fig. 1. Flies captured per trap per day in Jackson and McPhail traps at Sauce Huacho orchard, Tucumán, Argentina, from 1998 to 2006.

laying into avocado fruit used in cage test was not observed, the number of eggs laid on the agar oviposition substrate per female per day ranged from 28 to 38, demonstrating oviposition competence and fecundity of the tested females. The quality control evaluation at the rearing cages confirmed that the eggs were viable (egg hatch averaging 94.2%).

Fruit Sampling for Natural Infestation. During 11 seasons, 5,949 fruit in total were sampled from the trees, and 992 fruit were collected from the ground and processed, and in none of them were any live or dead fruit fly larvae found (Table 2). Trained experts with SENASA reported that thorough evaluation of 198,759 fruit from the packinghouse showed no fruit fly infestation.

Confidence Levels. The total number of gravid females used in cage testing was 11,250 (Table 3). The total number of fruit exposed to fruit flies was 2,250, and the total number of fruit inspected during fruit sampling from the field and packinghouse was 205,700. The total number of fruit evaluated considering infestation tests and fruit sampling (Table 3) was 207,950, so $C = 1 - (0.000032)^{207,950}$ for a confidence level of 99.99% assuming a required efficacy of 99.99%, or a confidence level of 99.87% assuming a required efficacy of 99.9968%. This means that we have 99.99 and 99.87% of confidence that the true survival of *C. capitata* on Hass avocado is no >0.0001 and 0.000032, respectively.

Discussion

Hass avocado harvested at the hard, mature green stage was not infested by *C. capitata.* Host resistance may act on one or more stages of the fruit fly. In nature, adult fruit flies must first be attracted to the host and then be capable of ovipositing successfully (Prokopy and Roitberg 1989). Eggs deposited in the host must hatch, and larvae must successfully feed and develop to maturity. Multiple factors probably contribute to Hass avocado fruit resistance to *C. capitata.* First, *C. capitata* is typically trapped at low levels in avocado orchards in Argentina, suggesting that avocado is not an attractive host. Higher population levels of *C. capi-*

Table 1. Exposure of Hass avocados from five localities to gravid female *C. capitata* in laboratory cages (24 and 48 h) and field cages, with potential number of eggs laid

Yr	Date	Locality	No. fruit evaluated[a]	Dry matter (%)[b]	No. females[c]	Infestation (%)
1998	18 Aug.	Sauce Huacho	150	26	750	0
1999	23 Aug.	Alpachiri	150	32	750	0
	12 Oct.	Sauce Huacho	150	32	750	0
2000	18 May	Yerba Buena	150	29	750	0
	13 Sept.	Sauce Huacho	150	28	750	0
2001	14 June	Taficillo	150	31	750	0
	02 Oct.	Taficillo	150	33	750	0
2002	18 April	Timbó Nuevo	150	23	750	0
	22 May	Taficillo	150	24	750	0
	12 June	Yerba Buena	150	26	750	0
	15 July	Timbó Nuevo	150	28	750	0
	13 Aug.	Taficillo	150	31	750	0
	10 Sept.	Timbó Nuevo	150	30	750	0
	07 Oct.	Sauce Huacho	150	32	750	0
2003	27 May	Taficillo	150	24	750	0

[a] In the three trials (field, 24-h laboratory infestation and 48-h laboratory infestation), 50 fruit were exposed.
[b] In all cases fruit hardness was >13 kg/cm^2.
[c] Each fruit was exposed to five females.

tata are found in orchards of preferred fruit hosts, and these fruit can be heavily infested (Ovruski et al. 2003, 2004; Segura et al. 2006; Oroño et al. 2008). Second, the hard exocarp poses a physical barrier to oviposition; *C. capitata* is known for its short aculei and Hass for its thick exocarp which may contribute to this "puncture resistance" (Follett 2009). Third, Hass avocado can form calluses and regenerate tissue at oviposition sites, making it less suitable for reproduction in several fruit fly species (Liquido et al. 1995, Aluja et al. 2004). Antibiosis mechanisms in the fruit pulp at the mature green stage also may limit larval development (Jang 1996). Under these conditions, Hass avocado qualifies as a conditional nonhost for *C. capitata,* and no postharvest treatments would be needed by importing countries.

In spite of this, Hass avocado may support *C. capitata* larval development under certain conditions. For example, De Graaf (2009) observed a low level of development to the adult stage in punctured Hass avocado fruit under the forced, artificial conditions in laboratory cage tests. Concurrently, Follett (2009) reported development to adult in 'Sharwil' avocado in punctured and unpunctured fruit 2–6 d after harvest regardless of fruit firmness. Under such situations, systems approaches such as those proposed by Armstrong (1991) and Follett and Vargas (2010) and used in the export program for Argentine Hass avocados to Chile provide postharvest safeguards (fruit harvested with stems attached and brought to the packinghouse within 12 h; culling to remove damaged fruit; and packing in fruit fly-proof cartons) that eliminate any possibility of postharvest infestation. In the particular case of the systems approach for exporting Hass avocados from Tucumán, Argentina, to Chile, it was only until recently that a postharvest quarantine treatment with methyl bromide to control any *C. capitata* was required.

The evidence presented in our work supports the conclusion that Hass avocado is not a natural host for *C. capitata.* De Graaf (2009) sampled 17,000 packing house fruit and 10,000 exported fruit and found no *C.*

Table 2. Inspection of fruit for *C. capitata* infestation from the field and packinghouse samplings at Famaillá, Tucumán, Argentina

Yr[a]	Sampling period	No. fruit from the tree	No. fruit from the ground	No. fruit from packing house	Infestation (%)
1998	April–Nov.	1,040	253	6,158	0
1999	April–Sept.	1,315	412	7,528	0
2000	April–July	643	20	4,754	0
2001	June	58	12	1,325	0
2002	May–July	423	177	42,404	0
2003	May–July	245	26	6,175	0
2004	April–June	112	23	1,224	0
2005	April	32	0	1,235	0
2006	April–June	198	7	18,501	0
2007	May	243	15	0	0
2008	May	200	19	0	0
2009	April–June	440	7	49,754	0
2010	April–Aug.	530	0	3,126	0
2011	April–July	470	21	56,575	0
Total		5,949	992	198,759	**0**

[a] Although in 2007 and 2008 there were no exports to Chile, fruit were sampled in the field.

Table 3. Confidence levels at two levels of required efficacy (99.99 and 99.9968%) for Hass avocado host status determination studies with *C. capitata* based on fruit and fruit fly sample sizes during testing

Situation	No.	Infestation/ survival (%)	Confidence levels (%)	
			99.99	99.9968
Infestation trials in the field				
Fruit	750	0	7.23	2.37
Females	3,750	0	31.27	11.31
Infestation trials in the laboratory				
Fruit	1,500	0	13.93	4.69
Females	7,500	0	52.77	21.34
Subtotal infestation trials				
Fruit	2,250	0	20.15	6.95
Females	11,250	0	67.54	30.23
Fruit samplings				
Fruit from the tree	5,949	0	44.84	17.33
Fruit from the ground	992	0	9.44	3.12
Fruit from the packinghouse	198,759	0	99.99	99.82
Subtotal fruit sampling	205,700	0	99.99	94.48
Total fruit evaluated	207,950	0	99.99	99.86

capitata infestation. Concurrently, after exposure of 30 Hass avocado fruit to 16,000 gravid females during laboratory and field experiments under forced artificial conditions, no survivors were obtained. Our study involved inspection of >200,000 fruit from field and packinghouse samples, and no infestation by *C. capitata* was found. In addition, exposure of 2,250 Hass avocado fruit to 11,250 gravid females under forced artificial conditions during laboratory and field cage experiments resulted in no survivors, being the absence of oviposition the most likely cause. These data exceed the standards for nonhost status published by Cowley et al. (1992), as well as the Probit 9 standard for development of quarantine treatments (Follett and Neven 2006, Follett and Hennessey 2007). In addition, our results served as a basis to eliminate the methyl bromide fumigation included in the trading protocol agreed between SENASA (Argentina) and SAG (Chile) (SENASA and SAG 2008). Since methyl bromide fumigations were stopped, no larval interceptions have occurred, reinforcing the fact that the quarantine risk is negligible.

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