

EVALUATION OF QUARANTINE RISK OF INTRODUCTION OF *Ceratitis capitata* IN HASS AVOCADOS FROM ARGENTINA

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Avocado is generically considered a fruit fly host, for that reason it is included as such in the lists developed by all National Plant Protection Organizations, but Hass has shown resistance to certain fruit fly species in Mexico and Guatemala. The most important economic fruit fly in some regions of Argentina is the medfly, *Ceratitis capitata*. For this reason, a quarantine treatment is mandatory to export to medfly-free countries. The objective of this study was to establish if there is a quarantine risk to export Hass avocados from Argentina without a quarantine treatment. The research included studies of resistance, monitoring adult populations in the field, and postharvest fruit sampling. To determine the host status of avocados, 15 resistance tests were made in different localities, including forced infestations of 750 fruits on plants and 1500 postharvest fruits with 23 to 33% dry matter content. Fruits were caged for 48 hours with 5 mature females per fruit. Seventy six McPhail and 76 Jackson traps for 36 ha were used to monitor adult population of medflies during the export season. Two percent of the fruits exported to Chile were checked in the packinghouse. Resistance trials showed no infestation. The flies/trap/day index during the export season hardly ever exceeded 0.14. Postharvest inspection of 85,520 fruits showed no infestation. All these factors allow to conclude that Hass avocados exported from Argentina without a quarantine treatment do not constitute a quarantine risk for medfly-free countries.

Keywords: Mediterranean fruitfly, pest, tefritid, infestation, resistance.

EVALUACIÓN DEL RIESGO CUARENTENARIO DE INTRODUCCIÓN DE *Ceratitis capitata* EN LA PALTA HASS DE ARGENTINA

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La palta es considerada genéricamente hospedero de moscas de los frutos, por lo que está incluida en los listados de las Organizaciones Nacionales de Protección Vegetal; pero el cv Hass mostró resistencia a ciertas especies de moscas de los frutos en México y Guatemala. *Ceratitis capitata* está presente en algunas regiones de la Argentina por lo que se exige un tratamiento cuarentenario para la exportación a países libres de esta mosca. El objetivo de este trabajo fue establecer si existe riesgo cuarentenario para exportar paltas

Hass de Argentina sin un tratamiento cuarentenario para *C. capitata*. Las investigaciones incluyeron estudios de resistencia, monitoreo de poblaciones de adultos en el campo y muestreo de frutos en poscosecha. Se realizaron 15 ensayos de resistencia de palta Hass en diferentes localidades, incluyendo infestaciones forzadas de 750 frutos en la planta y 1500 frutos en poscosecha con un porcentaje de materia seca del 23 al 33%. Se colocaron 5 hembras maduras durante 48 horas por fruto. Se monitoreó la población de adultos durante la temporada de exportación usando 76 trampas McPhail y 76 Jackson en 36 ha. Se muestreó en el empaque el 2 % de los frutos exportados a Chile. Los ensayos de resistencia no mostraron infestación. El índice de moscas/trampa/día casi nunca excedió 0,14. La revisión de 85.520 frutos en poscosecha tampoco mostró infestación. Todos estos factores permiten concluir que la exportación desde Argentina de palta Hass sin tratamiento cuarentenario no constituye un riesgo para países libres de *Ceratitis capitata*.

Palabras clave: mosca de la fruta del Mediterráneo, plaga, tefrítidos, infestación, resistencia.

Introduction

In order to establish the quarantine risk of a pest introduction on a commodity, it is important to consider the pest level at harvest, the importance of the commodity as a fruit fly host and the harvest, packing and transport procedures. A correct evaluation should be based on solid scientific evidence, packing inspection records, storage points, departure ports and, above all, should not include doubtful data.

Fruit flies of the Tephritidae family are considered one of the most important pests of fruit and vegetables in the world and represents a serious impediment for commodity trade between countries with different sanitary status (White y Elson-Harris, 1992). Within this family, *Ceratitis capitata*, the Mediterranean fruit fly has a widespread distribution and a very large number of hosts (Liquido *et al.*, 1991). In Argentina, *C. capitata* is present in some regions and for this reason; a quarantine treatment is required for fruit exports to countries free of this fruit fly. This requirement does not account for abundance levels of the fruit fly in the crop and surroundings, nor the quality of a certain fruit as a pest host.

The pest level in the commercial orchard is a very important factor that has to be considered for asses the quarantine risk, as low populations levels lower the pest pressure on the host and consequently the risk decreases. An example can be found in the elimination of a quarantine treatment for *Ragoletis completa* on stone fruit from California to New Zealand. Adult trapping during the harvest time showed a free fruit fly period. In addition, Yokoyama *et al.* (1993) showed that peaches and nectarines are poor hosts, while plums are not suitable hosts. In this case, the determination of a pest free period and the stone fruit host status, were enough facts to give quarantine security and allow commercialization without a quarantine treatment.

In relation to host status, different definitions can be found. Armstrong (1986) defines fruit fly hosts as "any fruit or vegetable in which fruit fly oviposit

under field conditions". Cowley (1992) defines host as "any fruit or vegetable on which the fruit fly is capable of oviposition under field conditions, the eggs hatch, the larvae feed to complete its development and from a viable pupae emerge an adult able to reproduce". The Comité de Sanidad Vegetal del Cono Sur (COSAVE) defines fruitfly hosts as "the vegetal species whose fruit are infested by the pest under field conditions" (COSAVE, 1995). In all the definitions, it is emphasized the fact that infestation should occur in the field.

Armstrong (1994) points out the fact that many cultivars, stages of maturity and growing periods had received little attention as nonhost. There are some hosts that can be artificially but not naturally infested, as happens with *Bactrocera latifrons* on papaya. This author also cites hosts with cultivars or hybrids that are not infested by fruit flies, as the "Smooth Cayene" cultivar of pineapple found to be a nonhost of *Bactrocera dorsalis* y *Bactrocera cucurbitae*. In other cases, the state of maturity does not allow infestation as on green mature tomatoes that is not infested by *Bactrocera tryoni* in Australia, or green mature bananas that is not infested by the medfly, *B. dorsalis*, *B. tryoni* and other fruit flies. Also, not all hosts offer the same condition for development, and primary, secondary or poor hosts can be found. In this last status, minimal development can be achieved producing individuals with low reproductive potential or survival capacity.

Avocado (*Persea americana*) is considered generically a fruit fly host, reason why it is included as such in the host lists developed by different National Phytosanitary Protection Organizations. However, avocado is actually considered a poor host as supported by United States Agricultural Department (USDA) interception records that allowed concluding that this fruit is not a preferential fruit fly host (Miller *et al.*, 1995). There are records of resistance in some avocado varieties to fruit fly (Diptera: Tephritidae) infestation. For instance, Armstrong *et al.* (1983) showed that green harvest mature 'Sharwil' avocados are resistant to *C. capitata*. Moreover, Armstrong (1991) demonstrated that when harvested with stems attached, this variety shows resistance to *C. capitata*, *Dacus cucurbitae* and *D. dorsalis* for 24 hours, and to *D. latifrons* for 12 hours after harvest. Hennessey *et al.* (1996) assessed *Anastrepha suspensa* resistance in 11 avocado cultivars, establishing three tolerance levels. Enkerlin *et al.* (1994) determined that under laboratory conditions and high fruit fly pressure, 'Hass' avocado is a good host for *Anastrepha ludens*, is a regular host for *A. serpentina* and a poor one for *A. striata*; and also that susceptibility increases as time elapses after harvest. They also demonstrated that 'Hass' avocado is resistant to forced field infestation using a high pressure of fruit flies, and that they are not infested under natural conditions. Aluja *et al.* (2004) continued these studies and concluded that commercial 'Hass' variety should not be considered as a natural host of *A. ludens*, *A. obliqua*, *A. striata* nor *A. serpentina* in México. At present, USDA does not consider 'Hass' avocado as a Mediterranean fruit fly host, and thus allows fruit to be imported from Guatemala and Mexico.

The objective of this work was to establish if there is a quarantine risk of introducing *C. capitata* by exporting Argentine 'Hass' avocados without a quarantine treatment. To accomplish this objective *C. capitata* population levels in the field during harvest time were determined, and the host status of avocado produced and harvested for export was evaluated.

Materials and methods

In order to assess the quarantine risk, three items were considered. First, the pest abundance in the field was determined. Second, the possibility that 'Hass' avocado harvested for export might be a host of *Ceratitis capitata* and finally, fruit infestation in the field and packing house was assessed.

I. Pest population levels in the field

Population levels were assessed in an export fruit crop located in Sauce Huacho, a locality in Famaillá, Tucumán, Argentina. Trapping began in April, one month before the beginning of export harvest, and lasted up to the end of August, when export harvest ends. Thirty two Jackson traps and 32 McPhail traps were distributed in 10 export parcels in a 36 ha area. Also, 44 Jackson traps and 44 McPhail traps were distributed in the surroundings of the former parcels. Jackson traps were baited with a sexual bait and the McPhail with a food bait. The traps were serviced weekly and sent to the Estación Experimental Agroindustrial Obispo Colombres laboratories where the fruit flies were identified and quantified. The values obtained were transformed to daily captures and informed as flies per trap per day (FTD) for each parcel.

II. Host status.

The host status of 'Hass' avocados was determined by forced no choice infestations trials or resistance trials at different locations.

Insects. The insects for the trials were obtained from the medfly colony held at the EEAOC insect rearing facility. The females used were sexually mature, at the oviposition highest peak, with 7 to 11 days after emergence. To determine fecundity of the flies used, 20 females were taken from the same group used for trials and placed in groups of 5 in plastic jars covered with voile. Inside each recipient, 4 cm diameter agar pieces with peach juice and wrapped with film paper were placed as oviposition substrate. After 48 hs, eggs were counted and the number of eggs per female was estimated for each trial. These estimates were done for the 8 trials made during 2002 and 2003.

Avocado maturity. The trials were made when the fruit achieved the physiological state of maturity. Physiological maturity was determined by dry matter content expressed as percentage. The fruit used achieved a minimum of 23% of dry matter content, being this value the minimum established to export 'Hass' variety. The dry matter content was analyzed at the Chemistry of Agroindustrial Products Laboratory at the EEAOC.

Study areas. The trials were located at the following localities in the Province of Tucumán: 1) Sauce Huacho, placed at 550 m.o.s.l., 26° 56'24.76" S and 65°28'14.72" W; Famaillá. 2) Taficillo, placed at 800 m.o.s.l., 26° 41'22.31" S and 65°16'57.41" W; Tafí Viejo. 3) Alpachiri, placed at 500 m.o.s.l., 27° 20'04.73"

S and 65°16'14.72" W, Chicligasta. 4) Yerba Buena, placed at 500 m.o.s.l., 26° 48'26.10" S and 65°19'41.06" W; Yerba Buena. 5) Timbó Nuevo, placed at 600 m.o.s.l., 26° 39'20.37" S and 65°03'57.29" W; Burruyacu.

Resistance trials. Between 1998 and 2003, 15 forced infestation trials in the different localities mentioned. Each trial included three different treatments: field forced infestations during 48 h of 50 fruits, laboratory forced infestations of 50 fruit during the first 24 h after harvest, and laboratory forced infestations of another 50 fruit during the first 48 h after harvest. Each fruit was exposed to five females. For field infestations, cages of wire frames of 40 cm diameter and 60 cm long covered with a voile mesh were placed covering avocado tree branches with fruits. Inside each cage female fruit flies were introduced according to the number of fruit engaged. The females were left for 48 hs with water and food. After that time, fruit were collected and taken to the laboratory and placed in individual plastic containers with sand in the bottom and covered by a voile mesh. The forced infestation methodology used in the laboratory was carried out basically in the same way as in the field. The differences were that the fruit were harvested from the field with the peduncle and taken promptly to the laboratory, to assure that within the first 2 hs after harvest, the avocados were exposed to the fruit flies. The flies were fed in the same way as in the field, and after the exposure time (24 or 48 hs) the fruit were introduced in the plastic containers with sand to allow development in case oviposition and development had taken place. The avocados were incubated at $25 \pm 2^\circ\text{C}$ and the relative humidity above 70% during three weeks. Every three or four days, the containers were checked for pupae. Once the three weeks period passed, the sand was sieved to detect the presence of pupae, and fruit were dissected to determine the presence of live or dead larvae of *C. capitata*.

Data analysis. To obtain a probability level that one avocado is infested by *C. capitata*, the non preference for avocado or a probable antibiosis was equaled to a quarantine treatment. The level of confidence was estimated following Couey and Chew (1986). The fecundity data were used to consider that value as the number of potential eggs that the females would have laid in the avocados if these were a good host. These data, together with the number of potential number of larvae or pupae recovered, were used for the Probit 9 analysis. Also the real proportion of survivors (i.e. pupae obtained) for a given 95% of confidence level was estimated.

III. Fruit sampling

In the field. Fruit sampling was done during the years 1998, 1999, 2000, 2002, 2003 and 2006, with a 20 days frequency during the whole export period on 12 plants in pre-established sites of the Sauce Huacho farm. At each site, 10 avocados cut from the trees and all avocado fruit fallen to the ground were collected. In the laboratory, the fruit were placed in the same recipients as used in the infestation trials and the fruit was incubated for three weeks before revision.

In packing house. In the packing house 2% of the 'Hass' avocado fruit for export were inspected. The methodology included a visual revision of all fruit for symptoms and chopping and thorough check of at least 1% of the fruits. These revisions were made by trained personnel of the Servicio Nacional de Sanidad y Calidad Agroalimentaria of Argentina (SENASA). The revisions were made on 1998, 1999, 2000, 2002, 2003 and 2006.

Results

Pest population in the field

The levels of population abundance obtained in Jackson traps are shown in Figure 1, and the values obtained with the McPhail traps are shown in Figure 2. Fruit flies were trapped all years with the exception of 2000 when no captures were registered neither in Jackson, nor McPhail traps. Captures were registered at least during one week during April, while in the other months captures were more sporadic. The fruit fly captures were obtained more frequently in non export parcels or in traps placed at the surroundings, specifically near houses. The highest levels were obtained for the years 2004 and 2005, years without fruit export. During the years when fruit were exported, the highest level of FTD index obtained were: 0.18; 0.14; 0.14; 0.06; 0.13 and 0.14 for 1998, 1999, 2001, 2002, 2003 and 2006 respectively.

Host status

Two thousand and two hundred and fifty 'Hass' avocado fruit were infested with 11,250 sexually mature *C. capitata* females between 1998 and 2003. These trials were made in the field, simulating a high fruit fly pressure (five fruit flies per fruit), and in the laboratory, placing the avocados at a high fruit fly pressure in optimal oviposition conditions. No signs of infestation were observed, and no pupae were recovered neither from the field infested nor from the laboratory infested fruit. Under laboratory conditions, the potential number of eggs laid by the females if avocados would have been a good host was 201,750 eggs. This value was obtained from the last eight trials, and the average value for each trial was 25,219 eggs, that is to say that 17 eggs were laid per female per day, or 85 eggs were laid in each avocado fruit. With this value, the Probit 9 confidence level was 0.9984. This is to say that 22 individuals would survive from one million eggs with a confidence level of 95%. If this value was extrapolated to all the trials done, the confidence levels would have been even higher and the number of survivors would have decreased.

Fruit sampling

The quantity of fruit sampled in the field, from the trees, fallen to the ground and checked in the packing houses during years when exports occurred, are shown in Table 1. A total of 3,864 fruit from the trees and 895 fallen to the ground were checked. No *C. capitata* larvae or pupae were recovered from these fruit. In packing houses 85,520 fruit were inspected and also no signs of infection was detected.

Discussion

In this work, the possibility that 'Hass' avocados cultivated in Tucumán, Argentina and harvested following the export procedures, would represent a quarantine risk for countries free of the medfly was assessed. So, the possibility that this variety acts as a natural host of the medfly under the mentioned harvest conditions was determined. These studies were confirmed with fruit sampling in the field and packing houses, from which no pupae were obtained.

Cowley *et al.* (1992) states that if in a forced infestation laboratory trial, exposing a total of 250 fruit to fruit flies for 24 hours, without development of the fruit flies, the non host condition is demonstrated. In our case, 15 trials in laboratory were made, exposing in each of them 100 fruit (50 for 24 hours and 50 for 48 hours). These trials involved avocados from five different localities within the province for five years and throughout the whole export season. The results of this trials results in a sampling effort five times greater than the recommended by Cowley *et al.* (1992). In no case infestation was recorded. The fecundity values obtained under forced infestation trials in the laboratory allowed a quarantine security above a Probit 9 level. This shows that avocados harvested for export, even in the presence of high density of fruit flies, is not capable to produce live insects.

In México, Enkerlin *et al.* (1994) and Aluja *et al.* (2004) analyzed the resistance of Hass avocados to *Anastrepha obliqua*, *A. striata*, *A. serpentina* and *A. ludens* and demonstrated that even though no infestation occurred in the field, after 24 hours harvest, the fruit became susceptible to infestation. In our case, the absence of infestation was maintained for 24 hours after harvest. If it is considered that the packing of avocados is done within the first six hours after harvest, and is immediately kept in cold conditions, the resistance of the fruit for 48 hours provides an important margin of security.

Cowley *et al.* (1992) states that when infestation is obtained in laboratory trials, to determine the real host status, trials have to made in the field. Even though this is not our case, to be on the safe side, we did field trials and fruit sampling at the same time we run the trials in the laboratory. The same results, that is no infestation, were obtained in all trials.

The results obtained along nine years of evaluations and sampling, lets us conclude that 'Hass' variety is not a host of *Ceratitis capitata* in the field; condition that is maintained 48 hours after harvest. This fact is confirmed by the sampling of 4,759 fruit in the field and 85,520 fruit in the packing house. Adding to this data the fact that during all the export harvest season medfly population levels are nul or very low, the absolute lack of quarantine risk of 'Hass' avocados produced in Tucumán, Argentina can be assured. Hence, the commercialization of 'Hass' avocados from Argentina to free *Ceratitis capitata* countries without a quarantine treatment, does not imply a risk for the introduction of this pest.

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Figure 1: Flies captured per trap per day in Jackson traps at Sauce Huacho plantation, Tucumán Argentina from 1998 to 2006.

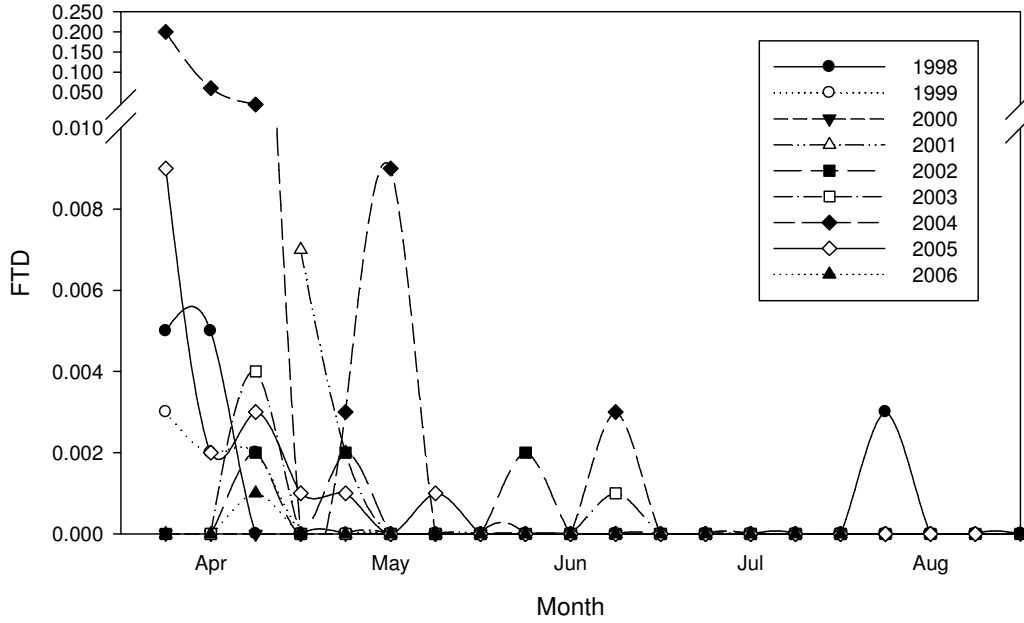


Figure 2: Flies captured per trap per day in McPhail traps at Sauce Huacho plantation, Tucumán Argentina from 1998 to 2006.

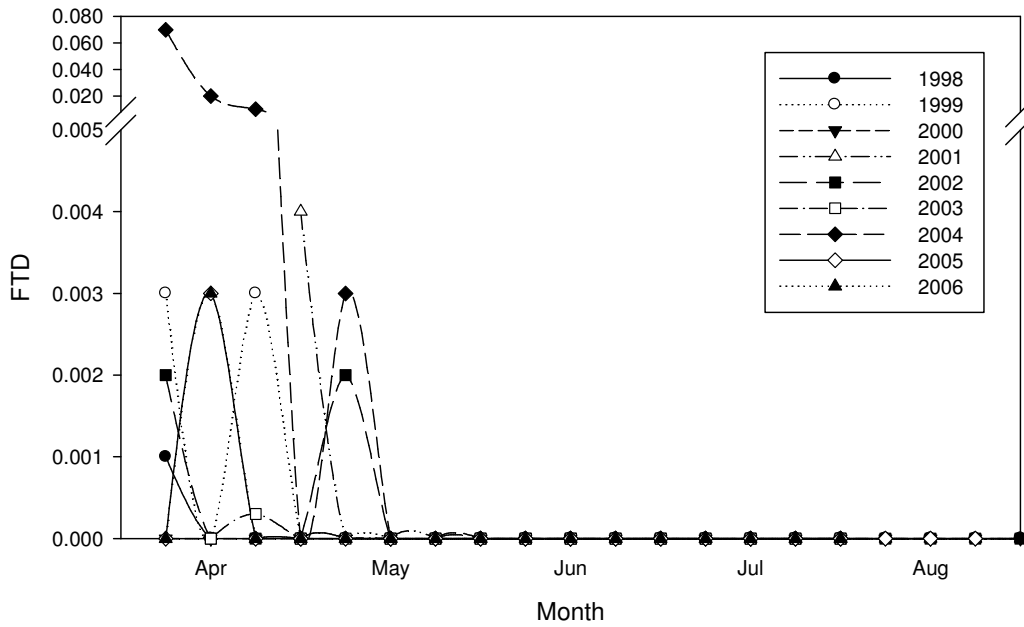


Table 1. Fruit sampled from the tree and fallen to the ground during six exportation seasons of 'Hass' avocados from Tucumán, Argentina.

Year	Fruit trees	from	Fruit to ground	fallen	Packing houses
1998	1,040		253		6,158
1999	1,315		412		7,528
2000	643		20		4,754
2002	423		177		42,404
2003	245		26		6,175
2006	198		7		18,501
Total	3,864		895		85,520