

Determination of the condition of lemons as host of *Ceratitis capitata* and *Anastrepha fraterculus*

G. Gastaminza¹, L. Augier¹, M. E. Villagrán¹, M. F. Villagrán¹ and E. Willink¹

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INTRODUCTION

A fruit fly host is defined as the vegetable or fruit in which the adult oviposits under natural conditions, the larvae eclode and acquire enough food substances to develop into the pupa stage, from which an adult capable of reproduction emerges (Armstrong, 1986). In this respect, Cowley *et al.* (1992) determined that if no adult emergence occurs, the fruit cannot be considered as a host.

Lemon (*Citrus limon* (L.) Burm. f.) status as a host for *Ceratitis capitata* (Wiedemann) is an example of controversy among different phytosanitary organizations. For example, El Comité de Sanidad Vegetal del Cono Sur (COSAVE) do not consider it as a host while Japan does. The USA did not consider it as a host until recently. At the international level, different works and regulations state the general and specific requirements to determine the host status of different species of tephritids for a certain variety of fruit and vegetables. Cowley *et al.* (1992) proposed a methodology to determine the host status that consists in forced infestation assays in the laboratory; and according to the results obtained, field complementary assays can be performed including adult trapping, fruit sampling and infestation trials (Fig. 1). The Regional Standards for Phytosanitary Measures (RSPM) N°4 of the Asia and Pacific Plant Protection Commission (APPPC) (2005) established guidelines for the determination of non host status of fruit and vegetables to tephritid fruit flies. This guide includes infestation tests in the laboratory with punctured fruit and infestation assays in the laboratory and the field with unpunctured fruit (Fig. 2).

The aim of this work was to determine the condition of lemons as host of *Ceratitis capitata* and *Anastrepha fraterculus* (Wiedemann).

MATERIALS AND METHODS

The assays to determine the host status of lemons were carried out in laboratory and in the field throughout the year, during fall / winter, fresh lemon export season, (April to September southern hemisphere) and spring / summer (October to March southern hemisphere), non export period The methodologies proposed by Cowley *et al.* (1992) and RSPM N°4 of the APPPC (2005) were followed.

¹Centro de Investigaciones Cuarentenarias, Sección Zoología Agrícola, Estación Experimental Agroindustrial Obispo Colombres (EEAOC). William Cross 3150, 4101, Las Talitas, Tucumán Argentina. E-mail: Imaugier@eeaoc.org.ar

Chapter IX

EEAOC - Chapter IX 1



Figure 1. Methodology proposed by Cowley *et al.* (1992) to determine the host status of multivoltine fruit fly species.

TRIALS WITH Ceratitis capitata

The lemon fruit used for field and laboratory trials were obtained from commercial lemon orchards that have not receive chemical sprays at least 30 days before the trials. The fruit that were used were mature lemon of 60 mm or more in diameter and had a minimum juice content of 35%, without any packing houses treatment.

I.- Assays of forced natural infestation of lemons to *C. Capitata*

A.- Laboratory assays

A. 1. - Laboratory assays with punctured fruit

A. 1. a - Materials

Forced natural infestations in the laboratory with punctured fruit were performed in cages with a metal structure of 70 cm depth, 40 cm width and 60 cm height (Fig. 3). *C. capitata* adults obtained from the rearing developed in the laboratory of the Estación Experimental Agroindustrial Obispo Colombres were used. This rearing is routinely subjected to quality controls following international standards.

A. 1. b - Methodology

The lemons used in the assays were artificially punctured 50 times with a N° 3 entomological pin, the wounds were distributed on the whole fruit surface (Fig. 4). Groups of 35 lemons with 175 gravid females (5 females per fruit) were then placed with water and food in the cages for 24 h. At the end of this period live



Figure 2. Diagram proposed by the RSPM N° 4 APPPC, 2005, to determine the host status of a vegetable species for fruit files.

flies were counted and, if mortality was over 10% the cage was discarded. The lemons were placed in individual containers with sterile sand and taken to the incubation chamber at $25 \pm 2^{\circ}$ C (Fig. 5). Half of the fruit were dissected after 10 days to check for the presence of live larvae and the other half were dissected after 20 days to detect the presence of larvae or pupae. A total of 70 lemons were infested per assay.

A. 1. c - Controls: oviposition and egg viability determination

For each trial, the number of viable eggs (a viable egg is the one from which a larva eclodes) laid per Medfly female under laboratory conditions was estimated. C. capitata females obtained from the same set of flies as the ones used in the infestation tests were placed with preferential hosts and/or artificial oviposition substrates (prepared on agar and fruit juices base) and covered with parafilm (Figs. 6-9). Preferential host varied according to the time of the year (peaches, plums, kiwis, etc.). Five replications were made in which 10 punctured fruit (following the same procedure described under A. 1.b) were placed in infestation cages with gravid females (5 flies per fruit), food and water. After 24 h the fruit were taken out of the cages, the eggs were counted, taken out and placed in an incubation chamber for 96 h at 25 ± 2°C to determinate viability (Fig. 10-11). With



Figure 3. Lemon fruit in the laboratory infestation cage.



Figure 5. Incubation chamber with lemon fruit.



Figure 7. Infestation of kiwi fruit.



Figure 9. Artificial hosts' infestation.



Figure 4. Lemon fruit punctured with entomological plns #3.



Figure 6. Kiwi fruit puntured with entomological pins #3.



Figure 8. Incubation of kiwi fruit.



Figure 10. Collection of eggs from kiwi to estimate egg viability.

EEAOC - Chapter IX 3



Figure 11. Collection of eggs from agar to estimate egg vlability

these data the number of viable eggs per female was determined.

A. 2.- Laboratory assays with unpunctured fruit

A. 2. a- Methodology:

For the laboratory tests, groups of 35 unpunctured lemon fruit were placed together with 175 gravid females (5 females per fruit), food and water, in infestation cages for 48 h. After this time, lemons were taken out of the cages, and live flies were counted and, if mortality was above 10%, the cage was discarded. The lemons were placed in individual containers with sand for incubation at $25 \pm 2^{\circ}$ C. A total of 210 fruit were infested per assay (70 lemons per storage period) and fruit with 2, 4 and 6 days of storage before infestation assays were performed.

Figure 12 shows the sequence of the different stages that make up the infestation tests in the laboratory with unpunctured fruit. In all cases the fruit used in the trials were collected 2, 4 and 6 storage days previous to the start of the infestation tests (X_{2} , X_{4} y X_{6}). The fruit used as controls are represented with the letter C.

A.2. b- Controls: estimation of oviposition and egg viability

Controls were carried out following the methodology described in A.1.c, with the exception that the fruit and/or artificial hosts used were unpunctured and the exposure period of the flies was 48 h.

Activities									Days						
Activities	0	1	2	3	4	5	6	7	8	9-11	12	13-17	18	19-27	28
А	X 6		X ₄		X 2										
В							X ₂ X ₄ X ₆ C								
С									X ₂ X ₄ X ₆ C						
D										X ₂ X ₄ X ₆ C	X ₂ X ₄ X ₆				
E											с				
F													X ₂ X ₄ X ₆		
G															X ₂ X ₄ X ₆

A. Fruit collection; B. Start of infestation of fruit (X) and controls (C); C. End of infestation of fruit and controls; D. Incubation of fruit and controls. E. Determination of control viability; F. Revision of 50% fruit, larvae detection; G. Revision of the remaining 50% fruit, detection of larvae and pupae.

Figure. 12. Sequence of the steps in the natural infestations forced in the laboratory with fruit with 2, 4 and 6 days after collection.

4 EEAOC - Determination of the condition of lemons as host of Ceratitis capitata and Anastrepha fraterculus

B.- Field assays with unpunctured fruit

B. 1. - Materials:

Forced field natural infestations were carried out in wire-frame cages approximately 40 cm long and 30 cm diameter covered with a "voile" cloth.

Assays were performed in lemon orchards that had not received insecticide application during the previous 30 days at least.

B.2.- Methodology:

In the lemon orchard branches with four or five fruit (diameter 60 mm or more and 35% juice content) were selected. They were covered with cages with five gravid C. capitata females per fruit, water and food. After exposure of the fruit to the flies for 48 hours, the branches with the fruit were cut off and taken to the laboratory, where live flies were counted. If mortality was above 10%, the cage was discarded. Then the fruit were placed in individual containers with sterile sand and incubated at 25 ± 2°C. After 10 days of incubation, half of the fruit were dissected to detect larvae, while after 20 days the remaining half was dissected to look for C. capitata larvae or pupae (see Fig. 4 in Chapter VII). A total of 70 lemons were infested per assay.

B.3.- Controls: estimation of oviposition and egg viability

In each assay the number of viable eggs laid per Medfly female under field conditions was estimated. Assays were carried out using *C. capitata* preferential hosts or artificial hosts (agar pieces flavored with fruit juice and covered with parafilm), which were placed on the branches of the lemon tree (without lemon fruit) in groups of five and were covered with a cage containing flies, water and food.

Five replications were made, each consisting in placing 10 fruit with 5 gravid females per fruit. After 48 h, the fruit were taken to the laboratory where the eggs removed from the fruit were counted and then incubated at $25 \pm 2^{\circ}$ C for 96h to determine the number of viable eggs per female.

RESULTS

A. 1.- Results of the laboratory assays with punctured fruit

In 2007 13 assays of forced natural infestation in the laboratory were performed. Out of 13 trials, 3 were conducted during the summer period (when no exports are made); 210 lemons were infested with 1,050 flies with an estimation of 25,658 viable eggs and 267 pupae were obtained from which 115 C. capitata adults emerged (Table 1).

The remaining 10 assays were performed during the export period (April to September). Seven hundred lemons were infested with 3,550 gravid females with an estimation of 81,074 viable eggs and 17 pupae were obtained from which 7 *C. capitata* adults emerged (Table 1).

A. 2.- Results of laboratory assays with unpunctured fruit

In 2004 eight trials were made in the laboratory where a total of 1,680 lemons (with 2, 4, and 6 days of storage) were infested with 8,400 gravid females with an estimation of 116,970 viable eggs without obtaining *C. capitata* larvae or pupae in the different lemon storage days (Table 2).

In 2005 13 trials were made in the laboratory where a total of 2,730 lemons (with 2, 4, and 6 days of storage) were infested with 13,650 gravid females with an estimation of 189,231 viable eggs without obtaining *C. capitata* larvae or pupae in the different lemon storage days (Table 3).

In 2006 12 trials were made in the laboratory where a total of 2,520 lemons (with 2, 4, and 6 days of storage) were infested with 12,600 gravid females with an estimation of 169,498 viable eggs without obtaining *C. capitata* larvae or pupae in the different lemon storage days (Table 4).

B.- Results of field assays with unpunctured fruit

In 2004 eight field trials were made where a total of 560 lemons were infested with 2,800 gravid females with an estimation of 84,224 viable eggs without obtaining *C. capitata* larvae or pupae (Table 5).

In 2005 13 field trials were made where a total of 910 lemons were infested with 4,550 gravid females with an estimation of 138,481 viable eggs without obtaining *C. capitata* larvae or pupae (Table 6).

In 2006 12 field trials were made where a total of 840 lemons were infested with 4,200 gravid females with an estimation of 121,527 viable eggs without obtaining *C. capitata* larvae or pupae (Table 7).

Summary of the results of the infestations in the field and in the laboratory with unpunctured lemons for *C. capitata*.

Table 8 shows the results obtained in the field and laboratory assays with unpunctured fruit carried out in 2004, 2005, and 2006 for *C. capitata* grouped by export (April to September, fall and winter in southern hemisphere) and non export periods (October to March, spring and summer in southern hemisphere).

Table 1. C. capitata pupae or adults found in the assays of forced natural infestation in the laboratory with artificially punctured fruit and determination of viable eggs obtained in the controls during 2007.

	INFESTA	TION O	F LEMON FRUIT				CONTROLS				
Periods (month)	Dates	№ of fruit	№ of females	Nº of pupae obtained	№ of adults obtained	N° of eggs/ flies/ day	Total № of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host	
Non ownert period	Jan 17, 2007	70	350	156	5	26	9,100	93	8,463	Artificial host	
(lap March)	Feb 02, 2007	70	350	111	110	25	8,750	95	8,312	Prunes	
	Mar 26, 2007	70	350	0	0	27	9,450	94	8,883	Prunes	
Subtot	al	210	1,050	267	115		27,300		25,658		
	Apr 23, 2007	70	350	0	0	25	8,750	96	8,400	Prunes	
	Jun 04, 2007	70	350	0	0	23	8,050	93	7,486	Prunes	
	Jun 27, 2007	70	350	17	7	26	9,100	94	8,554	Kiwis	
	Jul 23, 2007	70	350	0	0	25	8,750	92	8,050	Kiwis	
Export period	Jul 25, 2007	70	350	0	0	26	9,100	96	8,736	Kiwis	
(April – Sep) ++	Aug 06, 2007	70	350	0	0	24	8,400	95	7,980	Kiwis	
	Aug 07, 2007	70	350	0	0	25	8,750	98	8,575	Kiwis	
	Aug 07, 2007	70	350	0	0	23	8,050	97	7,808	Kiwis	
	Aug 09, 2007	70	350	0	0	22	7,700	92	7,084	Kiwis	
	Aug 15, 2007	70	350	0	0	25	8,750	96	8,400	Kiwis	
Subtot	al	700	3,500	17	7		85,400		81,074		
TOTA	L	910	4,550	284	122		112,700		106,732		

+ January - March summer in the southern hemisphere.

++ April - August autumn / winter in the southern hemispher.

Table 2. C. capitata larvae or pupae found in the assays of forced natural infestation with unpunctured fruit and estimation of viable eggs obtained from the controls in 2004.

	INFESTASTIO	N OF LEMC	N FRUIT		CONTROLS						
Periods (month)	Dates	№ of fruit*	Nº of females**	Nº of larvae or pupae obtained ***	N⁰of eggs/ flies/ 2 days	Total Nº of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host		
	Apr 21, 2004	210	1,050	0	48	16,800	92	15,456	Artificial host		
Export pariod	May 26, 2004	210	1,050	0	50	17,500	89	15,575	Artificial host		
	Jun 30, 2004	210	1,050	0	40	14,000	91	12,740	Artificial host		
(iviay – Sep)	Jul 28, 2004	210	1,050	0	46	16,100	90	15,295	Artificial host		
Ŧ	Aug 24, 2004	210	1,050	0	42	14,700	94	13,818	Artificial host		
	Sep 15, 2004	210	1,050	0	42	14,700	93	13,671	Artificial host		
Non export	Oct 06, 2004	210	1,050	0	46	16,100	90	14,490	Prunes		
(Oct – Dec) ++	Nov 23, 2004	210	1,050	0	50	17,500	91	15,925	Prunes		
TO	ΓAL	1,680	8,400	0		127,400		116,970			

*210 corresponds to the fruit subjected to forced infestation with 2, 4 and 6 storage days previous to infestation (70 fruit/ storage day). **1,050 corresponds to the female flies for assays with lemons with 2, 4 and 6 storage days previous to infestation (350 females/ storage day). ***Larvae or pupae obtained from fruit with 2, 4 and 6 storage days previous to infestation.

+ May - September autumn / winter in the southern hemisphere.

++ October - December spring in the southern hemisphere.

	INFESTATIO	N OF LEMO	N FRUIT		CONTROLS					
Periods (month)	Dates	№ of fruit*	№ of females**	№ of larvae or pupae obtained ***	N⁰of eggs/ flies/ 2 days	Total № of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host	
Non export	Jan 04, 2005	210	1,050	0	48	16,800	93	15,624	Prunes	
period	Feb 23, 2005	210	1,050	0	52	18,200	91	16,562	Prunes	
(Jan – March) +	Mar 28, 2005	210	1,050	0	44	15,400	95	14,630	Artificial host	
	Apr 06, 2005	210	1,050	0	46	16,100	92	14,812	Artificial host	
	Apr 27, 2005	210	1,050	0	48	16,800	92	15,456	Artificial host	
Export pariod	May 18, 2005	210	1,050	0	42	14,700	94	13,818	Artificial host	
	Jun 14, 2005	210	1,050	0	50	17,500	90	15,750	Artificial host	
(April – Sep) ++	Jul 11, 2005	210	1,050	0	44	15,400	91	11,011	Artificial host	
	Aug 08, 2005	210	1,050	0	40	14,000	96	13,440	Artificial host	
	Sep 05, 2005	210	1,050	0	46	16,100	94	15,134	Loquats	
Non export	Oct 14, 2005	210	1,050	0	44	15,400	92	14,168	Prunes	
period	Nov 15, 005	210	1,050	0	44	15,400	91	14,014	Prunes	
(Oct- Dec) +++	Dec 21, 2005	210	1,050	0	46	16,100	92	14,812	Peaches	
TOT	AL	2,730	13,650	0		207,900		189,231		

*210 corresponds to the fruit subjected to forced infestation with 2, 4 and 6 storage days previous to infestation (70 fruit/ storage day). **1,050 corresponds to the female flies for assays with lemons with 2, 4 and 6 storage days previous to infestation (350 females/ storage day). ***Larvae or pupae obtained from fruit with 2, 4 and 6 storage days previous to infestation.

+ January - March summer in the southern hemisphere.

++ April - September autumn / winter in the southern hemisphere.

+++ October - December spring in the southern hemisphere.

Table 4. C. capitata larvae or pupae found in the assays of forced natural infestation with unpunctured fruit and estimation of viable eggs obtained from the controls in 2006.

	INFESTATION	N OF LEMO	N FRUIT				CONTROLS		
Periods (month)	Dates	№ of fruit*	№ of females**	N⁰ of larvae or pupae obtained ***	N⁰of eggs/ flies/ 2 days	Total № of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host
Non export	Jan 09, 2006	210	1,050	0	42	14,700	92	13,524	Prunes
(Jan – March) +	Feb 02, 2006	210	1,050	0	44	15,400	91	14,014	Prunes
	Apr 03, 2006	210	1,050	0	44	15,400	93	14,322	Peaches
	May 24, 2006	210	1,050	0	46	16,100	90	14,490	Kiwis
Export poriod	Jun 13, 2006	210	1,050	0	42	14,700	91	13,377	Kiwis
(April Sop)	Jul 05, 2006	210	1,050	0	48	16,800	90	15,120	Kiwis
(April – 3cp) ++	Jul 26, 2006	210	1,050	0	44	15,400	95	14,630	Kiwis
	Aug 29, 2006	210	1,050	0	42	14,700	93	13,671	Prunes
	Sep 27, 2006	210	1,050	0	44	15,400	90	13,860	Loquats
Non export	Oct 24, 2006	210	1,050	0	42	14,700	91	13,377	Peaches
period	Nov 26, 2006	210	1,050	0	42	14,700	94	13,818	Peaches
(Oct- Dec) +++	Dec 21, 2006	210	1,050	0	46	16,100	95	15,295	Prunes
TOT	AL	2,520	12,600	0		184,100		169,498	

*210 corresponds to the fruit subjected to forced infestation with 2, 4 and 6 storage days previous to infestation (70 fruit/ storage day). **1,050 corresponds to the female flies for assays with lemons with 2, 4 and 6 storage days previous to infestation (350 females/ storage day).

***Larvae or pupae obtained from fruit with 2, 4 and 6 storage days previous to infestation.

+ January - March summer sin the outhern hemisphere. ++ April - September autumn / winter in the southern hemisphere.

+++ October - December spring in the southern hemisphere.

Table 5. C. capitata larvae or pupae found in the field assays of forced natural infestation with unpunctured fruit and estimation of viable eggs obtained in the controls in 2004.

	INFESTATIO	N OF LEMO	N FRUIT		CONTROLS					
Periods (month)	Dates	№ of fruit	№ of females	N⁰ of larvae or pupae obtained	N⁰of eggs/ flies/ 2 days	Total Nº of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host	
	Apr 21, 2004	70	350	0	30	10,500	90	9,450	Artificial host	
	May 26, 2004	70	350	0	32	11,200	92	10,304	Artificial host	
Export period	Jun 30, 2004	70	350	0	34	11,900	89	10,591	Artificial host	
(May – Sep) +	Jul 28, 2004	70	350	0	34	11,900	94	11,186	Artificial host	
	Aug 24, 2004	70	350	0	30	10,500	91	9,555	Artificial host	
	Sep 15, 2004	70	350	0	32	11,200	90	10,080	Artificial host	
Non export period	Oct 06, 2004	70	350	0	36	12,600	91	11,466	Prunes	
(Oct – Dec) ++	Nov 23, 2004	70	350	0	36	12,600	92	11,592	Prunes	
TOT	AL	560	2,800	0		92,400		84,224		

+ May - September autumn / winter in the southern hemisphere.

++ October - December spring in the southern hemisphere.

Table 6. C. capitata larvae or pupae found in the field assays of forced natural infestation with unpunctured fruit and estimation of viable eggs obtained in the controls in 2005.

	INFESTATIO	N OF LEMO	N FRUIT		CONTROLS				
Periods (month)	Dates	№ of fruit	№ of females	N⁰ of larvae or pupae obtained	N⁰of eggs/ flies/ 2 days	Total Nº of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host
Non export	Jan 04, 2005	70	350	0	34	11,900	93	11,067	Prunes
period (Jan – March)	Feb 23, 2005	70	350	0	30	10,500	94	9,870	Prunes
+	Mar 28, 2005	70	350	0	36	12,600	95	11,970	Artificial host
	Apr 06, 2005	70	350	0	32	11,200	91	10,192	Artificial host
	Apr 27, 2005	70	350	0	36	12,600	90	11,340	Artificial host
Export period	May 18, 2005	70	350	0	34	11,900	90	10,710	Artificial host
(April – Sep)	Jun 14, 2005	70	350	0	40	14,000	87	12,180	Artificial host
++	Jul 11, 2005	70	350	0	30	10,500	94	9,870	Artificial host
	Aug 08, 2005	70	350	0	32	11,200	93	10,416	Artificial host
	Sep 05, 2005	70	350	0	30	10,500	91	9,555	Loquats
Non export	Oct 14, 2005	70	350	0	32	11,200	90	10,080	Prunes
(Oct- Dec)	Nov 15, 005	70	350	0	34	11,900	89	10,591	Prunes
+++	Dec 21, 2005	70	350	0	32	11,200	95	10,640	Peaches
TO	TAL	910	4,550	0		151,200		138,481	

+ January - March summer in the southern hemisphere.

++ April - September autumn / winter in the southern hemisphere.

+++ October - December spring in the southern hemisphere.

CONCLUSIONS

On the basis of the results obtained from the assays for the determination of the condition of lemon as host of *C. capitata*, we arrived at the following conclusions:

1.- *C. capitata* develops in lemons artificially punctured and subjected to forced natural infestation in the laboratory.

2.- *C. capitata* does not develop in unpunctured lemons with 2, 4 and 6 storage days

and subjected to forced natural infestation in the laboratory.

3.- *C. capitata* does not develop in unpunctured lemons subjected to forced natural infestation in the field.

5.- Lemon (*Citrus limon*) is a conditional non host for *C. capitata* according to RSPIM N°4 APPPC, 2005.

4.- Lemon (*Citrus limon*) is not a host for *C. capitata* according to Cowley *et al.* (1992).

8 EEAOC - Determination of the condition of lemons as host of Ceratitis capitata and Anastrepha fraterculus

	INFESTATIO	N OF LEMON	N FRUIT		CONTROLS					
Periods (month)	Dates	№ of fruit	№ of females	N° of larvae or pupae obtained	N⁰of eggs/ flies/ 2 days	Total Nº of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host	
Non export period	Jan 09, 2006	70	350	0	34	11,900	91	10,829	Prunes	
(Jan – March) +	Feb 02, 2006	70	350	0	36	12,600	94	11,844	Prunes	
	Apr 03, 2006	70	350	0	32	11,200	94	10,528	Peaches	
	May 24, 2006	70	350	0	30	10,500	93	9,765	kiwis	
Export period	Jun 13, 2006	70	350	0	30	10,500	90	9,450	kiwis	
(April – Sep)	Jul 05, 2006	70	350	0	28	9,800	89	8,722	kiwis	
++	Jul 26, 2006	70	350	0	26	9,100	94	8,554	kiwis	
	Aug 29, 2006	70	350	0	32	11,200	93	10,416	Prunes	
	Sep 27, 2006	70	350	0	30	10,500	95	9,975	Loquats	
Non export	Oct 24, 2006	70	350	0	32	11,200	90	10,080	Peaches	
(Oct-Dec)	Nov 26, 2006	70	350	0	34	11,900	92	10,948	Peaches	
+++	Dec 21, 2006	70	350	0	32	11,200	93	10,416	Prunes	
TO	TAL	840	4,200	0		131,600		121,527		

+ January - March summer in the southern hemisphere.

++ April - September autumn / winter in the southern hemisphere.

+++ October - December spring in the southern hemisphere.

Table 8. Summary of the forced natural infestations in the field and in the laboratory with unpunctured fruit for C. capitata in 2004, 2005, and 2006.

Assay type	Periods	Number of assays	Number of fruit	Number of females	Adults obtained	Total viable eggs in the controls
Laboratory	Export period	20	4,150	21,000	0	285,446
(unpunctured fruit)	Non export period	13	2,730	13,650	0	190,253
Subtota	al	33	6,880	34,650	0	475,699
Field	Export period	20	1,400	7,000	0	202,839
(unpunctured fruit)	Non export period	13	910	4,550	0	141,393
Subtota	al	33	2,310	11,550	0	344,232
TOTAL		66	9,190	46,200	0	819,931

TRIALS WITH Anastrepha fraterculus II.- Lemon forced infestation assays for *A. fraterculus*

The trials were made following the same methodology described for *C. capitata*. The results are shown below in the text.

RESULTS

A. 1.- Results of the laboratory trials with punctured fruit

In 2007 13 forced natural infestation assays were carried out in the laboratory. Three assays were performed during the summer period (when no exports are made) in which 210 lemons were infested with 1,050 flies with an estimated of 14,297 viable eggs. The ten remaining assays were performed during the export period (April to September) in which 700 lemons were infested with 3,550 gravid females with an estimated 43,736 viable eggs. No *A. fraterculus* larvae or pupae were obtained in the 13 assays carried out (Table 9).

A. 2.- Results of the laboratory assays with unpunctured fruit

In 2004, eight assays were performed in the laboratory in which 1,680 lemons with 2, 4, and 6 days of storage were infested with 8,400 gravid females with an estimated 68,733 viable eggs without obtaining *A. fraterculus* larvae or pupae

in the different lemon storage days (Table 10).

In 2005, 13 assays were carried out in the laboratory in which 2,730 lemons were infested with 2, 4, and 6 days of storage with 13,650 gravid females with an estimated 117,244 viable eggs without obtaining *A. fraterculus* larvae or pupae in the different lemon storage days (Table 11).

In 2006, 12 assays were performed in the laboratory in which 2,520 lemons with 2, 4, and 6 days of storage were infested with 12,600 gravid females with an estimated 114,178 viable eggs without obtaining *A. fraterculus* larvae or pupae in the different lemon storage day (Table 12).

B.- Results of assays in the field with unpunctured fruit

In 2004, eight assays were carried out in the field in which 560 lemons were infested with 2,800 gravid females with an estimated 40,627 viable eggs without obtaining *A. fraterculus* larvae or pupae (Table 13).

In 2005 13 assays were carried out in the field in which a total of 910 lemons were

infested with 4,550 gravid females with an estimated 85,428 viable eggs without obtaining *A. fraterculus* larvae or pupae (Table 14).

In 2006 12 assays were performed in the field in which 840 lemons were infested with 4,200 gravid females with an estimated 78,971 viable eggs without obtaining *A. fraterculus* larvae or pupae (Table 15).

Summary of the results of the infestations in the field and in the laboratory with punctured and unpunctured lemons for *A. fraterculus*.

Table 16 summarizes the results obtained in the laboratory assays with punctured fruit carried out in 2007 for *A. fraterculus*, grouped according to export (April to September, fall and winter in southern hemisphere) and non export periods (October to March, spring and summer in southern hemisphere). Table 17 shows the results obtained in the field and laboratory assays with unpunctured fruit carried out in 2004, 2005 and 2006 for *A. fraterculus* grouped according to export and non export periods.

Table 9. A. fraterculus pupae or adults found in the assays of forced natural infestation in the laboratory with artificially punctured fruit and determination of viable eggs obtained in the controls during 2007.

	INFESTA	TION O	F LEMON FRUIT			CONTROLS				
Periods (month)	Dates	№ of fruit	N⁰ of females	№ of pupae obtained	Nº of adults obtained	N° of eggs/ flies/ day	Total № of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host
Non ownert period	Jan 17, 2007	70	350	0	0	17	5,950	85	5,057	Artificial host
(lap March)	Feb 02, 2007	70	350	0	0	16	5,600	88	4,928	Prunes
(Jali – March) +	Mar 26, 2007	70	350	0	0	16	5,600	77	4,312	Prunes
Subtot	al	210	1,050	0	0		17,150		14,297	
	Apr 23, 2007	70	350	0	0	17	5,950	83	4,938	Prunes
	Jun 04, 2007	70	350	0	0	15	5,250	80	4,200	Prunes
	Jun 27, 2007	70	350	0	0	15	5,250	84	4,410	Kiwis
Europet posical	Jul 23, 2007	70	350	0	0	16	5,600	83	4,648	Kiwis
(April September)	Jul 25, 2007	70	350	0	0	14	4,900	82	4,018	Kiwis
(April – September)	Aug 06, 2007	70	350	0	0	17	5,950	84	4,998	Kiwis
тт	Aug 07, 2007	70	350	0	0	15	5,250	79	4,147	Kiwis
	Aug 07, 2007	70	350	0	0	14	4,900	85	4,165	Kiwis
	Aug 09, 2007	70	350	0	0	13	4,550	82	3,731	Kiwis
	Aug 15, 2007	70	350	0	0	16	5,600	80	4,480	Kiwis
Subtot	al	700	3,500	0	0		53,200		43,736	
TOTA		910	4,550	0	0		70,350		58,033	

*210 corresponds to the fruit subjected to forced infestation with 2, 4 and 6 storage days previous to infestation (70 fruit/ storage day)

**1,050 corresponds to the female flies for assays with lemons with 2, 4 and 6 storage days previous to infestation (350 females/ storage day).
***Larvae or pupae obtained from fruit with 2, 4 and 6 storage days previous to infestation.

+ January - March summer in the southern hemisphere.

++ April - August autumn / winter in the southern hemispher.

10 EEAOC - Determination of the condition of lemons as host of Ceratitis capitata and Anastrepha fraterculus

	INFESTATIO	<mark>N OF LEMO</mark>	N FRUIT		CONTROLS					
Periods (month)	Dates	№ of fruit*	№ of females**	N° of larvae or pupae obtained ***	№of eggs/ flies/ 2 days	Total Nº of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host	
	Apr 21, 2004	210	1,050	0	48	10,500	81	8,505	Artificial host	
Evenant pariod	May 26, 2004	210	1,050	0	50	9,800	85	8,330	Artificial host	
	Jun 30, 2004	210	1,050	0	40	11,900	83	9,877	Artificial host	
(iviay – Sep)	Jul 28, 2004	210	1,050	0	46	9,100	84	7,644	Artificial host	
т	Aug 24, 2004	210	1,050	0	42	10,500	82	8,610	Artificial host	
	Sep 15, 2004	210	1,050	0	42	9,100	85	7,735	Artificial host	
Non export period	Oct 06, 2004	210	1,050	0	32	11,200	84	9,408	Prunes	
(Oct – Dec) ++	Nov 23, 2004	210	1,050	0	28	9,800	88	8,624	Prunes	
TO	TAL	1,680	8,400	0		81,900		68,733		

*210 corresponds to the fruit subjected to forced infestation with 2, 4 and 6 storage days previous to infestation (70 fruit/ storage day) **1,050 corresponds to the female flies for assays with lemons with 2, 4 and 6 storage days previous to infestation (350 females/ storage day). *** Larvae or pupae obtained from fruit with 2, 4 and 6 storage days previous to infestation.

+ May - September autumn / winter in the southern hemisphere.

++ October - December spring in the southern hemisphere.

Table 11. A. fraterculus larvae or pupae	found in the for	ced natural infestatio	n assays with unpuncture	d fruit and estimation	of viable eggs
obtained from the control in 2005.					

	INFESTASTIO	N OF LEMC	on Fruit		CONTROLS							
Periods (month)	Dates	Nº of fruit*	№ of females**	N° of larvae or pupae obtained***	N° of eggs/ flies/ 2 days	Total Nº of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host			
Non export	Jan 04, 2005	210	1,050	0	30	10,500	85	8,925	Prunes			
period	Feb 23, 2005	210	1,050	0	32	11,200	86	9,632	Prunes			
(Jan – March) +	Mar 28, 2005	210	1,050	0	26	9,100	89	8,009	Artificial host			
	Apr 06, 2005	210	1,050	0	28	9,800	84	8,232	Artificial host			
	Apr 27, 2005	210	1,050	0	34	11,900	87	10,353	Artificial host			
Export pariod	May 18, 2005	210	1,050	0	30	10,500	85	8,925	Artificial host			
(April Sop)	Jun 14, 2005	210	1,050	0	30	10,500	82	8,610	Artificial host			
(April – Sep) ++	Jul 11, 2005	210	1,050	0	28	9,800	84	8,232	Artificial host			
	Aug 08, 2005	210	1,050	0	30	10,500	82	8,610	Artificial host			
	Sep 05, 2005	210	1,050	0	32	11,200	89	9,968	Loquats			
Non export	Oct 14, 2005	210	1,050	0	30	10,500	82	8,610	Prunes			
period	Nov 15, 2005	210	1,050	0	28	9,800	86	8,428	Prunes			
(Oct- Dec) +++	Dec 21, 2005	210	1,050	0	34	11,900	90	10,710	Peaches			
TOTAL		2,730	13,650	0		137,200		117,244				

*210 corresponds to the fruit subjected to forced infestation with 2, 4 and 6 storage days previous to infestation (70 fruit/ storage day) **1,050 corresponds to the female flies for assays with lemons with 2, 4 and 6 storage days previous to infestation (350 females/ storage day).

***Larvae or pupae obtained from fruit with 2, 4 and 6 storage days previous to infestation.

+ January - March summer in the southern hemisphere. ++ April - September autumn / winter in the southern hemisphere.

+++ October - December spring in the southern hemisphere.

Table 12. A. fraterculus larvae or pupae found in the forced natural infestation assays with unpunctured fruit and estimation of viable eggs obtained from the control in 2006.

	INFESTATION	N OF LEMO	N FRUIT		CONTROLS						
Periods (month)	Dates	№ of fruit*	№ of females**	N° of larvae or pupae obtained***	№of eggs/ flies/ 2 days	Total Nº of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host		
Non export	Jan 09, 2006	210	1,050	0	36	12,600	87	10,962	Prunes		
(Jan – March) +	Feb 02, 2006	210	1,050	0	32	11,200	85	9,520	Prunes		
	Apr 03, 2006	210	1,050	0	34	11,900	87	10,353	Peaches		
	May 24, 2006	210	1,050	0	30	10,500	84	8,820	Kiwis		
- · · · ·	Jun 13, 2006	210	1,050	0	28	9,800	83	8,134	Kiwis		
Export period	Jul 05, 2006	210	1,050	0	32	11,200	80	8,960	Kiwis		
(April – Sep) ++	Jul 26, 2006	210	1,050	0	30	10,500	85	8,925	Kiwis		
	Aug 29, 2006	210	1,050	0	34	11,900	85	10,115	Prunes		
	Sep 27, 2006	210	1,050	0	32	11,200	83	9,269	Loquats		
Non export	Oct 24, 2006	210	1,050	0	30	10,500	88	9,240	Peaches		
period	Nov 26, 2006	210	1,050	0	34	11,900	88	10,472	Peaches		
(Oct- Dec) +++	Dec 21, 2006	210	1,050	0	32	11,200	84	9,408	Prunes		
TOTAL		2,520	12,600	0		134,400		114,178			

*210 corresponds to the fruit subjected to forced infestation with 2, 4 and 6 storage days previous to infestation (70 fruit/ storage day) **1,050 corresponds to the female flies for assays with lemons with 2, 4 and 6 storage days previous to infestation (76 females/ storage day)
***Larvae or pupae obtained from fruit with 2, 4 and 6 storage days previous to infestation.
+ January - March summer in the southern hemisphere.
++ April - September autumn / winter in the southern hemisphere.

+++ October - December spring in the southern hemisphere.

Table 13. A. fraterculus larvae or pupa	e found in the field assays	of forced natural infestation w	ith unpunctured fruit and estimation
of viable eggs obtained in the controls in	ו 2004.		

	INFESTATION	N OF LEMON	N FRUIT		CONTROLS					
Periods (month)	Dates	№ of fruit	N ^o of females	N° of larvae or pupae obtained	N⁰of eggs/ flies/ 2 days	Total Nº of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host	
	Apr 21, 2004	70	350	0	22	7,700	85	6,545	Artificial host	
Export pariod	May 26, 2004	70	350	0	18	6,300	80	5,040	Artificial host	
	Jun 30, 2004	70	350	0	20	7,000	83	5,810	Artificial host	
(iviay – Sep)	Jul 28, 2004	70	350	0	18	6,300	79	4,997	Artificial host	
Ŧ	Aug 24, 2004	70	350	0	16	5,600	84	4,704	Artificial host	
	Sep 15, 2004	70	350	0	22	7,700	83	6,391	Artificial host	
Non export period	Oct 06, 2004	70	350	0	24	8,400	85	7,140	Prunes	
(Oct- Dec) ++	Nov 23, 2004	70	350	0	22	7,700	86	6,622	Prunes	
TO	ΓAL	560	2,800	0		56,700		40,627		

+ May - September autumn / winter in the southern hemisphere.

++ October - December spring in the southern hemisphere.

	INFESTATIO	N OF LEMON	I FRUIT		CONTROLS						
Periods (month)	Dates	Nº of fruit	№ of females	Nº of larvae or pupae obtained	№of eggs/ flies/ 2 days	Total Nº of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host		
Non export	Jan 04, 2005	70	350	0	26	9,100	82	7,462	Prunes		
period (Ian – March)	Feb 23, 2005	70	350	0	24	8,400	83	6,972	Prunes		
+	Mar 28, 2005	70	350	0	28	9,800	83	8,134	Artificial host		
	Apr 06, 2005	70	350	0	22	7,700	78	6,006	Artificial host		
	Apr 27, 2005	70	350	0	24	8,400	84	7,056	Artificial host		
Export period	May 18, 2005	70	350	0	20	7,000	81	5,670	Artificial host		
(April – Sep)	Jun 14, 2005	70	350	0	18	6,300	80	5,040	Artificial host		
++	Jul 11, 2005	70	350	0	20	7,000	81	5,670	Artificial host		
	Aug 08, 2005	70	350	0	20	7,000	83	5,810	Artificial host		
	Sep 05, 2005	70	350	0	22	7,700	85	6,545	Loquats		
Non export	Oct 14, 2005	70	350	0	24	8,400	87	7,308	Prunes		
period (Oct- Dec)	Nov 15, 2005	70	350	0	24	8,400	84	7,056	Prunes		
+++	Dec 21, 2005	70	350	0	22	7,700	87	6,699	Peaches		
TO	TAL	910	4,550	0		102,900		85,428			

+ January - March summer in the southern hemisphere.
++ April - September autumn / winter in the southern hemisphere.
+++ October - December spring in the southern hemisphere.

Tab	le 1	5. /	А.	<i>fraterculus</i>	arvae	or	pupae	found	l in	the	field	assays	of	forced	natural	infestatio	ו with	unpunctured	l fruit	and	estimation
of v	iabl	e e	ggs	obtained i	in the c	ont	rols in 3	2006.													

	INFESTASTIO	N OF LEMO	n Fruit		CONTROLS						
Periods (month)	Dates	№ of fruit	№ of females	Nº of larvae or pupae obtained	N⁰of eggs/ flies/ 2 days	Total Nº of eggs	Viable eggs (%)	Total viable eggs	Fruit/ Artificial host		
Non export period	Jan 09, 2006	70	350	0	26	9,100	86	7,826	Prunes		
(Jan – March) +	Feb 02, 2006	70	350	0	28	9,800	83	8,134	Prunes		
	Apr 03, 2006	70	350	0	24	8,400	82	6,888	Peaches		
	May 24, 2006	70	350	0	22	7,700	80	6,160	Kiwis		
Export period	Jun 13, 2006	70	350	0	18	6,300	79	4,997	Kiwis		
(April – Sep)	Jul 05, 2006	70	350	0	20	7,000	81	5,670	Kiwis		
++	Jul 26, 2006	70	350	0	20	7,000	82	5,740	Kiwis		
	Aug 29, 2006	70	350	0	18	6,300	84	5,292	Prunes		
	Sep 27, 2006	70	350	0	22	7,700	86	6,622	Loquats		
Non export	Oct 24, 2006	70	350	0	24	8,400	83	6,972	Peaches		
period	Nov 26, 2006	70	350	0	24	8,400	87	7,208	Peaches		
(Oct- Dec) +++	Dec 21, 2006	70	350	0	26	9,100	82	7,462	Prunes		
TOT	AL	840	4,200	0		95,200		78,971			

+ January - March summer in the southern hemisphere. ++ April - September autumn / winter in the southern hemisphere. +++ October - December spring in the southern hemisphere.

Table16. Summary of the forced natural infestations in the laboratory with punctured fruit for A. fraterculus in 2007.

Assay type	Periods	Number of assays	Number of fruit	Number of females	Adults obtained	Total viable eggs in the controls
Laboratory	Export period	10	700	3,500	0	43,736
(punctured fruit)	Non export period	3	210	1,050	0	14,297
TOTAL		13	910	4,550	0	58,033

Table17. Summary of forced natural infestations in the field and in the laboratory with unpunctured fruit for *A. fraterculus* in 2004, 2005, and 2006.

Assay type	Periods	Number of assays	Number of fruit	Number of females	Adults obtained	Total viable eggs in the controls
Laboratory	Export period	20	4,150	2,100	0	178,207
(unpunctured fruit)	Non export period	13	2,730	13,650	0	121,948
Subtota	I	33	6,880	34,650	0	300, 155
Field	Export period	20	1,400	7	0	116,653
(unpunctured fruit)	Non export period	13	910	4,550	0	94,995
Subtota	I	33	2,310	11,550	0	211,648
TOTAL		66	9,190	46,200	0	511,803

CONCLUSIONS

On the basis of the results obtained from the assays for the determination of the condition of lemon as host of *A. fraterculus*, we arrived at the following conclusions:

1.- *A. fraterculus* did not develop in artificially punctured lemons subjected to forced natural infestation in the laboratory.

2.- A. fraterculus did not develop in unpunctured fruit with 2, 4 and 6 storage days and subjected to forced natural infestation in the laboratory.

3.- A. fraterculus did not develop in

unpunctured lemons subjected to forced natural infestation in the field.

4.- Lemons is not a host for *A.fraterculus*, according to RSPM N° 4 APPPC, (2005) and to Cowley *et al.* (1992).

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EEAOC - Chapter IX 15

Fruit flies and its quarantine relevance in the citriculture of Northwestern Argentina. Eleven years of research 1996-2007