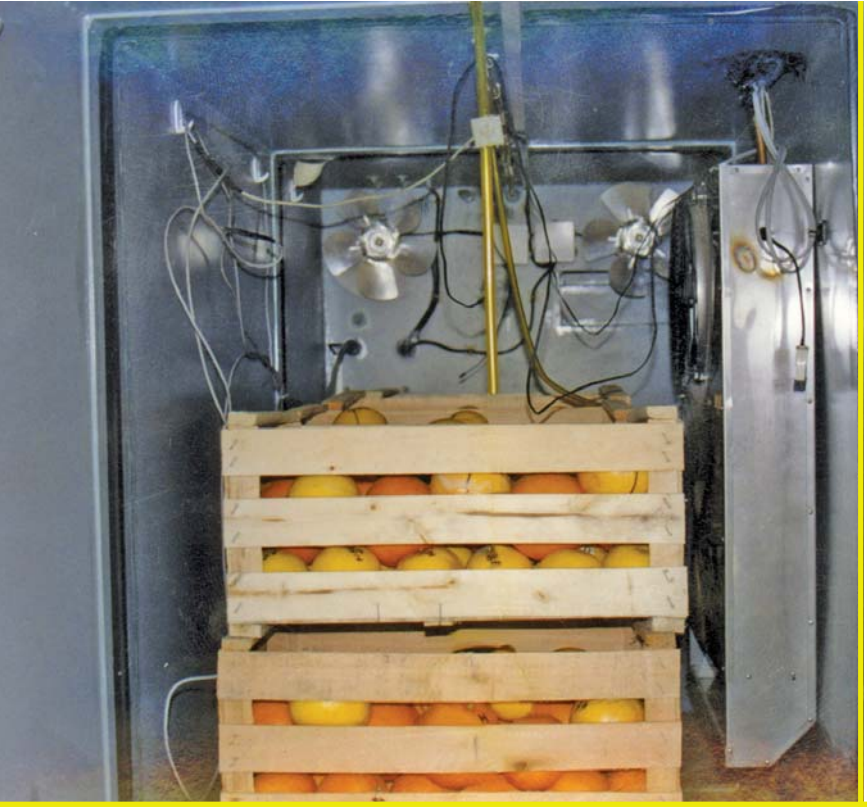

Quarantine treatments with Methyl Bromide for control of *Ceratitis capitata* and *Anastrepha fraterculus* in citrus fruit

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Key words: South American fruit fly, Mediterranean fruit fly.

Chapter V

INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and the South American fruit fly *Anastrepha fraterculus* (Wiedemann), are important pests present in the citrus growing regions of Argentina, which affect sweet citrus such as oranges (*Citrus sinensis* (L.) Osbeck), grapefruit (*C. paradisi* Macfadyn), tangerines (*C. reticulata* Blanco) and sour orange (*C. aurantium* L. (Rootstock)).

The Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA) from our country demands a quarantine treatment for the commercialization of those citrus fruit produced in regions whit the presence of *C. capitata* or *A. fraterculus* and with destiny to free areas or regions with ongoing control and eradication programs against fruit flies (namely Patagonia and Cuyo respectively). Under resolution 601/01 from SENASA, a treatment with a dose of 40g/m^3 of Methyl Bromide for 2 hours at 21 - 29ºC is established for citrus fruit.

Several authors have worked on the development of quarantine treatments for control *C. capitata* with Methyl Bromide in several fruit species such as cherry, peaches, nectarines, pears and plums, using the egg as the most tolerant developmental stage in Mediterranean fruit fly (Spitler and Couey, 1983; Armstrong and Couey, 1984). For *A. fraterculus* there are no published works respect to its response to Methyl Bromide.

The aim of the present work was to develop a quarantine treatment for the control of *C. capitata* and *A. fraterculus* in citrus fruit with Methyl Bromide at 15ºC.

MATERIALS AND METHODS

Fruit fly species and developmental stages

The biological material used in this work was the immature stages (eggs and larvae) of *C. capitata* and *A. fraterculus*, obtained from the laboratory rearing of the EEAOC, Tucumán, Argentina. The *C. capitata* colony was originated from the collection of infested fruit, mainly oranges and grapefruit from NW Argentina, while that of *A. fraterculus* came mainly from the collection of *Psidium guajava* L. from Tucumán, Argentina. Each summer wild blood was incorporated to the laboratory colony in four successive cross-breedings until 93.75% wild blood was obtained. Quality control parameters such as egg viability, egg to pupas recovery, pupal weight, sex ratio, flight ability, adult longevity, and female fecundity were assessed each generation.

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Citrus species and varieties

The citrus species used were: oranges, grapefruit, and tangerines with their hybrids. Within each species, several varieties were evaluated.

Inoculation methodology

Fruit were inoculated with different methodologies according to the fruit fly species. In the case of *A. fraterculus*, each fruit was artificially inoculated with 50 eggs or larvae. The fruit were cut in the upper part, the eggs or larvae were placed in the pulp and the fruit was then sealed with parafilm. Those fruit inoculated were placed in chambers at 15°C for 24 hours to allow larvae adaptation to the fruit pulp. After this time they were introduced in the Methyl Bromide fumigation chamber. In the case of *C. capitata*, the inoculation was performed as described above for larvae, whereas the eggs were inoculated naturally. To do so, 40 to 60 fruit were placed for two hours in a cage (0,70 m. long, 0,60 m. de width y 0,42 m. de height) with 2,000 gravid females. Fruit to be fumigated with eggs were incubated for 36 hours at 25°C.

Determination of viable insects

In order to determine the minimum number of viable insects per treatment, a sample of the fruit was taken before introducing the fruit in the fumigation chamber. These fruit were revised and the number of live individuals was determined. For the case of eggs, fruit were incubated for three days before being revised; only those eggs in which a larvae had eclode were considered viable. The number of viable individuals was estimated by subtracting the number of dead individuals to the number of inoculated individuals. Mortality was corrected with the Abbott's equation.

Treatment development and confirmation

Treatments were developed in three stages. In the first, sensibility tests were performed to determine the stage or instar most tolerant to Methyl Bromide. In the second, the conditions of the treatment such as doses and exposure times (small scale tests) were determinated. Lastly, in the third, the dose and the exposure time were confirmed analyzing more individuals (large scale tests).

I.- Sensibility tests to determine the most tolerant stage and/or instar of *C. capitata* and *A. fraterculus* to Methyl Bromide.

In order to determine the most tolerant stage and/or instar to Methyl Bromide of *C. capitata* and *A. fraterculus*, sensibility trials were performed for one variety for each citrus species. In the case of *A. fraterculus* eggs (with most of the half gestation period), immature larvae (L_1+L_2), recently matured larvae (L_3) and advanced mature larvae (L_3 about to pupate) were evaluated. In the case of *C. capitata* eggs, immature larvae (L_1+L_2), and mature larvae (L_3). were evaluated. No distinction was made within third instars larvae. For each stage or instar, more than 200 individuals were evaluated in each replicate, being each trial replicated three times. Four doses of Methyl Bromide were evaluated for *C. capiata* (16, 24, 32, and 40 g/m^3), while for *A. fraterculus* seven doses were tested (8, 12, 16, 24, 32, 40 and 48 g/m^3). In all cases the treatment exposure time was two hours.

II. Small scale tests

In order to determine the fumigation conditions to develop a quarantine treatment (doses and exposure time) small scale tests were performed. Fruit were inoculated with the developmental stage most tolerant to Methyl Bromide, wich were subjected to differente doses and exposure time. For the case of *C. capitata* a dose of 48 g/m^3 with two exposure times (3.5 and 4.0 hours) and a dose of 56 g/m^3 with three exposure times (3.0, 3.5 and 4.5 hours) were evaluated. In *A. fraterculus*, the dose of 40 g was evaluated with three exposure times (2.0, 3.0, and 3.5 hours), the dose of 48 g/m^3 with four exposure times (2.0, 3.0, 3.5 and 4.0 hours), and the dose of 56 g/m^3 for 2.5 hours. Each treatment was replicated three times with more than 3,000 viable insects evaluated in each replicate. Trials were performed for one variety in each citrus species.

III. Large scale tests

In order to confirm the doses and duration of the quarantine treatment large scale tests were performed in those treatments that required the minimum exposure time at the given doses with no live insects recovered at large scale. Trials were replicated three times and for each replicate more than 10,000 viable insects were evaluated.

Tests temperature

All the trials were performed at 15 ± 1°C.

Treatments evaluation

After the fumigation, the fruit containing

the larvae, either immature or mature, were incubated at 25°C and revised after 72 hours. Those larvae that presented no movement were considered dead. Fruit with eggs were dissected and the eggs collected and placed in humid chambers that were incubated at 25°C. Eggs were revised everyday and the number of unhatched eggs counted until two consecutive numbers were obtained.

Statistical analysis

Mortality data were analyzed using the Probit method, by comparing lethal dosis 50% (LD 50) with their respective 95% confidence intervals. If no overlapping was found, the intervals were considered different. The most tolerant stage and /or instar was the one showing the highest lethal dosis. In the trials aimed at determining the doses and duration of the treatments and its confirmation (small and large scale tests), no statistical analysis were performed since the objective was to obtain 100% mortality.

RESULTS

I.- Sensibility tests to determine the stage and/or instar most tolerant to Methyl Bromide

Results from the sensibility tests with their respective Probit analysis aiming at determining the stage or instar most tolerant to the treatment for the three citrus species (grapefruit, tangerines and oranges) analyzed are presented in Table 1 and 2 for *C. capitata* and *A. fraterculus* respectively.

Results from sensibility trails in *C. capitata* showed that for all the citrus species evaluated the eggs presented the highest LD50 with statistically significant differences between this stage and the larval stage (either immature or mature larvae). Besides, mature larvae presented a higher LD50 than the immature ones. No differences were detected in the eggs LD50 among the three citrus species.

Table 1. Sensibility of *C. capitata* different developmental stages or instars to Methyl Bromide in grapefruit, tangerines and oranges.

Citrus species	Replicate	Egg		Immature larvae (L1+L2)		Mature larvae (L3)	
		LD50	CI 95%	LD50	CI 95%	LD50	CI 95%
Grapefruit	I	22.14	16.78 - 28.63	8.56	7.30 - 9.64	12.20	11.17 - 13.24
	II	21.89	17.36 - 27.16	8.62	7.12 - 9.51	12.08	11.04 - 13.12
	III	20.98	16.89 - 25.46	8.25	7.80 - 8.68	12.24	11.20 - 13.29
Tangerine	I	21.19	16.06 - 27.18	9.14	7.80 - 10.33	11.59	10.32 - 12.86
	II	22.47	16.29 - 30.66	9.23	8.07 - 10.28	12.14	11.12 - 12.16
	III	20.84	15.91 - 29.19	8.88	8.18 - 9.53	11.50	10.63 - 12.40
Orange	I	20.47	14.59 - 27.49	8.77	6.16 - 10.77	12.16	11.05 - 13.28
	II	30.34	14.51 - 27.21	8.90	7.33 - 10.23	12.12	10.85 - 13.39
	III	20.56	14.50 - 27.88	8.74	6.82 - 10.30	12.35	11.33 - 13.38

LD: Letal dose; **CI:** Confidence interval.

Table 2. Sensibility of *A. fraterculus* different developmental stages or instars to Methyl Bromide in grapefruit, tangerines and oranges.

Citrus species	Replicate	Egg		Immature larvae (L1+L2)		Mature larvae (L3)		Mature larvae (L3)	
		LD50	CI 95%	LD50	CI 95%	LD50	CI 95%	LD50	CI 95%
Grapefruit	I	16,07	12.92 - 19.81	9,11	8.72 - 9.49	7,88	6.62 - 8.83	6,04	4.04 - 7.45
	II	15,68	10.76 - 22.43	8,02	7.42 - 8.49	6,84	0.27 - 10.30	5,99	2.87 - 8.05
	III	15,69	13.48 - 18.14	7,89	7.04 - 8.54	6,62	2.11 - 9.06	7,66	5.72 - 9.02
Tangerine	I	13,75	10.85 - 16.82	8,35	5.59 - 9.87	11,62	8.29 - 14.79	13,11	11.35 - 15.00
	II	14,16	10.99 - 17.47	7,82	5.11 - 9.23	10,26	7.79 - 12.27	11,62	10.23 - 12.93
	III	13,41	11.22 - 15.55	7,99	6.74 - 8.78	10,25	9.04 - 11.34	12,35	10.94 - 13.76
Orange	I	19,31	16.09 - 22.71	4,81	0.14 - 6.33	12,26	9.81 - 14.50	16,47	13.46 - 19.71
	II	19,03	16.95 - 21.21	5,94	2.86 - 6.89	11,19	8.42 - 13.54	16,45	14.82 - 18.14
	III	19,02	16.28 - 21.92	6,42	4.82 - 7.14	11,72	9.49 - 13.72	15,59	12.52 - 18.72

LD: Letal dose; **CI:** Confidence interval.

Results from sensibility tests in *A. fraterculus* for grape fruit showed that the egg were the most tolerant developmental stage to Methyl Bromide. In tangerines the LD50 for eggs was highest but it did not differ statistically from that of mature larvae (either young or advanced). In oranges there were no differences between the eggs and the advanced mature larvae (L_{3a}); however, the eggs presented the highest value. Similarly to *C. capitata*, there were no differences among the eggs LD50 from the three citrus species.

Results obtained for *A. fraterculus* showed the same pattern as in *C. capitata* in which the eggs was the most tolerant stage to Methyl Bromide.

II. Small scale tests

Results obtained in the small scale tests are presented in Table 3, 4 and 5 for *C. capitata* and Tables 6, 7, and 8 for *A. fraterculus*.

Results obtained in the small scale trials showed that it is possible to kill all *C. capitata* eggs in grapefruit, oranges and tagerines with doses of 48 g/m³ and 56 g/m³ for four and three hours respectively. No differences were found among the citrus.

Results obtained in the small scale trials showed that it is possible to kill all *A. fraterculus* eggs in grapefruit, tagerines and oranges with doses of 48 g/m³ and 56 g/m³ for 4 and 2.5 hours respectively. No differences were found among the citrus species.

III. Large scale tests

Tables 9 and 10 presents the results from the large scale tests for *C. capitata* and *A. fraterculus* respectively for different varieties of grapefruit, oranges and tangerines.

Table 3. Live individuals obtained in the small scale tests for *C. capitata* in grapefruit.

Treatment	Replicate	Treated individuals	Live individuals
48 g/m³ 3.5 h	I	3,374	0
	II	3,756	2
	III	3,344	0
48 g/m³ 4.0 h	I	3,753	0
	II	3,564	0
	III	3,839	0
56g/m³ 3.0 h	I	3,915	0
	II	3,608	0
	III	3,600	0
56 g/m³ 3.5 h	I	3,817	0
	II	3,640	0
	III	3,648	0
56 g/m³ 4.5 h	I	3,304	0
	II	4,050	0
	III	4,488	0

Table 4. Live individuals obtained in the small scale tests for *C. capitata* in oranges.

Treatment	Replicate	Treated individuals	Live individuals
48 g/m³ 4.0 h	I	3,735	0
	II	3,942	0
	III	4,815	0
56g/m³ 3.0 h	I	3,606	0
	II	3,745	0
	III	4,098	0

Table 5. Live individuals obtained in the small scale tests for *C. capitata* in tangerines.

Treatment	Replicate	Treated individuals	Live individuals
48 g/m³ 3.5 h	I	3,242	2
	II	3,815	1
	III	3,615	3
48 g/m³ 4.0 h	I	3,685	0
	II	3,735	0
	III	3,447	0
56g/m³ 3.0 h	I	3,645	0
	II	3,512	0
	III	3,773	0
56 g/m³ 3.5 h	I	3,701	0
	II	3,451	0
	III	3,576	0
56 g/m³ 4.5 h	I	3,680	0
	II	3,750	0
	III	3,780	0

Table 6. Live individuals obtained in the small scale tests for *A. fraterculus* in grapefruit.

Treatment	Replicate	Treated individuals	Live individuals
40 g/m³ 2.0 h	I	3,800	91
40 g/m³ 3.0 h	I	3,510	4
40 g/m³ 3.5 h	I	3,034	487
48 g/m³ 2.0 h	I	3,772	189
48 g/m³ 3.0 h	I	3,397	509
48 g/m³ 3.5 h	I	3,640	59
48 g/m³ 4.0 h	I	3,150	0
	II	3,230	0
	III	3,091	0
56 g/m³ 2.5 h	I	3,256	0
	II	3,015	0
	III	3,276	0

Results obtained in the large scale tests showed that treatments at 15°C with 48 g/m³ for four hours or 56 g/m³ for three hours are effective to eliminate 30,000 viable individuals of *C. capitata* developmental stage most tolerant to Methyl Bromide for the three citrus species evaluated. These values confer to the above mentioned treatments a 99.99% efficiency with a confidence higher than 95.1% in all cases.

Table 7. Live individuals obtained in the small scale tests for *A. fraterculus* in tangerines.

Treatment	Replicate	Treated individuals	Live individuals
40 g/m^3 2.0 h	I	3,028	77
40 g/m^3 3.0 h	I	3,706	6
40 g/m^3 3.5 h	I	3,036	56
48 g/m^3 2.0 h	I	3,124	50
48 g/m^3 3.0 h	I	3,225	574
48 g/m^3 3.5 h	I	3,886	64
48 g/m^3 4.0 h	I	3,412	0
	II	3,047	0
	III	3,189	0
56 g/m^3 2.5 h	I	3,600	0
	II	3,075	0
	III	3,036	0

Table 8. Live individuals obtained in the small scale tests for *A. fraterculus* in oranges.

Treatment	Replicate	Treated individuals	Live individuals
48 g/m^3 4.0 h	I	3,412	0
	II	3,047	0
	III	3,189	0
56 g/m^3 2.5 h	I	8,750	0
	II	6,300	0
	III	5,670	0
56 g/m^3 3.0 h	I	3,600	0
	II	3,075	0
	III	3,036	0

Table 9. Live individuals obtained in the large scale tests for *C. capitata* for grapefruit, oranges and tangerines.

Citrus species	Varieties	Treatments			
		48 g/m^3 – 4.0 h – 15ºC		56 g/m^3 – 3.0 h – 15ºC	
		Treated individuals	Live individuals	Treated individuals	Live individuals
Grapefruit	Río Red	30,532	0	31,122	0
	Marsh Seedless	30,914	0	30,183	0
	Henninger`s Ruby	30,888	0	31,484	0
Orange	Washington Navel	31,095	0	30,841	0
	Pinneaple	30,564	0	30,660	0
	Hamlin	30,301	0	31,436	0
	Westin	30,748	0	31,282	0
	Salustiana	31,134	0	31,122	0
	Valencia	31,022	0	30,828	0
Tangerine	Murcott	30,684	0	30,755	0
	Hernandina	31,350	0	31,130	0
	Clemenule	31,614	0	30,506	0
	Ellendale	31,641	0	31,466	0
	Satsuma	30,694	0	30,735	0
	Común	30,245	0	30,544	0

Table 10. Live individuals obtained in the large scale tests for *A. fraterculus* for grapefruit, oranges and tangerines.

Citrus species	Varieties	Treatments			
		48 g/m^3 – 4.0 h – 15ºC		56 g/m^3 – 2.5 h – 15ºC	
		Treated individuals	Live individuals	Treated individuals	Live individuals
Grapefruit	Río Red	33,787	0	32,361	0
	Marsh Seedless	34,048	0	N/D	0
	Henninger`s Ruby	31,548	0	N/D	0
	Rouge la Toma	33,083	0	N/D	0
	Star Ruby	32,331	0	N/D	0
	Shambar	31,812	0	N/D	0
Orange	Valencia	31,022	0	30,828	0
Tangerine	Satsuma	31,050	0	38,156	0
	Común	32,016	0	N/D	0
	Clementina	31,050	0	40,260	0

N/D: no data.

Results obtained in the large scale tests showed that treatments at 15°C with 48 g/m³ for four hours or 56 g/m³ for 2.5 hours are effective to eliminate 30,000 viable individuals of *A. fraterculus* developmental stage most tolerant to Methyl Bromide for the three citrus species evaluated. These values confer to the above mentioned treatments a 99.99% efficiency with a confidence higher than 95.4% in all cases.

CONCLUSIONS

The egg is the most tolerant developmental stage to Methyl Bromide in *C. capitata* and *A. fraterculus*.

Fumigations with Methyl Bromide at 15°C with doses of 48 and 56 g/m³ for four and three hours, respectively guarantee the elimination of all immature stages (i.e. eggs or larvae) of *C. capitata*. Hence, they can be used as quarantine treatments in grapefruit, oranges and tangerines.

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The efficiency on the quarantine treatment developed are independent of the citrus species and their varieties

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