Cold treatments for the control of *Ceratitis capitata* and *Anastrepha fraterculus* for citrus export

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Chapter IV

INTRODUCTION

The commercialization of fruit and vegetable products from regions with fruit flies such as *Ceratitis capitata* (Wiedemann) and *Anastrepha fraterculus* (Wiedemann) towards regions free from them is regulated by phytosanitary organizations in each country. In order to allow the entry of products considered as hosts, fly-free countries carry out pest risk analyses that may often culminate in the requirement for the implementing of systems for risk minimization or quarantine treatments by physical or chemical means. National organizations for plant protection have different policies to determine phytosanitary measures for the same pest. Recently, the US, through the Animal Plant and Inspection Service (APHIS, 2006), modified the quarantine cold treatments required from other countries. Such treatments are independent of the product to be exported or of the country of origin of the product for a certain species. Japan, through the Ministry of Agriculture, Forestry and Fisheries, (MAFF, 1996), requires each country to develop its own quarantine systems. This is why it approved different treatments for the same vegetable species. Some examples are the current treatments for oranges from different countries: for oranges from Spain, treatment is 17 days at 2°C, from Israel 14 or 16 days at 0.5°C or 1.5°C, respectively, while for oranges from Australia treatment is 16 days at 1°C. The aim of the present work was to develop a treatment at 2ºC for the quarantine control of *C. capitata* and *A. fraterculus* in different citrus species for export to Japan.

MATERIALS AND METHODS

The development of the quarantine treatment was carried out in two stages. During the first, called small-scale insect removal tests (desinsectation), the duration of the treatment was determined on the basis of the elimination of 3,000 viable insects at the stage most tolerant to cold (third instar larvae, see Chapter III). During the second, called large-scale insect removal test, the duration of the treatment was confirmed on the basis of the elimination of 30,000 viable third instar larvae.

Treatments

In the small-scale insect removal tests, six exposure time to cold (treatments) were assessed. Each treatment included over 3,000 viable individuals and the assays were repeated three times. Each fruit was artificially inoculated with 35 third instar larvae. The upper portion of each fruit was removed and the larvae were

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placed on the pulp, the fruit being later sealed with paraffin. The inoculated fruit were placed in chambers at 25ºC for 24 hours for the adaptation of the larvae inside the fruit, which were later placed inside the cold chamber. After the cold exposure period, the fruit containing the mature larvae were placed in chambers at 25ºC and checked after 48 h. Larvae showing no motion were considered not viable.

In the large-scale insect removal tests only one exposure period (treatment) was assessed, during which over 10,000 viable insects were treated by repetition. Each assay was replicated three times. The fruit inside the cold chamber were placed in cardboard boxes, constituting a pallet, which simulates the export conditions (Figures 1 and 2). The form of inoculation of the fruit, the period of adaptation of the larvae and the assessment of the treatments were similar to those described in the small-scale insect removal tests.

Determination of viable insects

In order to determine the minimum number of viable insects (3,000) per treatment, in the small-scale test a portion of the inoculated fruit were set apart as controls (100 fuits). They were checked on the same day as the fruit were placed in the cold chamber. The total number of viable insects was determined by substracting the number of dead insects in the control from the total number of inoculated insects. Mortality was corrected by Abbot´s method. For the large-scale insect removal tests, the same methodology was used, with the difference that the number of viable insects per treatment was above 10,000.

Figure 1. Inoculated fruit for the small scale tests.

Figure 2. Distribution of cajes with fruit and pulp sensors simulating an export pallet.

Fruit fly species and developmental stage

The biological material used in this work were third instar larvae of *C. capitata* and *A. fraterculus* obtained from the rearing at the EEAOC laboratory, Tucumán, Argentina.

Citrus species and varieties

The citrus species and varieties used in the small and large scale insect removal tests for *C. capitata* were: oranges (*Citrus sinensis* (L.) Osbeck) Valencia variety; grapefruit (*Citrus paradisi* Macfadyen) Rouge La Toma and Star Ruby varieties, and tangerines (*Citrus reticulata* Blanco) Murcott. For *A. fraterculus* Valencia oranges were used.

Temperature of the assays

The temperature of the assays was 2 ± 0.5ºC. Temperature was automatically registered every hour by means of eight pulp sensors per treatment. The assays were started when more than half the sensors recorded 2ºC or less.

RESULTS AND DISCUSSIONS

Small scale insect removal tests (determination of the duration of the treatment)

The results of the small scale insect removal tests for *C. capitata* are shown in Tables 1, 2, and 3 for grapefruit, oranges and tangerines, respectively. Results for *A. fraterculus* in oranges are shown in Table 4.

The results of the small scale insect removal tests for *C. capitata* in grapefruit showed no viable larvae from day 18 of treatment onwards.

The results of the small scale insect removal tests for *C. capitata* in oranges showed no viable larvae from day 20 of treatment onwards.

The results of the small scale insect removal tests for *C. capitata* in tangerines showed no viable larvae from day 22 of treatment onwards.

The results of the small scale insect removal tests for *A. fraterculus* in oranges showed no viable larvae from day 20 of treatment onwards.

Table 1. Small scale insect removal tests. Viable larvae of *C. capitata* in grapefruit.

Repetition	Viable insects	Treatments (days)					
		16	17	18	19	20	21
I	3,267	0	0	0	0	0	0
II	3,247	0	1	0	0	0	0
III	3,400	2	0	0	0	0	0

Table 2. Small scale insect removal tests. Viable larvae of *C. capitata* in oranges.

Repetition	Viable insects	Treatments (days)					
		17	18	19	20	21	22
I	3,198	1	1	0	0	0	0
II	3,215	1	0	1	0	0	0
III	3,328	3	0	0	0	0	0

Table 3. Small scale insect removal tests. Viable larvae of *C. capitata* in tangerines.

Repetition	Viable insects	Treatments (days)				
		20	21	22	23	24
I	3,451	2	1	0	0	0
II	3,377	1	0	0	0	0
III	3,419	1	0	0	0	0

Table 4. Small scale insect removal tests. Viable larvae of *A. fraterculus* in oranges.

Repetition	Viable insects	Treatments (days)				
		18	19	20	21	22
I	3,266	0	1	0	0	0
II	3,121	4	1	0	0	0
III	3,304	1	1	0	0	0

Large scale insect removal tests (confirmation of the duration of the treatment).

The results of the small scale insect removal tests for *C. capitata* are shown in Tables 5, 6 and 7 for grapefruit, oranges and tangerines, respectively. Results for *A. fraterculus* in oranges are shown in table 8.

The analysis of the data from the large scale insect removal tests in grapefruit showed that after treating 36,052 third instar larvae at 2 ± 0.5°C for 19 days no viable larvae were found. The efficacy of the treatment was 99.99% with a

Table 5. Large scale insect removal tests. Determination of third instar larvae mortality of *C. capitata* in grapefruit.

Repetition	Duration of the treatment (days)	Viable insects	Viable insects post treatment	% Mortality
I	19	11,592	0	100%
II	19	12,004	0	100%
III	19	12,456	0	100%
Total		36,052	0	100%

Table 6. Large scale insect removal tests. Determination of third instar larvae mortality of *C. capitata* in oranges.

Repetition	Duration of the treatment (days)	Viable insects	Viable insects post treatment	% Mortality
I	21	11,731	0	100%
II	21	12,051	0	100%
III	21	11,999	0	100%
Total		35,781	0	100%

Table 7. Large scale insect removal tests. Determination of third instar larvae mortality of *C. capitata* in tangerines.

Repetition	Duration of the treatment (days)	Viable insects	Viable insects post treatment	% Mortality
I	23	12,132	0	100%
II	23	12,060	0	100%
III	23	12,048	0	100%
Total		36,240	0	100%

Table 8. Large scale insect removal tests. Determination of third instar larvae mortality of *A. fraterculus* in oranges.

Repetition	Duration of the treatment (days)	Viable insects	Viable insects post treatment	% Mortality
I	21	11,366	0	100%
II	21	10,042	0	100%
III	21	10,602	0	100%
Total		32,010	0	100%

confidence level of 97.28%.

The analysis of the data from the large scale insect removal tests in oranges showed that after treating 35,781 third instar larvae at 2 ± 0.5°C for 21 days no viable larvae were found. The efficacy of the treatment was 99.99% with a confidence level of 97.21%.

The analysis of the data from the large scale insect removal tests in tangerines showed that after treating 36,240 third instar larvae at 2 ± 0.5°C for 23 days no viable larvae were found. The efficacy of the treatment was 99.99% with a confidence level of 97.33%.

The analysis of the data from the large scale insect removal tests in oranges showed that after treating 32,010 third instar larvae at 2 ± 0.5°C for 21 days no viable larvae were found. The efficacy of the treatment was 99.99% with a confidence level of 95.93%.

CONCLUSIONS

The results obtained in the present work allow us to conclude that:

A.- Treatment for 19 days at 2°C or less guarantees the removal of all immature stages of eggs and larvae *C. capitata* in grapefruit.

B.- Treatment for 21 days at 2°C or less guarantees the removal of all immature stages of eggs and larvae of *C. capitata* and *A. fraterculus* in oranges.

C.- Treatment for 23 days at 2°C or less guarantees the removal of all immature stages of eggs and larvae of *C. capitata* in tangerines.

D.- Cold quarantine treatments for *C. capitata* and *A. fraterculus* in oranges are the same.

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