Basic studies for the development of quarantine cold treatments for *Ceratitis capitata* and *Anastrepha fraterculus* in citrus in Argentina

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Chapter III

INTRODUCTION

The opening of potencial markets for citrus production in Argentina is restricted by the presence in our region of the Medfly *Ceratitis capitata* (Wiedemann), which is considered one of the most harmful pests in agriculture and, to a lesser extent, by the presence of the South American fruit fly *Anastrepha fraterculus* (Wiedemann).

The national organizations for plant protection have different policies to determine the phytosanitary measures for the same pest. The Animal Plant and Inspection Service (APHIS) of the USA has standardized cold treatments for the different fruit fly species, independently of the fruit varieties and of the country of origin. Japan, on the other hand, requires each country to develop its own quarantine treatments, including all the varieties to be exported.

Reports on cold treatments date back to the early 20th century, when Back and Pemberton (1916) studied the influence of low temperatures in the different developmental stages of *C. capitata* in peaches and apples; they concluded that the third instar larvae was the most tolerant stage to cold. Hill *et al.* (1988), working with Valencia oranges, arrived at the same conclusions. Gould (1996), working with carambolas infested with *Anastrepha suspensa* (Loew) eggs and larvae, found no differences between these stages of development. In contrast with the extensive research on *C. capitata*, no data on cold treatment for *A. fraterculus* has been reported.

Since 1996 the Estación Experimental Agroindustrial Obispo Colombres (EEAOC) Tucumán, Argentina, has developed different investigations on quarantine cold treatments for the control of *C. capitata* and *A. fraterculus* for the opening of new markets for its citrus production. In this work we analyzed the results obtained during the last 10 years in order to facilitate the development of citrus cold treatments in the future.

The aims of the present work were:

- To determine the developmental stage of *C. capitata* and *A. fraterculus* most tolerant to cold.
- To determine the influence of the varieties in cold tolerance within the same citrus species.
- To compare cold tolerance between *C. capitata* and *A. fraterculus*.

MATERIALS AND METHODS

In order to determine the developmental stage of *C. capitata* most tolerant to cold,

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sensitivity trials were carried out, comparing them in four varieties for different citrus species. The same trials were performed for *A. fraterculus* in a single variety within the same citrus species.

To find out whether the varieties within the same citrus species had an incidence on cold sensitivity in *C. capitata*, we compared the third instar larvae in four varieties of grapefruits in five of oranges and in six of tangerines and their hybrids. In the case of *A. fraterculus*, we compared the third instar larvae in three varieties of oranges, tangerines and their hybrids. Cold tolerance between *C. capitata* and *A. fraterculus* was compared for the three developmental stages in one variety per citrus species.

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 breeding of the EEAOC, Tucumán, Argentina. The *C. capitata* breeding originated in the collection of infested fruit, mainly oranges and grapefruits from NW Argentina, while that of *A. fraterculus* came mainly from the collection of guavas from Tucumán, Argentina. Each summer wild blood was incorporated to the laboratory breeding in four successive cross-breedings until 93.75% wild blood was obtained. Quality control was performed for each generation checking egg viability, hatch percentage, egg/pupae recovery, pupae weight, male/female ratio, flight ability, adult longevity and female fertility.

Developmental stages used in the trails were: eggs (with more than half of their completed embryonic development); immature larvae (first and second instars, L_1+L_2) and mature larvae (third instar larvae, L_3).

Citrus species and varieties

The citrus species and varieties used were: oranges (*Citrus sinensis* (L.) Osbeck) Washington Navel and Lanelate (within the navel group), Salustiana, Lue Gim Gong and Valencia varieties; red pulp grapefruits (*Citrus paradisi* Macfadyen), Rouge La Toma and Star Ruby, rosy pulp, Henninger's Ruby and white pulp, Marsh Seedless varieties; tangerines (*Citrus reticulata* Blanco) and their hybrids Clemenules, Marisol and Hernandina (within the Clementines group), Nova, Ellendale and Murcott.

Trial temperature

The temperature of the trials was 2±0.5ºC. Temperature was automatically registered every hour by eight pulp sensors per treatment. Trials started when over half the sensors recorded 2ºC or less.

Treatments

Six to eight cold treatments for the different developmental stages were assessed. Each treatment comprised over 200 viable individuals per each stage mentioned above; each trial was replicated three times. Fruit were artificially inoculated with 35 eggs or larvae per fruit. For inoculation the fruit upper part was removed, the eggs or larvae were placed on the fruit pulp, the cap being replaced and the whole fruit being finally sealed with paraffin (Figures 1, 2 and 3). The fruit inoculated with mature and immature larvae were placed in chambers at 25ºC for 24 hours to reach larvae adaptation inside the fruit; they were later introduced into the cold chamber. Eggs were inoculated on the same day they were introduced into the chamber. After exposure to cold, the fruit containing the mature and immature

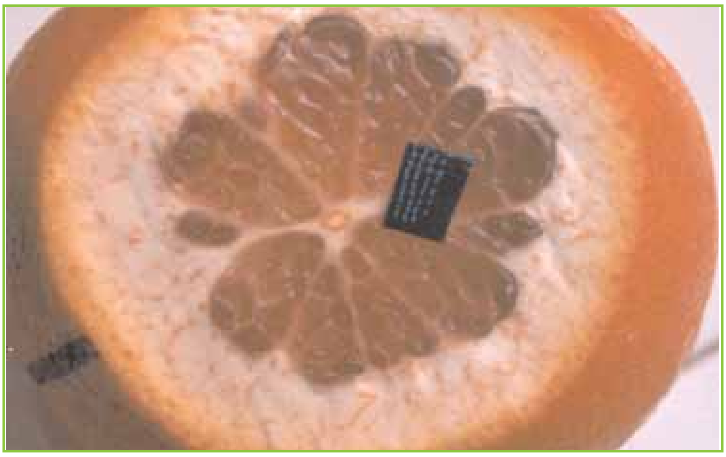

Figure 1. Fruit inoculation with eggs of *C. capitata*.

Figure 2. Detail of the inoculation technique.

Figure 3. Inoculated fruit with third instar larvae.

Figure 4. Third instar larvae killed by the cold treatment.

larvae were placed in chambers at 25ºC and checked after 48 hours; larvae exhibiting no motion were considered dead (Fig. 4). Fruit containing eggs were checked five days later. If larvae emergence occurred, the egg was considered viable.

Determination of viable insects

In order to determine the minimal number of 200 individuals viable per treatment, a portion of the inoculated fruit was set apart as controls before the remaining fruit were introduced into the cold chamber. Eggs were checked five days after keeping them at 25ºC, while larvae were checked on the same day the fruit were placed in the cold chamber. The total number of viable insects was calculated by substracting the proportion of dead insects in the control from the total number of inoculated insects. Mortality was corrected using Abbot's method.

Statistical analysis

Mortality data were analyzed using the Probit method (Finney, 1971), by comparing lethal times 50% (LT 50) with their respective 95% confidence intervals. If no overlapping was found, the intervals were considered different. The most tolerant stage or variety was the one showing the longest lethal time.

RESULTS AND DISCUSSION

The results of the Probit analyses for the different developmental stages of *C. capitata* in the different varieties of oranges, grapefruits and tangerines and their hybrids are shown in Tables 1, 2, and 3, respectively. The results of the Probit analyses for the developmental stages of *A. fraterculus* in oranges, grapefruits and tangerines and their hybrids are shown in Tables 4, 5, and 6, respectively.

Cold sensitivity trial for the developmental stages of *C. capitata*

The results of the sensitivity trials for the developmental stages of *C. capitata* in grapefruit showed no differences between eggs and immature larvae in all varieties; howevwer, there were differences between these stages and

Table 1. Sensibility to cold of different developmental stages of *C. capitata* in four orange varieties.

Stage		Eggs		Immature Larvae		Mature Larvae	
Variety	Rep.	LT 50	CI 95%	LT 50	CI 95%	LT 50	CI 95%
Valencia	I	1.205	0.715 - 1.621	3.966	3.659 - 4.229	7.400	6.181 - 8.364
	II	2.268	1.180 - 2.693	3.936	3.263 - 4.424	6.400	4.939 - 7.673
	III	1.036	0.747 - 1.290	4.162	3.792 - 4.485	6.283	5.371 - 7.122
Lue Gim Gong	I	1.261	1.036 - 1.463	4.665	4.336 - 4.967	5.829	5.415 - 6.241
	II	3.374	2.651 - 4.037	4.765	4.032 - 5.422	5.937	5.745 - 6.126
	III	3.839	3.266 - 4.321	4.524	3.892 - 5.067	5.541	5.070 - 5.982
Salustiana	I	1.425	0.856 - 1.885	3.712	3.373 - 4.054	5.667	4.919 - 6.295
	II	1.523	0.999 - 1.961	3.606	3.161 - 4.059	5.735	5.253 - 6.168
	III	2.104	1.717 - 2.429	5.348	4.647 - 5.925	5.775	5.333 - 6.172
Washington Navel	I	2.730	2.278 - 3.139	2.945	2.153 - 3.590	5.303	4.853 - 5.712
	II	2.647	1.893 - 3.250	2.768	2.007 - 3.377	5.203	4.960 - 5.432
	III	2.766	2.247 - 3.189	2.399	1.558 - 3.038	5.641	5.200 - 6.044

LT 50: Lethal Time 50
CI: Confidencial Interval

Table 2. Sensibility to cold of different developmental stages of *C. capitata* in four grapefruit varieties.

Stage		Eggs		Immature Larvae		Mature Larvae	
Variety	Rep.	LT 50	CI 95%	LT 50	CI 95%	LT 50	CI 95%
Marsh seedless	I	3.751	2.349 - 4.859	4.272	2.715 - 5.056	6.910	5.562 - 7.891
	II	4.032	3.480 - 4.796	3.618	3.152 - 3.965	6.765	6.391 - 7.111
	III	1.686	1.492 - 1865	3.788	2.710 - 4.441	6.961	6.674 - 7.225
Star Ruby	I	3.701	3.275 - 4.109	3.829	3.043 - 4.187	7.169	6.514 - 7.731
	II	3.376	2.622 - 4.056	3.473	2.827 - 3.911	6.724	5.746 - 7.485
	III	2.461	1.954 - 2.913	3.494	2.967 - 3.831	6.387	5.288 - 7.340
Henninger's Ruby	I	1.117	0.688 - 1.488	4.194	3.838 - 4.491	5.650	4.700 - 6.457
	II	1.230	0.666 - 1.704	4.113	3.932 - 4.279	6.133	5.630 - 6.622
	III	3.480	2.891 - 4.042	4.204	3.925 - 4.446	5.602	5.384 - 5.819
Rouge La Toma	I	2.036	1.315 - 2.703	3.534	2.881 - 4.004	5.646	5.035 - 6.233
	II	2.145	1.781 - 2.451	3.662	2.951 - 4.123	5.231	4.129 - 6.158
	III	1.877	1.145 - 2.540	2.888	2.386 - 3.251	5.387	4.379 - 6.267

Table 3. Sensibility to cold of different developmental stages of *C. capitata* in six tangerines and hybrid varieties.

Stage		Eggs		Immature Larvae		Mature Larvae	
Variety	Rep.	LT 50	CI 95%	LT 50	CI 95%	LT 50	CI 95%
Nova	I	1.690	1.432 - 1.927	3.254	2.381 - 4.022	5.888	5.016 - 6.648
	II	1.755	1.651 - 1.857	3.888	3.294 - 4.458	6.495	6.271 - 6.711
	III	1.818	1.600 - 2.023	4.702	4.313 - 5.053	5.965	5.115 - 6.695
Ellendale	I	0.915	0.326 - 1.405	3.409	3.040 - 3.757	4.782	4.174 - 5.305
	II	1.048	0.384 - 1.581	3.591	3.200 - 3.962	4.614	3.961 - 5.159
	III	1.188	0.522 - 1.713	3.234	2.493 - 3.889	5.443	5.021 - 5.823
Murcott	I	1.969	1.740 - 2.173	5.102	4.607 - 5.545	6.340	5.821 - 6.816
	II	1.974	1.847 - 2.093	4.965	4.143 - 5.662	6.530	5.732 - 7.234
	III	1.974	0.619 - 2.991	3.666	2.781 - 4.422	6.955	5.711 - 7.936
Clemenule	I	2.724	1.488 - 3.737	2.033	1.847 - 2.201	5.635	5.325 - 5.923
	II	2.323	1.536 - 2.931	2.217	1.588 - 2.724	4.454	3.965 - 4.860
	III	2.066	0.780 - 3.167	2.491	2.239 - 2.710	5.330	4.913 - 5.709
Hernandina	I	N / D		N / D		5.607	5.386 - 5.818
	II					5.513	5.140 - 5.856
	III					5.097	4.613 - 5.528
Marisol	I	N / D		N / D		5.282	4.833 - 5.690
	II					4.871	4.290 - 5.356
	III					5.334	4.903 - 5.716

N/D: No Data

Table 4. Sensibility to cold of different developmental stages of *A. fraterculus* in three orange varieties.

Stage		Eggs		Immature Larvae		Mature Larvae	
Variety	Rep.	LT 50	CI 95%	LT 50	CI 95%	LT 50	CI 95%
Valencia	I	1.634	0.871 - 2.212	3.859	3.527 - 4.133	5.866	5.488 - 6.210
	II	1.860	1.141 - 2.434	3.671	2.990 - 4.145	6.097	5.250 - 6.827
	III	1.775	1.054 - 2.339	3.892	3.513 - 4.201	5.368	4.663 - 5.964
Salustiana	I	N / D		N / D		4.382	3.204 - 5.265
	II					4.340	3.153 - 5.218
	III					4.535	3.369 - 5.410
Washington	I	N / D		N / D		5.821	5.317 - 6.267
	II					5.762	5.047 - 6.382
	III					5.425	4.982 - 5.823

Table 5. Sensibility to cold of different developmental stages of *A. fraterculus* in grapefruit.

Stage		Eggs		Immature Larvae		Mature Larvae	
Variety	Rep.	LT 50	CI 95%	LT 50	CI 95%	LT 50	CI 95%
Heninnger's Ruby	I	1.803	0.975 - 2.455	4.174	3.243 - 4.761	6.171	5.429 - 6.853
	II	2.510	1.531 - 3.250	3.591	2.488 - 4.120	6.279	5.859 - 6.863
	III	2.217	0.790 - 3.242	4.109	3.306 - 4.602	6.972	5.707 - 7.949

Table 6. Sensibility to cold of different developmental stages of *A. fraterculus* in three tangerines and hybrid varieties.

Stage		Eggs		Immature Larvae		Mature Larvae	
Variety	Rep.	LT 50	CI 95%	LT 50	CI 95%	LT 50	CI 95%
Murcott	I	4.902	1.011 - 6.986	3.724	2.787 - 4.598	6.031	4.252 - 7.439
	II	2.643	1.634 - 3.724	4.016	3.314 - 4.598	6.245	4.880 - 7.319
	III	2.366	1.636 - 2.971	2.993	2.361 - 3.529	5.788	5.220 - 6.290
Hernadine	I	4.957		4.249		6.106	3.967 - 7.584
	II	5.115		2.452		5.341	4.070 - 6.332
	III	4.840		3.120		5.888	4.890 - 6.722
Ellendale	I					5.96	4.874 - 6.690
	II	N / D		N / D		5.451	1.135 - 7.704
	III					5.166	4.837 - 5.464

mature larvae. In tangerines and their hybrids there were no differences between the stages of eggs and immature larvae in Murcott and Clemenule, while differences were found in Ellendale and Nova. All the varieties showed significant differences between the immature and mature larvae with the exception of Ellendale. In oranges, the comparison between eggs and immature larvae showed differences in the Valencia and Salustiana varieties, no differences being found in the other two varieties. When comparing immature with mature larvae, significant differences were found in all varieties with the exception of Salustiana.

The data obtained showed that the third instar larvae of *C. capitata* were the most tolerant to cold, in agreement with the results of Back and Pemberton (1916) and Hill *et al*. (1988).

Cold sensitivity trial for the developmental stages of *A. fraterculus*.

The results of the sensitivity trials for the developmental stages of *A. fraterculus* in grapefruits showed no differences between eggs and immature larvae; however, there were differences between these stages and mature larvae. In tangerines and their hybrids no differences were found between eggs, immature larvae and mature larvae. In oranges, the comparison between eggs and immature larvae showed significant differences, the same as between mature and immature larvae.

The results obtained showed the same pattern as for *C capitata*, where the third instar larvae was the most tolerant stage to cold.

Incidence of varieties on cold sensitivity of *C. capitata* mature larvae.

On comparing cold sensitivity of third instar larvae in different varieties within the same citrus species (six tangerine, five orange, and four grapefruit varieties) no differences were found between the varieties of the same citrus species.

Incidence of varieties on cold sensitivity of *A. fraterculus* mature larvae.

No differences were found in the sensitivity of third instar larvae of *A. fraterculus* in the three varieties of tangerines and their hybrids or in the three orange varieties.

Cold tolerance of *C. capitata* and *A. fraterculus*

No differences were found when comparing cold sensitivity between the *C. capitata* and *A. fraterculus* third instar larvae in each of the three citrus species analyzed. These results differ from what was stated by APHIS, which requires a longer treatment for fruit infested with different species of the *Anastrepha* genus as compared with that of *C. capitata* (APHIS 2006).

CONCLUSIONS

From the analysis of the results of the present work, we can conclude that:

A.- Third instar larvae of *C. capitata* and *A. fraterculus* are the most tolerant stage to cold.

B.-Varieties within the same citrus species had no incidence on cold sensitivity of third instar larvae of *C. capitata* or *A. fraterculus.*

C.- No differences in sensitivity to cold were found between third instar larvae of *C. capitata* and *A. fraterculus.*

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