Argentine citrus species and varieties variability to develop cold quarantine treatments for *Ceratitis capitata*

**Revision of related literature**

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Argentina developed cold quarantine treatments in order to be able to export citrus to Japan between 1996 and 2003. Different treatments were established for oranges (5 varieties) lemons (4 varieties), grapefruits (4 varieties) and mandarins and hybrids (6 varieties).

Considering that very different cold treatments schedules were established for medfly around the world, the literature was analyzed, compared with our data, and try to establish the origin of those differences.

**Inoculation**

Back and Pemberton, 1916: apples

Naturally infested in lab.

Mason and McBride 1934: apples, oranges and avocados

Natural, fruit collected from the field, and naturally infested fruit in lab.

Nel, 1936: nectarines, peaches, plums and grapes

Natural infestation in lab.

Sproul 1974: apples

Artificial (siringe, agar + water + eggs).

Hill et al 1988: Valencia and Navel oranges

Artificial (siringe, agar + water + eggs).

Jessup et al 1993: lemons (Eureka and Lisbon)

Artificial (siringe, agar + water + eggs).

Gastaminza, 2001: lemons (4 varieties)

Artificial.

Hashem et al 2004: (guava, oranges (Navel and Valencia) and mango

Artificial: siringe

Ware 2006: lemon (1), Grapefruit (1) and orange (1)

Artificial: fruit drilled, protein supplement and inoculation

Willink et al 2006: oranges (5), grapefruits (4), mandarins (6) and lemons (4).

Artificial.

De Lima et al 2007: oranges (2), Mandarins ((2) and lemon (1)

Artificial (siringe, agar + eggs).

Santaballa et al 2009: mandarins

Artificial (siringe, agar + water + eggs).

Grout et al 2011: Valencia orange

Artificial (siringe, water + eggs).

Hallman et al 2011: petri dishes

Artificial.

Fallik et al 2012: sweet pepper

Artificial (siringe, agar + water + eggs).

**Larval feeding**

Back and Pemberton 1916: apples

Feeding in natural host.

Mason and McBride 1934: apples, oranges and avocados

Feeding in natural host.

Nel, 1936: nectarines, peaches, plums and grapes

Feeding in natural hosts.

Sproul 1974: apples

Feeding in natural host.

Hill et al 1988: Valencia and Navel oranges

Feeding in natural host.

Jessup et al 1993: lemons (Eureka and Lisbon)

Feeding in natural host.

Gastaminza, 2001: lemons (4 varieties) 2001

Young larvae incubated in lemon and L3 with artificial diets

Hashem et al 2004: (guava, oranges (Navel and Valencia) and mango.

Feeding in natural host

Ware 2006: lemon (1), Grapefruit (1) and orange (1)

Feeding in natural host, but in inoculation hydrolyzed protein is added.

Willink et al 2006: oranges (5), grapefruits (4), mandarins (6) and lemons (4).

Young larvae and L3 with artificial diets

De Lima et al 2007: oranges (2), Mandarins ((2) and lemon (1)

Feeding in natural host

Santaballa et al 2009: mandarins

Feeding in natural host.

Grout et al 2011: Valencia orange

Feeding in natural host, but in inoculation hydrolyzed protein is added.

Hallman et al 2011: petri dishes

Artificial diet.

Fallik et al 2012: sweet pepper

Feeding in natural host.

**Number of individuals per fruit**

Back and Pemberton, 1916: apples

Does not specify

Mason and McBride 1934: apples, oranges and avocados

By estimation of pupae.

Nel, 1936: nectarines, peaches, plums and grapes

Very variable.

Sproul 1974: apples

150 – 200 eggs/fruit

Hill et al 1988: Valencia and Navel oranges

Eggs: 23,27 +/- 3.54.

Jessup et al 1993: lemons (Eureka and Lisbon)

Same as Hill et al 1988, obtaining an average 7.7 pupas.

Gastaminza, 2001: lemons (4 varieties)

Inoculates 35 individuals/fruit.

Hashem et al 2004: (guava, oranges (Navel and Valencia) and mango

Estimated from control pupariation

Guava: 41.15+/- 0.01 (egg viability 98.45%)

Navel orange: 39.03+/-0.12 (egg viability 98.12%)

Valencia Orange: 38.17+/-0.03 (egg viability 98.56%)

Mango 75.18+/-0.04 (egg viability 99.01%)

Ware 2006: lemon (1), Grapefruit (1) and orange (1)

Inoculates 40 eggs/fruit.

Willink et al 2006: oranges (5), grapefruits (4), mandarins (6) and lemons (4).

Inoculates 35 individuals/fruit.

De Lima et al 2007: oranges (2), Mandarins ((2) and lemon (1)

250 eggs of 6hs.

Santaballa et al 2009: mandarins

Inoculates 200 eggs/fruit (has a high natural mortality, obtaining around 10% of the inoculated).

Grout et al 2011: Valencia orange

40 eggs of up to 24hs.

Hallman et al 2011: petri dishes

102 +/- 6 per petri dish.

Fallik et al 2012: sweet pepper

Eggs: 26 +/- 7.8 (rep. 1); 32.3 +/- 11.3 (rep.2)

**Endpoint evaluation**

Back and Pemberton 1916: apples

Larvae eclosion – larvae with movement

Mason and McBride 1934: apples, oranges and avocados

Uses 2 methods: disection of fruit and pupariation.

Nel, 1936: nectarines, peaches, plums and grapes

Disection of fruits.

Sproul 1974: apples

Pupariation.

Hill et al 1988: Valencia and Navel oranges

Normal pupaes for the treatment. For stages, opening of fruit at different intervals.

Jessup et al 1993: lemons (Eureka and Lisbon)

Pupariation.

Gastaminza, 2001: lemons (4 varieties)

Disection of fruits

Hashem et al 2004: (guava, oranges (Navel and Valencia) and mango

Normal pupariation.

Ware 2006: lemon (1), Grapefruit (1) and orange (1)

Disection of fruit.

Willink et al 2006: oranges (5), grapefruits (4), mandarins (6) and lemons (4).

Disection of fruit.

De Lima et al 2007: oranges (2), Mandarins ((2) and lemon (1)

Pupariation.

Santaballa et al 2009: mandarins

Disection of fruit.

Grout et al 2011: Valencia orange

Larvae search by opening fruit.

Hallman et al 2011: petri dishes

Pupariation.

Fallik et al 2012: sweet pepper

Pupariation.

**Selection criteria**

Back and Pemberton 1916: apples

Mortality percentage.

Mason and McBride 1934: apples, oranges and avocados

Mortality percentage.

Nel, 1936: nectarines, peaches, plums and grapes

Mortality percentage.

Sproul 1974: apples

Mortality percentage.

Hill et al 1988: Valencia and Navel oranges

Mortality % and then logit comparison.

Jessup et al 1993: lemons (Eureka and Lisbon)

LT 99.9968.

Gastaminza, 2001: lemons (4 varieties)

Probit and calculates LT 50.

Hashem et al 2004: (guava, oranges (Navel and Valencia) and mango

LT 99.998

Ware 2006: lemon (1), Grapefruit (1) and orange (1)

Probit and calculates LT 50; 90 and 99 with an 90% confidence interval.

Willink et al 2006: oranges (5), grapefruits (4), mandarins (6) and lemons (4).

Probit and calculates LT 50.

De Lima et al 2007: oranges (2), Mandarins ((2) and lemon (1)

LD 95.

Santaballa et al 2009: mandarins

Compares LT at Probit 9 level

Grout et al 2011: Valencia orange

Mortality percentage, Probit 9.

Hallman et al 2011: petri dishes

Probit regression.

Fallik et al 2012: sweet pepper

Mortality percentage.

**Life stages**

Back and Pemberton 1916: apples

Uses all stages (eggs, L1, L2 and L3). L3 is the most tolerant to cold, and all larval instars are more tolerant to cold tan eggs.

Mason and McBride 1934: apples, oranges and avocados

Does not compare, uses all stages (eggs, L1, L2 and L3) in the final treatment.

Nel, 1936: nectarines, peaches, plums and grapes

Eggs less resistant than larvae comparing mortality percentage.

Sproul 1974: apples

Does not compare, uses all stages eggs, young larvae (L1 +L2) and old larvae (L3).

Hill et al 1988: Valencia and Navel oranges

Compares by mortality percentage eggs, L1+L2 and L3, determining that the eggs are the most sensible and young and old larvaes are equally tolerant to cold.

Jessup et al 1993: lemons (Eureka and Lisbon)

No differences between eggs, L1, L2 and L3 were found in Eureka using LT 99,9968. In Lisbon, the L2 is more tolerant that the egg, but equally tolerant to the other larval instars. L2 is chosen as it has the highest absolute values.

Gastaminza, 2002: lemons (4 varieties)

In Eureka the L3 was the most tolerant using LT 50; eggs and young larvae without differences. In Lisbon all stages were different, being L3 also the most tolerant.

In Genova and Limoneira the L3 differs from the other. Eggs and young larvae, sometimes with differences, but eggs always with the lowest LT.

Hashem et al 2004: (guava, oranges (Navel and Valencia) and mango

Compares eggs, young larvae (L1 +L 2) and L3.

Eggs and L1 are the most sensible, without differences betwwen them for the 4 hosts.

L2 y L3 most tolerant. In guava and Valencia Orange L3 most tolerant, while in Navel Orange and Mango no statistical differences. Always L3 with highest absolute values.

Ware 2006: lemon (1), Grapefruit (1) and orange (1)

In general, eggs are statistically the most sensitive to cold using LT 50 and 90, but not with LT 99. There are no differences between young larvae and L3 in all species and all LT. The highest absolute value in lemon and grapefruit using the LT 50 , 90 y 99 was for the L3. In oranges the same happened at the LT 50, and for higher LT´s the young larvae.

Willink et al 2006: oranges (5), grapefruits (4), mandarins (6) and lemons (4).

In oranges cv. Valencia, Salustiana, Washington and Lue Ging Gong L3 was the most tolerant. Eggs and young larvae with variable behaviour, but always the eggs hd the lowest LT.

In grapefruits, lemons and mandarins the L3 was always the most tolerant. Eggs and young larvae with variable behaviour, but always the eggs have the lowest LT.

De Lima et al 2007: oranges (2), Mandarins ((2) and lemon (1)

2ºC

* the second instar was in general the most tolerant stage, using a LD 95.

- Using LD 50, in oranges no differences between L1 and L2; but there were differences in lemon and in 2 mandarin varieties.

3ºC

* Using LD 95 the L2 is the most tolerant in 1 variety of orange, and 1of mandarin and lemon. No differences were found in 1orange variety and 1 mandarin.
* Using LD 50, L2 is the most tolerant in all varities of all species.

Santaballa et al 2009: mandarins

Compares eggs, young larvae (L1 +L 2) and L3. The eggs more sensible. Does not find differences between young and old larvae, but the last ones have the highest LT (probit 9) values.

Grout et al 2011: Valencia orange

Assumes by literature review that L2 is the most tolerant life stage (Ware et al 2006).

Hallman et al 2011: petri dishes

L3 is the most tolerant based on literature (Powell,2003).

Fallik et al 2012: sweet pepper

Does not compare, uses all stages (eggs, L1, L2 and L3) in the final treatment.

**Varieties**

Back and Pemberton 1916: apples

Does not compare.

Mason and McBride 1934: apples, oranges and avocados

Does not compare.

Nel, 1936: nectarines, peaches, plums and grapes

Does not compare.

Sproul 1974: apples

Does not compare.

Hill et al 1988: Valencia and Navel oranges

Does not compare.

Jessup et al 1993: lemons (Eureka and Lisbon)

Not stated, but data shows no differences.

Gastaminza, 2001: lemons (4 varieties) 2001

No differences between the 4 lemon varieties.

Hashem et al 2004: (guava, oranges (Navel and Valencia) and mango

No differences between the 2 orange varieties (comparing the same life stages)

Ware 2006: lemon (1), Grapefruit (1) and orange (1)

Does not compare.

Willink et al 2006: oranges (5), grapefruits (4), mandarins (6) and lemons (4).

Of all posible combinations of LT 50 replicates between varieties:

* Oranges: 9/30 had differences.
* Grapefruits: 5/18 had differences.
* Mandarins: 15/45 had differences.
* Lemons: 2/18 had differences.

De Lima et al 2007: oranges (2), Mandarins ((2) and lemon (1)

2ºC

* Using LD 95, no differences in mandarins, but differences in oranges.
* Using LD 50, differences in oranges and mandarins.

3ºC

* Using LD 95, no differences in mandarins, but differences in oranges.
* Using LD 50, no differences in mandarins and oranges.

Santablla et al 2009:

Does not compare.

Hallman et al 2011: petri dishes

Does not compare.

Grout et al 2011: Valencia orange

Does not compare.

Fallik et al 2012: sweet pepper

Does not compare.

**Host species**

Back and Pemberton 1916: apples

Does not compare

Mason and McBride 1934: apples, oranges and avocados

Does not compare.

Nel, 1936: nectarines, peaches, plums and grapes

Does not compare.

Sproul 1974: apples

Does not compare.

Hill et al 1988: Valencia and Navel oranges

Does not compare.

Jessup et al 1993: lemons (Eureka and Lisbon)

Does not compare.

Gastaminza, 2002: lemons (4 varieties)

Does not compare.

Hashem et al 2004: (guava, oranges (Navel and Valencia) and mango

No differences between the 4 species (comparing the same life stages)

Ware 2006: lemon (1), Grapefruit (1) and orange (1)

Does not compare, but from data, the eggs do not have differences in all LT´s for all host species.

Young larvae also have no differences in all LT´s in oranges and lemons, but the absolute values were always higher in oranges. The same happened with the L3. Grapefruits are not analyzed because they do not present confidence intervals.

Willink et al 2006: oranges (5), grapefruits (4), mandarins (6) and lemons (4).

Of all posible combinations of LT 50 replicates between species, 2/18 had differences (one lemon variety with 2 grapefruit varieties.

De Lima et al 2007: oranges (2), Mandarins ((2) and lemon (1)

2ºC

Not defined, but the data shows differences between the LD 95 and LD 50 of oranges, mandarins and lemons (in order of decreasing resistance).

3ºC

Not defined, but the data shows differences between the LD 95 and LD 50 of oranges, mandarins and lemons (in order of decreasing resistance).

Santaballa et al 2009: mandarins

Does not compare.

Hallman et al 2011: petri dishes

Does not compare.

Grout et al 2011: Valencia orange

Does not compare.

Fallik et al 2012: sweet pepper

Does not compare.

**Treatments**

Back and Pemberton 1916: apples

Does not calculate. Describes days at which ther are no survivors in relation to different temperatures.

Mason and McBride 1934: apples, oranges and avocados

Range of temperatures between 10 – 31ºF. Mortality from the 8th day.

Nel, 1936: nectarines, peaches, plums and grapes

9 days at 0.56ºC for all host species.

12 days at 1.11ºC for all host species.

16 days at 2.78ºC for all host species.

Sproul 1974: apples

- 14 días; 0.5 +/- 0.5 (48300 eggs, 88700 L1+L2 and 59500 L3; 196.500 in total.

- 16 días; 1.5 +/- 0.5). 46800 eggs, 38900 L1+L2 and 58000 L3; 143.700 in total.

Hill et al 1988: Valencia and Navel oranges

* 16 days; 1ºC +/- 0.5 (41.156 individuals)

Jessup et al 1993: lemons (Eureka and Lisbon)

* 14 days; 1.0 +/- 0.2 C (30,230 and 43,343 individuals) Eureka y Lisbon

Gastaminza, 2001: lemons (4 varieties) 2001

* 19 días; 2.0 +/- 0.5. 36.564 for lemons.

Hashem et al 2004: (guava, oranges (Navel and Valencia) and mango

16 days at 1.7ºC for all species

Willink et al 2006:

Does not analize. Figures taken from reports to Japan.

* 19 days; 2.0 +/- 0.5. 36.052 for grapefruits
* 19 days; 2.0 +/- 0.5. 36.564 for lemons
* 21 days; 2.0 +/- 0.5. 35.781 for oranges
* 23 days; 2.0 +/- 0.5. 36.240 for mandarins

De Lima et al 2007: oranges (2), Mandarins ((2) and lemon (1)

* 16 dyas; 2 ºC lemon, 165.894 individuals
* 18 days; 2 ºC oranges and mandarins (average more than 120.000)
* 18 días; 3 ºC limón 122.400 individuos
* 20 días; 3 ºC naranjas y mandarinas (promedio más de 120.000)

Santaballa et al 2009: mandarins

16 días; 2ºC. +/- 0.5. . 31.988 (Larvas) mandarinas

Grout et al 2011: Valencia orange

* 16 days; less than 1.4ºC (71.756 individuals)

Hallman et al 2011: petri dishes

Does not analize.

Fallik et al 2012: sweet pepper

* 21 days; 1.5ºC (1200 individuals)
* 21 days; 4ºC (1200 individuals)

**Final considerations**

**Inoculation and larval feeding**

The old experiments were done with natural infestation.

Afterwards, artificial inoculation introducing eggs with a siringe in the fruit (some researchers place also protein into the fruit) and allow the larvae develop to the larval instar desired to treat.

Our inoculation, direct counts of eggs or larvae that were fed with artificial diets were introduced directly into the fruit at the desired instar, and allowed a period of 24 hours for acclimatization before cooling the fruit. The quality of the food for the larvae can be one factor affecting survivorship to the cold treatment.

**Number of individual per fruit**

The numbers vary between 23 and 250. With high numbers, high mortality occurred.

**Endpoint evaluation**

Is done basically by two methods, pupariation and count live or dead larvae. The second method is more severe than the first one, as some of the larvae that are moving after the treatment may probably die before reaching the pupal stage. This difference may explain some of the differences in the length of the treatments.

**Life stages tolerance**

The eggs in almost all papers are the least tolerant to cold. In some of them the L3 is the instar most tolerant to cold, and in some the L2. In one paper, the 3 larval stages were equally tolerant. It is clear that the old larvae should be used to develop cold treatments with medflies.

**Varieties**

The tolerance to cold of the larvae in different varieties of a same host species (all citrus species) show, in general, no differences in their tolerance. This could mean that at least for citrus species, there would be no need to test all varieties.

**Host species**

The tolerance to cold of the larvae in different host species show no differences in 3 papers and had differences in one.

**Treatments**

The treatments differ quite a lot. Maybe some differences can be attributed to the factors mentioned earlier, as the feeding of the larvae and the endpoint. On the other hand, the fact that statistically the differences, or no differences between species at the level of small scale tests, finally do not show at large scale tests.

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