Comparison of Cold Tolerance of Eggs and Larvae of *Bactrocera dorsalis* (Diptera: Tephritidae) among Citrus Fruits

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Abstract: Citrus fruit ('Navel' and 'Valencia' oranges (*Citrus sinensis*), 'Murcott' (*C. reticulata* × *C. sinensis*), 'Satsuma' (*C. unshiu*), 'Marsh' grapefruit (*C. paradisi*) and 'Lisbon' lemon (*C. limon*) infested with immature stages (eggs and first, second and third instars) of the oriental fruit fly, *Bactrocera dorsalis* (Hendel), were subjected to cold treatment at 2°C for 3-15 days in order to determine the most cold-tolerant stage in each citrus fruit. Second instars were the most tolerant stage in lemons whereas third instars were in the other citrus fruit. 'Navel' and 'Valencia' orange infested with third instars were subjected to cold treatment at 2°C for 3-18 days and similar mortality were observed between the two cultivars. 'Murcott' and 'Satsuma' infested with third instars were subjected to cold treatment at 2°C for 3-14 days and there was significant difference in the estimated days until lethal between the two mandarin types. 'Valencia' orange, grapefruit and lemon infested with the most tolerant stage (third instars in orange and grapefruit, and second instars in lemon) were subjected to cold treatment at 2°C for 2-13 days and the estimated days until lethal of lemon was significantly shorter than those of orange and grapefruit.

Key words: oriental fruit fly, cold tolerance, quarantine treatment, citrus

Introduction

Phytosanitary cold treatment schedules for broad ranges of agricultural commodities against multiple quarantine insect pests are described in the treatment manual of the United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS 2019), while most of the phytosanitary cold treatment schedules of the International Plant Protection Convention are for one agricultural commodity and against one target insect pest (IPPC 2016abc, 2017abc). In developing a phytosanitary treatment, a large-scale disinfection test is conducted after determining the most cold-tolerant pest species and developmental stage among the target pests (De Lima et al. 2007; Gastaminza 2007; Grout et al. 2011; Heather and Hallman 2008; IPPC 2016a) requiring both longer periods of time and greater cost to conduct (De Lima et al. 2007; Willink et al. 2006). The IPPC has organized and supported researchers and regulators for the development of phytosanitary treatments and provided the forums for discussing and collaborating on more generic treatment schedules and rapid development of phytosanitary treatments (IPPC ECCT 2013; IPPC PTTEG 2015; IPPC PMRG 2017; Hallman et al. 2013ab).

The oriental fruit fly, *Bactrocera dorsalis*, is one of the most important fruit flies because it attacks many kinds of fruits and vegetables in Southeast Asia; furthermore, it has invaded and spread in the continent of Africa over the last 15 years. Therefore, phytosanitary treatments against *B. dorsalis* are needed for the international trade of agricultural products; however, mortality data of *B. dorsalis* by cold treatment for the developing treatment schedule is limited (Dohino et al. 2017; Grout et al. 2011; Sugimoto and Hurusawa 1982; Yamamoto et al. 2017).

In the present study, two experiments were conducted to determine the most cold-tolerant stage among the immature stages of *B. dorsalis*.

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in some citrus types and to compare the mortality of *B. dorsalis* among the different citrus types.

**Materials and Methods**

**Test insects and preparation of infested fruit**
A laboratory colony of the *B. dorsalis* maintained at the Research Center of Yokohama Plant Protection Station was used. The colony was originally from Thailand in 2005 (Export Permit No.17Y566) as the fly was eradicated in Japan in 1986 (Yoshizawa 1997). Flies were kept at 26±1°C, 65±10%RH and a photoperiod of 13L: 11D and given an artificial diet and water in the adult cage (30 × 30 × 45 cm).

Eggs were obtained from gravid females by placing a polyethylene receptacle (8 cm in diameter, 13 cm in height) with small oviposition holes into the adult cage for a period of one hour. The inner surface of the receptacle was moistened with orange juice. In preparation for infested fruit, 50 or 100 eggs were counted on black filter paper (1.3 × 1.3 cm) and placed on white filter paper saturated with water in Petri dishes using a fine brush under the microscope. A triangular piece was placed back on the top of the lower part and affixed with surgical tape, to restore the same appearance it had before being separated. Infested fruit were kept at 26±1°C and 65±10%RH, and fruit flies were allowed to grow until the target stage inside the fruit in which the duration period (day) was determined from the result of the preliminary tests.

**Cold treatment and temperature recording**

A cold treatment room (Nikkei Panel System Co., Ltd, Japan; 21.3 m³) was used for cold treatment of infested fruit at 2°C (fruit pulp temperature). Infested fruit and uninfested fruit for measuring fruit pulp temperature were placed in a plastic container (30 × 22 × 10 cm) and loaded into the cold treatment room. Temperatures inside the room and fruit pulp were measured using sensor probes (T&D Co., Ltd., Japan; Model: RTR-52) and recorded every 5 minutes.

**Determination of the most cold-tolerant stage in citrus fruit**

To determine the most cold-tolerant stage among the immature stages of *B. dorsalis* in various citrus types, the following citrus were used and the scientific names are after Cottin (2002): ‘Navel’ orange (*Citrus sinensis*) (weight per fruit: 208.2±15.4 g) imported from California, USA; ‘Valencia’ orange (*Citrus sinensis*) (262.6±19.7 g) from California, USA, ‘Murcott’ (*C. reticulata × C. sinensis*) (135.8±6.0 g) from Australia; ‘Satsuma’ (*C. unshiu*) (93.3±8.9 g) from Ehime Prefecture, Japan; ‘Marsh’ grapefruit (*C. paradisi*) (380.3±23.1 g) from Florida, USA; and ‘Lisbon’ lemon (*C. limon*) (120.1±10.9 g) from California, USA. Except for ‘Satsuma’, ‘Orthophenyl phenol (OPP)’, ‘Imazalil’ and ‘Thiabenzole (TBZ)’ were applied to the citrus as a food preservative and fungicide in the exporting countries (‘OPP’ was not used on ‘Valencia’ orange).

One hundred eggs were placed into the fruit by the infestation method mentioned above. After infestation, each citrus type was incubated in the rearing room at 26±1°C and 65±10%RH for 1, 2, 3

**Table 1. Development of Bactrocera dorsalis in orange, mandarin, grapefruit and lemon stored at 26°C for 1-8 days**

<table>
<thead>
<tr>
<th>Citrus</th>
<th>Developmental stage</th>
<th>1 d</th>
<th>2 d</th>
<th>3 d</th>
<th>4 d</th>
<th>5 d</th>
<th>6 d</th>
<th>7 d</th>
<th>8 d</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Citrus sinensis</em> (orange) ‘Navel’</td>
<td>Egg</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>First instar</td>
<td>0</td>
<td>100</td>
<td>12.5</td>
<td>1.1</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Second instar</td>
<td>0</td>
<td>0</td>
<td>87.5</td>
<td>67.6</td>
<td>9.2</td>
<td>1.1</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Third instar</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>31.3</td>
<td>90.1</td>
<td>98.9</td>
<td>99.3</td>
<td>99.7</td>
</tr>
<tr>
<td><em>Citrus sinensis</em> (orange) ‘Valencia’</td>
<td>Egg</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>First instar</td>
<td>0</td>
<td>100</td>
<td>7.2</td>
<td>0.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>Second instar</td>
<td>0</td>
<td>0</td>
<td>92.8</td>
<td>56.7</td>
<td>2.4</td>
<td>0.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Third instar</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>42.7</td>
<td>97.6</td>
<td>99.6</td>
<td>99.3</td>
<td>99.7</td>
</tr>
<tr>
<td><em>Citrus reticulata x C. sinensis</em> ‘Murcott’</td>
<td>Egg</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
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<td>4.5</td>
<td>1.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Second instar</td>
<td>0</td>
<td>0</td>
<td>95.5</td>
<td>24.0</td>
<td>1.3</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Third instar</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>74.9</td>
<td>98.7</td>
<td>98.4</td>
<td>98.4</td>
<td>98.4</td>
</tr>
<tr>
<td><em>Citrus unshiu</em> ‘Satsuma’</td>
<td>Egg</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>First instar</td>
<td>0</td>
<td>100</td>
<td>27.5</td>
<td>8.6</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Second instar</td>
<td>0</td>
<td>0</td>
<td>72.5</td>
<td>48.6</td>
<td>9.4</td>
<td>2.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Third instar</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>42.8</td>
<td>90.3</td>
<td>97.4</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>Citrus paradisi</em> (grapefruit) ‘Marsh’</td>
<td>Egg</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>First instar</td>
<td>0</td>
<td>100</td>
<td>9.2</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Second instar</td>
<td>0</td>
<td>0</td>
<td>90.8</td>
<td>34.7</td>
<td>4.9</td>
<td>10.1</td>
<td>2.4</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Third instar</td>
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<td>0</td>
<td>0</td>
<td>65.3</td>
<td>95.1</td>
<td>89.6</td>
<td>97.6</td>
<td>99.7</td>
</tr>
<tr>
<td><em>Citrus limon</em> (lemon) ‘Lisbon’</td>
<td>Egg</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>First instar</td>
<td>0</td>
<td>100</td>
<td>52.9</td>
<td>5.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Second instar</td>
<td>0</td>
<td>0</td>
<td>47.1</td>
<td>83.0</td>
<td>32.7</td>
<td>22.1</td>
<td>7.7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Third instar</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11.1</td>
<td>67.3</td>
<td>77.9</td>
<td>92.3</td>
<td>100</td>
</tr>
</tbody>
</table>
(4 in lemon) and 5 days (6 in lemon), resulting in fruit infested with eggs and first, second and third instars, respectively (Table 1). Thus, four different immature stages in infested fruit were prepared for each citrus type at the same time for each replication of the cold treatment. Five infested fruits were assigned in each citrus type, each developmental stage, each of the five exposure period treatment groups and an untreated control group for each replication. Five uninfested fruit in each citrus type were used to monitor the fruit core temperatures during the treatment and sensor probes were inserted into the center of the fruit.

The infested fruit and uninfested fruit with probes were placed in a plastic container (30 × 23 × 10 cm) and loaded into the cold treatment room at 2.1°C. Cold treatment was commenced when the fruit were placed in the cold treatment room. The infested fruit were removed from the cold room after 3, 6, 9, 12 and 15 days and transferred to the rearing room at 26°C. The treated fruit infested with eggs and first instars were kept at 26°C for 4-5 days. The treated fruit infested with second and third instars were kept at 26°C for 3-4 days. Then the treated fruit were dissected for mortality assessment. Mobile insects were counted as survivors. Mortality assessment of untreated controls for each developmental stage in each citrus type and each replication was conducted after keeping infested fruit at 26°C for 5-6 days after egg inoculation. The experiment with each citrus type was replicated twice.

Comparison of mortality effect between different citrus cultivar or citrus species (citrus types)

Comparison of B. dorsalis mortality between ‘Murcott’ and ‘Satsuma’ in mandarin type

‘Murcott’ from Australia and ‘Satsuma’ produced in Ehime Prefecture, Japan were used because these two citrus have similar shape and character. The weight per fruit of ‘Murcott’ and ‘Satsuma’ was 136.9±6.4 g and 98.2±9.7 g, respectively. ‘OPP’, ‘Imazalil’ and ‘TBZ’ have been applied to ‘Murcott’ as a food preservative and fungicide in the exporting country.

Fifty eggs were inoculated to one fruit by the method mentioned above. After egg inoculation, each citrus type was incubated at 26°C for 5 days and the most cold-tolerant stage (third instars) infested fruit were obtained. Twenty fruits infested with third instars were assigned to each citrus type, each of six exposure period treatment groups and untreated-control group and each replication. Five uninfested fruits in each citrus were used to monitor the fruit core temperatures during the treatment and sensor probes were inserted into the center of the fruit.

The infested fruit and uninfested fruit with probes of each citrus type were placed in the plastic containers and both citrus types were loaded into the cold treatment room. Cold treatment was commenced when three of five probes in each citrus type reached ≤ 2.1°C. The infested fruit of each citrus type were removed from the cold treatment room after 3, 6, 8, 10, 12 and 14 days. Mortality assessments of the treatment groups and untreated control group were conducted in the same manner described in the two orange cultivars test. The experiment was replicated three times.

Comparison of B. dorsalis mortality among orange, grapefruit and lemon

‘Valencia’ orange from California, ‘Marsh’ grapefruit from Florida, and ‘Lisbon’ lemon from California were used, and the weight per fruit was 256.4±21.6 g, 385.3±23.1 g and 124.4±9.3 g, respectively. ‘Imazalil’ and ‘TBZ’ have been applied to all citrus types as a food preservative and fungicide in the exporting county. In addition, ‘OPP’ was applied to grapefruit.

One hundred eggs were placed in each fruit by the method mentioned above. After infestation, orange and grapefruit were incubated at 26°C for 5 days and the most cold-tolerant stage (third instars) infested fruit were obtained while lemon was incubated for 4 days in order to obtain the most tolerant stage (second instars) infested fruit. Ten fruit infested with the most tolerant stage were assigned in each citrus type, each of six exposure period treatment groups and untreated-control group and each replication. Five uninfested fruit in each citrus type were used to monitor the fruit core temperatures during the treatment and sensor probes were inserted into the center of the fruit.

The infested fruit and uninfested fruit with probes of each cultivar were placed in the plastic containers and both cultivars were loaded into the cold treatment room. Cold treatment commenced when three of five probes in each cultivar reached ≤ 2.1°C. The infested fruit of each cultivar were removed from the cold treatment room after 3, 6, 8, 10, 12 and 14 days. Each treated fruit was kept at 26°C for 4 days and dissected for mortality assessment. The number of survivors was counted. Mortality assessment of the untreated control was conducted on the day of starting the cold treatment. Larvae with mobility were considered survivors. The experiment was replicated three times.
into the center of the fruit.

The infested fruit and uninfested fruit with probes of each citrus type were placed in the plastic containers and these three citrus types were loaded into the cold treatment room. Cold treatment was commenced when three of five probes in each citrus type reached ≤ 2.1°C. The infested fruit of each citrus type were removed from the cold treatment room after 2, 5, 7, 9, 11 and 13 days. Each treated fruit was kept at 26°C for two days and dissected for mortality assessment. The number of survivors was counted. Mortality assessment of the untreated control was conducted on the day of starting the cold treatment. Larvae with mobility were considered survivors. The experiment was replicated three times.

Data Analysis

All mortality data from these experiments was corrected for natural mortality using Abbott’s formula (Abbott, 1925). Mortality data of the most tolerant stage infesting different citrus types was subjected to Probit analysis using SAS® University Edition (SAS Institute Inc., Cary, NC) to estimate the days until lethal (exposure period) for mortality of 95% (LD₉₅) and 99% (LD₉₉), respectively and to compare the treatment efficacy between different citrus types.

Results and Discussion

Determination of the most cold-tolerant stage in citrus fruit

In each citrus type, the cold treatment profile including the precooling (the mean fruit core temperature before cooling, the mean precooling time to achieve 2.1°C and the mean fruit temperature during cold treatment of two replications) was shown in Table 2. In all of the fruits studied except lemons, third instars were clearly the most cold-tolerant stage because 100% mortality was not observed in third instars up to 12 days treatment but was observed in the other stages after 6-12 days treatment (Table 3). Studies comparing cold tolerance among immature stages of fruit fly species infesting citrus and the methods and results are summarized in Table 4.

Regarding the cold treatment experiments with ‘Navel’ orange

<table>
<thead>
<tr>
<th>Citrus</th>
<th>Mean fruit temperature (°C) before cooling1)</th>
<th>Mean precooling time (hour) to achieve 2.1°C1)</th>
<th>Mean fruit temperature (°C) during cold treatment1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus sinensis (orange)‘Navel’</td>
<td>23.2</td>
<td>23.5</td>
<td>2.04</td>
</tr>
<tr>
<td>Citrus sinensis (orange)‘Valencia’</td>
<td>25.4</td>
<td>20.5</td>
<td>1.95</td>
</tr>
<tr>
<td>Citrus reticulata x C. sinensis ‘Murcott’</td>
<td>23.3</td>
<td>13.3</td>
<td>1.97</td>
</tr>
<tr>
<td>Citrus unshiu ‘Satsuma’</td>
<td>19.5</td>
<td>14.2</td>
<td>2.05</td>
</tr>
<tr>
<td>Citrus paradisi (grapefruit)‘Marsh’</td>
<td>23.1</td>
<td>32.9</td>
<td>2.02</td>
</tr>
<tr>
<td>Citrus limon (lemon) ‘Lisbon’</td>
<td>24.1</td>
<td>18.0</td>
<td>2.04</td>
</tr>
</tbody>
</table>

1) Mean of two replications. Five uninfested fruit in each citrus type were used to monitor the fruit core temperature each replication.

Table 3. Corrected mortality of immature stages of Bactrocera dorsalis in various citrus after cold treatment at 2°C for 3, 6, 9, 12 and 15 days

<table>
<thead>
<tr>
<th>Citrus</th>
<th>Developmental stage</th>
<th>No. of fruit in each group1)</th>
<th>No. of test insects in each group1)</th>
<th>No. of survivors in control group1)</th>
<th>Corrected mortality (%) in each treatment group2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus sinensis (orange)‘Navel’</td>
<td>Egg</td>
<td>10</td>
<td>1,000</td>
<td>649</td>
<td>27.4 (100.0) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>First instar</td>
<td>10</td>
<td>1,000</td>
<td>572</td>
<td>16.8 (88.8) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>Second instar</td>
<td>10</td>
<td>1,000</td>
<td>673</td>
<td>37.0 (93.8) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>Third instar</td>
<td>10</td>
<td>1,000</td>
<td>572</td>
<td>38.8 (85.1) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td>Citrus sinensis (orange)‘Valencia’</td>
<td>Egg</td>
<td>10</td>
<td>1,000</td>
<td>686</td>
<td>29.2 (100.0) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>First instar</td>
<td>10</td>
<td>1,000</td>
<td>706</td>
<td>38.8 (98.3) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>Second instar</td>
<td>10</td>
<td>1,000</td>
<td>742</td>
<td>30.1 (94.1) 100.0 100.0 100.0 100.0</td>
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<tr>
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<td>Third instar</td>
<td>10</td>
<td>1,000</td>
<td>706</td>
<td>35.0 (79.2) 100.0 100.0 100.0 100.0</td>
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<tr>
<td>Citrus reticulata x C. sinensis ‘Murcott’</td>
<td>Egg</td>
<td>10</td>
<td>1,000</td>
<td>720</td>
<td>18.5 (99.4) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>First instar</td>
<td>10</td>
<td>1,000</td>
<td>679</td>
<td>56.6 (99.0) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>Second instar</td>
<td>10</td>
<td>1,000</td>
<td>687</td>
<td>29.7 (86.8) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
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<td>Third instar</td>
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<td>1,000</td>
<td>679</td>
<td>68.8 (88.1) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td>Citrus unshiu ‘Satsuma’</td>
<td>Egg</td>
<td>10</td>
<td>1,000</td>
<td>636</td>
<td>46.1 (99.8) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>First instar</td>
<td>10</td>
<td>1,000</td>
<td>587</td>
<td>51.4 (99.3) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>Second instar</td>
<td>10</td>
<td>1,000</td>
<td>616</td>
<td>35.4 (89.9) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>Third instar</td>
<td>10</td>
<td>1,000</td>
<td>587</td>
<td>76.1 (91.1) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td>Citrus paradisi (grapefruit)‘Marsh’</td>
<td>Egg</td>
<td>10</td>
<td>1,000</td>
<td>703</td>
<td>26.9 (99.9) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>First instar</td>
<td>10</td>
<td>1,000</td>
<td>646</td>
<td>30.3 (97.7) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>Second instar</td>
<td>10</td>
<td>1,000</td>
<td>643</td>
<td>28.3 (89.4) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>Third instar</td>
<td>10</td>
<td>1,000</td>
<td>646</td>
<td>55.1 (86.2) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td>Citrus limon (lemon) ‘Lisbon’</td>
<td>Egg</td>
<td>10</td>
<td>1,000</td>
<td>646</td>
<td>35.8 (100.0) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>First instar</td>
<td>10</td>
<td>1,000</td>
<td>601</td>
<td>93.8 (100.0) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>Second instar</td>
<td>10</td>
<td>1,000</td>
<td>644</td>
<td>34.2 (85.2) 100.0 100.0 100.0 100.0</td>
</tr>
<tr>
<td></td>
<td>Third instar</td>
<td>10</td>
<td>1,000</td>
<td>601</td>
<td>49.5 (92.8) 100.0 100.0 100.0 100.0</td>
</tr>
</tbody>
</table>

1) Total number of two replications.
2) Calculated from the data of two replications.
Table 4. Summary of studies on cold-tolerance comparison among developmental stages of Tephritidae in citrus fruits

<table>
<thead>
<tr>
<th>Fruit fly species</th>
<th>Publication</th>
<th>Scientific name 1)</th>
<th>Cultivar 1)</th>
<th>Developmental stage 2)</th>
<th>Infestation technique</th>
<th>Treatment condition (fruit temperature and [exposure days])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anastrepha fraterculus</td>
<td>Willink et al. (2006)</td>
<td>Citrus sinensis (orange)</td>
<td>'Valencia'</td>
<td>E, L1-2, L3</td>
<td>Egg, young, old larvae inoculation</td>
<td>2°C [0-8 days]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrus reticulata x C. sinensis (tangerine)</td>
<td>'Morcott'</td>
<td>E, L1-2, L3</td>
<td>Egg, young, old larvae inoculation</td>
<td>2°C [0-8 days]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrus clementina (clementine)</td>
<td>'Hernandine'</td>
<td>E, L1-2, L3</td>
<td>Egg, young, old larvae inoculation</td>
<td>2°C [0-8 days]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrus paradisii (grapefruit)</td>
<td>'Henninger's Ruby'</td>
<td>E, L1-2, L3</td>
<td>Egg, young, old larvae inoculation</td>
<td>2°C [0-8 days]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrus limon (lemon)</td>
<td>'Eureka'</td>
<td>E, L1-2, L3</td>
<td>Egg, young, old larvae inoculation</td>
<td>2°C [0-8 days]</td>
</tr>
<tr>
<td>Bactrocera dorsalis</td>
<td>Sugimoto and Harasawa (1982)</td>
<td>Citrus reticulata x C. paradisi (tangelo)</td>
<td>'Seminole'</td>
<td>E, L1-2, L3</td>
<td>Punctured-natural infestation</td>
<td>-0.6°C [0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 days]</td>
</tr>
<tr>
<td></td>
<td>Yamamoto et al. (2017)</td>
<td>Citrus tankan (tankan)</td>
<td></td>
<td>E, L1-2, L3</td>
<td>Egg inoculation (hole)</td>
<td>1.15°C [0, 3, 6, 9, 12, 15 days]</td>
</tr>
<tr>
<td>Bactrocera invadens (= B. dorsalis)</td>
<td>Groot et al. (2011)</td>
<td>Citrus sinensis (orange)</td>
<td>'Valencia'</td>
<td>E, L1-2, L3</td>
<td>Egg inoculation (hole)</td>
<td>1.1°C [0, 3, 5, 7, 9, 11, 13 days]</td>
</tr>
<tr>
<td>Bactrocera tryoni</td>
<td>De Lima et al. (2007)</td>
<td>Citrus reticulata x C. paradisi (tangerine)</td>
<td>'Ellendale', 'Morcott'</td>
<td>E, L1-2, L3</td>
<td>Punctured-natural infestation</td>
<td>2°C [0, 1, 3, 5, 7, 9, 11, 13, 15 days]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrus limon (lemon)</td>
<td>'Lisbon'</td>
<td>E, L1-2, L3</td>
<td>Punctured-natural infestation</td>
<td>2°C [0, 1, 3, 5, 7, 9, 11, 13, 15 days]</td>
</tr>
<tr>
<td></td>
<td>Hill et al. (1988)</td>
<td>Citrus sinensis (orange)</td>
<td>'Navel'</td>
<td>E, L1-2, L3</td>
<td>Punctured-natural infestation</td>
<td>1°C [0, 10 days]</td>
</tr>
<tr>
<td></td>
<td>Jessup et al. (1993)</td>
<td>Citrus limon (lemon)</td>
<td>'Eureka', 'Lisbon'</td>
<td>E, L1-2, L3</td>
<td>Punctured-natural infestation</td>
<td>1°C [0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 days]</td>
</tr>
<tr>
<td>Bactrocera zonata</td>
<td>Hashern et al. (2004)</td>
<td>Citrus sinensis (orange)</td>
<td>'Navel', 'Valencia'</td>
<td>E, L1-2, L3</td>
<td>Egg-injection (cap)</td>
<td>1.7°C [0, 1, 3, 5, 7, 10, 12, 14, 16, 18 days]</td>
</tr>
<tr>
<td></td>
<td>Mohamed and El -Wakkad (2009)</td>
<td>Citrus sinensis (orange)</td>
<td>'Valencia'</td>
<td>E, L1-2, L3</td>
<td>Egg inoculation (cap)</td>
<td>1.7°C [0, 1, 3, 5, 7, 10, 14 days]</td>
</tr>
<tr>
<td>Ceratitis capitata</td>
<td>De Lima et al. (2007)</td>
<td>Citrus sinensis (orange)</td>
<td>'Navel', 'Valencia'</td>
<td>E, L1-2, L3</td>
<td>Egg-injection</td>
<td>2°C [0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 18 days]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrus reticulata x C. sinensis (tangor)</td>
<td>'Ellendale', 'Morcott'</td>
<td>E, L1-2, L3</td>
<td>Egg-injection</td>
<td>2°C [0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 18 days]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrus limon (lemon)</td>
<td>'Lisbon'</td>
<td>E, L1-2, L3</td>
<td>Egg-injection</td>
<td>2°C [0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 18 days]</td>
</tr>
<tr>
<td></td>
<td>Hallman et al. (2019)</td>
<td>Citrus reticulata -</td>
<td>-</td>
<td>E, L1-2, L3</td>
<td>Natural infestation</td>
<td>1.1°C [9 days]</td>
</tr>
<tr>
<td></td>
<td>Hashern et al. (2004)</td>
<td>Citrus sinensis (orange)</td>
<td>'Navel', 'Valencia'</td>
<td>E, L1-2, L3</td>
<td>Egg-injection</td>
<td>1.7°C [0, 1, 3, 5, 7, 10, 12, 14, 16, 18 days]</td>
</tr>
<tr>
<td></td>
<td>Hill et al. (1988)</td>
<td>Citrus sinensis (orange)</td>
<td>'Valencia'</td>
<td>E, L1-2, L3</td>
<td>Egg-injection</td>
<td>1.5°C [0, 16 days]</td>
</tr>
<tr>
<td></td>
<td>Jessup et al. (1993)</td>
<td>Citrus limon (lemon)</td>
<td>'Eureka', 'Lisbon'</td>
<td>E, L1-2, L3</td>
<td>Egg-injection</td>
<td>1°C [0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14 days]</td>
</tr>
<tr>
<td></td>
<td>Quenta Cherre (2013)</td>
<td>Citrus unshiu</td>
<td>'Satsuma'</td>
<td>E, L1-2, L3</td>
<td>Egg inoculation (cap)</td>
<td>2°C [0, 2, 4, 6, 8, 10, 12, 14 days]</td>
</tr>
<tr>
<td></td>
<td>Santaballa et al. (2009)</td>
<td>Citrus clementina (clementine)</td>
<td>'Clementina'</td>
<td>E, L1-2, L3</td>
<td>Egg-injection</td>
<td>2°C [0, 2, 4, 6, 8, 10, 12, 14, 16 days]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrus reticulata</td>
<td>'Nova'</td>
<td>E, L1-2, L3</td>
<td>Egg, young, old larvae inoculation</td>
<td>2°C [0-8 days]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrus reticulata x C. sinensis (tangor)</td>
<td>'Ellendale', 'Morcott'</td>
<td>E, L1-2, L3</td>
<td>Egg, young, old larvae inoculation</td>
<td>2°C [0-8 days]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrus clementina (clementine)</td>
<td>'Clemenules'</td>
<td>E, L1-2, L3</td>
<td>Egg, young, old larvae inoculation</td>
<td>2°C [0-8 days]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrus paradisi (grapefruit)</td>
<td>'Marsh seedless', 'Henninger's Ruby', 'Star Ruby'</td>
<td>E, L1-2, L3</td>
<td>Egg, young, old larvae inoculation</td>
<td>2°C [0-8 days]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrus limon (lemon)</td>
<td>'Eureka', 'Lisbon', 'Lishbone Limoneira 8 A', 'Genoa'</td>
<td>E, L1-2, L3</td>
<td>Egg, young, old larvae inoculation</td>
<td>2°C [0-8 days]</td>
</tr>
</tbody>
</table>

1) Cultivars in publication were classified by Cottin (2002).
2) Developmental stage means; E (Egg), L1 (1st instar), L2 (2nd instar), L1-2 (1st and 2nd instar or young larva), L3 (3rd instar, old larva or mature larva).
Life stage with underline means the most cold-tolerant stage which authors mentioned in their publication.
and fruit fly, third instars were found as the most tolerant stage in the peach fruit fly, Bactrocera zonata (Saunders) (Hashem et al. 2004) and Mediterranean fruit fly, Ceratitis capitata (Wiedemann) (Hashem et al. 2004; Willink et al. 2006). Whereas, first and second instars were reported as the most tolerant stage in the Queensland fruit fly, Bactrocera tryoni (Frogatt) and C. capitata, respectively (De Lima et al. 2007). Furthermore, Hill et al. (1988) reported no significant difference in cold tolerance was seen between young and old larvae of B. tryoni. The difference of the most cold-tolerant stage might have arisen from the difference of the experimental methodology such as infestation techniques and the exposure interval of cold treatment (Table 4).

Our result in the most tolerant stage of B. dorsalis in 'Valencia' orange coincides with Grout et al. (2011). Regarding the cold treatment experiments with ‘Valencia’ orange and fruit flies, third instars were found as the most tolerant stage in B. zonata (Hashem et al. 2004; Mohamed & El-Wakkad, 2009), C. capitata (Hashem et al. 2004; Willink et al. 2006) and the South American fruit fly, Anastrepha fraterculus (Wiedemann) (Willink et al. 2006). Whereas, first and second instars were reported as the most tolerant stage in B. tryoni and C. capitata, respectively (De Lima et al. 2007). Furthermore, Hill et al. (1988) reported that no significant difference in cold tolerance was seen between young and old larvae of C. capitata. The difference of the most cold-tolerant stage might have arisen from the difference of the experimental methodology mentioned above.

Third instars were found as the most tolerant stage in ‘Murcott’ infested with C. capitata and A. fraterculus by Willink et al. 2006, whereas, first and second instar were reported as the most tolerant stages in B. tryoni and C. capitata, respectively, by De Lima et al. 2007.

Third instars were found as the most tolerant stage in ‘Satsuma’ infested by C. capitata (Quenta Cherre 2013).

Third instars were found as the most tolerant stage in ‘Marsh’ grapefruit infested by C. capitata (Willink et al. 2006).

In ‘Lisbon’ lemon, second instars were the most cold-tolerant stage based on the corrected mortality after nine-day treatment which was slightly lower than that of third instars (Table 3). Second instars were found as the most tolerant stage in lemons infested with C. capitata by Jessup et al. 1993 and De Lima et al. 2007, whereas, first and third instars were reported as the most tolerant stages in B. tryoni (Jessup et al. 1993, De Lima et al. 2007) and C. capitata (Willink et al. 2006), respectively.

### Table 5. Temperature profile of the cold treatment and corrected mortality of the most tolerant immature stage: third instars of Bactrocera dorsalis in ‘Navel’ and ‘Valencia’ oranges after cold treatment at 2°C for 3-18 days

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fruit temp. (°C) before cooling</th>
<th>Cooling time (hours)</th>
<th>Fruit temp. (°C) during cold treatment</th>
<th>No. of fruit in each group</th>
<th>No. of test insects in each group</th>
<th>No. of survivors in control group</th>
<th>Corrected mortality (%) of in each treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Navel’</td>
<td>24.0</td>
<td>23.4</td>
<td>2.02</td>
<td>30</td>
<td>3.000</td>
<td>2,065</td>
<td>46.97</td>
</tr>
<tr>
<td>‘Valencia’</td>
<td>24.3</td>
<td>21.8</td>
<td>1.96</td>
<td>30</td>
<td>3.000</td>
<td>2,104</td>
<td>53.42</td>
</tr>
</tbody>
</table>

1) Mean of three replications. Five uninsected fruit in each citrus type were used to monitor the fruit core temperature each replication.
2) Total number of three replications
3) Calculated from the data of three replications

### Table 6. Estimates and 95% confidence limits (CL) of days exposure at 2°C required to kill 95% (LD95) and 99% (LD99) of the most tolerant stage: third instars of Bactrocera dorsalis in ‘Navel’ and ‘Valencia’ oranges

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>LD95 (95%CL)</th>
<th>LD99 (95%CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Navel’</td>
<td>7.423 (7.235-7.624)</td>
<td>10.495 (10.137-10.893)</td>
</tr>
</tbody>
</table>

Comparison of mortality effect between different citrus cultivar or species

Comparison of B. dorsalis mortality between ‘Navel’ and ‘Valencia’ orange

In each cultivar, the cold treatment profile including the precooling (the mean fruit core temperature before cooling, the mean precooling time to achieve 2.1°C and the mean fruit temperature during cold treatment of three replications) was shown in Table 5. Corrected mortality of third instars of B. dorsalis in ‘Navel’ and ‘Valencia’ oranges are shown in Table 5. LD95 and LD99 in each cultivar are shown in Table 6. The confidence limits of each LD value of two cultivars overlapped, indicating no significant differences among the two.

Our result showing similar mortality of B. dorsalis in ‘Navel’ and ‘Valencia’ orange is consistent with previous studies. Hashem et al. (2004) found 100% mortality of the third instars of B. zonata in both cultivars was obtained after cold treatment at 1.7°C for 10 days. One hundred percent mortality of the most cold-tolerant stage of C. capitata in both cultivars was obtained after cold treatment at 2°C for 16 days (De Lima et al. 2007) and at 1.7°C for 16 days (Hashem et al. 2004).

Comparison of B. dorsalis mortality between ‘Murcott’ and ‘Satsuma’ in mandarin type

In each citrus type, the cold treatment profile including the precooling (the mean fruit core temperature before cooling, the mean precooling time to achieve 2.1°C and the mean fruit temperature during cold treatment of three replications) was shown in Table 7. The number of the survivors in the untreated control in ‘Murcott’ was more than that of ‘Satsuma’ (Table 7). Corrected
mortality of third instars of *B. dorsalis* in the two mandarin types are shown in Table 7. LD₉₅ and LD₉₉ are shown in Table 8. The values of LD₉₅ and LD₉₉ indicate that a longer treatment period is required for ‘Murcott’ and a significant difference was observed between the two mandarin types.

The data of De Lima et al. (2007) shows that there is a significant difference in the estimated exposure days at 2°C requiring 50% mortality (LD₅₀) of the most tolerant stage of *C. capitata* between ‘Murcott’ and ‘Ellendale’ (*C. reticulata × C. sinensis*), however, the confidence limits of LD₅₀ of these two cultivars overlapped. Furthermore, in *B. tryoni*, the 95% confidence limit of LD₅₀ and LD₉₅ of these two cultivars overlapped; however, 100% mortality of the most tolerant stage was obtained in 7 days at 2°C in ‘Murcott’ while 11 days at 2°C was required for ‘Ellendale’ (De Lima et al. 2007).

**Table 7.** Temperature profile of the cold treatment and corrected mortality of the most tolerant immature stage: third instars of *Bactrocera dorsalis* in ‘Murcott’ and ‘Satsuma’ in mandarin type after cold treatment at 2°C for 3-14 days

<table>
<thead>
<tr>
<th>Citrus</th>
<th>Fruit temp. (°C) before cooling(1)</th>
<th>Precooling time (hour) to achieve 2.1°C(1)</th>
<th>Fruit temp. (°C) during cold treatment(1)</th>
<th>No. of fruit in each group(2)</th>
<th>No. of test insects in each group(2)</th>
<th>No. of survivors in control group(3)</th>
<th>Corrected mortality (%) of in each treatment group(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murcott</td>
<td>24.3</td>
<td>24.0</td>
<td>2.03</td>
<td>60</td>
<td>3,000</td>
<td>3,000</td>
<td>2,280</td>
</tr>
<tr>
<td>Satsuma</td>
<td>24.4</td>
<td>21.0</td>
<td>1.97</td>
<td>60</td>
<td>3,000</td>
<td>3,000</td>
<td>1,854</td>
</tr>
</tbody>
</table>

1) Mean of three replications. Five uninfested fruit in each citrus type were used to monitor the fruit core temperature each replication.
2) Total number of three replications
3) Calculated from the data of three replications

**Table 8.** Estimates and 95% confidence limits (CL) of days exposure at 2°C required to kill 95% (LD₉₅) and 99% (LD₉₉) of the most tolerant stage: third instars of *Bactrocera dorsalis* in ‘Murcott’ and ‘Satsuma’ in mandarin type

<table>
<thead>
<tr>
<th>Citrus</th>
<th>LD₉₅ (95%CL)</th>
<th>LD₉₉ (95%CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murcott</td>
<td>5.465 (5.309-5.636)</td>
<td>7.660 (7.345-8.023)</td>
</tr>
<tr>
<td>Satsuma</td>
<td>4.126 (3.958-4.309)</td>
<td>6.346 (5.964-6.828)</td>
</tr>
</tbody>
</table>

**Comparison of B. dorsalis mortality among orange, grapefruit and lemon**

In each citrus type, the cold treatment profile including the precooling (the mean fruit core temperature before cooling, the mean precooling time to achieve 2.1°C and the mean fruit temperature during cold treatment of three replications) was shown in Table 9. The number of the survivors in the untreated control and the corrected mortality of the most tolerant stage of *B. dorsalis* treated at 2°C in each citrus type are shown in Table 9. The most tolerant stage (second instars) in lemon was clearly more susceptible to cold treatment than the most tolerant stage (third instars) in orange and grapefruit because 100% mortality was observed after 7 days in lemon but not observed up to 13 days treatment in both orange and grapefruit (Table 9). LD₉₅ and LD₉₉ in lemon is significantly shorter than those in orange and grapefruit (Table 10).

The data of De Lima et al. (2007) shows that 100% mortality of the most tolerant stage of *C. capita* and *B. tryoni* was obtained in a shorter treatment period at 2°C in lemon than in the two orange cultivars (‘Valencia’ and ‘Naval’) and a shorter LD₅₀ of the most tolerant stage was observed in lemon compared to those in the two orange cultivars. Papachristos et al. (2008) found that survival of *C. capitata* larvae reared in lemon pulp was lower than those reared in

**Table 9.** Temperature profile of the cold treatment and corrected mortality of the most tolerant immature stage: third instars of *Bactrocera dorsalis* in orange and grapefruit, and second instars in lemon after cold treatment at 2°C for 2-13 days

<table>
<thead>
<tr>
<th>Citrus</th>
<th>Cultivar</th>
<th>Fruit temp. (°C) before cooling(1)</th>
<th>Precooling time (hour) to achieve 2.1°C(1)</th>
<th>Fruit temp. (°C) during cold treatment(1)</th>
<th>No. of fruit in each group(2)</th>
<th>No. of test insects in each group(2)</th>
<th>No. of survivors in control group(3)</th>
<th>Corrected mortality (%) of in each treatment group(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Citrus sinensis</em> (orange)</td>
<td>‘Valencia’</td>
<td>25.7</td>
<td>22.0</td>
<td>1.99</td>
<td>30</td>
<td>3,000</td>
<td>3,000</td>
<td>2,195</td>
</tr>
<tr>
<td><em>C. paradisi</em> (grapefruit)</td>
<td>‘Marsh’</td>
<td>25.3</td>
<td>30.9</td>
<td>2.02</td>
<td>30</td>
<td>3,000</td>
<td>3,000</td>
<td>2,321</td>
</tr>
<tr>
<td><em>C. limon</em> (lemon)</td>
<td>‘Lisbon’</td>
<td>25.8</td>
<td>12.5</td>
<td>2.01</td>
<td>30</td>
<td>3,000</td>
<td>3,000</td>
<td>2,091</td>
</tr>
</tbody>
</table>

1) Mean of three replications. Five uninfested fruit in each citrus type were used to monitor the fruit core temperature each replication.
2) Total number of three replications
3) Calculated from the data of three replications

**Table 10.** Estimates and 95% confidence limits (CL) of days exposure at 2°C required to kill 95% (LD₉₅) and 99% (LD₉₉) of the most tolerant stage of *Bactrocera dorsalis*; third instars in orange and grapefruit, and second instars in lemon

<table>
<thead>
<tr>
<th>Citrus</th>
<th>Cultivar</th>
<th>LD₅₀ (95%CL)</th>
<th>LD₉₉ (95%CL)</th>
</tr>
</thead>
</table>
the pulp of the two orange cultivars (‘Merlin’ and ‘Xino Artas’).

In fact, the cold treatment schedules for *C. capitata* and *B. tryoni* adopted in the annexes to ISPM 28 show short cold treatment periods in lemon compared to those in orange or grapefruit (IPPC 2015, 2017).

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**References**


PT 27: Cold treatment for *Ceratitis capitata* on *Citrus paradisi*. Produced by the Secretariat of the IPPC. Adopted 2017. Published 2017. (Food and Agriculture Organization of the United Nations: Rome, Italy).


和文摘要

かんきつ類数種におけるミカンコミバエ卵及び幼虫の低温耐性の比較

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かんきつ類の品種・品目間におけるミカンコミバエ Bactrocera dorsalis の低温最耐性態の相違及び殺虫効果の有無を調査した。
ミカンコミバエ各態（卵、1、2、3 齢幼虫）の寄生したネーブル種及びバレンシア種オレンジ（C. sinensis）、マーコット（C. reticulata × C. sinensis）及びうんしゅうみかん（C. unshiu）、グレープフルーツ（C. paradisi）、レモン（C. limon）を低温処理（2℃、3 ～ 15 日間）し、各かんきつ類での各態間の低温耐性を比較した。レモンでは2 齢幼虫が最耐性であり、その他は3 齢幼虫が低温最耐性であった。
低温最耐性ステージであった3 齢幼虫の寄生したネーブル種及びバレンシア種オレンジを同時に低温処理（2℃、3 ～ 18 日間）し、両者の殺虫率を比較した結果、類似した殺虫率を示した。
低温最耐性ステージであった3 齢幼虫の寄生したマーコット及びうんしゅうみかんを同時に低温処理（2℃、3 ～ 14 日間）し、両者のLD₉₅ 及びLD₉₉ 値を比較した結果、マーコットのLD 値はうんしゅうみかんのそれよりも有意に短かった。
低温最耐性ステージの寄生したバレンシア種オレンジ（3 齢幼虫）、グレープフルーツ（3 齢幼虫）、レモン（2 齢幼虫）を低温処理（2℃、2 ～ 13 日間）し、LD₉₅ 及びLD₉₉ 値を比較した結果、レモンのLD 値は他の2 品目よりも有意に短かった。

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