

**Cold treatment of Australian cherries  
infested with eggs and larvae of the Queensland fruit fly  
( *Bactrocera tryoni* (Froggatt) ) Diptera : Tephritidae .**

\* \* \* \* \*

**PART ONE - MAIN REPORT**

OF THE MOST TOLERANT STAGE AND LARGE SCALE TRIAL  
PROTOCOLS FOR COLD DISINFESTATION OF  
QUEENSLAND FRUIT FLY

**CONDUCTED AT**

NEW SOUTH WALES DEPARTMENT OF PRIMARY INDUSTRIES,  
GOSFORD, NSW. AUSTRALIA 2250

**Bcj Ya VYf &\$%&**

## **i. Format of this Report**

This report, on experiments carried out to prove the efficacy of storage at 1°C or 3°C against immature life-stages of Queensland fruit fly (*Bactrocera tryoni* (Froggatt)) in Australian cherries, is in four parts.

<b>Part One: Main Report:</b>	Description of experimental facilities, materials and methods, results and discussion.
<b>Part Two: Photos and Diagrams:</b>	Photos and diagrams of experimental facilities, procedures and equipment.
<b>Part Three: Summary Tables:</b>	Tables for each replicate experiment showing dates of activities, results of experiments, summaries of temperature records.
<b>Part Four: Tables of Temperature Data:</b>	Tables of raw temperature data for each replicate for the large scale trials.

## Table of Contents

i. Format of this Report.....	2
1. Abstract .....	4
2. Introduction.....	5
3. General information .....	6
3.1. Test insect.....	6
3.2. Origin .....	6
3.3. General information on rearing methods for <i>B. tryoni</i> .....	6
3.4. Adults .....	7
3.5. Larvae.....	7
3.6. Pupae .....	7
3.7. Test fruit.....	8
3.8. Research laboratory .....	8
3.9. Specifications of the cold treatment facilities.....	9
3.10. Description of hot storage room .....	11
4. Materials and methods.....	12
4.1. Plan of the trials .....	12
4.2. Method of fruit storage before infestation .....	12
4.3. Method of infesting test fruits .....	12
4.4. Storage conditions of infested fruit prior to treatment.....	13
4.5. Fruit handling .....	13
4.6. Storage conditions of infested test fruit.....	14
4.7. Storage conditions of control (untreated) fruit.....	14
4.8. Description of fruit cartons.....	14
4.9. Larval development study .....	14
4.10. Determination of the most tolerant stage .....	15
4.11. Large scale trials .....	15
4.12. Method of calibration of temperature probes before and after each trial.....	16
4.13. Recording of temperature.....	16
4.14. Placement of temperature data logger probes.....	17
4.15. Experimental cool room loading factor .....	17
4.16. Layout of pallets and test fruit in cool rooms.....	17
4.17. Trial schedule .....	18
4.18. Data analysis .....	29
4.19. Quality of fruit after treatment .....	29
5. Results.....	30
5.1. Larval development .....	30
5.2. Most tolerant stage.....	30
5.3. Large scale confirmation of treatment efficacy.....	31
5.4. Temperature records.....	32
5.5. Fruit quality .....	32
6. Discussion .....	33
6.1. Larval development .....	33
6.2. Most treatment tolerant life-stage .....	33
6.3. Large scale trials .....	33
6.4. Fruit quality .....	33
7. Product specifications.....	34

## 1. Abstract

Australian cherries were subjected to cold storage treatment at temperatures of  $1.0\pm0.5^{\circ}\text{C}$  and  $3.0\pm0.5^{\circ}\text{C}$  for disinfestation of Queensland fruit fly (*Bactrocera tryoni* (Froggatt)).

Larval development studies were conducted to determine the course of development of each immature life-stage and the dates when each stage (i.e. eggs, first instar, second instar and third instar) should be tested.

The most tolerant life-stage trials were conducted by exposing each stage to cold treatment for 3, 4, 5, 6, 7, 8, 9, 10, 12 and 14 days.

Data showed that in cherries, the first instar Queensland fruit fly larvae is more tolerant of storage at  $1^{\circ}\text{C}$  and at  $3^{\circ}\text{C}$  than Queensland fruit fly eggs, second instar larvae and third instar larvae. When large scale trials were carried out on first instar larvae in cherries there were no survivors following storage at either  $1.0\pm0.5^{\circ}\text{C}$  for a period of 14 days, or at  $3.0\pm0.5^{\circ}\text{C}$  for a period of 14 days.

All trials were replicated three or four times to expose a sufficient number of insects to the treatments.

In summary:

$1^{\circ}\text{C}$  for **14 days**: 0 survivors from a total of 64,119 insects treated

$3^{\circ}\text{C}$  for **14 days**: 0 survivors from a total of 102,372 insects treated

Results from the work described here have proven that:

Storage treatments at  $1^{\circ}\text{C}$  for 14 days or at  $3^{\circ}\text{C}$  for 14 days for Australian cherries are suitable as quarantine treatments against Queensland fruit fly.

## 2. Introduction

Australian producers are in a position to supply fresh, high quality cherries to markets in the Northern Hemisphere when local supplies in those countries are not available because of the different growing seasons.

Cold treatments offer a commercially viable method for quarantine disinfestation of cherries, since cherries maintain their quality during cold storage. Previous studies have demonstrated that cold disinfestation is an effective treatment against Queensland fruit fly in oranges, lemons, tangerines and tangelos for export to Japan and other markets.

DAFF would like to present this trial protocol that demonstrates treatment efficacy of cold disinfestation on the most treatment tolerant life stage of Queensland fruit fly. The most tolerant stage is determined by subjecting all life-stages of *B. tryoni* to different durations of cold storage, and determining the percent mortality for each life-stage.

The life-stage that is found to be the most tolerant of the treatment is used in a series of large scale trials conducted under simulated export conditions. These large scale trials comprise at least three replicates of the treatment applied to the most tolerant stage. To be considered successful, the large scale trial must demonstrate that there are no survivors when at least 30,000 insects are exposed to the treatment.

The trials using *B. tryoni* were conducted in New South Wales, on the east coast of Australia, at New South Wales Department of Primary Industries, Gosford Horticultural Institute (GHI). All trial techniques are fully explained in the report.

### 3. General information

This section contains general background information on test insects, test fruit, research laboratories, cold room facilities, temperature monitoring devices and methods used in the trials.

#### 3.1. Test insect

Queensland fruit fly *Bactrocera tryoni* (Froggatt)

[For more information refer to Part 2 – Figures 1 – 4, Photos and diagrams]

#### 3.2. Origin

Test insects were sourced from a laboratory colony of *B. tryoni* that is managed by scientists from New South Wales Department of Primary Industries, GHI. Scientists at the GHI laboratories have been managing different colonies of *B. tryoni* since 1956.

Wild characteristics of the *B. tryoni* colony are maintained through regular introductions of *B. tryoni* reared from field collected fruit from the Central and North Coasts of New South Wales. Four to six times a year, infested fruit is brought into the GHI laboratory from these areas and flies are reared out, sorted and checked to ensure 100 percent are *B. tryoni*. They are then reared under laboratory conditions, separate from the established laboratory colony, for six generations. At the sixth generation these flies are used to replace the old colony.

#### 3.3. General information on rearing methods for *B. tryoni*

Eggs are collected from adult *B. tryoni* flies in plastic cups that have been pierced with more than 200 fine holes, through which the fly oviposits. The egg cups are removed after 24 hours and eggs are placed on artificial larval rearing medium using a pipette. The medium comprises diced dried carrot, yeast hydrolysate, water and preservatives.

Developing larvae are kept in the insectary at  $26\pm 2^{\circ}\text{C}$  and  $65\pm 5\%$  relative humidity (RH). Larvae pupate in trays of slightly dampened vermiculite. Mature pupae are separated from the vermiculite and placed into cages (1665 x 510 x 250 mm high) where they emerge as adults.

At any time there are 16 cages of adult flies aged between one and four weeks housed in the insectary. At the end of each week, the four cages of oldest flies are killed off and four new cages of flies are prepared to replace them. Eggs are collected from 2 to 3 week-old adults for subsequent laboratory generations.

Each cage is lit from above by a single 1200 mm long 40W fluorescent light tube which allows at least 2000 Lux of light into the cage. The lighting regime in the insectary is 12 hours full light, then 1 hour slowly dimming to full dark, then 10 hours full dark, then 1 hour slowly brightening to full light.

[For more information refer to Part 2 – Figures 1 – 4, Photos and diagrams]

### 3.4. Adults

The adult flies are fed on a diet of yeast hydrolysate, sugar and water. Each of the 16 cages houses about 15,000 to 20,000 flies. Fly populations in the laboratory have a sex ratio of approximately 1:1 (male:female).

**Quantity of eggs collected:** Approximately 10 mL per egg cup per day.

### 3.5. Larvae

Larval rearing is done on an artificial medium based on diced dried carrot. Special larval rearing cages are used to house the developing larvae. Mature larvae are channelled via a chute into a pupation tray (530 x 350 x 195 mm) containing damp vermiculite. Larvae are reared in the insectary. The following ingredients are mixed in the specified proportions into a homogenous mix which is then spread on plastic covered trays for egg deposition.

**Medium:**

2.7 L Dried carrot  
20 g Sodium benzoate  
480 g Yeast hydrolysate  
72 g Citric acid  
7 L Water

**Quantity of medium:** Approximately 16 trays of medium is made in each mix. It is distributed in the following way: 4 trays/chute. (tray size: 295 x 195 x 30 mm).

**Quantity of eggs inoculated:** 0.4 mL/tray x 16 trays/cage. This gives approximately 15,000 to 20,000 adult flies per cage or a total of 60,000 to 80,000 flies per week.

**Insectary conditions:** 26±2°C: 65±5% RH

**Development duration of different life stages of *B. tryoni* in the insectary:**

Eggs: between 0 and 2 days

Larvae: between 5 and 7 days.

Pupae: between 10 and 12 days.

Adults are culled 4 weeks after emergence.

Rate of egg hatch: approx. 70 - 90%

Rate of larval pupation (of eggs that hatch): 80 - 90%

Note: development times in artificial media differ from those in infested fruit.

### 3.6. Pupae

**Maturation of pupae:** The trays containing larvae in vermiculite (530 x 350 x 195 mm) remain underneath the chutes until all larvae have left the medium. The resulting trays of pupae are removed from underneath the chutes and are stacked and stored in the same insectary as the larvae. Thereafter, they are transferred to the adult

colony cages for emergence as described above.

**Number of pupae reared:** Approx. 3750 – 5000 pupae/tray x 4 trays x 4 chutes  
x 1 cage = approx. 60,000 – 80,000 pupae in total.

**Rate of adult emergence:** Approx.: 90 - 98%.

### **3.7. Test fruit**

Insecticide-free, Class 1 cherries for these trials were sourced from Tasmania, Australia. *B. tryoni* is not present in Tasmania, thus the cherries for these experiments had not been sprayed for fruit flies. Orchards from which fruit used in these experiments were harvested had not been sprayed with insecticides during the fruit growth and maturation stage. The fruit was therefore suitable for the survival of immature stages of *B. tryoni*.

Produce met specification as per FreshSpecs product specifications (Part 1, Table 6).

For verification purposes, small scale studies were conducted upon receipt of fruit to confirm infestation was possible, prior to the large scale trials. The fruit was of excellent quality in terms of maturity and was suitable for the survival of immature stages of *B. tryoni* at the time of infestation.

### **3.8. Research laboratory**

#### **Organisation**

New South Wales Department of Primary Industries.

#### **Location of trials**

The *B. tryoni* trials were conducted at the disinfestation laboratory, Gosford, NSW. This laboratory is part of the Postharvest Section of the GHI. The Postharvest Section is responsible for the research and advisory work on disinfestation and other related *B. tryoni* work in NSW.

#### **Facilities of the postharvest disinfestation laboratory**

The postharvest disinfestation facilities include the following:

- *B. tryoni* insectary
- Cold room for fruit holding
- 3 cold rooms for cold disinfestation trials
- 3 hot storage rooms for insect development
- Fruit fly preparation room
- Disinfestation studies laboratory

### **3.9. Specifications of the cold treatment facilities**

[For more information refer to Part 2 – Photos and diagrams]

#### **3.9.1. Description of cold treatment facilities**

The cold treatment facilities are situated at GHI. The treatment facilities were constructed by Thermoline Australia in 2004.

#### **3.9.2. Number of cold rooms**

Three cold rooms were used for these trials. The rooms have the designated labels cold treatment room (CTR) 7, CTR 8 and CTR 9.

#### **3.9.3. Room construction**

The three temperature and humidity controlled rooms are located within a larger freestanding building. All rooms are manufactured from structural, polystyrene sandwich panels for high level thermal resistance. The rooms are formed from 75 mm panels, with all internal and external seams sealed. Floors in the rooms are formed from 75 mm thick concrete finished with welded vinyl flooring.

#### **3.9.4. Performance**

Rooms CTR 7 and CTR 9 have been designed to provide temperature and humidity control (0.0°C to 45.0°C and 30 - 95% RH). CTR 8 has been designed to provide temperature and humidity control (-10.0°C to 45.0°C and 30 - 95% RH).

#### **3.9.5. Air circulation**

Five axial type fans attached to the refrigeration unit provide air circulation in each room.

#### **3.9.6. Refrigeration**

Air cooling is achieved by means of direct expansion refrigeration evaporators in the fan coil assembly attached to the roof of each room. An air cooled, hermetically sealed condensing unit, using R404a refrigerant, is located outside the rear of each room.

#### **3.9.7. Cooling regulation**

Time proportional signals from the temperature controllers switch liquid line solenoid valves. These regulate the flow of refrigerant into the evaporators; thereby controlling the cooling effect.

### **3.9.8. Humidification**

Humidification is generated by a 'Defensor' atomiser type humidification system. This introduces water vapour without the addition of heat, as is usually done via steam injection.

### **3.9.9. Dehumidification**

Dehumidification is by water condensation on the surface of the direct injection refrigeration evaporator. The liquid line solenoid valves, also used to regulate cooling, are switched by time proportional pulsed signals from the humidity controllers.

### **3.9.10. Sensors**

All rooms are fitted with Rototronic Hygroclip C temperature and humidity sensors, capable of monitoring temperature in the range of 0.0 to 85.0°C and humidity in the range of 0% RH to 100% RH. CTR 8 is fitted with a DIN standard pt100 resistance sensor, which will measure temperature down to -10°C.

### **3.9.11. Size and capacity of each room**

The three cold rooms measure 3750 x 2400 x 2800 mm with a total volume of 25.5 m<sup>3</sup>. Door openings are 1000 mm by 2005 mm. The door is fitted on all four sides with double neoprene labyrinth gaskets. The door overlaps the opening by 75 mm and is 120 mm thick.

### **3.9.12. Description of temperature recorders**

Core temperatures of fruit were monitored with a Grant 2040 series Squirrel Data Logger with metal oxide 2 K Ohm thermistor probes. Probes have a temperature range between -50°C and 150°C with an accuracy of  $\pm 0.2^\circ\text{C}$ . The thermistor probes are connected to the logger by a factory built and calibrated cable of 5 m length. The Grant 2040 series Squirrel data Logger has 32 channels available for temperature input. The summary details are as follows:

- 1) Type: Grant 2040 series Squirrel Data Logger with 32 channels
- 2) Temperature sensor: U 2 K Ohm thermistor probes
- 3) Number of probes: 8
- 4) Logger accuracy:  $\pm 0.05\%$

Temperature sensors were calibrated before the trial to verify that they were functioning according to specification. Each probe was held in ice slurry (melting ice and distilled water). Recordings were taken on each probe, each minute, for 60 minutes. The readings were stored on disk via a USB interface.

[For more information refer to Part 3 – Table 18 Calibration for data loggers and Part 2, Figure 27] ]

### **3.9.13. Recording intervals for temperature and relative humidity**

Fruit core temperatures were recorded every 30 minutes during the cool down and cold storage periods of the treatment.

[For more information refer to Part 2, Figures 28,29 and 35 – Photos and diagrams]

## **3.10. Description of hot storage room**

### **3.10.1. Temperature and relative humidity of hot storage room**

The hot storage room was set at  $26\pm0.5^{\circ}\text{C}$  and 50 - 60% relative humidity.

### **3.10.2. Duration of storage after cold treatment**

After cold treatment, cherries were stored in the hot storage room for up to 18 days with periodic inspection and counting of surviving insects.

### **3.10.3. Description of hot storage room**

The hot storage room is constructed from panels of 125 mm expanded polystyrene with internal and external skins of polished 22 gauge, 0.75 hardness marine grade aluminium sheeting permanently bonded to the polystyrene. External joints are caulked with silicone rubber adhesive. The floor consists of an insulated layer (100 mm expanded polyurethane) covered with 75 mm concrete. All sheet aluminium is finished with white baked enamel.

The door openings are 1350 mm clear width by 2100 mm. The door is fitted on all four sides with a double neoprene labyrinth gasket. The door overlaps the opening by 75 mm, the door is 125 mm thick.

## 4. Materials and methods

### 4.1. Plan of the trials

The trials were conducted in the following manner in three stages:

**STAGE 1 : Larval development studies** were conducted first to determine the rate at which *B. tryoni* larvae develop at 26°C. This determined when treatment for each life-stage should commence in subsequent trials.

**STAGE 2:** The **most tolerant life-stage** trials were conducted by exposing more than 200 insects of each life-stage to a series of treatment periods (from 3 to 14 days) at 1°C and 3°C. From these studies it was determined which life-stage of *B. tryoni* was most tolerant to the specific cold treatment. The data obtained from the most tolerant stage trials were subjected to Probit analysis to calculate Probit 9 for each stage. The life stage with the highest Probit 9 value was selected for the large scale trials.

**STAGE 3:** The **large scale trials** were conducted by exposing at least 30 000 larvae (at the life-stage demonstrated to be most tolerant to the treatment in stage 2) to cold storage at 1°C or 3°C for a period of 14 days. This was done across several replicates.

### 4.2. Method of fruit storage before infestation

After harvest, fruit were packed and stored under normal commercial conditions. Fruit was held in a cold room until required for the trials.

#### 4.2.1. Storage temperature after harvest

Fruit were harvested into field bins which were placed into cold storage at 5°C to 7°C. They were sorted and packed into cartons on pallets within the next 5 days and returned to 5°C storage.

#### 4.2.2. Duration of storage after harvest

Fruit in cartons, on pallets, were stored at 5°C for between 3 and 10 days before being transported to GHI for the commencement of the trials.

#### 4.2.3. Storage temperature and duration prior to start of trials

Upon receipt at GHI, cherries were stored at 0°C, 95% relative humidity, in export fibreboard cartons, in 5 kg units for up to 3 days until ready to start the trials. Cartons had a low-density polyethylene (LDPE) liner. Cherries were then allowed to warm up to the optimum fruit fly infestation temperature of 26±2°C in the insectary. The fruit in cartons were protected from contaminant insects by covering with a fine terylene cloth.

### 4.3. Method of infesting test fruits

After overnight adjustment to  $26\pm 2^{\circ}\text{C}$  (the optimum temperature for *B. tryoni* egg-laying) the cherries were placed on top of cages of 2 to 4 week old *B. tryoni*. For the larval development studies and the most tolerant stage studies fruit remained on the cages for 2 to 4 hours. For large scale trials fruit remained on the cages overnight (16 hours). A longer time period was required to infest fruit for the large scale trials due to the large number of fruit being infested. Flies infested the fruit by ovipositing eggs through the gauze roof of the cage into the fruit.

[For more information refer to Part 2, Figures 14 - 20 – Photos and diagrams]

#### **4.4. Storage conditions of infested fruit prior to treatment**

Infested fruit were placed in plastic racks measuring 350 x 430 x 60 mm, lined with 2 mm mesh, in the hot storage room. To prevent desiccation of *B. tryoni* eggs, infested fruit were covered with damp material, and kept damp until treatment commenced.

#### **4.5. Fruit handling**

##### **4.5.1. During treatment**

Once insects in the treated fruit had reached the target immature life-stage, the treated fruit was labelled and repacked into export cherry cartons. Filler fruit (non-infested apple fruit) were used to fill the cold room to the specified loading factor ( $> 35\%$ ). Apples were chosen as filler fruit due to their low cost and excellent ability to store. Temperature probes were inserted into several non-infested cherries to monitor fruit core temperatures throughout the cool-down, the cold storage and the warm-up periods of the treatment. Temperature monitored fruit were placed throughout the load of fruit to provide data on temperature variations within the cold rooms.

##### **4.5.2. After treatment**

Treated fruit were removed from the cold room and placed in the hot storage room. Infested fruit were removed from their cartons and placed in 60 x 40 x 250 mm plastic boxes. Each box was fitted with a wood-framed plastic mesh insert which allowed treated fruit to be suspended above dampened vermiculite contained in the bottom of the box. Vermiculite was the pupation medium for surviving insects. The box containing the infested fruit was covered with terylene cloth to allow air exchange and prevent post-treatment contamination with *B. tryoni* and *Drosophila* spp. The fruit were held in the hot storage room to enable any potential survivors to emerge.

##### **4.5.3. Sieving**

Vermiculite beneath control fruit was sieved approximately 10 days after infestation, another sieve was conducted after approximately another 7 days. Vermiculite beneath treated fruit was sieved at approximately 7 days after removal from cold storage and again approximately every week until no survivors were found. Surviving pupae and larvae in the vermiculite and fruit remains were collected and counted.

[For more information refer to Part 2, Figure 33 – Photos and diagrams]

#### **4.6. Storage conditions of infested test fruit**

Test fruit infested with the most tolerant stage *B. tryoni* were labelled and placed in cherry export cartons in amongst non-infested fruit.

The temperature of the cold rooms was set so that fruit temperature was maintained within the range of  $1.0\pm0.5^{\circ}\text{C}$  for 14 days for the  $1^{\circ}\text{C}$  trials; and at  $3.0\pm0.5^{\circ}\text{C}$  for 14 days for the  $3^{\circ}\text{C}$  trials. Temperature data loggers monitored fruit core temperatures throughout the duration of the treatments. The relative humidity in each cold room was between 50% and 60%. Relative humidity was monitored but not logged.

#### **4.7. Storage conditions of control (untreated) fruit**

Control fruit were stored in the hot storage room and were covered with fine terylene cloth to prevent contamination by *Drosophila* spp. (vinegar flies).

#### **4.8. Description of fruit cartons**

Fruit were received at GHI in export fibreboard cartons on wooden pallets. Test fruit and filler fruit were placed in the cold storage treatment rooms in their export cartons.

Four pallets of filler fruit were placed in each of the cool rooms. Each pallet was comprised of 60 cartons (5 kg cartons measuring 370 mm long x 270 mm wide x 115 mm high).

Infested fruit were placed within each stack of filler fruit according to a system. Certain cartons of filler fruit were removed from certain rows of each pallet and replaced with a labelled carton of test fruit in which a temperature probe was inserted.

[For more information refer to Part 2, Figures 6 – 13, 35 and 36 – Photos and diagrams]

#### **4.9. Larval development study**

To determine the course of development of the immature stages of *B. tryoni* in the cherries, test fruit was infested by placing them on top of a wire mesh cage housing 15,000 to 20,000 sexually-mature *B. tryoni*.

Approximately 200 fruit were selected and placed on cages of mature (1 to 4 weeks from adult eclosion) *B. tryoni* adults for about 2 to 4 hours for adequate infestation to occur. These fruit were then placed in containers and covered with fine mesh bags to prevent contamination with other fruit flies and placed in the hot storage room to allow eggs to hatch and larvae to develop. Every 2 to 3 days a sample of 5 fruit were removed from storage. Eggs and larvae were removed from each fruit after dissection of the fruit by washing. Eggs and larvae were counted and larval instars (life-stages) were identified by examination of mouthparts under a dissecting microscope. Each

larval stage has distinctive mouthparts, which enable identification of the different life-stages [For more information refer to Part 2, Figure 34 – Photos and diagrams].

The proportion of insects at each life-stage was determined at set periods after infestation.

These data enabled treatment of infested fruit to commence at each target life-stage.

[For more information refer to Part 3, Table 1 – Summary tables].

#### **4.10. Determination of the most tolerant stage**

Each insect life-stage must be tested with a range of doses (days of cold treatment). This will result in a dose-mortality response which can be evaluated by probit analysis to determine which life-stage is most tolerant. This most tolerant stage is then the subject in the large scale trials.

At each replicate treatment, fruit was sampled and divided into control (infested but untreated) fruit and treated (infested and cold-stored) fruit. Approximately 300 fruit were labelled as ‘controls’ and approximately 1300 to 1500 as ‘treated’.

Controls were infested and placed in trays on mesh sub-trays suspended over a pupation medium consisting of damp Grade 1 (fine particles) vermiculite. The hot storage room was maintained at a temperature of  $26 \pm 0.5^\circ\text{C}$  and 50 – 60% RH. Each tray of control fruit was enclosed in a fine mesh bag to prevent contamination by other fruit flies and *Drosophila* spp.

Treated fruit were randomly divided into sub-groups representing the four insect life-stages (eggs, first instar, second instar or third instar) to be tested. A sample of each sub-group was removed from cold treatment on days 3, 4, 5, 6, 7, 8, 9, 10, 12 and 14. Three replicates were conducted for each life-stage, each replicate was treated in a separate cold room.

At completion of the treatment, fruit was removed from the cold treatment room and stored as described in section 4.5. Fruit was then sieved as described in section 4.5. The criterion for survival was the formation of an apparently normal puparium.

The numbers of surviving insects per fruit from the controls were used to estimate the numbers of insects that would be in the treated fruit before treatment. Mortality at each dose was estimated by comparing the number of surviving insects in the treatments with the number of surviving insects in the controls. A dose-mortality curve was produced and analysed to predict Probit 9 (or 99.99683% mortality, the parameter for quarantine security). These data were used to determine the insect life-stage that was most tolerant of cold storage.

See Part 1, Tables 1 and 2 for the experimental schedules and treatment dates for the most tolerant stage trials.

#### **4.11. Large scale trials**

Large scale trials were conducted over two growing seasons of Australian cherries from March 2006 to April 2007 at GHI. Cherries were infested with Queensland fruit flies (*B. tryoni*) reared at GHI. Replication occurred over time and across three different treatment rooms. Four replicate experiments were performed at 3°C to achieve the required number of treated insects, and three replicate experiments were performed at 1°C.

Between 440 and 520 fruit per replicate were used as controls and the rest were treated. All treated fruit were infested and placed in the hot storage room to allow development to the most tolerant stage. Prior to being placed in treatment, a sample of fruit was dissected and examined to confirm that the larvae were at the most tolerant life-stage at the time of treatment. If larvae had not yet developed to the most tolerant stage, fruit for treatment was returned to the hot storage room and sampled periodically until the most tolerant life-stage was reached. Treated fruit were then placed into the cold treatment room as described in section 4.5. At completion of the treatment, fruit were removed from the cold treatment room and stored as described in section 4.5. The control and treated fruit were then sieved as described in section 4.5.

See Part 1, Tables 3, 4 and 5 for the experimental schedules and treatment dates for the large scale trial.

#### **4.12. Method of calibration of temperature probes before and after each trial**

Temperature sensors were calibrated before each trial to verify that they were functioning according to specifications. Each probe was submerged in ice slurry. Recordings were taken at 10 minute intervals.

[For more information refer to Part 2, Figure 27 – Photos and diagrams]

#### **4.13. Recording of temperature**

Temperature data logger probes were placed so that the measurement surface of the probe was in the centre of test fruit. Data loggers measured fruit core temperature every 30 minutes for the duration of the experiments.

Probes were placed in non-infested filler fruit (cherries) placed near the infested fruit within a pallet of apples. For the most tolerant stage studies, infested fruit were removed from cold storage at intervals (i.e. 3, 4, 5, 6, 7, 8, 9, 10, 12 and 14 days) after more than 50% of probes reached the target temperature (i.e. 1.5°C or 3.5°C). If there were 6 probes used in one of the most tolerant stage studies at 1°C then treatment commenced from the time 4 probes read < 1.5°C.

Filler fruit with probes remained in the cold room after each removal of test fruit for the most tolerant stage trials.

Staff on site were able to read temperature records while the data logger was recording to determine when treatment commenced. Between 7 and 10 probes were used for each experiment.

#### **4.14. Placement of temperature data logger probes**

Probes were placed in the centre (i.e. against the seed) of each fruit to be logged. Logged fruit were placed randomly within each carton of treated fruit. Cartons were marked and placed in various positions around the cold treatment room and within each pallet load of fruit.

[For more information refer to Part 2, Figures 8 – 12, 35 and 36 – Photos and diagrams]

#### **4.15. Experimental cool room loading factor**

The loading factor was greater than 35 percent. The bulk of fruit required to make up that loading factor was pallets of cartons of second quality apples. Three cold treatment rooms were used.

#### **4.16. Layout of pallets and test fruit in cool rooms**

Pallets of filler fruit plus test fruit were placed so that there was a 10 cm gap between the cartons of fruit and the cool room walls and the next pallet. There was a gap of 40 cm down the middle of the room to allow access to each pallet of fruit. The gap between the top of the fruit and the cool room ceiling was about 100 cm. Each pallet of fruit sat on a wooden pallet 20 cm above the floor.

[For more information refer to Part 2, Figures 7, 12 and 13– Photos and diagrams]

#### 4.17. Trial schedule

**Table 1. Experimental schedule of activities for most treatment tolerant life-stage trials at 1°C (1STS: first instar larvae; 2NDS: second instar larvae; and 3RDS: third instar larvae).**

Date	Temperature	Life-stage	Activity
27 Mar 2006	1°C	EGGS	Infest approx. 1600 fruit on cages at GHI.
28 Mar 2006	1°C	EGGS	Remove fruit from cages, randomly select sample of 276 fruit as controls (non-cold treated fruit). Control fruits into hot storage room.
29 Mar 2006	1°C	EGGS	Treatment units into treatment at 1°C.
30 Mar 2006	1°C	EGGS	Day 1 of treatment.
31 Mar 2006	1°C	EGGS	Day 2 of treatment.
01 Apr 2006	1°C	EGGS	Day 3 of treatment. Remove 3 samples of 44 fruit.
02 Apr 2006	1°C	EGGS	Day 4 of treatment. Remove 3 samples of 44 fruit.
03 Apr 2006	1°C	EGGS	Day 5 of treatment. Remove 3 samples of 44 fruit.
04 Apr 2006	1°C	EGGS	Day 6 of treatment. Remove 3 samples of 44 fruit.
05 Apr 2006	1°C	EGGS	Day 7 of treatment. Remove 3 samples of 44 fruit.
06 Apr 2006	1°C	EGGS	Day 8 of treatment. Remove 3 samples of 44 fruit.
07 Apr 2006	1°C	EGGS	Day 9 of treatment. Remove 3 samples of 44 fruit. 1st sieving of control fruits.
08 Apr 2006	1°C	EGGS	Day 10 of treatment. Remove 3 samples of 44 fruit.
09 Apr 2006	1°C	EGGS	Day 11 of treatment.
10 Apr 2006	1°C	EGGS	Day 12 of treatment. Remove 3 samples of 44 fruit.
11 Apr 2006	1°C	EGGS	1st sieving of Day 3 treatments.
12 Apr 2006	1°C	EGGS	Day 14 of treatment. Remove 3 samples of 44 fruit. 1st sieving of Day 4 treatments.
13 Apr 2006	1°C	EGGS	1st sieving of Day 5 treatments.
14 Apr 2006	1°C	EGGS	1st sieving of Day 6 treatments.
15 Apr 2006	1°C	EGGS	1st sieving of Day 7 treatments.
16 Apr 2006	1°C	EGGS	1st sieving of Day 8 treatments.
17 Apr 2006	1°C	EGGS	1st sieving of Day 9 treatments. 2nd sieving of control fruits.
18 Apr 2006	1°C	EGGS	1st sieving of Day 10 treatments. 2nd sieving of Day 3 treatments.
19 Apr 2006	1°C	EGGS	2nd sieving of Day 4 treatments.
20 Apr 2006	1°C	EGGS	1st sieving of Day 12 treatments. 2nd sieving of Day 5 treatments.
21 Apr 2006	1°C	EGGS	2nd sieving of Day 6 treatments.
22 Apr 2006	1°C	EGGS	1st sieving of Day 14 treatments. 2nd sieving of Day 7 treatments.
23 Apr 2006	1°C	EGGS	2nd sieving of Day 8 treatments.
24 Apr 2006	1°C	EGGS	2nd sieving of Day 9 treatments.
25 Apr 2006	1°C	EGGS	2nd sieving of Day 10 treatments.
26 Apr 2006	1°C	EGGS	No activity.
27 Apr 2006	1°C	EGGS	2nd sieving of Day 12 treatments.
28 Apr 2006	1°C	EGGS	No activity.
29 Apr 2006	1°C	EGGS	2nd sieving of Day 14 treatments.
<hr/>			
27 Mar 2006	1°C	1STS	Infest approx. 1600 fruit on cages at GHI.
28 Mar 2006	1°C	1STS	Remove fruit from cages, randomly select sample of 276 fruit as controls (non-cold treated fruit). Control fruits into hot storage room.

29 Mar 2006	1°C	1STS	Treatment units into hot storage room.
30 Mar 2006	1°C	1STS	Larvae developing in fruit.
31 Mar 2006	1°C	1STS	Larvae developing in fruit.
01 Apr 2006	1°C	1STS	Treatment units into treatment at 1°C.
02 Apr 2006	1°C	1STS	Day 1 of treatment.
03 Apr 2006	1°C	1STS	Day 2 of treatment.
04 Apr 2006	1°C	1STS	Day 3 of treatment. Remove 3 samples of 44 fruit.
05 Apr 2006	1°C	1STS	Day 4 of treatment. Remove 3 samples of 44 fruit.
06 Apr 2006	1°C	1STS	Day 5 of treatment. Remove 3 samples of 44 fruit.
07 Apr 2006	1°C	1STS	Day 6 of treatment. Remove 3 samples of 44 fruit.
08 Apr 2006	1°C	1STS	Day 7 of treatment. Remove 3 samples of 44 fruit.
			Day 8 of treatment. Remove 3 samples of 44 fruit.
			1st sieving of control fruits.
09 Apr 2006	1°C	1STS	Day 9 of treatment. Remove 3 samples of 44 fruit.
10 Apr 2006	1°C	1STS	Day 10 of treatment. Remove 3 samples of 44 fruit.
11 Apr 2006	1°C	1STS	Day 11 of treatment.
12 Apr 2006	1°C	1STS	Day 12 of treatment. Remove 3 samples of 44 fruit.
13 Apr 2006	1°C	1STS	1st sieving of Day 3 treatments.
14 Apr 2006	1°C	1STS	Day 14 of treatment. Remove 3 samples of 44 fruit.
			1st sieving of Day 4 treatments.
15 Apr 2006	1°C	1STS	1st sieving of Day 5 treatments.
16 Apr 2006	1°C	1STS	1st sieving of Day 6 treatments.
17 Apr 2006	1°C	1STS	1st sieving of Day 7 treatments.
			2nd sieving of control fruits.
18 Apr 2006	1°C	1STS	1st sieving of Day 8 treatments.
19 Apr 2006	1°C	1STS	1st sieving of Day 9 treatments.
20 Apr 2006	1°C	1STS	1st sieving of Day 10 treatments.
			2nd sieving of Day 3 treatments.
21 Apr 2006	1°C	1STS	2nd sieving of Day 4 treatments.
22 Apr 2006	1°C	1STS	1st sieving of Day 12 treatments.
			2nd sieving of Day 5 treatments.
23 Apr 2006	1°C	1STS	2nd sieving of Day 6 treatments.
24 Apr 2006	1°C	1STS	1st sieving of Day 14 treatments.
			2nd sieving of Day 7 treatments.
25 Apr 2006	1°C	1STS	2nd sieving of Day 8 treatments.
26 Apr 2006	1°C	1STS	2nd sieving of Day 9 treatments.
27 Apr 2006	1°C	1STS	2nd sieving of Day 10 treatments.
28 Apr 2006	1°C	1STS	No activity.
29 Apr 2006	1°C	1STS	2nd sieving of Day 12 treatments.
30 Apr 2006	1°C	1STS	No activity.
02 May 2006	1°C	1STS	2nd sieving of Day 14 treatments.
-----			
29 Mar 2006	1°C	2NDS	Infest approx. 1600 fruit on cages at GHI.
30 Mar 2006	1°C	2NDS	Remove fruit from cages, randomly select sample of 276 fruit as controls (non-cold treated fruit).
			Control fruits into hot storage room.
			Treatment units into hot storage room.
31 Mar 2006	1°C	2NDS	Larvae developing in fruit.
01 Apr 2006	1°C	2NDS	Larvae developing in fruit.
02 Apr 2006	1°C	2NDS	Larvae developing in fruit.
03 Apr 2006	1°C	2NDS	Treatment units into treatment at 1°C.
04 Apr 2006	1°C	2NDS	Day 1 of treatment.
05 Apr 2006	1°C	2NDS	Day 2 of treatment.
06 Apr 2006	1°C	2NDS	Day 3 of treatment. Remove 3 samples of 44 fruit.
07 Apr 2006	1°C	2NDS	Day 4 of treatment. Remove 3 samples of 44 fruit.
08 Apr 2006	1°C	2NDS	Day 5 of treatment. Remove 3 samples of 44 fruit.

09 Apr 2006	1°C	2NDS	Day 6 of treatment. Remove 3 samples of 44 fruit.
10 Apr 2006	1°C	2NDS	Day 7 of treatment. Remove 3 samples of 44 fruit. 1st sieving of control fruits.
11 Apr 2006	1°C	2NDS	Day 8 of treatment. Remove 3 samples of 44 fruit.
12 Apr 2006	1°C	2NDS	Day 9 of treatment. Remove 3 samples of 44 fruit.
13 Apr 2006	1°C	2NDS	Day 10 of treatment. Remove 3 samples of 44 fruit.
14 Apr 2006	1°C	2NDS	Day 11 of treatment.
15 Apr 2006	1°C	2NDS	Day 12 of treatment. Remove 3 samples of 44 fruit. 1st sieving of Day 3 treatments.
16 Apr 2006	1°C	2NDS	1st sieving of Day 4 treatments.
17 Apr 2006	1°C	2NDS	Day 14 of treatment. Remove 3 samples of 44 fruit. 1st sieving of Day 5 treatments. 2nd sieving of control fruits.
18 Apr 2006	1°C	2NDS	1st sieving of Day 6 treatments.
19 Apr 2006	1°C	2NDS	1st sieving of Day 7 treatments.
20 Apr 2006	1°C	2NDS	1st sieving of Day 8 treatments.
21 Apr 2006	1°C	2NDS	1st sieving of Day 9 treatments.
22 Apr 2006	1°C	2NDS	1st sieving of Day 10 treatments. 2nd sieving of Day 3 treatments.
23 Apr 2006	1°C	2NDS	2nd sieving of Day 4 treatments.
24 Apr 2006	1°C	2NDS	1st sieving of Day 12 treatments. 2nd sieving of Day 5 treatments.
25 Apr 2006	1°C	2NDS	2nd sieving of Day 6 treatments.
26 Apr 2006	1°C	2NDS	1st sieving of Day 14 treatments. 2nd sieving of Day 7 treatments.
27 Apr 2006	1°C	2NDS	2nd sieving of Day 8 treatments.
28 Apr 2006	1°C	2NDS	2nd sieving of Day 9 treatments.
29 Apr 2006	1°C	2NDS	2nd sieving of Day 10 treatments.
30 Apr 2006	1°C	2NDS	No activity.
01 May 2006	1°C	2NDS	2nd sieving of Day 12 treatments.
02 May 2006	1°C	2NDS	No activity
03 May 2006	1°C	2NDS	2nd sieving of Day 14 treatments.
<hr/>			
02 May 2006	1°C	3RDS	Infest approx. 1800 fruit on cages at GHI.
03 May 2006	1°C	3RDS	Remove fruit from cages, randomly select sample of 300 fruit as controls (non-cold treated fruit). Control fruits into hot storage room. Treatment units into hot storage room.
04 May 2006	1°C	3RDS	Larvae developing in fruit.
05 May 2006	1°C	3RDS	Larvae developing in fruit.
06 May 2006	1°C	3RDS	Larvae developing in fruit.
07 May 2006	1°C	3RDS	Larvae developing in fruit.
08 May 2006	1°C	3RDS	Larvae developing in fruit.
09 May 2006	1°C	3RDS	Treatment units into treatment at 1°C.
10 May 2006	1°C	3RDS	Day 1 of treatment.
11 May 2006	1°C	3RDS	Day 2 of treatment.
12 May 2006	1°C	3RDS	Day 3 of treatment. Remove 3 samples of 50 fruit. 1st sieving of control fruits.
13 May 2006	1°C	3RDS	Day 4 of treatment. Remove 3 samples of 50 fruit.
14 May 2006	1°C	3RDS	Day 5 of treatment. Remove 3 samples of 50 fruit.
15 May 2006	1°C	3RDS	Day 6 of treatment. Remove 3 samples of 50 fruit.
16 May 2006	1°C	3RDS	Day 7 of treatment. Remove 3 samples of 50 fruit.
17 May 2006	1°C	3RDS	Day 8 of treatment. Remove 3 samples of 50 fruit.
18 May 2006	1°C	3RDS	Day 9 of treatment. Remove 3 samples of 50 fruit.
19 May 2006	1°C	3RDS	Day 10 of treatment. Remove 3 samples of 50 fruit.
20 May 2006	1°C	3RDS	Day 11 of treatment.

21 May 2006	1°C	3RDS	Day 12 of treatment. Remove 3 samples of 50 fruit.
22 May 2006	1°C	3RDS	1st sieving of Day 3 treatments.
23 May 2006	1°C	3RDS	Day 14 of treatment. Remove 3 samples of 50 fruit.
			1st sieving of Day 4 treatments.
24 May 2006	1°C	3RDS	1st sieving of Day 5 treatments.
25 May 2006	1°C	3RDS	1st sieving of Day 6 treatments.
26 May 2006	1°C	3RDS	1st sieving of Day 7 treatments.
			2nd sieving of control fruits.
27 May 2006	1°C	3RDS	1st sieving of Day 8 treatments.
28 May 2006	1°C	3RDS	1st sieving of Day 9 treatments.
29 May 2006	1°C	3RDS	1st sieving of Day 10 treatments.
			2nd sieving of Day 3 treatments.
30 May 2006	1°C	3RDS	2nd sieving of Day 4 treatments.
31 May 2006	1°C	3RDS	2nd sieving of Day 5 treatments.
			1st sieving of Day 12 treatments.
01 Jun 2006	1°C	3RDS	2nd sieving of Day 6 treatments.
02 Jun 2006	1°C	3RDS	2nd sieving of Day 7 treatments.
			1st sieving of Day 14 treatments.
03 Jun 2006	1°C	3RDS	2nd sieving of Day 8 treatments.
04 Jun 2006	1°C	3RDS	2nd sieving of Day 9 treatments.
05 Jun 2006	1°C	3RDS	2nd sieving of Day 10 treatments.
06 Jun 2006	1°C	3RDS	No activity.
07 Jun 2006	1°C	3RDS	2nd sieving of Day 12 treatments.
08 Jun 2006	1°C	3RDS	No activity.
09 Jun 2006	1°C	3RDS	2nd sieving of Day 14 treatments.

**Table 2. Experimental schedule of activities for most tolerant life-stage trials at 3°C (1STS: first instar larvae; 2NDS: second instar larvae; and 3RDS: third instar larvae).**

Date	Temperature	Life-stage	Activity
13 Mar 2006	3°C	EGGS	Infest approx. 1800 fruit on cages at GHI.
14 Mar 2006	3°C	EGGS	Remove fruit from cages, randomly select sample of 300 fruit as Controls (non-cold treated fruit). Control fruits into hot storage room. Treatment units into hot storage room.
15 Mar 2006	3°C	EGGS	Treatment units into treatment at 3°C.
16 Mar 2006	3°C	EGGS	Day 1 of treatment.
17 Mar 2006	3°C	EGGS	Day 2 of treatment.
18 Mar 2006	3°C	EGGS	Day 3 of treatment. Remove 3 samples of 46 fruit.
19 Mar 2006	3°C	EGGS	Day 4 of treatment. Remove 3 samples of 46 fruit.
20 Mar 2006	3°C	EGGS	Day 5 of treatment. Remove 3 samples of 46 fruit.
21 Mar 2006	3°C	EGGS	Day 6 of treatment. Remove 3 samples of 46 fruit.
22 Mar 2006	3°C	EGGS	Day 7 of treatment. Remove 3 samples of 46 fruit.
23 Mar 2006	3°C	EGGS	Day 8 of treatment. Remove 3 samples of 46 fruit.
24 Mar 2006	3°C	EGGS	Day 9 of treatment. Remove 3 samples of 46 fruit. 1st sieving of control fruits.
25 Mar 2006	3°C	EGGS	Day 10 of treatment. Remove 3 samples of 46 fruit.
26 Mar 2006	3°C	EGGS	Day 11 of treatment.
27 Mar 2006	3°C	EGGS	Day 12 of treatment. Remove 3 samples of 46 fruit.
28 Mar 2006	3°C	EGGS	Day 13 of treatment. 1st sieving of Day 3 treatments.
29 Mar 2006	3°C	EGGS	Day 14 of treatment. Remove 3 samples of 46 fruit. 1st sieving of Day 4 treatments.
30 Mar 2006	3°C	EGGS	1st sieving of Day 5 treatments.
31 Mar 2006	3°C	EGGS	1st sieving of Day 6 treatments. 2nd sieving of control fruits.
01 Apr 2006	3°C	EGGS	1st sieving of Day 7 treatments.
02 Apr 2006	3°C	EGGS	1st sieving of Day 8 treatments.
03 Apr 2006	3°C	EGGS	1st sieving of Day 9 treatments.
04 Apr 2006	3°C	EGGS	1st sieving of Day 10 treatments. 2nd sieving of Day 3 treatments.
05 Apr 2006	3°C	EGGS	2nd sieving of Day 4 treatments.
06 Apr 2006	3°C	EGGS	1st sieving of Day 12 treatments. 2nd sieving of Day 5 treatments.
07 Apr 2006	3°C	EGGS	2nd sieving of Day 6 treatments.
08 Apr 2006	3°C	EGGS	1st sieving of Day 14 treatments. 2nd sieving of Day 7 treatments.
09 Apr 2006	3°C	EGGS	2nd sieving of Day 8 treatments.
10 Apr 2006	3°C	EGGS	2nd sieving of Day 9 treatments.
11 Apr 2006	3°C	EGGS	2nd sieving of Day 10 treatments.
12 Apr 2006	3°C	EGGS	No activity.
13 Apr 2006	3°C	EGGS	2nd sieving of Day 12 treatments.
14 Apr 2006	3°C	EGGS	No activity.
15 Apr 2006	3°C	EGGS	2nd sieving of Day 14 treatments.
<hr/>			
13 Mar 2006	3°C	1STS	Infest approx. 1800 fruit on cages at GHI.
14 Mar 2006	3°C	1STS	Remove fruit from cages, randomly select sample of 300 fruit as controls (non-cold treated fruit).

			Control fruits into hot storage room. Treatment units into hot storage room.
15 Mar 2006	3°C	1STS	Larvae developing in fruit.
16 Mar 2006	3°C	1STS	Larvae developing in fruit.
17 Mar 2006	3°C	1STS	Treatment units into treatment at 3°C.
18 Mar 2006	3°C	1STS	Day 1 of treatment.
19 Mar 2006	3°C	1STS	Day 2 of treatment.
20 Mar 2006	3°C	1STS	Day 3 of treatment. Remove 3 samples of 46 fruit.
21 Mar 2006	3°C	1STS	Day 4 of treatment. Remove 3 samples of 46 fruit.
22 Mar 2006	3°C	1STS	Day 5 of treatment. Remove 3 samples of 46 fruit.
23 Mar 2006	3°C	1STS	Day 6 of treatment. Remove 3 samples of 46 fruit.
24 Mar 2006	3°C	1STS	Day 7 of treatment. Remove 3 samples of 46 fruit. 1st sieving of control fruits.
25 Mar 2006	3°C	1STS	Day 8 of treatment. Remove 3 samples of 46 fruit.
26 Mar 2006	3°C	1STS	Day 9 of treatment. Remove 3 samples of 46 fruit.
27 Mar 2006	3°C	1STS	Day 10 of treatment. Remove 3 samples of 46 fruit.
28 Mar 2006	3°C	1STS	Day 11 of treatment.
29 Mar 2006	3°C	1STS	Day 12 of treatment. Remove 3 samples of 46 fruit.
30 Mar 2006	3°C	1STS	Day 13 of treatment. 1st sieving of Day 3 treatments.
31 Mar 2006	3°C	1STS	Day 14 of treatment. Remove 3 samples of 46 fruit. 1st sieving of Day 4 treatments. Sieve control fruits.
01 Apr 2006	3°C	1STS	1st sieving of Day 5 treatments.
02 Apr 2006	3°C	1STS	1st sieving of Day 6 treatments.
03 Apr 2006	3°C	1STS	1st sieving of Day 7 treatments.
04 Apr 2006	3°C	1STS	1st sieving of Day 8 treatments.
05 Apr 2006	3°C	1STS	1st sieving of Day 9 treatments.
06 Apr 2006	3°C	1STS	1st sieving of Day 10 treatments. 2nd sieving of Day 3 treatments.
07 Apr 2006	3°C	1STS	2nd sieving of Day 4 treatments.
08 Apr 2006	3°C	1STS	1st sieving of Day 12 treatments. 2nd sieving of Day 5 treatments.
09 Apr 2006	3°C	1STS	2nd sieving of Day 6 treatments.
10 Apr 2006	3°C	1STS	1st sieving of Day 14 treatments. 2nd sieving of Day 7 treatments.
11 Apr 2006	3°C	1STS	2nd sieving of Day 8 treatments.
12 Apr 2006	3°C	1STS	No activity.
13 Apr 2006	3°C	1STS	2nd sieving of Day 9 treatments.
14 Apr 2006	3°C	1STS	No activity.
15 Apr 2006	3°C	1STS	2nd sieving of Day 10 treatments.
17 Apr 2006	3°C	1STS	2nd sieving of Day 12 treatments.
19 Apr 2006	3°C	1STS	2nd sieving of Day 14 treatments.
<hr/>			
16 Mar 2006	3°C	2NDS	Infest approx. 1800 fruit on cages at GHI.
17 Mar 2006	3°C	2NDS	Remove fruit from cages, randomly select sample of 300 fruit as controls (non-cold treated fruit). Control fruits into hot storage room. Treatment units into hot storage room.
18 Mar 2006	3°C	2NDS	Larvae developing in fruit.
19 Mar 2006	3°C	2NDS	Larvae developing in fruit.
20 Mar 2006	3°C	2NDS	Larvae developing in fruit.
21 Mar 2006	3°C	2NDS	Treatment units into treatment at 3°C.
22 Mar 2006	3°C	2NDS	Day 1 of treatment.
23 Mar 2006	3°C	2NDS	Day 2 of treatment.
24 Mar 2006	3°C	2NDS	Day 3 of treatment. Remove 3 samples of 46 fruit.

25 Mar 2006	3°C	2NDS	Day 4 of treatment. Remove 3 samples of 46 fruit.
26 Mar 2006	3°C	2NDS	Day 5 of treatment. Remove 3 samples of 46 fruit.
27 Mar 2006	3°C	2NDS	Day 6 of treatment. Remove 3 samples of 46 fruit.
28 Mar 2006	3°C	2NDS	Day 7 of treatment. Remove 3 samples of 46 fruit.
29 Mar 2006	3°C	2NDS	Day 8 of treatment. Remove 3 samples of 46 fruit.
			1st sieving of control fruits.
30 Mar 2006	3°C	2NDS	Day 9 of treatment. Remove 3 samples of 46 fruit.
31 Mar 2006	3°C	2NDS	Day 10 of treatment. Remove 3 samples of 46 fruit.
01 Apr 2006	3°C	2NDS	Day 11 of treatment.
02 Apr 2006	3°C	2NDS	Day 12 of treatment. Remove 3 samples of 46 fruit.
03 Apr 2006	3°C	2NDS	Day 13 of treatment.
04 Apr 2006	3°C	2NDS	Day 14 of treatment. Remove 3 samples of 46 fruit.
			1st sieving of Day 3 treatments.
05 Apr 2006	3°C	2NDS	2nd sieving of control fruits.
			1st sieving of Day 4 treatments.
06 Apr 2006	3°C	2NDS	1st sieving of Day 5 treatments.
07 Apr 2006	3°C	2NDS	1st sieving of Day 6 treatments.
08 Apr 2006	3°C	2NDS	1st sieving of Day 7 treatments.
09 Apr 2006	3°C	2NDS	1st sieving of Day 8 treatments.
10 Apr 2006	3°C	2NDS	1st sieving of Day 9 treatments.
	3°C	2NDS	1st sieving of Day 10 treatments.
11 Apr 2006			2nd sieving of Day 3 treatments.
	3°C	2NDS	1st sieving of Day 12 treatments.
12 Apr 2006			2nd sieving of Day 4 treatments.
	3°C	2NDS	1st sieving of Day 14 treatments.
13 Apr 2006			2nd sieving of Day 5 treatments.
14 Apr 2006	3°C	2NDS	2nd sieving of Day 6 treatments.
15 Apr 2006	3°C	2NDS	2nd sieving of Day 7 treatments.
16 Apr 2006	3°C	2NDS	2nd sieving of Day 8 treatments.
17 Apr 2006	3°C	2NDS	2nd sieving of Day 9 treatments.
18 Apr 2006	3°C	2NDS	2nd sieving of Day 10 treatments.
19 Apr 2006	3°C	2NDS	No activity.
20 Apr 2006	3°C	2NDS	2nd sieving of Day 12 treatments.
21 Apr 2006	3°C	2NDS	No activity.
22 Apr 2006	3°C	2NDS	2nd sieving of Day 14 treatments.
<hr/>			
16 Mar 2006	3°C	3RDS	Infest approx. 1800 fruit on cages at GHI.
17 Mar 2006	3°C	3RDS	Remove fruit from cages, randomly select sample of 300 fruit as controls (non-cold treated fruit). Control fruits into hot storage room. Treatment units into hot storage room.
18 Mar 2006	3°C	3RDS	Larvae developing in fruit.
19 Mar 2006	3°C	3RDS	Larvae developing in fruit.
20 Mar 2006	3°C	3RDS	Larvae developing in fruit.
21 Mar 2006	3°C	3RDS	Larvae developing in fruit.
22 Mar 2006	3°C	3RDS	Larvae developing in fruit.
23 Mar 2006	3°C	3RDS	Treatment units into treatment at 3°C.
24 Mar 2006	3°C	3RDS	Day 1 of treatment.
25 Mar 2006	3°C	3RDS	Day 2 of treatment.
26 Mar 2006	3°C	3RDS	Day 3 of treatment. Remove 3 samples of 46 fruit.
27 Mar 2006	3°C	3RDS	Day 4 of treatment. Remove 3 samples of 46 fruit.
28 Mar 2006	3°C	3RDS	Day 5 of treatment. Remove 3 samples of 46 fruit.
29 Mar 2006	3°C	3RDS	Day 6 of treatment. Remove 3 samples of 46 fruit.
			1st sieving of control fruits.
30 Mar 2006	3°C	3RDS	Day 7 of treatment. Remove 3 samples of 46 fruit.
31 Mar 2006	3°C	3RDS	Day 8 of treatment. Remove 3 samples of 46 fruit.

01 Apr 2006	3°C	3RDS	Day 9 of treatment. Remove 3 samples of 46 fruit.
02 Apr 2006	3°C	3RDS	Day 10 of treatment. Remove 3 samples of 46 fruit.
03 Apr 2006	3°C	3RDS	Day 11 of treatment.
04 Apr 2006	3°C	3RDS	Day 12 of treatment. Remove 3 samples of 46 fruit.
05 Apr 2006	3°C	3RDS	Day 13 of treatment.
06 Apr 2006	3°C	3RDS	2nd sieving of control fruits. Day 14 of treatment. Remove 3 samples of 46 fruit.
07 Apr 2006	3°C	3RDS	1st sieving of Day 3 treatments.
08 Apr 2006	3°C	3RDS	1st sieving of Day 4 treatments.
09 Apr 2006	3°C	3RDS	1st sieving of Day 5 treatments.
10 Apr 2006	3°C	3RDS	1st sieving of Day 6 treatments.
11 Apr 2006	3°C	3RDS	1st sieving of Day 7 treatments.
12 Apr 2006	3°C	3RDS	1st sieving of Day 8 treatments.
13 Apr 2006	3°C	3RDS	1st sieving of Day 9 treatments.
14 Apr 2006	3°C	3RDS	1st sieving of Day 10 treatments.
15 Apr 2006	3°C	3RDS	2nd sieving of Day 3 treatments.
16 Apr 2006	3°C	3RDS	2nd sieving of Day 4 treatments.
17 Apr 2006	3°C	3RDS	1st sieving of Day 12 treatments.
18 Apr 2006	3°C	3RDS	2nd sieving of Day 5 treatments.
19 Apr 2006	3°C	3RDS	2nd sieving of Day 6 treatments.
20 Apr 2006	3°C	3RDS	1st sieving of Day 14 treatments.
21 Apr 2006	3°C	3RDS	2nd sieving of Day 7 treatments.
22 Apr 2006	3°C	3RDS	2nd sieving of Day 8 treatments.
23 Apr 2006	3°C	3RDS	2nd sieving of Day 9 treatments.
24 Apr 2006	3°C	3RDS	2nd sieving of Day 10 treatments.
25 Apr 2006	3°C	3RDS	No activity.
			2nd sieving of Day 12 treatments.
			No activity.
			2nd sieving of Day 14 treatments.

**Table 3. Experimental schedule of activities for large scale trial on Sweetheart cherries at 1°C conducted using first instar larvae.**

Date	Replicate	Activity
26 Feb 2007	1	Infest fruit on cages at GHI.
27 Feb 2007	1	Remove fruit from cages, place in hot storage room.
02 Mar 2007	1	Larval development study 88% first instars (see Part 3, Table 4).
03 Mar 2007	1	Test fruit into 1°C, controls into hot storage room. Test fruit reaches target temperature, day zero of treatment.
09 Mar 2007	1	1st sieving of controls.
16 Mar 2007	1	2nd sieving of controls, dissect fruit for survivors.
16 Mar 2007	1	14 day treatment ends. Into hot storage room.
27 Mar 2007	1	1st sieving of treated fruits.
03 Apr 2007	1	2nd sieving of treated fruits, dissect fruits for survivors.
.....		
06 Mar 2007	2	Infest fruit on cages at GHI.
07 Mar 2007	2	Remove fruit from cages, place in hot storage room.
10 Mar 2007	2	Larval development study 88% first instars (see Part 3, Table 4).
11 Mar 2007	2	Test fruit into 1°C, controls into hot storage room. Test fruit reaches target temperature, day zero of treatment.
16 Mar 2007	2	1st sieving of controls.
24 Mar 2007	2	2nd sieving of controls, dissect fruit for survivors.
25 Mar 2007	2	14 day treatment ends. Into hot storage room.
04 Apr 2007	2	1st sieving of treated fruits.
11 Apr 2007	2	2nd sieving of treated fruits, dissect fruits for survivors.
.....		
12 Mar 2007	3	Infest fruit on cages at GHI.
13 Mar 2007	3	Remove fruit from cages, place in hot storage room.
16 Mar 2007	3	Larval development study 86% first instars (see Part 3, Table 4).
17 Mar 2007	3	Test fruit into 1°C, controls into hot storage room. Test fruit reaches target temperature, day zero of treatment.
23 Mar 2007	3	1st sieving of controls.
30 Mar 2007	3	2nd sieving of controls, dissect fruit for survivors.
31 Mar 2007	3	14 day treatment ends. Into hot storage room.
11 Apr 2007	3	1st sieving of treated fruits.
18 Apr 2007	3	2nd sieving of treated fruits, dissect fruits for survivors.

**Table 4. Experimental schedule of activities for large scale trial on Sweetheart cherries at 3°C conducted using first instar larvae.**

Date	Replicate	Activity
02 Jan 2007	1	Infest 2840 fruit on cages at GHI.
03 Jan 2007	1	Remove fruit from cages, place in hot storage room.
08 Jan 2007	1	Larval development study 86% first instars (see Part 3, Table 11).
		Test fruit into 3°C, controls into hot storage room.
09 Jan 2007	1	Test fruit reaches target temperature, day zero of treatment.
15 Jan 2007	1	1st sieving of controls.
22 Jan 2007	1	2nd sieving of controls, dissect fruit for survivors.
23 Jan 2007	1	14 day treatment ends. Into hot storage room.
02 Feb 2007	1	1st sieving of treated fruits.
09 Feb 2007	1	2nd sieving of treated fruits, dissect fruits for survivors.
.....		
09 Jan 2007	2	Infest 3102 fruit on cages at GHI.
10 Jan 2007	2	Remove fruit from cages, place in hot storage room.
15 Jan 2007	2	Larval development study 80% first instars (see Part 3, Table 11).
		Test fruit into 3°C, controls into hot storage room.
16 Jan 2007	2	Test fruit reaches target temperature, day zero of treatment.
22 Jan 2007	2	1st sieving of controls.
29 Jan 2007	2	2nd sieving of controls, dissect fruit for survivors.
30 Jan 2007	2	14 day treatment ends. Into hot storage room.
09 Feb 2007	2	1st sieving of treated fruits.
16 Feb 2007	2	2nd sieving of treated fruits, dissect fruits for survivors.
.....		
06 Feb 2007	3	Infest 2840 fruit on cages at GHI.
07 Feb 2007	3	Remove fruit from cages, place in hot storage room.
10 Feb 2007	3	Larval development study 67% first instars (see Part 3, Table 11).
		Test fruit into 3°C, controls into hot storage room.
11 Feb 2007	3	Test fruit reaches target temperature, day zero of treatment.
19 Feb 2007	3	1st sieving of controls.
25 Feb 2007	3	14 day treatment ends. Into hot storage room.
26 Feb 2007	3	2nd sieving of controls, dissect fruit for survivors.
07 Mar 2007	3	1st sieving of treated fruits.
14 Mar 2007	3	2nd sieving of treated fruits, dissect fruits for survivors.
.....		
20 Feb 2007	4	Infest 2840 fruit on cages at GHI.
21 Feb 2007	4	Remove fruit from cages, place in hot storage room.
23 Feb 2007	4	Larval development study 88% first instars (see Part 3, Table 11).
		Test fruit into 3°C, controls into hot storage room.
24 Feb 2007	4	Test fruit reaches target temperature, day zero of treatment.
05 Mar 2007	4	1st sieving of controls.
10 Mar 2007	4	14 day treatment ends. Into hot storage room.
12 Mar 2007	4	2nd sieving of controls, dissect fruit for survivors.
20 Mar 2007	4	1st sieving of treated fruits.
27 Mar 2007	4	2nd sieving of treated fruits, dissect fruits for survivors.

**Table 5. Key dates and location of large scale trials at 1°C and 3°C.**

<i>Fruit</i>	<i>Replicate</i>	<i>Treatment</i>	<i>Infestation date</i>	<i>Fruit into treatment</i>	<i>Fruit reaches target temperature</i>	<i>Treatment ends</i>	<i>Room number</i>
<b><i>Cherries</i></b>	1	14 d at 1°C	26 Feb 2007	02 Mar 2007	03 Mar 2007	17 Mar 2007	CTR 9
	2	14 d at 1°C	06 Mar 2007	10 Mar 2007	11 Mar 2007	25 Mar 2007	CTR 9
	3	14 d at 1°C	12 Mar 2007	16 Mar 2007	17 Mar 2007	31 Mar 2007	CTR 9

<i>Fruit</i>	<i>Replicate</i>	<i>Treatment</i>	<i>Infestation date</i>	<i>Fruit into treatment</i>	<i>Fruit reaches target temperature</i>	<i>Treatment ends</i>	<i>Room number</i>
<b><i>Cherries</i></b>	1	14 d at 3°C	02 Jan 2007	08 Jan 2007	09 Jan 2007	23 Jan 2007	CTR 8
	2	14 d at 3°C	09 Jan 2007	15 Jan 2007	16 Jan 2007	30 Jan 2007	CTR 8
	3	14 d at 3°C	06 Feb 2007	10 Feb 2007	11 Feb 2007	25 Feb 2007	CTR 8
	4	14 d at 3°C	20 Feb 2007	23 Feb 2007	24 Feb 2007	10 Mar 2007	CTR 8

#### 4.18. Data analysis

Bioassay data were obtained from the most tolerant life-stage studies where cherry fruit containing the four immature *B. tryoni* life-stages - eggs, first, second, and third instars – were subjected to sub-lethal and lethal periods of storage at  $1.0\pm0.5^{\circ}\text{C}$  or at  $3.0\pm0.5^{\circ}\text{C}$ .

These data were analysed by probit regression analysis. Values for Probit 9 mortality were determined.

#### 4.19. Quality of fruit after treatment

Fifty non-infested cherry fruit were randomly chosen from each of the three replicates and assessed for quality attributes prior to storage and then again after 14 and 21 days in cold storage. These fruit were stored with infested fruit in replicates 1, 2 and 3 of the large scale trials at  $1^{\circ}\text{C}$  and  $3^{\circ}\text{C}$ .

Cherry fruit were assessed for these quality attributes:

- a) **Peduncle discoloration:** scored from 1 to 10, where 1 indicated 0-10% discoloration or damage, and 10 indicated 90-100% discoloration or damage). Fruit with a peduncle discoloration score greater than 5 (40-50% of the peduncle was brown, or dried out) were classed as unmarketable.
- b) **External damage** i.e. pitting, browning or splitting: scored from 1 (0% surface area damaged) to 5 (100% surface area damaged).
- c) **Degree of peduncle abscission** i.e. a measure of how susceptible the stem is to becoming dislodged from the fruit: quantified using a clamping device which held the fruit while the peduncle was removed. The force required to remove the peduncle was measured in grams.
- d) **Skin colour:** scored from 1 (completely black) to 5 (completely red).

Data were analysed by analysis of variance where differences between treatment means were significant if  $P<0.05$ . K-lsd values were calculated at  $k=100$  using the Duncan-Waller Bayesian k-ratio test.

## 5. Results

### 5.1. Larval development

Larvae developed normally at 26°C (Part 1, Table 6). Eggs and first instar larvae were always the dominant life-stages within infested fruit at 1 and 3 days after infestation respectively. Second instar larvae and third instar larvae often occurred together in infested cherry fruit, but on days 5 and 7 after infestation there were more than 50% at each stage respectively.

**Table 6. Approximate days post-infestation at 26°C for insects to develop to target life-stage for most tolerant life-stage trial.**

Fruit	Egg	1 <sup>st</sup> instar	2 <sup>nd</sup> Instar	3 <sup>rd</sup> Instar
Cherries	0-2	2-4	4-6	6+

[For more information refer to Part 3 – Summary tables – as below]

**Table numbers for larval development studies for most tolerant stage and large scale trials in Part 3**

Fruit	Larval development studies	
	1°C	3°C
Cherries	Most tolerant stage: Part 3, Table 1	Most tolerant stage: Part 3, Table 1
	Large scale trials Part 3, Table 4	Large scale trials Part 3, Table 11

### 5.2. Most tolerant stage

First instar larvae were identified as the most tolerant stage for both the 1°C and 3°C treatments (Part 1, Table 7).

**Table 7. Probit mortality (Probit 9) against days in cold storage (non-transformed) shows the most tolerant stage of the fruit fly.**

Sweetheart Cherries	REGRESSION FORMULA	REGRESSION CO-EFF (R <sup>2</sup> )	SLOPE	Y- INTERCEPT	Est. Probit 9 <sup>a</sup>
<b>1°C</b>					
<b>EGG</b>	y=0.6171x+5.0300	0.9591	0.6171	5.0300	6.43
<b>FIRST</b>	<b>y=0.3875x+5.0525</b>	<b>0.8820</b>	<b>0.3875</b>	<b>5.0525</b>	<b>10.19</b>
<b>SECOND</b>	y=0.4802x+5.1014	0.9850	0.4802	5.1014	8.12
<b>THIRD</b>	y=0.5077x+5.5889	0.9062	0.5077	5.5889	7.62
<b>3°C</b>					
<b>EGG</b>	y=0.5268x+4.7457	0.9097	0.5268	4.7470	8.07
<b>FIRST</b>	<b>y=0.3077x+5.6389</b>	<b>0.9084</b>	<b>0.3077</b>	<b>5.6389</b>	<b>10.92</b>
<b>SECOND</b>	y=0.3123x+5.5935	0.8966	0.3123	5.9350	9.81
<b>THIRD</b>	y=0.3323x+5.8511	0.8650	0.3323	5.8511	9.48

<sup>a</sup> Est. Probit 9: Estimate of the number of days at the target temperature to reach Probit 9 (i.e. 99.99683%) mortality

[For more information refer to Part 3 – Summary tables – as below]

**Table numbers referring to most tolerant stage studies in Part 3**

Fruit	Most tolerant stage trials	
	1°C	3°C
Cherries	Part 3, Table 2, 3	Part 3, Table 9, 10

### 5.3. Large scale confirmation of treatment efficacy

The authorised experimental protocols state that there should be nil survival from replicate trials on a total of 30,000 insects treated at the most tolerant stage. Part 1, Table 8 shows that the number of insects treated exceeded the authorised experimental protocols. All large scale trials resulted in nil survival.

**Table 8. Demonstration of treatment efficacy**

<i><b>Fruit (Replicate)</b></i>	<i><b>No. fruit treated at 1°C</b></i>	<i><b>Average no. of insects treated per fruit <math>\pm</math> SD*</b></i>	<i><b>Estimated no. of insects treated at 1°C</b></i>	<i><b>No. insects surviving treatment at 1°C</b></i>
<i>Cherries (1)</i>	1600	20.06 $\pm$ 1.48	32101	0
<i>Cherries (2)</i>	1494	8.80 $\pm$ 0.43	13154	0
<i>Cherries (3)</i>	2086	9.05 $\pm$ 1.24	18864	0
<b>Total</b>	<b>5180</b>	-	<b>64119</b>	<b>0</b>

<i><b>Fruit (Replicate)</b></i>	<i><b>No. fruit treated at 3°C</b></i>	<i><b>Average no. of insects treated per fruit <math>\pm</math> SD *</b></i>	<i><b>Estimated no. of insects treated at 3°C</b></i>	<i><b>No. insects surviving treatment at 3°C</b></i>
<i>Cherries (1)</i>	2400	7.92 $\pm$ 0.76	18998	0
<i>Cherries (2)</i>	2582	9.77 $\pm$ 0.57	25229	0
<i>Cherries (3)</i>	2400	2.42 $\pm$ 0.86	5798	0
<i>Cherries (4)</i>	2400	21.81 $\pm$ 2.63 *	52347	0
<b>Total</b>	<b>9782</b>	-	<b>102372</b>	<b>0</b>

\* The average number of insects per fruit in the controls has been rounded to 2 decimal places

[For more information refer to Part 3 – Summary tables – as below]

**Table numbers referring to large scale trial studies in Part 3**

Fruit	Large scale trials	
	1°C	3°C
Cherries	Part 3, Tables 5, 6, 7, 8	Part 3, Tables 12, 13, 14, 15, 16

## 5.4 Temperature records

Summaries of the temperature records are presented in Part 3 as referred to in the table below. There was some temperature variation in the cold rooms with some temperature probes recording above the target temperatures, even after calibration correction. Data loggers recorded the vast bulk of the time within the target temperature  $\pm 0.5^{\circ}\text{C}$ . Temperatures very rarely went below  $0.5^{\circ}\text{C}$ .

Temperature variation within the room is due mainly to the position of the test fruit in the room and the efficiency of air circulation around the room.

Because temperature data logger probes were placed at the centre of fruit, randomly within each carton of treated fruit, we have a close estimate of the temperature that the insects in the infested fruit were exposed to. The large scale trials showed nil (0) survival from all experiments. Temperature records show that some fruit did not experience 14 days at exactly  $1^{\circ}\text{C}$  or  $3^{\circ}\text{C}$  but at a slightly higher temperature, at least for part of that period. Treatment efficacy records show that there were no survivors from those fruit.

[For more information refer to Part 3 – Summary tables – as below]

**Table numbers referring to temperature records in Part 3**

Fruit	Temperature records	
	1°C	3°C
Cherries	Part 3, Tables 19, 20, 21	Part 3, Tables 22, 23, 24, 25

## 5.5. Fruit quality

Treating cherry fruit for 14 days at  $1^{\circ}\text{C}$  had no significant effect on quality attributes when compared to fruit quality prior to storage. A very minimal loss in fruit quality (slight peduncle discolouration and peduncle more easily removed) was observed in cherry fruit stored at  $3^{\circ}\text{C}$  for 14 days but fruit were still of a Class 1 marketable quality.

[For more information refer to Part 3, Table 17 – Summary tables]

## **6. Discussion**

### **6.1. Larval development**

There were differences in the rate of larval development in the cherries for the most tolerant stage and large scale trials. To verify that the correct life-stage was being treated in the large scale trials, a sample of five fruit were dissected and evaluated for larval development just prior to infested fruit being placed into cold storage. This ensured that the target life-stage was the dominant life-stage being treated.

### **6.2. Most treatment tolerant life-stage**

The first instar larvae were most cold-tolerant life-stage based on estimation of Probit 9 (LD<sub>99.99683</sub>) (Part 1, Table 7). Estimates of Probit 9 (LD<sub>99.99683</sub>) of dose / mortality responses of insects in these fruit showed that first instar larvae were the most cold tolerant life-stage.

### **6.3. Large scale trials**

First instar larvae were treated with cold storage at 1°C and 3°C. No insects survived treatment at either temperature when stored at that temperature for 14 days (Part 1, Table 8).

The results offer proof of efficacy for treatment at either 1°C for 14 days or 3°C for 14 days as a quarantine treatment against *B. tryoni* eggs and larvae for cherries.

### **6.4. Fruit quality**

Standard fruit descriptions are available for cherries (Part 1, Table 9). All fruit satisfied these quality parameters before and after the cold treatment and were classed as acceptable for export.

## 7. Product specifications

**Table 9. Product specifications for Sweetheart cherries**

**FreshSpecs**

**Produce Specifications**

PRODUCE: CHERRY

TYPE: Red

VARIETY: Sweetheart

CLASS: One

GENERAL APPEARANCE CRITERIA	
COLOUR	Mid red, light green stalks
VISUAL APPEARANCE	Full bodied; plump; stalks intact; sutures not excessively deep; no foreign matter.
SENSORY	With medium to firm, slightly raised skin texture; not soft or shrivelled; sweet to very sweet juicy flavour; no 'off' odours or tastes.
SHAPE	Round to heart shaped berries.
SIZE	Minimum 22mm diameter; Uniform size per package with a maximum of 2mm variance.
MATURITY	Firm and full coloured at receipt.
MAJOR DEFECTS	
INSECTS	With evidence of live insects, eg. Larvae.
DISEASES	With evidence of fungal or bacterial rots. (eg. Decaying areas).
PHYSICAL / PEST DAMAGE	With cuts or punctures (that break the skin) (wounds or pest damage). With rain or post harvest split
PHYSIOLOGICAL DISORDER	With skin softening, darkening, pitting (irregular brownish spots) (cool storage breakdown)
TEMPERATURE INJURY	With tissue shrivelling, softening and browning. (heat stress) With soft, dark water-soaked areas. (freeze injury)
MINOR DEFECTS	
DISEASES	With bacterial spot >2 dry spots (1mm); not sunken and water soaked. With superficial skin scarring due to hail, insect, bird damage >0.5 sq; no broken skin.
PHYSICAL / PEST DAMAGE	With slight depression/flattening of skin affecting > half visible surface. With surface pitting (circular depressions, surface wrinkling, impact bruising) >0.5 sq cm. With point cracking > 2 mm With ring split > 1mm width; > half surface area of cherry affected in length.
PHYSIOLOGICAL DISORDERS	With healed stem end cracks, vertical cracks > 1mm deep, wide; no unhealed splits/cracks. With post harvest unhealed point splits less than 2 mm.
SKIN MARKS / BLEMISHES	With superficial skin marks/blemish, eg. limb rub, leaf rub, scattered spot, speck >0.5 sq cm.
CONSIGNMENT CRITERIA	
TOLERANCE PER CONSIGNMENT	Total minor defects (within allowance limit) to be < 2 defects per item Total minor defects (outside allowance limit) must not exceed 10% of consignment. Total major defects must not exceed 2 % of consignment. Combined Total not to exceed 10%.
PACKAGING & LABELLING	Packaging manufactured from new food grade materials or sanitised returnable crates. All labelling must meet the current legislative requirements. Labelling to identify grower's name/brand (plus growers name/code if via a packhouse), address, contents, class, size and/or minimum net weight. Produce to identify Country of Origin (eg. Produce of Australia) on outer container.
SHELF LIFE	Produce must provide not less than 14 days clear shelf life from date of receipt.
RECEIVAL CONDITIONS	Compliance with Quarantine Treatments (if required) for Interstate Consignment. Stacked onto a stabilised pallet. Refrigerated van with air bag suspension, unless otherwise approved. Pulp Temperature 6 - 10°C for Receipt.
CHEMICAL & CONTAMINANT RESIDUES	All chemicals used pre/postharvest must be registered and approved for use in accordance with the requirements of the APVMA regulatory system. Residues, Contaminants and Heavy Metals to comply to the FSANZ Food Standards Code ML's and MRL's.
FOOD SAFETY REQUIREMENTS	Produce is to be grown and packed under a HACCP based food safety program that is subject to an annual third-party audit. A copy of current certification to be forwarded to receiver.
Specifications reviewable: eg. to account for specific regional effects or adverse seasonal impacts on quality or early or late seasonal variances as agreed and communicated formally in writing.	

Specifications released by the Australian Chamber of Fruit and Vegetable Industries (<http://www.freshmarkets.com.au/FreshSpec/freshspecs.html>)



**Cold treatment of Australian cherries  
infested with eggs and larvae of the Queensland fruit fly  
( *Bactrocera tryoni* (Froggatt) ) Diptera : Tephritidae .**

\* \* \* \* \*

**PART TWO – PHOTOS and DIAGRAMS**

OF THE MOST TOLERANT STAGE AND LARGE SCALE TRIAL  
PROTOCOLS FOR COLD DISINFESTATION OF  
QUEENSLAND FRUIT FLY

**CONDUCTED AT**

NEW SOUTH WALES DEPARTMENT OF PRIMARY INDUSTRIES,  
GOSFORD, NSW. AUSTRALIA 2250

**November 2012**

## TABLE OF CONTENTS – FIGURES

<b>Figure 1</b>	Adult Queensland fruit fly lay eggs which, at 26°C in the laboratory, hatch as 1st instar larvae within 2 to 4 days after infestation.....	4
<b>Figure 2</b>	By day 6 after egg laying, at 26°C, larvae have moulted through 1st and 2nd instars and have reached the mature 3rd instar.....	4
<b>Figure 3</b>	Mature 3 <sup>rd</sup> instar larvae pupate. They remain in the pupal state for 10 to 12 days at 26°C and then eclose as adult flies. ....	4
<b>Figure 4</b>	Adult flies must become sexually mature (takes about 5 to 7 days at 26°C) before they can lay eggs. The whole cycle from egg to egg takes, at 26°C, about 22 to 29 days.....	4
<b>Figure 5</b>	Some of the fruit cartons used in these trials. ....	5
<b>Figure 6</b>	Diagram of the fruit carton used in these trials showing its dimensions. ....	5
<b>Figure 7</b>	The layout of pallets in the three cold rooms was identical. Equivalent cartons of filler fruit in each stack and each room were replaced with cartons of test fruit throughout the trials.....	6
<b>Figure 8</b>	PALLET 1: Placement of infested (TEST) fruit and temperature data logger probes. Please refer to the room diagram for the pallet's location and alignment.....	7
<b>Figure 9</b>	PALLET 2: Placement of infested (TEST) fruit and temperature data logger probes. Please refer to the room diagram for the pallet's location and alignment.....	8
<b>Figure 10</b>	PALLET 3: Placement of infested (TEST) fruit and temperature data logger probes. Please refer to the room diagram for the pallet's location and alignment.....	9
<b>Figure 11</b>	PALLET 4: Placement of infested (TEST) fruit and temperature data logger probes. Please refer to the room diagram for the pallet's location and alignment.....	10
<b>Figure 12</b>	Filler fruit, apples in citrus cartons surround cherry cartons with infested cherries and non-infested cherries that are monitored for temperature (the temperature probe cables connecting the cherries to the data logger can be seen suspended from the ceiling).....	11
<b>Figure 13</b>	At the correct time (14 days at 1°C or 3°C) during the cold storage treatment in the large scale trials, fruit are removed from their position embedded in the pallet of apples (filler fruit).....	11
<b>Figure 14</b>	Female <i>Bactrocera tryoni</i> preparing to lay eggs into a cherry in the laboratory.....	12
<b>Figure 15</b>	Infested fruit with typical sunken appearance.....	12
<b>Figure 16</b>	Often bacterial / fungal rotting develops in conjunction with fruit fly infestation.....	12
<b>Figure 17</b>	Here are the fruit laid out on top of the fruit fly cages in the Gosford facility. The fruit remain there for up to 16 hours before being removed at random and allocated to life stage and treatment dose.....	13
<b>Figure 18</b>	Fruit laid out on fruit fly cages for infestation prior to treatment.....	13
<b>Figure 19</b>	Fruit flies can lay their eggs into test fruit through the cage mesh.....	13
<b>Figure 20</b>	Fruit laid out on fruit fly cages for infestation prior to treatment.....	13

<b>Figure 21</b>	
After a suitable period of being infested the fruit was removed at random. The fruit was then stored at 26°C until the insects infesting it had reached the egg, 1 <sup>st</sup> instar, 2 <sup>nd</sup> instar or 3 <sup>rd</sup> instar life stages. Then these fruit, with their insects at a certain life stage were exposed to cold storage temperatures. These photos show the method of holding infested fruit in cold storage for the most tolerant life-stage trials.....	14
<b>Figure 22</b>	
Infested fruit being loaded into plastic bags prior to treatment for most tolerant life-stage trials.....	14
<b>Figure 23</b>	
Infested fruit (50 per treatment unit) being loaded into plastic bags prior to treatment for most tolerant life-stage trials.....	14
<b>Figure 24</b>	
The front, bottom air circulation fan.....	15
<b>Figure 25</b>	
The rear, top cooling fans.....	15
<b>Figure 26</b>	
The bank of three cool rooms CTR7, CTR8 and CTR9 used in these trials.....	15
<b>Figure 27</b>	
Squirrel Data Logger probes being calibrated in an ice/water slurry prior to treatment commencement.....	16
<b>Figure 28</b>	
The Squirrel Data Logger.....	16
<b>Figure 29</b>	
Data logger probes were placed into the centre of test fruit, randomly throughout each carton of test fruit inside each pallet of filler fruit. The positions of the cartons of test fruit varied depending on which pallet they were in.....	16
<b>Figure 30</b>	
After cold storage treatment, for both the most tolerant life-stage trials and the large scale trials, test fruit and control fruit were placed over a pupation medium of damp vermiculite in plastic trays at 26°C.....	17
<b>Figure 31</b>	
Each treatment unit of fruit was placed in separate trays at 26°C to allow the pupation of any surviving Queensland fruit fly.....	17
<b>Figure 32</b>	
Trays of fruit were covered with bags made of fine terylene which allowed air movement around the fruit but stopped contamination by other insects.....	17
<b>Figure 33</b>	
After sieving the vermiculite at least two times (once a week for two weeks) after the conclusion of the cold treatment any remaining fruit were dissected and checked for live larvae or pupae.....	18
<b>Figure 34</b>	
Diagram showing the mouthparts of each larval stage of <i>B. tryoni</i> . A – 1 <sup>st</sup> instar, B – 2 <sup>nd</sup> instar, C – 3 <sup>rd</sup> instar.....	18
<b>Figure 35</b>	
The data logger probes were inserted into two cherries alongside the stone.....	19
<b>Figure 36</b>	
The data logger probes with the cherries attached were then placed in the centre of the carton and an attempt was made to pack other cherries tightly around the probe.....	19



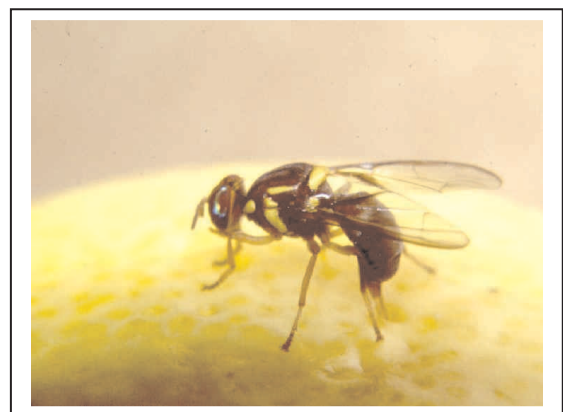
**Figure 1**  
Adult Queensland fruit fly lay eggs which, at 26°C in the laboratory, hatch as 1st instar larvae within 2 to 4 days after infestation.



**Figure 2**  
By day 6 after egg laying, at 26°C, larvae have moulted through 1st and 2nd instars and have reached the mature 3rd instar.

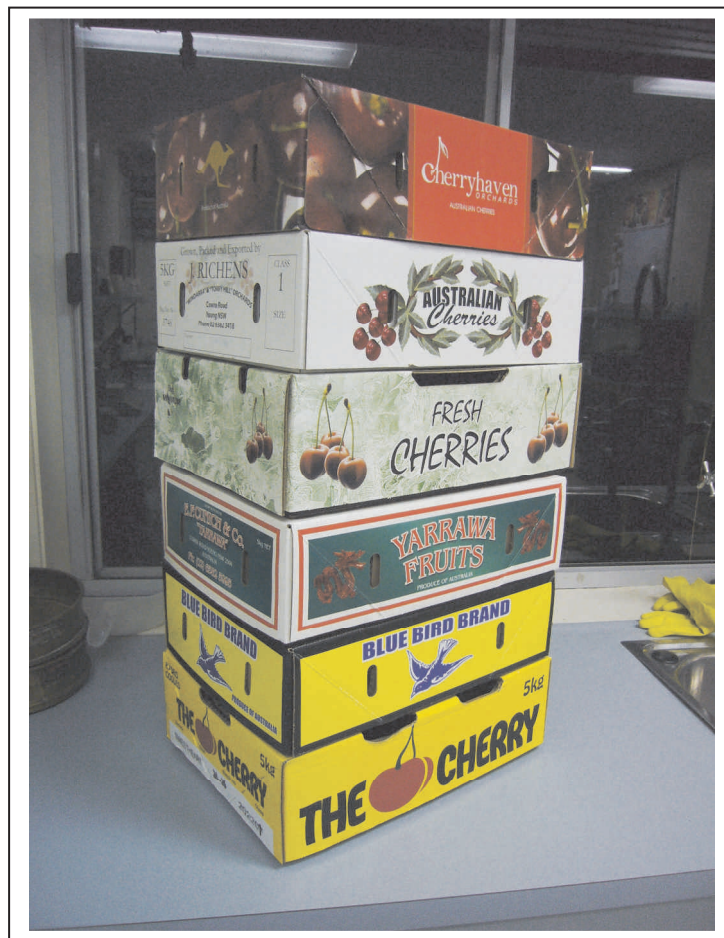


**Figure 3**  
Mature 3<sup>rd</sup> instar larvae pupate. They remain in the pupal state for 10 to 12 days at 26°C and then eclose as adult flies.



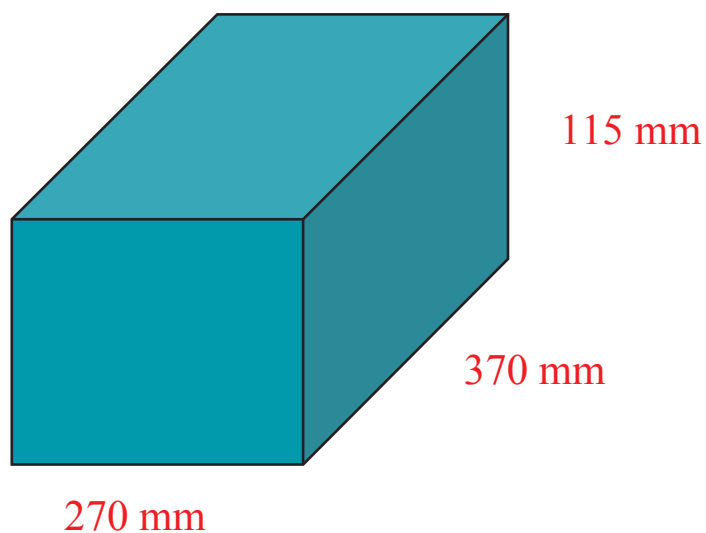
**Figure 4**  
Adult flies must become sexually mature (takes about 5 to 7 days at 26°C) before they can lay eggs. The whole cycle from egg to egg takes, at 26°C, about 22 to 29 days.

**Figure 5**  
Some of the fruit cartons  
used in these trials.

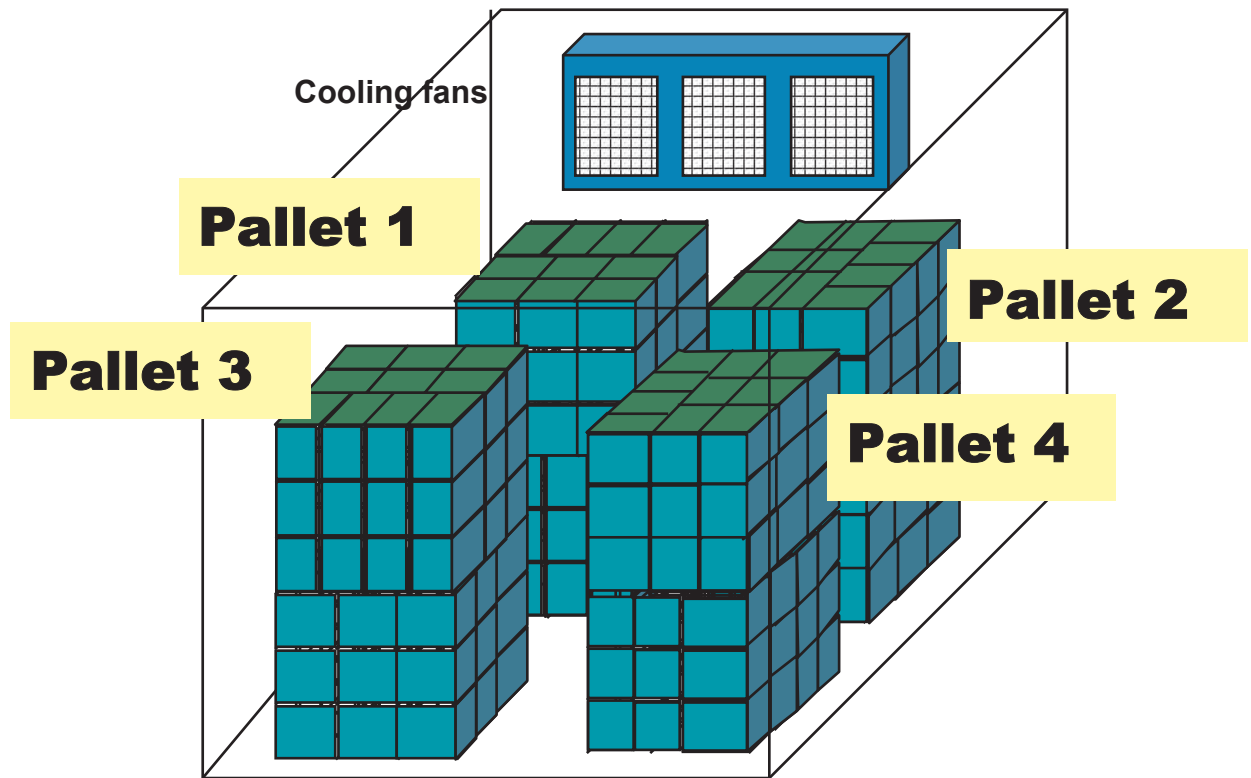


**5kg**  
**Carton**

**Weight with  
fruit is about 5  
kg**



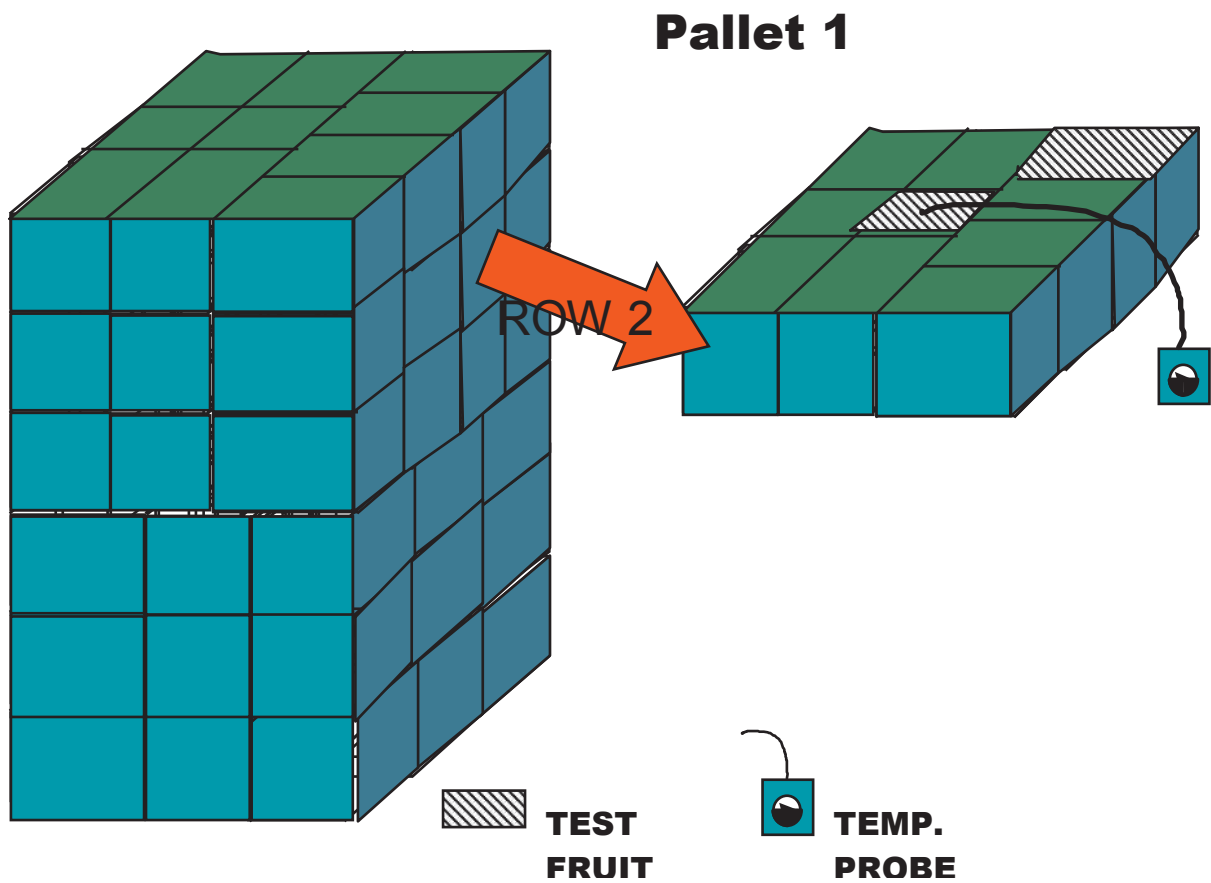
**Figure 6**  
Diagram of the fruit carton used in these trials showing its dimensions.



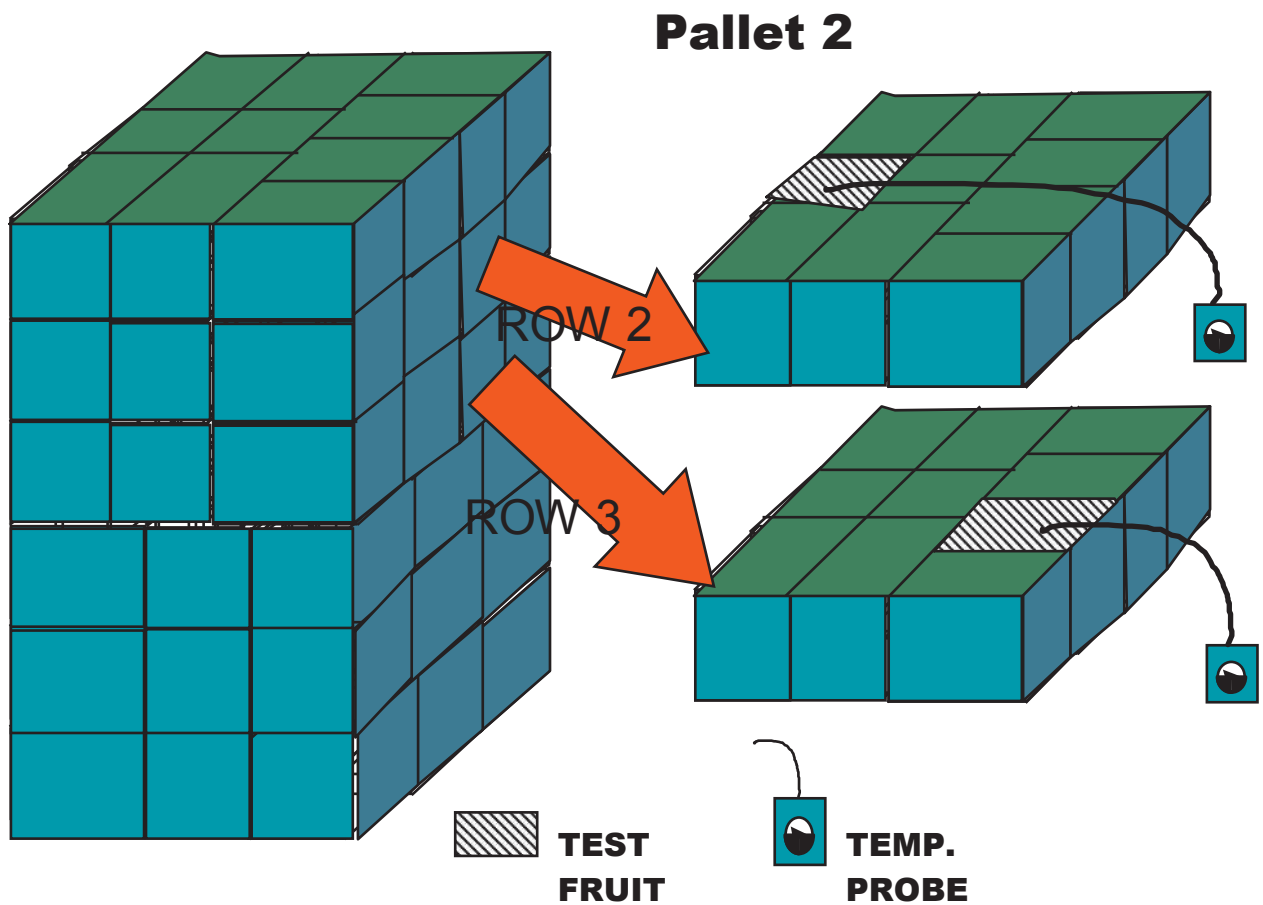
Layout of cold rooms 1, 2 and 3 showing placement of pallet loads of fruit in cartons. Pallets are numbered from 1 to 4. The layouts in rooms 1, 2 and 3 are identical.

**Figure 7**

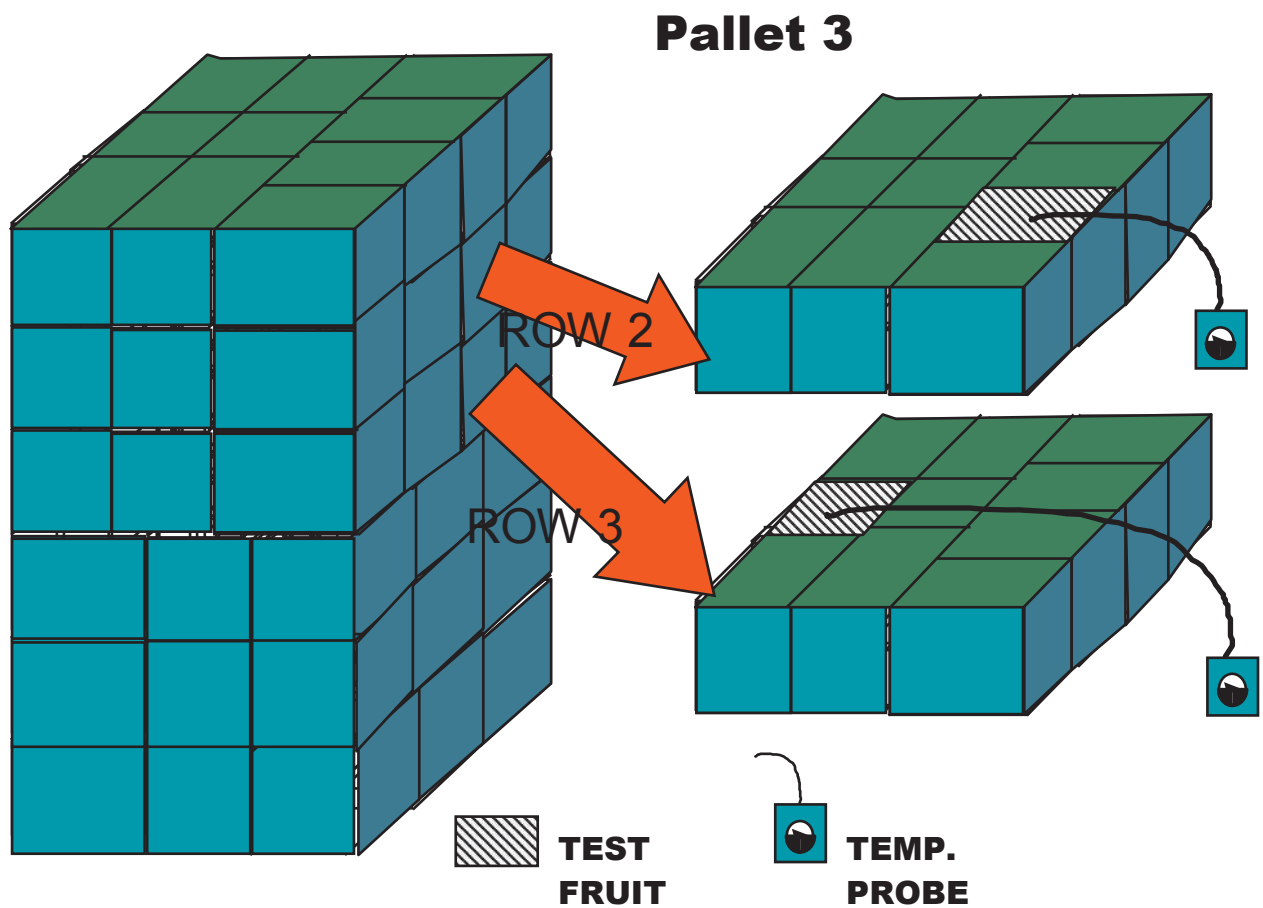
The layout of pallets in the three cold rooms was identical. Equivalent cartons of filler fruit in each stack and each room were replaced with cartons of test fruit throughout the trials.



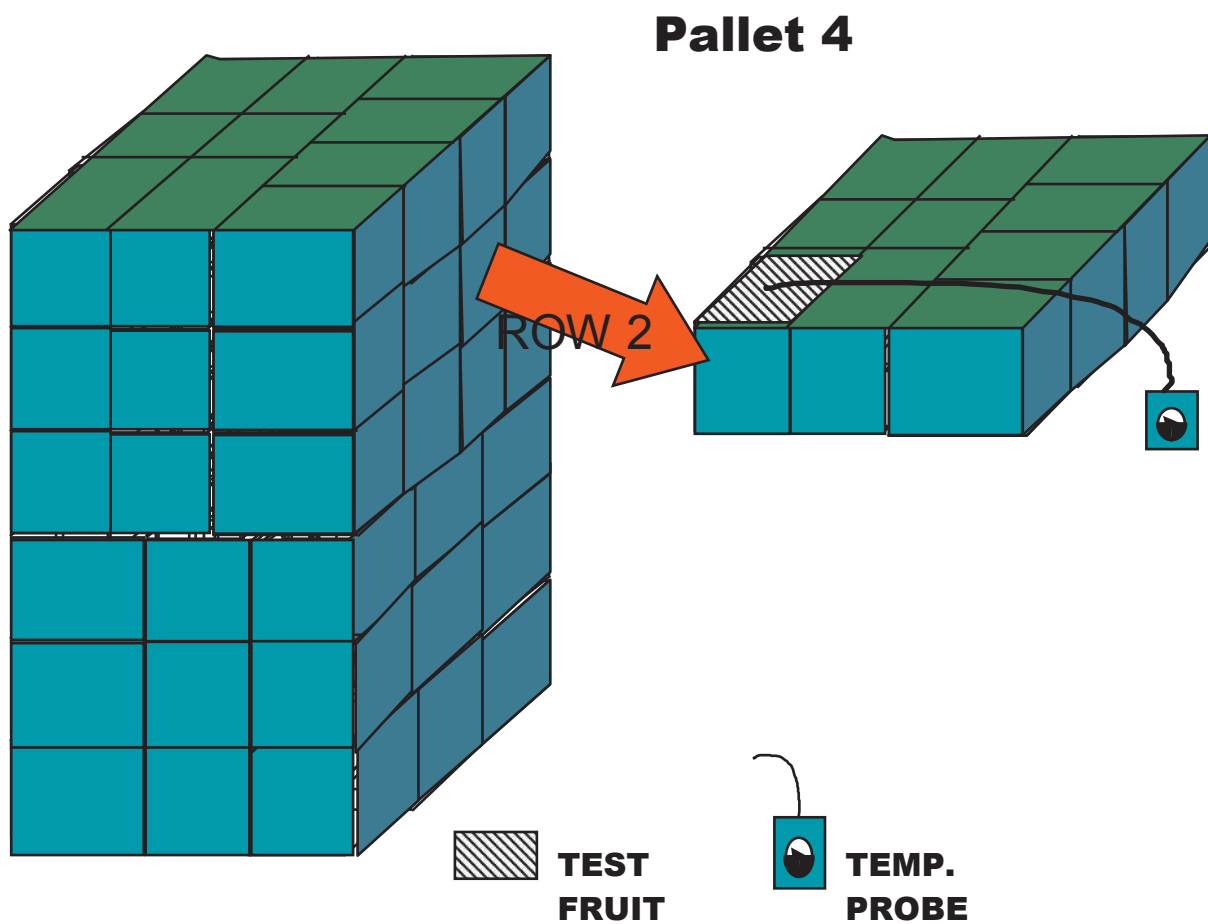
**Figure 8**  
**PALLET 1: Placement of infested (TEST) fruit and temperature data logger probes.**  
 Please refer to the room diagram for the pallet's location and alignment.



**Figure 9**  
**PALLET 2: Placement of infested (TEST) fruit and temperature data logger probes.**  
Please refer to the room diagram for the pallet's location and alignment.



**Figure 10**  
**PALLET 3: Placement of infested (TEST) fruit and temperature data logger probes.**  
Please refer to the room diagram for the pallet's location and alignment.



**Figure 11**  
**PALLET 4: Placement of infested (TEST) fruit and temperature data logger probes.**  
Please refer to the room diagram for the pallet's location and alignment.



**Figure 12**  
 Filler fruit, apples in citrus cartons surround cherry cartons with infested cherries and non-infested cherries that are monitored for temperature (the temperature probe cables connecting the cherries to the data logger can be seen suspended from the ceiling).



**Figure 13**  
 At the correct time (14 days at 1°C or 3°C) during the cold storage treatment in the large scale trials, fruit are removed from their position embedded in the pallet of apples (filler fruit).



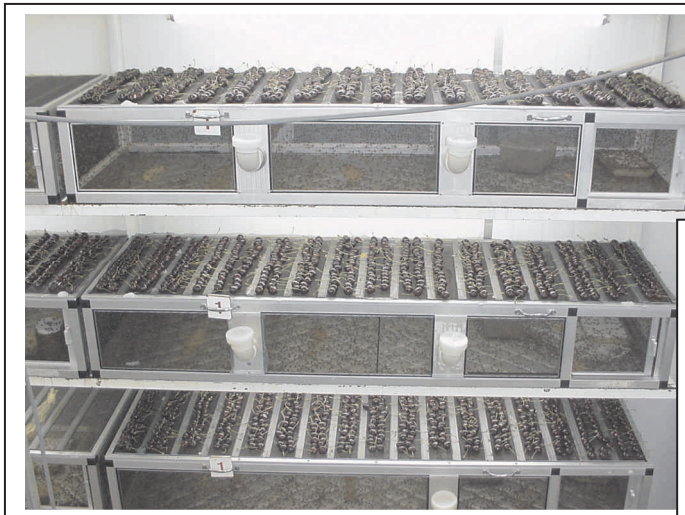
**Figure 14**  
Female *Bactrocera tryoni* preparing to lay eggs into a cherry in the laboratory.



**Figure 15**  
Infested fruit with typical sunken appearance.



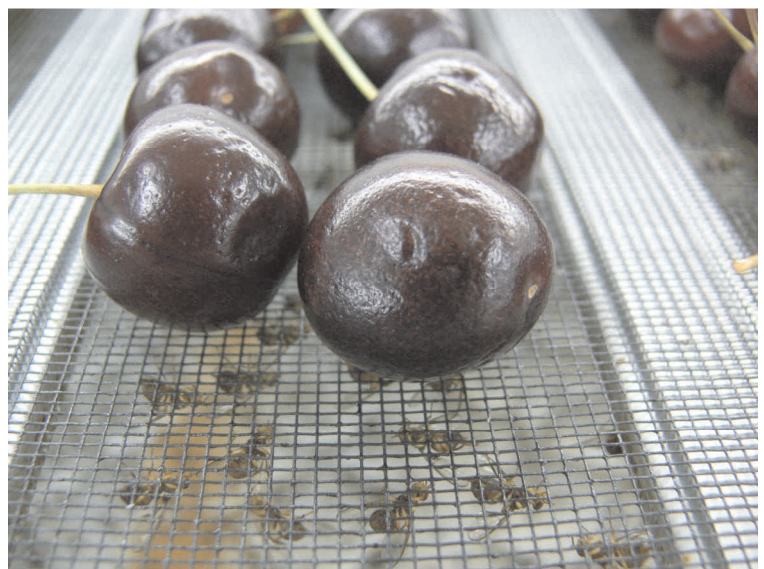
**Figure 16**  
Often bacterial / fungal rotting develops in conjunction with fruit fly infestation.



**Figure 17**  
Here are the fruit laid out on top of the fruit fly cages in the Gosford facility. The fruit remain there for up to 16 hours before being removed at random and allocated to life stage and treatment dose.



**Figure 18**  
Fruit laid out on fruit fly cages for infestation prior to treatment.



**Figure 19**  
Fruit flies can lay their eggs into test fruit through the cage mesh.



**Figure 20**  
Fruit laid out on fruit fly cages for infestation prior to treatment.

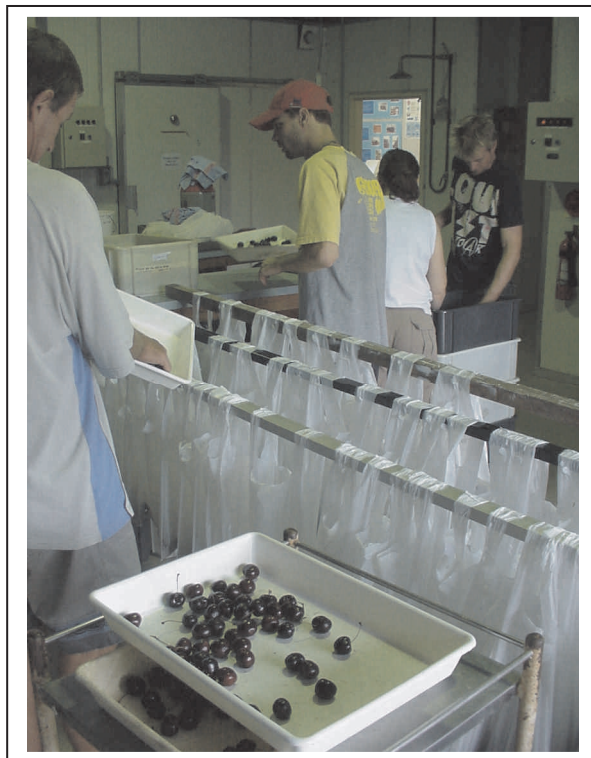


**Figure21**

After a suitable period of being infested the fruit was removed at random. The fruit was then stored at 26°C until the insects infesting it had reached the egg, 1<sup>st</sup> instar, 2<sup>nd</sup> instar or 3<sup>rd</sup> instar life stages. Then these fruit, with their insects at a certain life stage were exposed to cold storage temperatures. These photos show the method of holding infested fruit in cold storage for the most tolerant life-stage trials. See Figs 30, 31 and 32 for photos of containers used to hold infested fruit for the large scale trials.



**Figure 22**  
Infested fruit being loaded into plastic bags prior to treatment for most tolerant life-stage trials.



**Figure23**  
Infested fruit (between 44 and 50 per treatment unit) being loaded into plastic bags prior to treatment for most tolerant life-stage trials.



**Figure 24**  
The front, bottom air circulation fan.



**Figure 25**  
The rear, top cooling fans.



**Figure 26**  
The bank of three cool rooms CTR7, CTR8 and CTR9 used in these trials.



**Figure 27**  
Squirrel Data Logger probes being calibrated in an ice/water slurry prior to treatment commencement.



**Figure 28**  
The Squirrel Data Logger.

**Figure 29**  
Data logger probes were placed into the centre of test fruit, randomly throughout each carton of test fruit inside each pallet of filler fruit. The positions of the cartons of test fruit varied depending on which pallet they were in.





**Figure 30**

After cold storage treatment, for both the most tolerant life-stage trials and the large scale trials, test fruit and control fruit were placed over a pupation medium of damp vermiculite in plastic trays at 26°C.



**Figure 31**

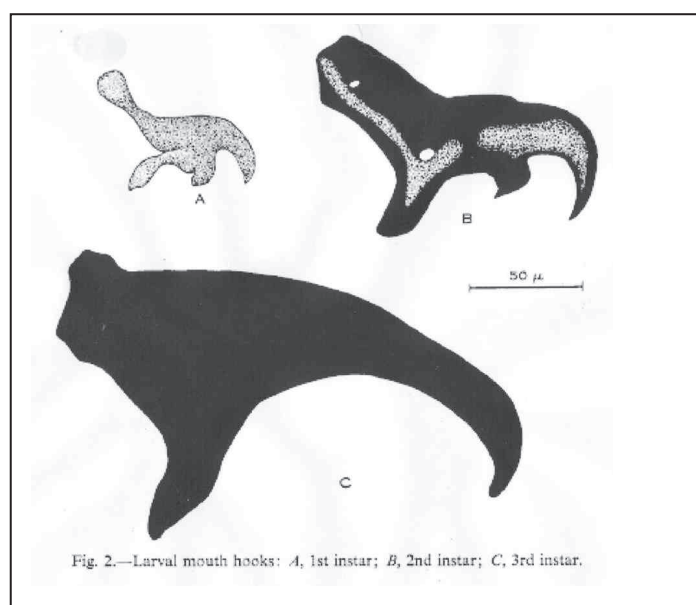
Each treatment unit of fruit was placed in separate trays at 26°C to allow the pupation of any surviving Queensland fruit fly.



**Figure 32**

Trays of fruit were covered with bags made of fine terylene which allowed air movement around the fruit but stopped contamination by other insects.

**Figure 33**  
After sieving the vermiculite at least two times (once a week for two weeks) after the conclusion of the cold treatment any remaining fruit were dissected and checked for live larvae or pupae.



**Figure 34**  
Diagram showing the mouthparts of each larval stage of *B. tryoni*. A – 1<sup>st</sup> instar, B – 2<sup>nd</sup> instar, C – 3<sup>rd</sup> instar.



**Figure 35**  
The data logger probes were inserted into two cherries alongside the stone.



**Figure 36**  
The data logger probes with the cherries attached were then placed in the centre of the carton and an attempt was made to pack other cherries tightly around the probe

**Cold treatment of Australian cherries  
infested with eggs and larvae of the Queensland fruit fly  
( *Bactrocera tryoni* (Froggatt) ) Diptera : Tephritidae .**

\* \* \* \* \*

**PART THREE – SUMMARY TABLES**

OF THE MOST TOLERANT STAGE AND LARGE SCALE TRIAL  
PROTOCOLS FOR COLD DISINFESTATION OF  
QUEENSLAND FRUIT FLY

**CONDUCTED AT**

NEW SOUTH WALES DEPARTMENT OF PRIMARY INDUSTRIES,  
GOSFORD, NSW. AUSTRALIA 2250

**November 2012**

## TABLE OF CONTENTS – PART 3

Table 1: Larval development studies conducted at 26°C (replicates 1, 2 and 3) – eggs, first, second and third instars.....	3
Table 2: Most tolerant stage at 1°C – Untreated (control) fruit .....	4
Table 3: Most tolerant stage at 1°C – Treated fruit.....	5
Table 4: Larval development studies at 1°C for large scale trials (replicates 1, 2 and 3).....	9
Table 5: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 1°C (replicate 1) .....	10
Table 6: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 1°C (replicate 2) .....	11
Table 7: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 1°C (replicate 3) .....	12
Table 8: Large scale trials at 1°C (replicates 1, 2 and 3) – Treated fruit .....	13
Table 9: Most tolerant stage at 3°C – Untreated (control) fruit .....	14
Table 10: Most tolerant stage at 3°C – Treated fruit.....	15
Table 11: Larval development studies for large scale trials at 3°C (replicates 1, 2, 3 and 4).....	19
Table 12: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 3°C (replicate 1) .....	20
Table 13: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 3°C (replicate 2) .....	21
Table 14: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 3°C (replicate 3) .....	22
Table 15: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 3°C (replicate 4) .....	23
Table 16: Large scale trials at 3°C (replicates 1, 2, 3 and 4) – Treated fruit .....	24
Table 17: Effects of cold storage at 1°C and 3°C on quality of Sweetheart cherries.....	26
Table 18: Data logger calibration, statistics and graphs.....	27
Table 19: Data logger statistics for large scale trials at 1°C (replicate 1) ....	29
Table 20: Data logger statistics for large scale trials at 1°C (replicate 2) ....	30
Table 21: Data logger statistics for large scale trials at 1°C (replicate 3) ....	31
Table 22: Data logger statistics for large scale trials at 3°C (replicate 1) ....	32
Table 23: Data logger statistics for large scale trials at 3°C (replicate 2) ....	33
Table 24: Data logger statistics for large scale trials at 3°C (replicate 3) ....	34
Table 25: Data logger statistics for large scale trials at 3°C (replicate 4) ....	35

**Table 1: Larval development studies conducted at 26°C (replicates 1, 2 and 3) – eggs, first, second and third instars**

Date of ovipositing	Date of larval dev.	Sample unit	NUMBERS OF EACH LIFESTAGE				TOTAL	PERCENT AT EACH LIFESTAGE			
			EGGS	1st instar	2nd instar	3rd instar		Eggs	1st instar	2nd instar	3rd instar
1 to 2 March 2006 (overnight)	3 March 2006	1	325	52	0	0	377	86	14	0	0
		2	344	26	0	0	370	93	7	0	0
		3	338	89	0	0	427	79	21	0	0
		4	402	54	0	0	456	88	12	0	0
		5	258	12	0	0	270	96	4	0	0
							AVERAGE	88	12	0	0
	5 March 2006	1	12	123	1	0	136	<1	98	<1	0
		2	41	205	0	0	246	17	83	0	0
		3	16	89	5	0	110	15	81	4	0
		4	5	112	1	0	118	4	95	1	0
		5	21	92	2	0	115	18	80	2	0
							AVERAGE	11	87	2	0
	7 March 2006	1	0	12	25	1	38	0	31	66	3
		2	0	2	48	0	50	0	4	96	0
		3	0	5	32	2	39	0	13	82	5
		4	0	12	35	0	47	0	25	75	0
		5	0	6	19	4	29	0	21	66	13
							AVERAGE	0	19	77	4
	9 March 2006	1	0	0	3	15	18	0	0	17	87
		2	0	0	9	18	27	0	0	33	67
		3	0	0	2	14	16	0	0	12	88
		4	0	0	4	9	13	0	0	31	69
		5	0	0	5	21	26	0	0	19	81
							AVERAGE	0	0	22	78

**Table 2: Most tolerant stage at 1°C – Untreated (control) fruit**

<b>LIFE STAGE</b>	<b>Sample</b>	<b>SIEVE 1</b>	<b>SIEVE 2</b>	<b>Total</b>	<b>Insects per fruit</b>	<b>No. Fruit</b>	<b>Average insects per fruit ± SD</b>
EGGS	1	722	34	756	16.43	46	
EGGS	2	1162	31	1193	25.93	46	
EGGS	3	1109	13	1122	24.39	46	
EGGS	4	1124	32	1156	25.13	46	
EGGS	5	851	43	894	19.43	46	
EGGS	6	941	25	966	21.00	46	22.05 ± 3.73
1STS	1	1101	33	1134	24.65	46	
1STS	2	778	18	796	17.30	46	
1STS	3	935	14	949	20.63	46	
1STS	4	812	17	829	18.02	46	
1STS	5	632	18	650	14.13	46	
1STS	6	1081	20	1101	23.93	46	19.78 ± 4.07
2NDS	1	861	40	901	19.59	46	
2NDS	2	1592	73	1665	36.20	46	
2NDS	3	1003	17	1020	22.17	46	
2NDS	4	612	42	654	14.22	46	
2NDS	5	709	56	765	16.63	46	
2NDS	6	1203	82	1285	27.93	46	22.79 ± 8.10
3RDS	1	615	42	657	13.14	50	
3RDS	2	824	27	851	17.02	50	
3RDS	3	632	18	650	13.00	50	
3RDS	4	479	24	503	10.06	50	
3RDS	5	613	18	631	12.62	50	
3RDS	6	469	33	502	10.04	50	12.65 ± 2.57

**Table 3: Most tolerant stage at 1°C – Treated fruit**

Life stage	Treatment days	Replicate No.	Sieve 1	Sieve 2	TOTAL PUPAE	No. Fruit	Insects per fruit	Average insects per fruit $\pm$ Stdev	Insects per fruit $\pm$ Stdev (controls)	% mortality
EGGS	3	1	115	58	173	44	3.93			
EGGS	3	2	213	39	252	44	5.73			
EGGS	3	3	104	0	104	44	2.36	4 $\pm$ 1.68	22.05 $\pm$ 3.73	81.82
EGGS	4	1	127	11	138	44	3.14			
EGGS	4	2	160	0	160	44	3.64			
EGGS	4	3	156	14	170	44	3.86	3.55 $\pm$ 0.37	22.05 $\pm$ 3.73	83.92
EGGS	5	1	749	11	760	44	17.27			
EGGS	5	2	709	4	713	44	16.20			
EGGS	5	3	523	4	527	44	11.98	15.15 $\pm$ 2.80	22.05 $\pm$ 3.73	31.29
EGGS	6	1	15	0	15	44	0.34			
EGGS	6	2	28	5	33	44	0.75			
EGGS	6	3	26	0	26	44	0.59	0.56 $\pm$ 0.20	22.05 $\pm$ 3.73	97.46
EGGS	7	1	7	1	8	44	0.18			
EGGS	7	2	4	0	4	44	0.09			
EGGS	7	3	5	4	9	44	0.20	0.16 $\pm$ 0.06	22.05 $\pm$ 3.73	99.28
EGGS	8	1	0	0	0	44	0			
EGGS	8	2	3	0	3	44	0.07			
EGGS	8	3	3	0	3	44	0.07	0.05 $\pm$ 0.04	22.05 $\pm$ 3.73	99.79
EGGS	9	1	0	0	0	44	0			
EGGS	9	2	0	0	0	44	0			
EGGS	9	3	0	0	0	44	0	0	22.05 $\pm$ 3.73	100
EGGS	10	1	0	0	0	44	0			
EGGS	10	2	0	0	0	44	0			
EGGS	10	3	0	0	0	44	0	0	22.05 $\pm$ 3.73	100
EGGS	12	1	0	0	0	44	0			
EGGS	12	2	0	0	0	44	0			
EGGS	12	3	0	0	0	44	0	0	22.05 $\pm$ 3.73	100
EGGS	14	1	0	0	0	44	0			
EGGS	14	2	0	0	0	44	0			
EGGS	14	3	0	0	0	44	0	0	22.05 $\pm$ 3.73	100

Life stage	Treatment days	Replicate No.	Sieve 1	Sieve 2	TOTAL PUPAE	No. Fruit	Insects per fruit	Average insects per fruit $\pm$ Stdev	Insects per fruit $\pm$ Stdev (controls)	% mortality
1STS	3	1	88	29	117	44	2.66			
1STS	3	2	75	27	102	44	2.32			
1STS	3	3	87	35	122	44	2.77	2.58 $\pm$ 0.24	19.78 $\pm$ 4.07	86.94
1STS	4	1	265	14	279	44	6.34			
1STS	4	2	419	20	439	44	9.98			
1STS	4	3	275	16	291	44	6.61	7.64 $\pm$ 2.03	19.78 $\pm$ 4.07	61.35
1STS	5	1	165	4	169	44	3.84			
1STS	5	2	104	16	120	44	2.73			
1STS	5	3	89	1	90	44	2.05	2.87 $\pm$ 0.91	19.78 $\pm$ 4.07	85.48
1STS	6	1	48	5	53	44	1.20			
1STS	6	2	88	2	90	44	2.05			
1STS	6	3	32	6	38	44	0.86	1.37 $\pm$ 0.61	19.78 $\pm$ 4.07	93.07
1STS	7	1	8	1	9	44	0.20			
1STS	7	2	17	3	20	44	0.45			
1STS	7	3	10	1	11	44	0.25	0.30 $\pm$ 0.13	19.78 $\pm$ 4.07	98.47
1STS	8	1	7	1	8	44	0.18			
1STS	8	2	11	0	11	44	0.25			
1STS	8	3	1	0	1	44	0.027	0.15 $\pm$ 0.12	19.78 $\pm$ 4.07	99.23
1STS	9	1	5	0	5	44	0.11			
1STS	9	2	2	1	3	44	0.07			
1STS	9	3	11	0	11	44	0.25	0.14 $\pm$ 0.09	19.78 $\pm$ 4.07	99.27
1STS	10	1	0	0	0	44	0			
1STS	10	2	0	4	4	44	0.09			
1STS	10	3	0	0	0	44	0	0.03 $\pm$ 0.05	19.78 $\pm$ 4.07	99.85
1STS	12	1	0	0	0	44	0			
1STS	12	2	0	0	0	44	0			
1STS	12	3	0	0	0	44	0	0	19.78 $\pm$ 4.07	100
1STS	14	1	0	0	0	44	0			
1STS	14	2	0	0	0	44	0			
1STS	14	3	0	0	0	44	0	0	19.78 $\pm$ 4.07	100

Life stage	Treatment days	Replicate No.	Sieve 1	Sieve 2	TOTAL PUPAE	No. Fruit	Insects per fruit	Average insects per fruit $\pm$ Stdev	Insects per fruit $\pm$ Stdev (controls)	% mortality
2NDS	3	1	402	12	414	44	9.41			
2NDS	3	2	307	9	316	44	7.18			
2NDS	3	3	216	2	218	44	4.95	7.18 $\pm$ 2.23	22.79 $\pm$ 8.10	68.49
2NDS	4	1	168	5	173	44	3.93			
2NDS	4	2	129	5	134	44	3.05			
2NDS	4	3	173	6	179	44	4.07	3.68 $\pm$ 0.56	22.79 $\pm$ 8.10	83.84
2NDS	5	1	16	1	17	44	0.39			
2NDS	5	2	51	7	58	44	1.32			
2NDS	5	3	68	3	71	44	1.61	1.11 $\pm$ 0.64	22.79 $\pm$ 8.10	95.15
2NDS	6	1	12	1	13	44	0.30			
2NDS	6	2	21	3	24	44	0.55			
2NDS	6	3	2	1	3	44	0.07	0.30 $\pm$ 0.24	22.79 $\pm$ 8.10	98.67
2NDS	7	1	3	0	3	44	0.07			
2NDS	7	2	13	1	14	44	0.32			
2NDS	7	3	2	0	2	44	0.05	0.14 $\pm$ 0.15	22.79 $\pm$ 8.10	99.37
2NDS	8	1	0	1	1	44	0.02			
2NDS	8	2	0	0	0	44	0			
2NDS	8	3	0	2	2	44	0.05	0.02 $\pm$ 0.02	22.79 $\pm$ 8.10	99.90
2NDS	9	1	2	0	2	44	0.05			
2NDS	9	2	0	0	0	44	0			
2NDS	9	3	0	0	0	44	0	0.02 $\pm$ 0.03	22.79 $\pm$ 8.10	99.93
2NDS	10	1	0	0	0	44	0			
2NDS	10	2	0	0	0	44	0			
2NDS	10	3	0	0	0	44	0	0	22.79 $\pm$ 8.10	100
2NDS	12	1	0	0	0	44	0			
2NDS	12	2	0	0	0	44	0			
2NDS	12	3	0	0	0	44	0	0	22.79 $\pm$ 8.10	100
2NDS	14	1	0	0	0	44	0			
2NDS	14	2	0	0	0	44	0			
2NDS	14	3	0	0	0	44	0	0	22.79 $\pm$ 8.10	100

Life stage	Treatment days	Replicate No.	Sieve 1	Sieve 2	TOTAL PUPAE	No. Fruit	Insects per fruit	Average insects per fruit $\pm$ Stdev	Insects per fruit $\pm$ Stdev (controls)	% mortality
3RDS	3	1	70	1	71	50	1.42			
3RDS	3	2	79	0	79	50	1.58			
3RDS	3	3	148	0	148	50	2.96	1.99 $\pm$ 0.85	12.65 $\pm$ 2.57	84.29
3RDS	4	1	80	3	83	50	1.66			
3RDS	4	2	37	0	37	50	0.74			
3RDS	4	3	42	0	42	50	0.84	1.08 $\pm$ 0.51	12.65 $\pm$ 2.57	91.46
3RDS	5	1	10	2	12	50	0.24			
3RDS	5	2	18	0	18	50	0.36			
3RDS	5	3	19	0	19	50	0.38	0.33 $\pm$ 0.08	12.65 $\pm$ 2.57	97.42
3RDS	6	1	14	3	17	50	0.34			
3RDS	6	2	6	0	6	50	0.12			
3RDS	6	3	4	0	4	50	0.08	0.18 $\pm$ 0.14	12.65 $\pm$ 2.57	98.58
3RDS	7	1	0	0	0	50	0			
3RDS	7	2	2	0	2	50	0.04			
3RDS	7	3	0	0	0	50	0	0.01 $\pm$ 0.02	12.65 $\pm$ 2.57	99.89
3RDS	8	1	0	0	0	50	0			
3RDS	8	2	0	0	0	50	0			
3RDS	8	3	0	0	0	50	0	0	12.65 $\pm$ 2.57	100
3RDS	9	1	1	0	1	50	0.02			
3RDS	9	2	0	0	0	50	0			
3RDS	9	3	0	0	0	50	0	0.01 $\pm$ 0.01	12.65 $\pm$ 2.57	99.95
3RDS	10	1	0	0	0	50	0			
3RDS	10	2	0	0	0	50	0			
3RDS	10	3	0	0	0	50	0	0	12.65 $\pm$ 2.57	100
3RDS	12	1	0	0	0	50	0			
3RDS	12	2	0	0	0	50	0			
3RDS	12	3	0	0	0	50	0	0	12.65 $\pm$ 2.57	100
3RDS	14	1	0	0	0	50	0			
3RDS	14	2	0	0	0	50	0			
3RDS	14	3	0	0	0	50	0	0	12.65 $\pm$ 2.57	100

**Table 4: Larval development studies at 1°C for large scale trials (replicates 1, 2 and 3)**

**1°C Replicate 1 Larval development**

Date of infestation	Sample unit	NUMBERS OF EACH LIFESTAGE				TOTAL	PERCENT AT EACH LIFESTAGE			
		EGGS	1st instar	2nd instar	3rd instar		Eggs	1st instar	2nd instar	3rd instar
26 February 2007	1	9	89	1	0	99	9	90	1	0
Date of larval assessment	2	12	68	0	0	80	15	85	0	0
	3	20	112	2	0	134	15	84	1	0
	4	5	77	0	0	82	6	94	0	0
2 March 2007	5	14	102	0	0	116	12	88	0	0
AVERAGE							11	88	<1	0

**1°C Replicate 2 Larval development**

Date of infestation	Sample unit	NUMBERS OF EACH LIFESTAGE				TOTAL	PERCENT AT EACH LIFESTAGE			
		EGGS	1st instar	2nd instar	3rd instar		Eggs	1st instar	2nd instar	3rd instar
6 March 2007	1	15	132	0	0	147	10	90	0	0
Date of larval assessment	2	12	97	1	0	110	10	89	<1	0
	3	9	108	3	0	120	8	90	2	0
	4	21	84	0	0	105	20	80	0	0
10 March 2007	5	8	89	1	0	98	8	91	1	0
AVERAGE							11	88	<1	0

**1°C Replicate 3 Larval development**

Date of infestation	Sample unit	NUMBERS OF EACH LIFESTAGE				TOTAL	PERCENT AT EACH LIFESTAGE			
		EGGS	1st instar	2nd instar	3rd instar		Eggs	1st instar	2nd instar	3rd instar
12 March 2007	1	12	81	2	0	95	13	85	2	0
Date of larval assessment	2	14	78	0	0	92	15	85	0	0
	3	10	95	0	0	105	9	91	0	0
	4	25	89	0	0	114	22	78	0	0
16 March 2007	5	3	78	0	0	81	4	96	0	0
AVERAGE							13	86	<1	0

**Table 5: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 1°C (replicate 1)**

2040 cherries were infested on the cages at GHI. 440 fruit (CONTROL) were randomly chosen and placed in 4 pupation trays and held at 26°C until larvae had “hopped” from the fruit. These were counted to obtain an average number of survivors per fruit. The remaining 1600 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 <sup>st</sup> instar larval stage for the 1°C disinfestation treatment.							
<b>LARGE SCALE TRIAL AT 1°C SWEETHEART REPLICATE 1 – Infested 26 Feb 2007</b>							
Sample unit (110 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	No. fruit	Pupae per fruit	Average	Stdev
Sieve date	09 Mar 2007	16 Mar 2007					
1	1455	926	2381	110	21.65		
2	1400	684	2084	110	18.95		
3	1385	926	2311	110	21		
4	1394	658	2052	110	18.65	20.06	1.48

**Table 6: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 1°C (replicate 2)**

1934 cherries were infested on the cages at GHI. 440 fruit (CONTROL) were randomly chosen and placed in 4 pupation trays and held at 26°C until larvae had “hopped” from the fruit. These were counted to obtain an average number of survivors per fruit. The remaining 1494 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 <sup>st</sup> instar larval stage for the 1°C disinfestation treatment.								
<b>LARGE SCALE TRIAL AT 1°C SWEETHEART REPLICATE 2 – Infested 06 Mar 2007</b>								
Sample unit (110 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	No. pupae (sieve 3)	Total pupae	No. fruit	Pupae per fruit	Average	Stdev
Sieve date	16 Mar 2007	24 Mar 2007	31 Mar 2007					
1	606	387	7	1000	110	9.09		
2	678	235	10	923	110	8.39		
3	612	302	19	933	110	8.48		
4	727	278	13	1018	110	9.25	8.8	0.43

**Table 7: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 1°C (replicate 3)**

2526 cherries were infested on the cages at GHI. 440 fruit (CONTROL) were randomly chosen and placed in 4 pupation trays and held at 26°C until larvae had “hopped” from the fruit. These were counted to obtain an average number of survivors per fruit. The remaining 2086 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 <sup>st</sup> instar larval stage for the 1°C disinfestation treatment.							
<b>LARGE SCALE TRIAL AT 1°C SWEETHEART REPLICATE 3 – Infested 12 March 2007</b>							
Sample unit (110 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	No. fruit	Pupae per fruit	Average	Stdev
Sieve date	23 Mar 2007	30 Mar 2007					
1	1073	19	1092	110	9.93		
2	904	56	960	110	8.73		
3	749	68	817	110	7.43		
4	1038	72	1110	110	10.09	9.9	1.24

**Table 8: Large scale trials at 1°C (replicates 1, 2 and 3) – Treated fruit**

VARIETY	REPLICATE	TREATMENT	TREATMENT UNIT	No. FRUIT	No. PUPAE SIEVE 1	No. PUPAE SIEVE 2
SWEETHEART	1	14 DAYS @ 1°C	1	200	0	0
SWEETHEART	1	14 DAYS @ 1°C	2	200	0	0
SWEETHEART	1	14 DAYS @ 1°C	3	200	0	0
SWEETHEART	1	14 DAYS @ 1°C	4	200	0	0
SWEETHEART	1	14 DAYS @ 1°C	5	200	0	0
SWEETHEART	1	14 DAYS @ 1°C	6	200	0	0
SWEETHEART	1	14 DAYS @ 1°C	7	200	0	0
SWEETHEART	1	14 DAYS @ 1°C	8	200	0	0
<b>SUB TOTAL</b>		14 DAYS @ 1°C		<b>1600</b>	<b>0</b>	<b>0</b>
SWEETHEART	2	14 DAYS @ 1°C	1	200	0	0
SWEETHEART	2	14 DAYS @ 1°C	2	200	0	0
SWEETHEART	2	14 DAYS @ 1°C	3	200	0	0
SWEETHEART	2	14 DAYS @ 1°C	4	200	0	0
SWEETHEART	2	14 DAYS @ 1°C	5	200	0	0
SWEETHEART	2	14 DAYS @ 1°C	6	200	0	0
SWEETHEART	2	14 DAYS @ 1°C	7	200	0	0
SWEETHEART	2	14 DAYS @ 1°C	8	94	0	0
<b>SUB TOTAL</b>		14 DAYS @ 1°C		<b>1494</b>	<b>0</b>	<b>0</b>
SWEETHEART	3	14 DAYS @ 1°C	1	200	0	0
SWEETHEART	3	14 DAYS @ 1°C	2	200	0	0
SWEETHEART	3	14 DAYS @ 1°C	3	200	0	0
SWEETHEART	3	14 DAYS @ 1°C	4	200	0	0
SWEETHEART	3	14 DAYS @ 1°C	5	200	0	0
SWEETHEART	3	14 DAYS @ 1°C	6	200	0	0
SWEETHEART	3	14 DAYS @ 1°C	7	200	0	0
SWEETHEART	3	14 DAYS @ 1°C	8	200	0	0
SWEETHEART	3	14 DAYS @ 1°C	9	200	0	0
SWEETHEART	3	14 DAYS @ 1°C	10	200	0	0
SWEETHEART	3	14 DAYS @ 1°C	11	86	0	0
<b>SUB TOTAL</b>				<b>2086</b>	<b>0</b>	<b>0</b>
<b>GRAND TOTAL</b>				<b>5180</b>	<b>0</b>	<b>0</b>

**Table 9: Most tolerant stage at 3°C – Untreated (control) fruit**

LIFE STAGE	Sample	SIEVE 1	SIEVE 2	Total	Adjusted total	No. Fruit	Insects per fruit	Average insects per fruit $\pm$ SD
EGGS	1	456	0	456	623.8	50	12.48	
EGGS	2	595	5	600	767.8	50	15.36	
EGGS	3	327	8	335	502.8	50	10.06	
EGGS	4	963	10	973	1141	50	22.82	
EGGS	5	756	49	805	972.8	50	19.46	
EGGS	6	242	6	248	415.8	50	8.32	14.75 $\pm$ 5.59
1STS	1	423	17	440	607.8	50	12.16	
1STS	2	333	7	340	507.8	50	10.16	
1STS	3	436	8	444	611.8	50	12.24	
1STS	4	312	3	315	482.8	50	9.66	
1STS	5	121	7	128	295.8	50	5.92	
1STS	6	569	9	578	745.8	50	14.92	10.84 $\pm$ 3.05
2NDS	1	580	32	612		50	12.24	
2NDS	2	998	10	1008		50	20.16	
2NDS	3	640	172	812		50	16.24	
2NDS	4	754	34	788		50	15.76	
2NDS	5	1142	17	1159		50	23.18	
2NDS	6	1020	21	1041		50	20.82	18.07 $\pm$ 4.02
3RDS	1	825	37	862		50	17.24	
3RDS	2	907	34	941		50	18.82	
3RDS	3	692	19	711		50	14.22	
3RDS	4	818	57	875		50	17.5	
3RDS	5	801	40	841		50	16.82	16.92 $\pm$ 1.68

**Note adjusted total – In the control samples for eggs and first instars, pupae climbed out of the plastic trays and made their way into the bottom of the protective bag. These pupae were collected, counted, and added to the totals from sieve 1 and sieve 2. This did not occur in subsequent studies.**

Table 10: Most tolerant stage at 3°C – Treated fruit

Life stage	Treatment days	Replicate No.	Sieve 1	Sieve 2	TOTAL PUPAE	No. Fruit	Insects per fruit	Average insects per fruit ± Stdev	Insects per fruit ± Stdev (controls)	% mortality
EGGS	3	1	265	21	286	46	6.22			
EGGS	3	2	184	15	199	46	4.33			
EGGS	3	3	359	19	378	46	8.22	6.25 ± 1.95	14.75 ± 5.59	57.59
EGGS	4	1	179	49	228	46	4.96			
EGGS	4	2	295	32	327	46	7.11			
EGGS	4	3	200	30	230	46	5	5.69 ± 1.23	14.75 ± 5.59	61.43
EGGS	5	1	115	10	125	46	2.72			
EGGS	5	2	219	30	249	46	5.41			
EGGS	5	3	118	35	153	46	3.33	3.82 ± 1.41	14.75 ± 5.59	74.10
EGGS	6	1	55	48	103	46	2.24			
EGGS	6	2	58	24	82	46	1.78			
EGGS	6	3	77	39	116	46	2.52	2.18 ± 0.37	14.75 ± 5.59	85.21
EGGS	7	1	3	0	3	46	0.07			
EGGS	7	2	26	2	28	46	0.60			
EGGS	7	3	17	1	18	46	0.39	0.36 ± 0.27	14.75 ± 5.59	97.59
EGGS	8	1	0	0	0	46	0			
EGGS	8	2	1	0	1	46	0.02			
EGGS	8	3	0	0	0	46	0	0.01 ± 0.01	14.75 ± 5.59	99.95
EGGS	9	1	0	0	0	46	0			
EGGS	9	2	2	2	4	46	0.09			
EGGS	9	3	2	3	5	46	0.11	0.07 ± 0.06	14.75 ± 5.59	99.56
EGGS	10	1	0	0	0	46	0			
EGGS	10	2	0	0	0	46	0			
EGGS	10	3	0	2	2	46	0.04	0.01 ± 0.03	14.75 ± 5.59	99.90
EGGS	12	1	0	0	0	46	0			
EGGS	12	2	0	0	0	46	0			
EGGS	12	3	0	0	0	46	0	0	14.75 ± 5.59	100
EGGS	14	1	0	0	0	46	0			
EGGS	14	2	0	0	0	46	0			
EGGS	14	3	*	*	*	0	*	0	14.75 ± 5.59	100

Life stage	Treatment days	Replicate No.	Sieve 1	Sieve 2	TOTAL PUPAE	No. Fruit	Insects per fruit	Average insects per fruit ± Stdev	Insects per fruit ± Stdev (controls)	% mortality
1STS	3	1	132	28	160	46	3.48			
1STS	3	2	216	12	228	46	4.96			
1STS	3	3	352	26	378	46	8.22	5.56 ± 2.42	10.84 ± 3.05	48.79
1STS	4	1	149	26	175	46	3.80			
1STS	4	2	81	14	95	46	2.07			
1STS	4	3	89	15	104	46	2.26	2.71 ± 0.95	10.84 ± 3.05	74.0
1STS	5	1	56	10	66	46	1.43			
1STS	5	2	56	9	65	46	1.41			
1STS	5	3	65	10	75	46	1.63	1.49 ± 0.12	10.84 ± 3.05	86.23
1STS	6	1	12	0	12	46	0.26			
1STS	6	2	10	0	10	46	0.22			
1STS	6	3	9	0	9	46	0.20	0.22 ± 0.03	10.84 ± 3.05	97.93
1STS	7	1	8	0	8	46	0.17			
1STS	7	2	9	1	10	46	0.22			
1STS	7	3	10	2	12	46	0.26	0.22 ± 0.04	10.84 ± 3.05	97.99
1STS	8	1	5	1	6	46	0.13			
1STS	8	2	7	1	8	46	0.17			
1STS	8	3	4	4	8	46	0.17	0.16 ± 0.03	10.84 ± 3.05	98.53
1STS	9	1	2	0	2	46	0.04			
1STS	9	2	3	0	3	46	0.07			
1STS	9	3	2	0	2	46	0.04	0.05 ± 0.01	10.84 ± 3.05	99.53
1STS	10	1	3	0	3	46	0.07			
1STS	10	2	1	1	2	46	0.04			
1STS	10	3	1	0	1	46	0.02	0.04 ± 0.02	10.84 ± 3.05	99.60
1STS	12	1	1	0	1	46	0.02			
1STS	12	2	0	0	0	46	0.00			
1STS	12	3	0	1	1	46	0.02	0.015 ± 0.01	10.84 ± 3.05	99.87
1STS	14	1	0	0	0	46	0.00			
1STS	14	2	0	0	0	46	0.00			
1STS	14	3	0	0	*	0	*	0	10.84 ± 3.05	100

Life stage	Treatment days	Replicate No.	Sieve 1	Sieve 2	TOTAL	No. Fruit	Insects per fruit	Average insects per fruit $\pm$ STDEV	Insects per fruit (controls) $\pm$ STDEV	% mortality
2NDS	3	1	51	5	56	46	1.22			
2NDS	3	2	54	6	60	46	1.30			
2NDS	3	3	46	10	56	46	1.22	1.25 $\pm$ 0.05	18.06 $\pm$ 4.02	93.10
2NDS	4	1	102	6	108	46	2.35			
2NDS	4	2	78	17	95	46	2.07			
2NDS	4	3	109	16	125	46	2.72	2.38 $\pm$ 0.33	18.06 $\pm$ 4.02	86.84
2NDS	5	1	38	1	39	46	0.85			
2NDS	5	2	34	0	34	46	0.74			
2NDS	5	3	18	2	20	46	0.43	0.68 $\pm$ 0.21	18.06 $\pm$ 4.02	96.27
2NDS	6	1	7	3	10	46	0.22			
2NDS	6	2	0	3	3	46	0.07			
2NDS	6	3	4	0	4	46	0.09	0.12 $\pm$ 0.08	18.06 $\pm$ 4.02	99.32
2NDS	7	1	2	0	2	46	0.04			
2NDS	7	2	1	0	1	46	0.02			
2NDS	7	3	1	1	2	46	0.04	0.04 $\pm$ 0.01	18.06 $\pm$ 4.02	99.80
2NDS	8	1	1	1	2	46	0.04			
2NDS	8	2	2	0	2	46	0.04			
2NDS	8	3	3	2	5	46	0.11	0.07 $\pm$ 0.04	18.06 $\pm$ 4.02	99.64
2NDS	9	1	0	0	0	46	0			
2NDS	9	2	2	1	3	46	0.07			
2NDS	9	3	1	0	1	46	0.02	0.03 $\pm$ 0.03	18.06 $\pm$ 4.02	99.84
2NDS	10	1	1	0	1	46	0.02			
2NDS	10	2	0	1	1	46	0.02			
2NDS	10	3	0	0	0	46	0	0.01 $\pm$ 0.01	18.06 $\pm$ 4.02	99.92
2NDS	12	1	0	0	0	46	0			
2NDS	12	2	0	0	0	46	0			
2NDS	12	3	0	0	0	46	0	0	18.06 $\pm$ 4.02	100
2NDS	14	1	0	0	0	46	0			
2NDS	14	2	0	0	0	46	0			
2NDS	14	3	0	0	0	0	*	0	18.06 $\pm$ 4.02	100

Life stage	Treatment days	Replicate No.	Sieve 1	Sieve 2	TOTAL	No. Fruit	Insects per fruit	Average insects per fruit $\pm$ STDEV	Insects per fruit (controls) $\pm$ STDEV	% mortality
3RDS	3	1	399	10	409	46	8.89			
3RDS	3	2	342	3	345	46	7.5			
3RDS	3	3	447	17	464	46	10.09	8.83 $\pm$ 1.29	16.92 $\pm$ 1.68	47.84
3RDS	4	1	155	0	155	46	3.37			
3RDS	4	2	69	4	73	46	1.59			
3RDS	4	3	122	1	123	46	2.67	2.54 $\pm$ 0.90	16.92 $\pm$ 1.68	84.97
3RDS	5	1	48	0	48	46	1.04			
3RDS	5	2	33	0	33	46	0.72			
3RDS	5	3	28	0	28	46	0.61	0.79 $\pm$ 0.23	16.92 $\pm$ 1.68	95.33
3RDS	6	1	8	0	8	46	0.17			
3RDS	6	2	18	0	18	46	0.39			
3RDS	6	3	9	0	9	46	0.20	0.25 $\pm$ 0.12	16.92 $\pm$ 1.68	98.50
3RDS	7	1	22	0	22	46	0.48			
3RDS	7	2	5	0	5	46	0.11			
3RDS	7	3	3	0	3	46	0.07	0.22 $\pm$ 0.23	16.92 $\pm$ 1.68	98.72
3RDS	8	1	5	3	8	46	0.18			
3RDS	8	2	12	0	12	46	0.27			
3RDS	8	3	12	5	17	46	0.37	0.27 $\pm$ 0.10	16.92 $\pm$ 1.68	98.42
3RDS	9	1	0	0	0	46	0			
3RDS	9	2	3	1	4	46	0.09			
3RDS	9	3	1	1	2	46	0.04	0.04 $\pm$ 0.04	16.92 $\pm$ 1.68	99.74
3RDS	10	1	1	0	1	46	0.02			
3RDS	10	2	2	0	2	46	0.04			
3RDS	10	3	4	0	4	46	0.09	0.05 $\pm$ 0.03	16.92 $\pm$ 1.68	99.70
3RDS	12	1	0	0	0	46	0			
3RDS	12	2	0	0	0	46	0			
3RDS	12	3	0	0	0	46	0	0	16.92 $\pm$ 1.68	100
3RDS	14	1	0	0	0	46	0			
3RDS	14	2	0	0	0	46	0			
3RDS	14	3	0	0	0	46	*	0	16.92	100

\* No data for this entry.

**Table 11: Larval development studies for large scale trials at 3°C (replicates 1, 2, 3 and 4)**

**3°C Replicate 1 Confirmatory trial Larval development**

Date of infestation	Sample unit	NUMBERS OF EACH LIFESTAGE				TOTAL	PERCENT AT EACH LIFESTAGE			
		EGGS	1st instar	2nd instar	3rd instar		Eggs	1st instar	2nd instar	3rd instar
2 January 2007	1	21	59	0	0	80	26	74	0	0
Date of larval assessment	2	5	102	1	0	108	5	94	1	0
	3	11	78	2	0	91	12	86	2	0
8 January 2007	4	10	88	1	0	99	10	89	1	0
	5	9	74	0	0	83	11	89	0	0
AVERAGE							13	86	1	0

**3°C Replicate 2 Confirmatory trial Larval development**

Date of infestation	Sample unit	NUMBERS OF EACH LIFESTAGE				TOTAL	PERCENT AT EACH LIFESTAGE			
		EGGS	1st instar	2nd instar	3rd instar		Eggs	1st instar	2nd instar	3rd instar
9 January 2007	1	10	62	1	0	73	14	85	1	0
Date of larval assessment	2	25	51	0	0	76	33	67	0	0
	3	21	98	2	0	121	17	81	2	0
15 January 2007	4	18	87	0	0	105	17	83	0	0
	5	19	102	0	0	121	16	84	0	0
AVERAGE							19	80	<1	0

**3°C Replicate 3 Confirmatory trial Larval development**

Date of infestation	Sample unit	NUMBERS OF EACH LIFESTAGE				TOTAL	PERCENT AT EACH LIFESTAGE			
		EGGS	1st instar	2nd instar	3rd instar		Eggs	1st instar	2nd instar	3rd instar
6 February 2007	1	12	28	0	0	40	30	70	0	0
Date of larval assessment	2	15	32	0	0	47	32	68	0	0
	3	8	12	0	0	20	40	60	0	0
10 February 2007	4	9	21	0	0	20	45	55	0	0
	5	4	19	0	0	23	17	83	0	0
AVERAGE							33	67	0	0

**3°C Replicate 4 Confirmatory trial Larval development**

Date of infestation	Sample unit	NUMBERS OF EACH LIFESTAGE				TOTAL	PERCENT AT EACH LIFESTAGE			
		EGGS	1st instar	2nd instar	3rd instar		Eggs	1st instar	2nd instar	3rd instar
20 February 2007	1	6	81	0	0	87	7	93	0	0
Date of larval assessment	2	14	85	0	0	99	14	86	0	0
	3	15	69	2	0	86	17	80	3	0
23 February 2007	4	5	77	1	0	83	6	93	1	0
	5	10	91	0	0	101	10	90	0	0
AVERAGE							11	88	1	0

**Table 12: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 3°C (replicate 1)**

2840 cherries were infested on the cages at GHI. 440 fruit (CONTROL) were randomly chosen and placed in 4 pupation trays and held at 26°C until larvae had “hopped” from the fruit. These were counted to obtain an average number of survivors per fruit. The remaining 2400 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 <sup>st</sup> instar larval stage for the 3°C disinfestation treatment.							
<b>LARGE SCALE TRIAL AT 3°C SWEETHEART REPLICATE 1 – Infested 02 Jan 2007</b>							
Sample unit (110 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Number of fruit	Pupae per fruit	Average	Stdev
Sieve date	15 Jan 2007	22 Jan 2007					
1	791	59	850	110	7.73		
2	888	99	987	110	8.97		
3	759	98	857	110	7.91		
4	749	40	789	110	7.17	7.92	0.76

**Table 13: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 3°C (replicate 2)**

3102 cherries were infested on the cages at GHI. 520 fruit (CONTROL) were randomly chosen and placed in 4 pupation trays and held at 26°C until larvae had “hopped” from the fruit. These were counted to obtain an average number of survivors per fruit. The remaining 2582 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 <sup>st</sup> instar larval stage for the 3°C disinfestation treatment.							
<b>LARGE SCALE TRIAL AT 3°C SWEETHEART REPLICATE 2 – Infested 09 Jan 2007</b>							
Sample unit (130 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Number of fruit	Pupae per fruit	Average	Stdev
Sieve date	22 Jan 2007	29 Jan 2007					
1	1127	52	1179	130	9.07		
2	1276	75	1351	130	10.39		
3	1257	46	1303	130	10.02		
4	1191	57	1248	130	9.6	9.77	0.57

**Table 14: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 3°C (replicate 3)**

2840 cherries were infested on the cages at GHI. 440 fruit (CONTROL) were randomly chosen and placed in 4 pupation trays and held at 26°C until larvae had “hopped” from the fruit. These were counted to obtain an average number of survivors per fruit. The remaining 2400 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 <sup>st</sup> instar larval stage for the 3°C disinfestation treatment.							
<b>LARGE SCALE TRIAL AT 3°C SWEETHEART REPLICATE 3 – INFESTED 06 FEB 2007</b>							
Sample unit (110 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Number of fruit	Pupae per fruit	Average	Stdev
Sieve date	19 Feb 2007	26 Feb 2007					
1	257	111	368	110	3.35		
2	138	65	203	110	1.85		
3	220	103	323	110	2.94		
4	110	59	169	110	1.54	2.42	0.86

**Table 15: Survival of insects in untreated (control) fruit – Sweetheart large scale trial at 3°C (replicate 4)**

2840 cherries were infested on the cages at GHI. 440 fruit (CONTROL) were randomly chosen and placed in 4 pupation trays and held at 26°C until larvae had “hopped” from the fruit. These were counted to obtain an average number of survivors per fruit. The remaining 2400 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 <sup>st</sup> instar larval stage for the 3°C disinfestation treatment.							
<b>LARGE SCALE TRIAL AT 3°C SWEETHEART REPLICATE 4 – Infested 20 Feb 2007</b>							
Sample unit (110 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Number of fruit	Pupae per fruit	Average	Stdev
Sieve date	05 Mar 2007	12 Mar 2007					
1	2170	18	2188	110	19.89		
2	2080	32	2112	110	19.2		
3	2600	45	2645	110	24.05		
4	2630	22	2652	110	24.11	21.81	2.63

**Table 16: Large scale trials at 3°C (replicates 1, 2, 3 and 4) – Treated fruit**

VARIETY	REPLICATE	TREATMENT	TREATMENT UNIT	No. FRUIT	No. PUPAE SIEVE 1	No. PUPAE SIEVE 2
SWEETHEART	1	14 DAYS @ 3°C	1	200	0	0
SWEETHEART	1	14 DAYS @ 3°C	2	200	0	0
SWEETHEART	1	14 DAYS @ 3°C	3	200	0	0
SWEETHEART	1	14 DAYS @ 3°C	4	200	0	0
SWEETHEART	1	14 DAYS @ 3°C	5	200	0	0
SWEETHEART	1	14 DAYS @ 3°C	6	200	0	0
SWEETHEART	1	14 DAYS @ 3°C	7	200	0	0
SWEETHEART	1	14 DAYS @ 3°C	8	200	0	0
SWEETHEART	1	14 DAYS @ 3°C	9	200	0	0
SWEETHEART	1	14 DAYS @ 3°C	10	200	0	0
SWEETHEART	1	14 DAYS @ 3°C	11	200	0	0
SWEETHEART	1	14 DAYS @ 3°C	12	200	0	0
<b>SUB TOTAL</b>				<b>2400</b>	<b>0</b>	<b>0</b>
SWEETHEART	2	14 DAYS @ 3°C	1	200	0	0
SWEETHEART	2	14 DAYS @ 3°C	2	200	0	0
SWEETHEART	2	14 DAYS @ 3°C	3	200	0	0
SWEETHEART	2	14 DAYS @ 3°C	4	200	0	0
SWEETHEART	2	14 DAYS @ 3°C	5	200	0	0
SWEETHEART	2	14 DAYS @ 3°C	6	200	0	0
SWEETHEART	2	14 DAYS @ 3°C	7	200	0	0
SWEETHEART	2	14 DAYS @ 3°C	8	200	0	0
SWEETHEART	2	14 DAYS @ 3°C	9	200	0	0
SWEETHEART	2	14 DAYS @ 3°C	10	200	0	0
SWEETHEART	2	14 DAYS @ 3°C	11	200	0	0
SWEETHEART	2	14 DAYS @ 3°C	12	200	0	0
SWEETHEART	2	14 DAYS @ 3°C	13	182	0	0
<b>SUB TOTAL</b>				<b>2582</b>	<b>0</b>	<b>0</b>
SWEETHEART	3	14 DAYS @ 3°C	1	200	0	0
SWEETHEART	3	14 DAYS @ 3°C	2	200	0	0
SWEETHEART	3	14 DAYS @ 3°C	3	200	0	0

SWEETHEART	3	14 DAYS @ 3°C	4	200	0	0
SWEETHEART	3	14 DAYS @ 3°C	5	200	0	0
SWEETHEART	3	14 DAYS @ 3°C	6	200	0	0
SWEETHEART	3	14 DAYS @ 3°C	7	200	0	0
SWEETHEART	3	14 DAYS @ 3°C	8	200	0	0
SWEETHEART	3	14 DAYS @ 3°C	9	200	0	0
SWEETHEART	3	14 DAYS @ 3°C	10	200	0	0
SWEETHEART	3	14 DAYS @ 3°C	11	200	0	0
SWEETHEART	3	14 DAYS @ 3°C	12	200	0	0
<b>SUB TOTAL</b>				<b>2400</b>	<b>0</b>	<b>0</b>
SWEETHEART	4	14 DAYS @ 3°C	1	200	0	0
SWEETHEART	4	14 DAYS @ 3°C	2	200	0	0
SWEETHEART	4	14 DAYS @ 3°C	3	200	0	0
SWEETHEART	4	14 DAYS @ 3°C	4	200	0	0
SWEETHEART	4	14 DAYS @ 3°C	5	200	0	0
SWEETHEART	4	14 DAYS @ 3°C	6	200	0	0
SWEETHEART	4	14 DAYS @ 3°C	7	200	0	0
SWEETHEART	4	14 DAYS @ 3°C	8	200	0	0
SWEETHEART	4	14 DAYS @ 3°C	9	200	0	0
SWEETHEART	4	14 DAYS @ 3°C	10	200	0	0
SWEETHEART	4	14 DAYS @ 3°C	11	200	0	0
SWEETHEART	4	14 DAYS @ 3°C	12	200	0	0
<b>SUB TOTAL</b>				<b>2400</b>	<b>0</b>	<b>0</b>
<b>GRAND TOTAL</b>				<b>9782</b>	<b>0</b>	<b>0</b>

**Table 17: Effects of cold storage at 1°C and 3°C on quality of Sweetheart cherries.**

<b>Attribute</b>	<b>On receipt from grower</b>	<b>After 14 days at 1°C</b>	<b>After 21 days at 1°C</b>
<b>Peduncle discoloration <sup>x</sup></b>	1.05 <sup>a</sup> <sup>A</sup>	1.08 <sup>a</sup>	1.07 <sup>a</sup>
<b>Skin colour <sup>y</sup></b>	1.83 <sup>a</sup>	1.84 <sup>a</sup>	1.82 <sup>a</sup>
<b>External damage <sup>z</sup></b>	1.00 <sup>a</sup>	1.09 <sup>a</sup>	1.17 <sup>a</sup>
<b>Force to remove peduncle (g)</b>	10.7 <sup>a</sup>	10.3 <sup>a</sup>	10.4 <sup>a</sup>

<b>Attribute</b>	<b>On receipt from grower</b>	<b>After 14 days at 3°C</b>	<b>After 21 days at 3°C</b>
<b>Peduncle discoloration</b>	1.05 <sup>a</sup>	2.22 <sup>b</sup>	2.23 <sup>b</sup>
<b>Skin colour</b>	1.83 <sup>a</sup>	1.82 <sup>a</sup>	1.82 <sup>a</sup>
<b>External damage</b>	1.00 <sup>a</sup>	1.10 <sup>a</sup>	1.21 <sup>a</sup>
<b>Force to remove peduncle (g)</b>	10.7 <sup>a</sup>	9.1 <sup>b</sup>	8.5 <sup>b</sup>

**A** Means in each row followed by the same letter do not significantly differ ( $P < 0.05$ ) based on the Duncan-Waller Bayesian k- ratio test ( $k=100$ ). Each figure represents the mean of five replicates of 50 fruit each.

**x** Scored from 1: completely green to 10: completely discoloured.

**y** Scored from 1: completely black to 5: completely red.

**z** Scored from 1: 0% surface area damaged to 5: 100% surface area damaged.

**Table 18: Data logger calibration, statistics and graphs**

Four Grant 2040 series Squirrel Data Loggers with metal oxide 2 K Ohm thermistor probes were used with serial numbers KV0529008, KV0624006, KV0624007, KV0445002. Probes have a temperature range between -50 and 150 °C with an accuracy of  $\pm 0.2^\circ\text{C}$ . The thermistor probes are connected to the logger by a factory built and calibrated cable of 5m. The Grant 2040 series Squirrel Data Logger has 32 channels available for temperature input. Loggers were calibrated by placing probes in ice slurry in an insulated vessel for 1 hour and logging the temperature at 10 minute intervals. The calibration details are as follows:

**Calibration for data loggers for large scale cold trial**

<b>LOGGER KV0529008</b>								
<b>DATE / TIME</b>	<b>CH 1</b>	<b>CH 2</b>	<b>CH 3</b>	<b>CH 4</b>	<b>CH 5</b>	<b>CH 6</b>	<b>CH 7</b>	<b>CH 8</b>
26/03/2007 10:41:31.090	-0.01	0.03	-0.01	-0.01	0	0.05	0.11	-0.09
26/03/2007 10:51:31.090	-0.01	0.03	-0.01	-0.01	0	0.04	0.11	-0.08
26/03/2007 11:01:31.090	-0.01	0.03	-0.01	-0.01	0	0.04	0.1	-0.08
26/03/2007 11:11:31.090	-0.02	0.03	-0.01	-0.01	-0.01	0.04	0.1	-0.08
26/03/2007 11:21:31.090	-0.02	0.03	-0.01	-0.01	-0.01	0.04	0.1	-0.08
26/03/2007 11:31:31.090	-0.02	0.03	-0.01	-0.01	-0.01	0.04	0.09	-0.08
26/03/2007 11:41:31.090	-0.02	0.02	-0.01	-0.01	-0.01	0.03	0.09	-0.08
Calibration (°C)	-0.02	0.03	-0.01	-0.01	-0.01	0.04	0.1	-0.08

**Calibration for data loggers for large scale cold trial**

<b>LOGGER KV0624006</b>								
<b>DATE / TIME</b>	<b>CH 1</b>	<b>CH 2</b>	<b>CH 3</b>	<b>CH 4</b>	<b>CH 5</b>	<b>CH 6</b>	<b>CH 7</b>	<b>CH 8</b>
21/03/2007 13:17:51.090	0.13	0.2	-0.09	0.09	0.09	0.1	0.13	0.14
21/03/2007 13:27:51.090	0.15	0.1	-0.11	0.08	0.07	0.07	0.12	0.11
21/03/2007 13:37:51.090	0.14	0.1	-0.1	0.08	0.07	0.07	0.11	0.11
21/03/2007 13:47:51.090	0.14	0.1	-0.1	0.09	0.07	0.07	0.11	0.09
21/03/2007 13:57:51.090	0.14	0.11	-0.1	0.1	0.07	0.07	0.11	0.09
21/03/2007 14:07:51.090	0.15	0.12	-0.1	0.1	0.07	0.07	0.1	0.09
21/03/2007 14:16:51.090	0.14	0.12	-0.1	0.1	0.07	0.06	0.1	0.08
Calibration (°C)	0.14	0.12	-0.1	0.09	0.07	0.07	0.11	0.10

### Calibration for data loggers for large scale cold trial

<b>LOGGER KV0624007</b>								
<b>DATE / TIME</b>	<b>CH 1</b>	<b>CH 2</b>	<b>CH 3</b>	<b>CH 4</b>	<b>CH 5</b>	<b>CH 6</b>	<b>CH 7</b>	<b>CH 8</b>
26/03/2007 12:10:50.090	0.07	0	0.22	0.36	0.1	0.04	0.32	0.15
26/03/2007 12:20:50.090	0.04	-0.02	-0.12	0.11	0.04	0.01	0.09	0.01
26/03/2007 12:30:50.090	0.03	-0.02	-0.13	0.11	0.04	0.01	0.09	0.01
26/03/2007 12:40:50.090	0.04	-0.02	-0.13	0.11	0.04	0.01	0.09	0.01
26/03/2007 12:50:50.090	0.03	-0.02	-0.13	0.11	0.04	0.02	0.09	0.01
26/03/2007 13:00:50.090	0.03	-0.02	-0.13	0.11	0.04	0.02	0.09	0.01
26/03/2007 13:10:50.090	0.04	-0.02	-0.13	0.11	0.04	0.03	0.09	0.01
Calibration (°C)	0.04	-0.02	-0.07	0.15	0.05	0.02	0.13	0.03

### Calibration for data loggers for large scale cold trial

<b>LOGGER KV0445002</b>								
<b>DATE / TIME</b>	<b>CH 1</b>	<b>CH 2</b>	<b>CH 3</b>	<b>CH 4</b>	<b>CH 5</b>	<b>CH 6</b>	<b>CH 7</b>	<b>CH 8</b>
02/05/2007 08:51:42.090	0.03	-0.01	-0.14	0.12	0.06	0.21	-0.01	0.02
02/05/2007 09:01:42.090	0.02	-0.01	-0.14	0.12	0.06	0.18	-0.04	0.03
02/05/2007 09:11:42.090	0.02	-0.01	-0.13	0.12	0.06	0.19	-0.02	0.04
02/05/2007 09:21:42.090	0.02	0	-0.13	0.12	0.06	0.19	-0.01	0.04
02/05/2007 09:31:42.090	0.02	0	-0.12	0.13	0.06	0.2	0	0.05
02/05/2007 09:41:42.090	0.02	0	-0.12	0.13	0.06	0.2	0.01	0.06
02/05/2007 09:51:42.090	0.02	0.01	-0.11	0.14	0.07	0.21	0.02	0.07
Calibration (°C)	0.02	0.00	-0.13	0.13	0.06	0.20	0.00	0.04

Table 19: Data logger statistics for large scale trials at 1°C (replicate 1)

<b>Cherries large scale trial at 1°C replicate 1 (Sweetheart)</b>								
<b>Logger KV0445002</b>	<b>CH1</b>	<b>CH2</b>	<b>CH3</b>	<b>CH4</b>	<b>CH5</b>	<b>CH6</b>	<b>CH7</b>	<b>CH8</b>
<b>Total treatment time(hrs)</b>	357	357	357	357	357	357	357	357
<b>Average temp (°C)</b>	1.56	1.36	1.37	1.13	1.26	1.15	1.01	1.24
<b>no. readings above 1.5°C</b>	646	16	15	1	5	0	1	0
<b>no. readings below 0.5°C</b>	0	0	0	0	0	0	0	0
<b>Total number of readings</b>	715	715	715	715	715	715	715	715
<b>Time &gt; 1.5°C (hrs)</b>	323.00	8.00	7.50	0.50	2.50	0.00	0.50	0.00
<b>Time &lt; 0.5°C (hours)</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>% time &gt; 1.5°C</b>	90.48	2.24	2.10	0.14	0.70	0.00	0.14	0.00
<b>% time &lt; 0.5°C</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>% time within 0.5°C - 1.5°C</b>	9.52	97.76	97.90	99.86	99.30	100.00	99.86	100.00

Table 20: Data logger statistics for large scale trials at 1°C (replicate 2)

<b>Cherries large scale trial at 1°C replicate 2 (Sweetheart)</b>								
<b>LOGGER KV0529008</b>	<b>CH1</b>	<b>CH2</b>	<b>CH3</b>	<b>CH4</b>	<b>CH5</b>	<b>CH6</b>	<b>CH7</b>	<b>CH8</b>
<b>Total treatment time(hrs)</b>	349.00	349.00	349.00	349.00	349.00	*	349.00	349.00
<b>Average temp (°C)</b>	1.39	1.08	1.05	1.08	1.43	*	1.23	1.47
<b>no. readings above 1.5°C</b>	35	3	0	2	96	*	12	179
<b>no. readings below 0.5°C</b>	0	9	0	1	0	*	0	0
<b>Total number of readings</b>	698	698	698	698	698	*	698	698
<b>Time &gt; 1.5°C (hrs)</b>	17.50	1.50	0.00	1.00	48.00	*	6.00	89.50
<b>Time &lt; 0.5°C (hours)</b>	0.00	4.50	0.00	0.50	0.00	*	0.00	0.00
<b>% time &gt; 1.5°C</b>	5.01	0.43	0.00	0.29	13.75	*	1.72	25.64
<b>% time &lt; 0.5°C</b>	0.00	1.29	0.00	0.14	0.00	*	0.00	0.00
<b>% time within 0.5°C - 1.5°C</b>	94.99	98.28	100.00	99.57	86.25	*	98.28	74.36

Table 21: Data logger statistics for large scale trials at 1°C (replicate 3)

<b>Cherries large scale trial at 1°C replicate 3 (Sweetheart)</b>								
<b>LOGGER KV0624007</b>	<b>CH1</b>	<b>CH2</b>	<b>CH3</b>	<b>CH4</b>	<b>CH5</b>	<b>CH6</b>	<b>CH7</b>	<b>CH8</b>
<b>Total treatment time(hrs)</b>	365.00	365.00	365.00	365.00	365.00	365.00	365.00	*
<b>Average temp (°C)</b>	1.22	1.28	1.25	0.87	1.26	1.22	0.93	*
<b>no. readings above 1.5°C</b>	9	9	14	0	3	3	7	*
<b>no. readings below 0.5°C</b>	1	0	0	7	0	0	0	*
<b>Total number of readings</b>	731	731	731	731	731	731	731	*
<b>Time &gt; 1.5°C (hrs)</b>	4.5	4.5	7	0	1.5	1.5	3.5	*
<b>Time &lt; 0.5°C (hours)</b>	0.5	0	0	3.5	0	0	0	*
<b>% time &gt; 1.5°C</b>	1.23	1.23	1.92	0.00	0.41	0.41	0.96	*
<b>% time &lt; 0.5°C</b>	0.14	0.00	0.00	0.96	0.00	0.00	0.00	*
<b>% time within 1.5°C - 0.5°C</b>	98.63	98.77	98.08	99.04	99.59	99.59	99.04	*

**Table 22: Data logger statistics for large scale trials at 3°C (replicate 1)**

<b>Cherries large scale trial at 3°C replicate 1 (Sweetheart)</b>								
	<b>CH1</b>	<b>CH2</b>	<b>CH3</b>	<b>CH4</b>	<b>CH5</b>	<b>CH6</b>	<b>CH7</b>	<b>CH8</b>
<b>Total treatment time(hrs)</b>	357.5	357.5	357.5	357.5	357.5	357.5	357.5	357.5
<b>Average temp (°C)</b>	3.29	3.26	3.31	3.31	3.14	3.20	2.87	3.25
<b>no. readings above 3.5°C</b>	14	12	18	15	1	2	0	11
<b>no. readings below 2.5°C</b>	0	0	0	0	0	0	1	0
<b>Total number of readings</b>	715	715	715	715	715	715	715	715
<b>Time &gt; 3.5°C (hrs)</b>	7	6	9	7.5	0.5	1	0	5.5
<b>Time &lt; 2.5°C (hours)</b>	0	0	0	0	0	0	0.5	0
<b>% time &gt; 3.5°C</b>	1.96	1.68	2.52	2.10	0.14	0.28	0.00	1.54
<b>% time &lt; 2.5°C</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00
<b>% time within 2.5°C - 3.5°C</b>	98.04	98.32	97.48	97.90	99.86	99.72	99.86	98.46

**Table 23: Data logger statistics for large scale trials at 3°C (replicate 2)**

<b>Cherries large scale trial at 3°C replicate 2 (Sweetheart)</b>								
<b>LOGGER KV0624007</b>	<b>CH1</b>	<b>CH2</b>	<b>CH3</b>	<b>CH4</b>	<b>CH5</b>	<b>CH6</b>	<b>CH7</b>	<b>CH8</b>
<b>Total treatment time(hrs)</b>	358.00	358.00	358.00	358.00	358.00	358.00	358.00	358.00
<b>Average temp (°C)</b>	3.23	3.25	3.39	3.32	3.29	3.35	3.34	3.60
<b>no. readings above 3.5°C</b>	5	6	44	19	11	32	27	671
<b>no. readings below 2.5°C</b>	0	0	0	0	0	0	0	0
<b>Total number of readings</b>	717	717	717	717	717	717	717	717
<b>Time &gt; 3.5°C (hrs)</b>	2.5	3	22	9.5	5.5	16	13.5	335.5
<b>Time &lt; 2.5°C (hours)</b>	0	0	0	0	0	0	0	0
<b>% time &gt; 3.5°C</b>	0.70	0.84	6.15	2.65	1.54	4.47	3.77	93.72
<b>% time &lt; 2.5°C</b>	0	0	0	0	0	0	0	0
<b>% time within 2.5°C - 3.5°C</b>	99.30	99.16	93.85	97.35	98.46	95.53	96.23	6.28

**Table 24: Data logger statistics for large scale trials at 3°C (replicate 3)**

<b>Cherries large scale trial at 3°C replicate 3 (Sweetheart)</b>								
	<b>CH1</b>	<b>CH2</b>	<b>CH3</b>	<b>CH4</b>	<b>CH5</b>	<b>CH6</b>	<b>CH7</b>	<b>CH8</b>
<b>Total treatment time (hrs)</b>	357.50	357.50	357.50	357.50	357.50	357.50	357.50	357.50
<b>Average temp (°C)</b>	2.94	2.82	2.87	2.81	3.13	2.83	2.91	2.97
<b>no. readings above 3.5°C</b>	0	2	3	3	2	0	0	0
<b>no. readings below 2.5°C</b>	0	0	0	0	0	13	7	0
<b>Total number of readings</b>	716	716	716	716	716	716	716	716
<b>Time &gt; 3.5°C (hrs)</b>	0	1	1.5	1.5	1	0	0	0
<b>Time &lt; 2.5°C (hours)</b>	0	0	0	0	0	6.5	3.5	0
<b>% time &gt; 3.5°C</b>	0.00	0.28	0.42	0.42	0.28	0.00	0.00	0.00
<b>% time &lt; 2.5°C</b>	0.00	0.00	0.00	0.00	0.00	1.82	0.98	0.00
<b>% time within 2.5°C - 3.5°C</b>	100.00	99.72	99.58	99.58	99.72	98.18	99.02	100.00

Table 25: Data logger statistics for large scale trials at 3°C (replicate 4)

<b>Cherries large scale trial at 3°C replicate 4 (Sweetheart)</b>								
	<b>CH1</b>	<b>CH2</b>	<b>CH3</b>	<b>CH4</b>	<b>CH5</b>	<b>CH6</b>	<b>CH7</b>	<b>CH8</b>
<b>Total treatment time (hrs)</b>	354.5	354.5	354.5	354.5	354.5	354.5	*	*
<b>Average temp (°C)</b>	3.21	3.11	3.13	3.27	3.30	3.03	*	*
<b>no. readings above 3.5°C</b>	1	1	4	2	8	5	*	*
<b>no. readings below 2.5°C</b>	0	0	0	0	0	0	*	*
<b>Total number of readings</b>	710	710	710	710	710	710	*	*
<b>Time &gt; 3.5°C (hrs)</b>	0.5	0.5	2	1	4	2.5	*	*
<b>Time &lt; 2.5°C (hours)</b>	0	0	0	0	0	0	*	*
<b>% time &gt; 3.5°C</b>	0.14	0.14	0.56	0.28	1.13	0.71	*	*
<b>% time &lt; 2.5°C</b>	0	0	0	0	0	0	*	*
<b>% time within 2.5°C - 3.5°C</b>	99.86	99.86	99.44	99.72	98.87	99.29	*	*