

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

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PART ONE – GENERAL INFORMATION AND TEST METHODS

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

CONDUCTED AT

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Summary

This report presents data which proves the efficacy of cold storage as a quarantine treatment against Queensland fruit fly (*Bactrocera tryoni*) in five varieties of Australian table grapes (Flame Seedless, Red globes, Ruby Seedless, Crimson seedless, and Thompson seedless). These data form the basis of a submission to the importing authorities to approve the importation of Australian table grapes.

The proposed treatments are:

- *Storage of fruit at a core temperature of 1°C or below for 12 days*
- *Storage of fruit at a core temperature of 2°C or below for 14 days*
- *Storage of fruit at a core temperature of 3°C or below for 14 days*

A series of replicated experiments to prove treatment efficacy were conducted by scientists from the NSW Department of Primary Industries (NSWDPI), Gosford New South Wales. All experiments conducted at 1°C were carried out from 1986 to 1990 and all experiments conducted at 2°C and 3°C were carried out from 2005 to 2007.

Following is the report on the experiments which describes the Experimental Plan, Experimental Details, Results, Discussion and Conclusions, Appendices and References.

Table i. Summary of Experimental Results – 1°C Tests

<i>Variety (Replicate)</i>	<i>No .fruit treated at 1°C</i>	<i>Estimated no. of insects treated at 1°C</i>	<i>No. insects surviving treatment for 12 days at 1°C</i>
Total Flame Seedless	56752	66895	0
Total Ruby seedless	25955	47341	0
Total Thompson seedless	80284	39829	0

Table ii. Summary of Experimental Results – 2°C Tests

<i>Variety (Replicate)</i>	<i>No. fruit treated at 2°C</i>	<i>Av. No. of insects treated per fruit</i>	<i>Estimated no. of insects treated at 2°C</i>	<i>No. insects surviving treatment for 14 Days at 2°C</i>
<i>Red Globes (1)</i>	3300	5.4	17827	0
<i>Red Globes (2)</i>	2096	0.34	714	0
<i>Red Globes (3)</i>	4000	2.53	10120	0
<i>Red Globes (4)</i>	1050	23.3	24474	0
Total Red Globes	10446		53136	0
<i>Crimson seedless (1)</i>	9000	0.62	5568	0
<i>Crimson seedless (2)</i>	2400	3.28	7888	0
<i>Crimson seedless (3)</i>	4000	5.35	21392	0
<i>Crimson seedless (4)</i>	4000	3.34	13348	0
Total Crimson seedless	19400		48196	0
<i>Thompson seedless(1)</i>	5400	1.5	8136	0
<i>Thompson seedless(2)</i>	2278	5.64	12841	0
<i>Thompson seedless(3)</i>	2237	7.34	16413	0
<i>Thompson seedless(4)</i>	2125	0.712	1513	0
Total Thompson seedless	12040		38903	0

Table iii. Summary of Experimental Results – 3°C Tests

<i>Variety (Replicate)</i>	<i>No .fruit treated at 3°C</i>	<i>Av. No. of insects treated per fruit</i>	<i>Estimated no. of insects treated at 3°C</i>	<i>No. insects surviving treatment for 14 days at 3°C</i>
<i>Red Globes (1)</i>	3028	2.6	7878	0
<i>Red Globes(2)</i>	2000	6.7	13444	0
<i>Red Globes(3)</i>	4000	3.695	14780	0
<i>Red Globes (4)</i>	3000	2.5	7520	0
Total Red Globes	12028	-	43622	0
<i>Crimson seedless (1)</i>	6050	0.092	559	0
<i>Crimson seedless (2)</i>	4000	6.9	27776	0
<i>Crimson seedless (3)</i>	4000	4.477	17908	0
<i>Crimson seedless (4)</i>	3000	8.196	24588	0
Total Crimson seedless	17050	-	70831	0
<i>Thompson seedless(1)</i>	5000	6.17	30898	0
<i>Thompson seedless(2)</i>	4000	15.82	63248	0
<i>Thompson seedless(3)</i>	2000	9.337	18674	0
<i>Thompson seedless(4)</i>	2000	10.42	20842	0
Total Thompson seedless	13000	-	133663	0

1 General information

1.1 Test insects:

1.1.1 Origin (Location and date of collection, host plant, etc.)

1.1.1.1 Queensland fruit fly - *Bactrocera tryoni* - 1°C Tests

The laboratory colony of *B. tryoni* was established at the Gosford Postharvest Laboratory in 1956. Since then numerous injections of wild flies trapped in fruit from several districts on the coast of New South Wales have been made to preserve the colony's natural character. Each year since 1988 wild fruit fly are bred from field-infested fruit brought into the laboratory. These fruit are placed on mesh suspended over a pupation medium (sand, damp hardwood sawdust or damp fine-grade (Number 1) vermiculite and stored at 26°C. Insects which have pupated in the pupation medium are collected over a four week period for each batch of field-infested fruit and caged for emergence of adult insects. These insects are chilled at 0°C to 1°C for 2 to 4 minutes until they are immobilised. They are then sorted as to insect species. All *B. tryoni* are then caged. These “wild” fruit flies are kept separate from the laboratory colony for four generations and then incorporated into the laboratory colony after that.

1.1.1.2 Queensland fruit fly - *Bactrocera tryoni* - 2°C and 3°C Tests

Test insects were sourced from a laboratory colony of *Bactrocera tryoni* (Queensland fruit fly) at Elizabeth McArthur Agricultural Institute (EMAI), Camden New South Wales. This laboratory is maintained by scientists from the New South Wales Department of Primary Industries. The laboratory at EMAI produces approximately 800,000 insects per week and was able to supply an abundance of fruit fly eggs for the experiments performed at GHI.

The wild characteristics of the laboratory colony are maintained with introductions of *Bactrocera tryoni* reared from wild-infested fruit collected from growing regions around New South Wales.

The most treatment tolerant life stage at 3°C study was performed in March 2005 and the most treatment tolerant life stage at 2°C study was performed in March 2006. These studies confirmed that the most treatment tolerant life stage was First Instar larva – that is newly hatched *Bactrocera tryoni* larvae between 48 to 96 hours after eggs have been deposited by the adult insect.

The life stage present at the commencement of treatment was confirmed by fruit dissection, larval recovery, and examination of the mouth parts under the dissecting microscope.

1.1.2 Rearing method (Composition of larval medium and adults' food, temperature and humidity in the rearing rooms, etc

1.1.2.1 Queensland fruit fly - *Bactrocera tryoni* - 1°C Tests

Adult Cages: The adult mother colony cages were made by Zeee Industries. They are constructed from an aluminium box section (1665 mm x 510 mm x 250 mm high) using three-way corner inserts and spline groove extrusion which are attached for nylon meshing. The floor is composed of embossed aluminium sheeting for rigidity. The top of the cage has aluminium strips which run parallel to the width of the cage and are 70 mm apart. These strips are required to support fruit when infesting. Nylon flymesh is splined into the sides and top of the cage.

There is a sliding mesh-covered door (260 mm wide) which is sealed tightly to prevent flies from escaping. There are two holes in the aluminium sheets on the long side of the cage to support a 65 mm diameter piece of polypipe (waterer for flies). The newer cages have four holes which support two water pipes. On both long sides of the cage there are two chrome handles.

Setting up adult cages:

Number of adults per cage:

The stocking rate of the mother colony cage is approximately 15,000 to 20,000 flies which have a 1:1 sex ratio. The new cages are stocked with pupae produced from the mother colony.

Three small plastic boxes (180 mm x 120 mm x 80 mm high) of pupae and sawdust produced from the mother colony are placed into three separate cages. The number is not calculated weekly and is based upon past productions using 0.4 mL of eggs per tray.

Three new cages are made up weekly to maintain a constant number of mating and egg-laying flies. Cages of flies are kept for five weeks, killed and replaced with a new set. Flies are killed by starving then spraying with hot water. Hot water is used to clean the cage and it is then left outside to dry. Food and water containers are washed, soaked in a chlorine solution and are again washed and dried.

Feeding:

A small tray of sugar is placed into each of the three cages set up each week. Yeast hydrolysate is introduced into the diet once the flies begin to emerge. The measured amount of yeast is sprinkled into the cages through the flyscreen section on the top of the cage using a teaspoon. A thin layer of the yeast is dispensed in a line running across the width of the cage. If too much yeast is applied it becomes sticky due to the high humidity and flies would not eat it and become stuck to it and die.

Yeast hydrolysate and sugar are given three times per week during normal production (eg. colony maintenance). When large quantities of eggs are required (SIR production or infestation), sugar and yeast hydrolysate are given daily. If a little extra sugar is required it is sprinkled onto the floor of the cage through the flyscreen on the top.

Watering:

Water is provided from one or two lengths of 65 mm polypipe containing a slit. Inside the polypipe there is a V-shaped piece of perspex containing holes which are sealed at either end by polyurethane foam. Attapulgate is placed inside the polypipe above the perspex to prevent flies from drowning. An external inlet allows for refilling of the pipe and a lid is placed onto this to prevent flies from escaping. The new cages have two waterers whereas the older cages have only one. An extra waterer is added to those cages which only have one pipe waterer. A small plastic box is filled with attapulgate and water is poured in until it is just visible on the surface of the attapulgate. The box is then placed inside the cage.

Lighting:

Cages of flies are maintained in a fly room with fluorescent (40 W, 1.2 m) ceiling and wall lights. A light is located above each cage and is controlled by a wall clock. Lights begin to brighten at 7.30 a.m. and are fully bright by 8.30 a.m. Dimming of lights begins at 7.30 p.m. and are completely out by 8.30 p.m.

Heating:

Temperature in the fly room is thermostatically-controlled to 26°C.

Humidity:

Humidity in the fly room is automatically kept between 55 and 60% RH.

Egg Collection: Egging receptacles are identical to those used for SIR production (500 mL clear polyurethane cups pierced on sides with small holes and the outside surface roughened). A small piece of orange is threaded into a stainless steel screw inserted in the lid of the jar. A small quantity of water (20 mm) is placed in the egg cups.

Two egg cups are placed in each of the two- and three-week-old fly cages the day prior to egging the medium. When placing egg cups in cages a hairdryer is used to blow hot air into the doorway of the cage to prevent flies from escaping while the door is open. The same procedure is followed for removing egg cups.

Egg cups are removed after 24 hours. Female flies are attracted to the egg cups by the humidity in the cup and the orange. Once there, female flies oviposited eggs through the holes in the egg cup. Eggs are then washed from the egg cup into a beaker and allowed to settle. Excess water is then titred off.

Larvae:**Growth medium:**

The artificial diet consisted of:

- 6 L hot water from a tap
- 15 g sodium benzoate
- 54 g citric acid
- 180 g torula yeast
- 2 L minced dry carrot

Place hot water into a stainless steel container, add sodium benzoate and stir to dissolve. Add citric acid and stir to dissolve. Then add torula yeast, mix and then add dried, minced carrot and stir until thoroughly mixed. The lid is placed on the stainless steel container and left overnight at room temperature. Medium is stirred throughout the day to ensure that all

ingredients are mixed and carrot absorbs all the water. If by the following day the carrot has not fully expanded, more hot water is added. Medium is then left to absorb the extra water.

Plastic larval medium trays (300 mm x 200 mm x 30 mm high) with clear plastic lids are used in which to grow larvae. Twelve trays are encased in plastic bags (400 mm x 300 mm) to enable easier disposal of medium and cleaning of trays.

Medium is dispensed evenly onto the 12 trays and spread out within each tray.

Egging the Medium:

0.4 mL of settled eggs are pipetted onto the carrot medium. Eggs are then washed into the medium with a little water. Lids are placed on the trays and they are placed inside a woven terylene and material bag and moved to the fly room (26°C, 55-60% RH).

A sample of two x 100 eggs is taken and placed on dampened charcoal-impregnated black filter paper (9 cm diameter) and placed in a plastic Petrie dish with the lid on. The Petrie dish is then put in a lidded, clear plastic container (140 mm diameter x 97 mm high) in the fly room and left for four days. The number of eggs that hatch are recorded.

Larval Rearing/Incubation:

The larval medium is left in the fly room for four days and during this time eggs hatch and larvae develop to early third instar. On the fourth day the trays are removed from the bag, the lids taken off and the trays of carrot medium and larvae are placed into a "hopper" in the fly room.

The hopper is an open rectangular box on stilts (310 mm) and is made from marine stainless steel (316 mm). The rectangular box section (630 mm x 360 mm x 180 mm high) has mesh sectioned into each side and a hinged lid for aeration to prevent pupae from escaping. The hopper has a rectangular funnel in the centre at the base of the rectangular box which channels hopping larvae out of the hopper. Inside the hopper are two stainless steel grids, one above the other, on which the larval medium trays are placed.

Four larval medium trays are placed in each of the three hoppers and the lid on the hopper closed. A large plastic box (520 mm x 340 mm x 190 mm high) containing dampened sawdust (approximately 1.5 L) is placed underneath the funnel of the hopper to collect the larvae. A white terylene bag is placed over the hopper to prevent *Drosophila* from getting entering. The lids of the trays are then washed and left to dry.

Pupae:

Storage:

After six days most of the pupae have hopped from the medium and pupated in the sawdust. Boxes of pupae and sawdust are then placed into small plastic boxes (180 mm x 120 mm x 80 mm high) and placed on a scratch tray (448 mm x 315 mm x 58 mm high) inside a woven terylene bag and placed on the top shelf in the fly room (26°C, 55-60% RH).

The spent medium is disposed of and the trays washed and dried. The hopper cover is also washed and dried. Benches, walls and shelves surrounding the hoppers are also washed.

After four days the boxes of pupae are removed from the bag and one box is placed in each of three clean cages with food and water.

Weighing:

A sample of two x 50 pupae is taken, weighed and from this the average weight per pupa is determined.

Samples are placed in BACTO 250 mL sample jars containing a small quantity of slightly damp vermiculite. The lid of the jar is placed on loosely to allow air to enter. Pupae are left until emergence is completed (approximately 10 days) and the number of fully-emerged flies counted. The percentage emergence is then calculated.

Pupal weight and pupal emergence results are obtained for every batch of pupae produced (ie. weekly).

1.1.2.2 Queensland fruit fly - *Bactrocera tryoni* - 2°C and 3°C Tests

Methods of rearing *Bactrocera tryoni*

The *Bactrocera tryoni* colony at EMAI is reared on a medium comprising lucerne chaff, hydrolysed yeast, sugar, water and preservatives. Eggs are collected in perforated plastic cups that have been pierced by in excess of 200 fine holes through which the fly oviposits. Eggs are then dispensed over the medium by way of a pipette.

Developing larvae are kept at $26^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and $60\% \pm 5\%$ relative humidity. Larvae pupate in trays of slightly dampened vermiculite.

Emergent flies are also maintained at $26^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and $60\% \pm 5\%$ relative humidity in cages measuring 2000 x 1500 x 400mm.

Each cage is illuminated from above by a single 40 watt cool white fluorescent tube. The insectary is also illuminated by incandescent light.

Environmental conditions within the rearing facility

Temperature:	$26^{\circ}\text{C} \pm 1^{\circ}\text{C}$
Relative humidity:	$60\% \pm 5\%$
Light:	12 hr light (fluorescent + incandescent light): 1 hr dusk (incandescent light): 10 hr dark: 1 hr dawn (incandescent light)
Size of cage:	2m long X 1.5m high X 0.3m wide
Flies per cage:	200,000 (1:1 male: female ratio)

All other rearing methods are the same as for the Gosford laboratory.

1.2 Test fruits:

1.2.1 Origin (Location and date of harvesting, etc.)

1.2.1.1 Queensland fruit fly - *Bactrocera tryoni* - 1°C Tests

‘Thompson’s Seedless’ grapes (produced without the use of insecticides) were purchased from Flemington Produce Markets in Sydney, New South Wales. ‘Ruby Seedless’ and ‘Flame Seedless’ grapes, also harvested from unsprayed vineyards, were obtained from the NSW Agriculture research stations at Griffith and Dareton. After harvest, grapes were packaged and placed in storage at 1°C.

Cultivar : ‘Flame Seedless’

Origin : Dareton (about 1,100km by road SW of Gosford), New South Wales

Date of picking : March, 1988 / March, 1990

Cultivar : ‘Thompson’s Seedless’

Origin : Irymple (about 1,100km by road SW of Gosford), Victoria

Date of picking : March, 1986 / March, 1987

Cultivar : ‘Ruby Seedless’

Origin : Griffith (about 650km by road SW of Gosford), New South Wales

Date of picking : March, 1988 / March, 1990

1.2.1.2 Queensland fruit fly - *Bactrocera tryoni* - 2°C and 3°C Tests

The three varieties of table grapes, ‘Red Globe’, ‘Crimson Seedless’, and ‘Thompson’s Seedless’ were sourced from orchards in Victoria’s Murray Valley. This region falls within the fruit fly exclusion zone (FFEZ), and is thus classified as fruit fly free. Unless there is a fruit fly outbreak, grapes are not sprayed with insecticides. Orchards from which fruit used in these experiments were harvested, had not been sprayed with insecticides during fruit growth and maturation stage. ‘Red Globe’ and ‘Crimson Seedless’ grapes were grown and packed by T&S Romeo Pty Ltd, Cardross, Victoria (34° 17' South, 142°8' East). ‘Thompson’s Seedless’ were grown and packed by M.P. Dicheria & sons, Birdwoodton, Victoria (34° 11' South, 142°03' East).

For verification purposes, small scale studies were conducted upon receipt of fruit to confirm infestation was possible, prior to the large scale trials. After harvest, grapes were packaged and placed in storage at 1°C.

Cultivar : ‘Crimson Seedless’

Origin : Cardross, Victoria (about 1,120km SW of Gosford by road)

Date of picking : March, 2006 / April, 2006 / February, 2007

Cultivar : ‘Thompson’s Seedless’

Origin : Birdwoodton, Victoria (about 1,100km SW of Gosford by road)

Date of picking : March, 2006 / May, 2007 / February, 2007

Cultivar : ‘Red Globe’

Origin : Cardross, Victoria (about 1,120km SW of Gosford by road)

Date of picking : March, 2006 / February and April, 2007

1.2.2 Varieties, size, weight and maturity of the fruits, etc.

1.2.2.1 Queensland fruit fly - *Bactrocera tryoni* - 1°C Tests

Cultivar : ‘Flame Seedless’	Characteristics of test fruits:	Characteristics of test fruits:
Characteristics of test fruits:	Colour: cream / green	Colour: red
Colour: cherry red	Condition: firm	Condition: firm
Condition: firm	Shape: elongated, seedless	Shape: round, seedless
Shape: round, seedless	Weight: 5 - 7 g	Weight: 4 - 6.5 g
Weight: 4 - 5 g	Acidity (pH) : 3.5 - 3.6	Acidity (pH) : 3.8
Acidity (pH) : 3.6 - 3.7	Sugar (%Brix) : 17 - 18	Sugar (%Brix) : 16.5 - 17.5
Sugar (%Brix) : 17 - 18		
Cultivar : ‘Thompson’s Seedless’	Cultivar : ‘Ruby Seedless’	

1.2.2.2 Queensland fruit fly - *Bactrocera tryoni* - 2°C and 3°C Tests

Cultivar : ‘Crimson Seedless’	Cultivar : ‘Thompson’s Seedless’	Sugar (%Brix) : 17 - 18
Characteristics of test fruits:	Characteristics of test fruits:	Cultivar : ‘Red Globe’
Colour: red	Colour: cream / green	Characteristics of test fruits:
Condition: firm	Condition: firm	Colour: red
Shape: elongated, seedless	Shape: elongated, seedless	Condition: firm
Weight: 3 - 4 g	Weight: 5 - 7 g	Shape: round, seedless
Acidity (pH) : 3.6 - 3.7	Acidity (pH) : 3.5 - 3.6	Weight: 5 - 7 g
Sugar (%Brix) : 17 - 18		Acidity (pH) : 3.2 - 3.7
		Sugar (%Brix) : 17 - 18

1.2.3 Spray data of insecticides and fungicide before harvesting

1.2.3.1 Queensland fruit fly - *Bactrocera tryoni*

‘Thompson’s Seedless’ grapes used in these experiments were produced without the use of insecticides or fungicides. ‘Ruby Seedless’ and ‘Flame Seedless’ grapes were also harvested from unsprayed vineyards,

1.2.4 Data on postharvest treatment and storage conditions such as temperatures and humidity

1.2.4.1 Queensland fruit fly - *Bactrocera tryoni*

Table grapes used in these experiments were not treated with any postharvest fungicides or insecticides. They were packaged in plastic liners (folded over) inside unvented fibreboard cartons as they would be for export.

On arrival at the Gosford laboratory fruit were stored at 1°C and 50% Relative Humidity until ready for use in experiments. Fruit were not stored for longer than four weeks before commencement of the experiments.

1.3 Laboratory where experiments are conducted:

1.3.1 Location and organisation of the laboratory

1.3.1.1 Queensland fruit fly - *Bactrocera tryoni*

This organisation, administered by the New South Wales State Government, has its Postharvest Laboratory situated at Gosford on the Central Coast of New South Wales about 80km north of Sydney, the State capital.

This facility is operated by New South Wales Department of Primary Industries. The Gosford laboratory disinfestation research unit comprises one research horticulturist, one technical officer and two assistants and up to five temporary assistants as required for various disinfestation projects.

1.3.2 Dates of each experiment

1.3.2.1 Queensland fruit fly - *Bactrocera tryoni* - 1°C Tests

Table 1. Commencing dates for table grape experiments on *B. tryoni*

Experiment	Replicate	Thompson's Seedless	Flame Seedless	Ruby Seedless
Larval development 1	-	8 March 1986	2 March 1988	2 March 1988
Most tolerant life stage	1	21 March 1986	14 March 1988	14 March 1988
	2	21 March 1986	14 March 1988	14 March 1988
	3	21 March 1986	14 March 1988	14 March 1988
Larval development 2	-	20 March 1987	12 March 1990	12 March 1990
Large scale confirmatory	1	1 April 1987	20 March 1990	20 March 1990
	2	1 April 1987	20 March 1990	20 March 1990
	3	1 April 1987	20 March 1990	20 March 1990

1.3.2.2 Queensland fruit fly - *Bactrocera tryoni* - 2°C Tests

Table 2. Commencing dates for table grape experiments on *B. tryoni* - 2°C Tests

Experiment	Replicate	Thompson's Seedless	Crimson Seedless	Red Globe
Larval development 1	-	2 March 2005	2 March 2005	2 March 2005
Most tolerant life stage	1	9 March 2005	9 March 2005	9 March 2005
	2	9 March 2005	9 March 2005	9 March 2005
	3	9 March 2005	9 March 2005	9 March 2005
Larval development 2	-	1 March 2006	1 March 2006	1 March 2006
Larval development 3	-	9 March 2007	9 March 2007	9 March 2007
Large scale confirmatory	1	8 March 2006	8 March 2006	8 March 2006
	2	8 March 2006	8 March 2006	8 March 2006
	3	15 March 2007	15 March 2007	15 March 2007
	4	22 March 2007	22 March 2007	22 March 2007

1.3.2.3 Queensland fruit fly - *Bactrocera tryoni* - 3°C Tests

Table 3. Commencing dates for table grape experiments on *B. tryoni* - 3°C Tests

Experiment	Replicate	Thompson's Seedless	Crimson Seedless	Red Globe
Larval development 1	-	9 March 2005	9 March 2005	9 March 2005
Most tolerant life stage	1	30 March 2005	30 March 2005	30 March 2005
	2	30 March 2005	30 March 2005	30 March 2005
	3	30 March 2005	30 March 2005	30 March 2005
Larval development 2	-	10 May 2006	4 April 2006	5 April 2006
Larval development 3	-	15 Feb 2007	15 Feb 2007	15 Feb 2007
Large scale confirmatory	1	17 May 2006	11 April 2006	12 April 2006
	2	22 Feb 2007	22 Feb 2007	22 Feb 2007
	3	02 March 2007	02 March 2007	02 March 2007
	4	08 March 2007	08 March 2007	08 March 2007

1.4 Cold treatment facilities: 1°C Tests

1.4.1 Dimensions and capacities of the cold treatment chambers used for each test

1.4.1.1 Queensland fruit fly - *Bactrocera tryoni* - 1°C Tests

Dimensions

The cold room facilities at Gosford were designed for research into the development of quarantine commodity treatments for fruit likely to be infested with insects of quarantine significance. The room used in the grapes trials has the dimensions 3145mm x 2600mm x 2825mm (23.10 cubic metre capacity).

1.4.2 Specifications of the cold treatment chambers

1.4.2.1 Queensland fruit fly - *Bactrocera tryoni*

The cold room is prefabricated with walls and ceiling made of 125mm 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 is insulated with 100mm expanded polyurethane and covered with 75mm concrete cored at each edge and reinforced to tolerate four 1 tonne pallets of produce measuring 1200mm x 1200mm each. The joint between the floor core and wall is sealed with mastic and the floor level is graded to the door.

The door opening is 1350mm clear width by 2100mm high and is fitted with a low voltage heater. The door is fitted on all four sides with double neoprene labyrinth gaskets. The door overlaps the opening by 75mm. The door is 125mm thick, slides on robust guides and is fitted with a triple-glazed Austwin observation window.

All sheet aluminium is finished with white baked enamel.

Refrigeration for the cold room is supplied by a forced draft cooler with a capacity of 12,000 British thermal units/hour, a thermal depression of 5.6°C. Air circulates at approximately 96.3m³/minute. The coil is prewired with replaceable electric heaters. The unit is fitted with a removable heated drain pan and is drained to waste via a 25mm insulated and dropped copper drain pipe. Defrosting of the unit is controlled by a time-clocked defrost cycle controller.

1.5 Cold treatment facilities: 2°C and 3°C Tests

1.5.1 Description of cold treatment facilities

The cold treatment facilities are situated at New South Wales Department of Primary Industries' Horticultural Research and Advisory Station at Gosford in the State of New South Wales. The treatment facilities were constructed by Thermoline Australia in 2004.

1.5.2 Number of cold rooms

Three cold rooms were used for these trials. The rooms have the designated labels CTR7, CTR8, and CTR9.

1.5.3 Rooms construction

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

1.5.4 Performance

Rooms CTR7 and CTR9 have been designed to provide temperature and humidity control (0.0°C to 45°C and 30% - 95% RH).

CTR8 has been designed to provide temperature and humidity control (-10.0°C to 45°C and 30% - 95% RH).

1.5.5 Air circulation

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

1.5.6 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.

1.5.7 Cooling regulation

Time proportional signals, from the temperature controllers, switch liquid line solenoid valves, and regulate the flow of refrigerant into the evaporators therefore controlling the cooling effect.

1.5.8 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.

1.5.9 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 also switched by time proportional pulsed signals from the humidity controllers.

1.5.10 Sensors

All rooms are fitted with Rototronic Hygroclip C, temperature and humidity sensors, capable of monitoring temperature in the range of 0.0°C to 85.0°C and humidity in the range of 0% RH to 100% RH. CTR8 is fitted with a DIN standard, pt100 resistance sensor, which will measure temperature down to -10°C.

1.5.11 Size and capacity of each room

The three cold rooms measure 3750x2400x2800mm with a total volume of 25.5 m³. Door openings are 1000mm by 2005 mm. The door is fitted on all four sides with double neoprene labyrinth gaskets. The door overlaps the opening by 75mm and is 120mm thick.

1.5.12 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 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. The summary details are as follows:

- (1) Type: Grant 2040 series Squirrel data Logger with 32 channels
- (2) Temperature sensor: U 2 K Ohm thermistors
- (3) Number of probes: 8
- (4) 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 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.

1.5.13 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.

2 Test methods

The trials were conducted in the following manner:

- (i) All fruit that was received from the farms were held in a separate cold room until required for the trials.
- (ii) A life history study was conducted before each series of trials for each cultivar to determine the rate of development of immature stages to be tested.
- (iii) From the data obtained the most tolerant life stage trials were prepared by exposing more than 200 insects at each stage to a series of treatment periods at 1°C.
- (iv) The data obtained from the most tolerant stage trials were subjected to Probit analysis to compare Probit 9 [LD_{99.99683}] values for each stage
- (v) The stage with the highest LD₅₀ was selected for large scale trials described in **section ***.

2.1 Details of preparation of infested fruits:

2.1.1 Methods (artificial infestation or natural infestation)

2.1.1.1 Queensland fruit fly - *Bactrocera tryoni* - 1°C Tests

Until April 1989, grapes were infested by placing the fruit on top of nylon mesh cages containing 10,000 to 20,000 mature *B. tryoni* for 4 hours so that eggs could be laid in the fruit. Each cage had about equal numbers of males and females. Random samples of fruit were removed from the cages and incubated at 26°C for various times before disinfestation treatments were applied.

During the course of these experiments it was found that first instar larvae were more tolerant of cold storage than eggs or second or third instar larvae in terms of the quantity of larvae that survived to pupation, but many eggs laid in the grapes failed to hatch. In order to obtain sufficient numbers of insects for large scale trials an alternative technique for infestation was used. First instar larvae were raised outside the fruit on damp filter paper at 26°C then placed in the wound on the fruit caused by pulling the berry from its peduncle. The flat end of a 5 mm wide stainless steel spatula was used as a scoop. Fruit infested this way were stored at 26°C for a further 24 hours to allow the larvae to burrow into the fruit before cold storage treatments took place.

2.1.1.2 Queensland fruit fly - *Bactrocera tryoni* - 2°C and 3°C Tests

Individual berries were removed from bunches and allowed to warm to optimum infestation temperature of 26°C in the laboratory 24 hours prior to infestation. After overnight equilibrium to

26°C (the optimum temperature for *Bactrocera tryoni* egg laying), berries were punctured in the stem end region with a hand held wooden block studded with fine pins. A maximum of four individual punctures were made in each berry. Punctured berries were then pressed in slurry of *Bactrocera tryoni* eggs and water.

2.1.2 Number of infesting insects per fruit and survival

2.1.2.1 Queensland fruit fly - *Bactrocera tryoni* - 1°C Tests

Table 4. Number of insects per fruit surviving to pupation - *B. tryoni* - 1°C Tests

Grape cultivar	Cage infested fruit	Artificially infested fruit
Thompson's Seedless	0.03 to 1.80	Not done for this cultivar
Ruby Seedless	0.10 to 0.21	2.59 to 5.06
Flame Seedless	0.06 to 0.83	2.34 to 3.57

2.1.2.2 Queensland fruit fly - *Bactrocera tryoni* - 2°C Tests

Table 5. Number of insects per fruit surviving to pupation - *B. tryoni* - 2°C Tests

Grape cultivar	Artificially infested fruit
Thompson's Seedless	2.41 to 6.11
Red Globe	6.76 to 11.66
Crimson Seedless	2.39 to 3.44

2.1.2.3 Queensland fruit fly - *Bactrocera tryoni* - 3°C Tests

Table 6. Number of insects per fruit surviving to pupation - *B. tryoni* - 3°C Tests

Grape cultivar	Artificially infested fruit
Thompson's Seedless	4.43 to 17.00
Red Globe	1.60 to 7.30
Crimson Seedless	0.01 to 12.07

2.1.3 Storage condition of infested fruits

2.1.3.1 Queensland fruit fly - *Bactrocera tryoni* - 1°C Tests Prior to Treatment

The fruit was stored at $1.0 \pm 0.5^{\circ}\text{C}$. after being received from the farm or the produce markets to preserve its quality in good condition before the trials were conducted. This was necessary because the trials were conducted over several weeks and good quality fruit was required for each trial. Before egg inoculation the fruit was removed from the cold room and taken to the fruit holding room for 24 hours where the temperature and humidity for storage is maintained at : $26 \pm 1^{\circ}\text{C}$; 60-65% RH.

2.1.3.2 Queensland fruit fly - *Bactrocera tryoni* - 2°C and 3°C Tests Prior to Treatment

Infested fruit were placed in plastic racks measuring 350mm x 430mm x 60mm, lined with 2mm mesh. Racks were stored at $26^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, 50% – 60 % relative humidity. To prevent desiccation of *Bactrocera tryoni* eggs, infested fruit were covered with damp material, and kept damp until treatment commenced (when more than 60% of insects inside the fruit were at first instar).

2.1.4 Data of development of insects in infested fruit.

2.1.4.1 Queensland fruit fly - *Bactrocera tryoni*

Experiments were done according to the protocol used for trials involving export submissions to the Japanese Government in order to fulfil Japanese requirements for importation of Australian produce. The first step for each grape cultivar was to determine the period taken under laboratory conditions (26°C , 60-70% RH and 14:10 hours day/night cycle) for eggs to hatch and develop to the three larval instars: 26°C is the optimal temperature for egg hatch and larval development (Hill et al. 1988).

In 1°C Tests five hundred fruit of each table grape cultivar were cage infested by placing them on top of the gauze-covered cages housing 10,000 to 20,000 3 week old to 5 week old adult fruit flies, as described previously. Female adult fruit flies were able to oviposit into the fruit through the gauze of the cage. Fruit were left in contact with the fruit flies for 6 to 7 hours and then removed and place in storage at 26°C and 60% R.H.

Random samples of 50 fruit of each cultivar were dissected daily by cutting open under a slow stream of water from a tap into a series of beakers.

Using a binocular microscope larvae were recovered from the beakers and fixed in 70% alcohol. Then their mouth hooks were examined to identify life stage (Anderson 1963).

In 2°C and 3°C Tests five hundred fruit of each table grape cultivar were artificially infested as previously described. Infested fruit were held and assessed as described above.

2.2 Fundamental (basic, Most tolerant life stage) test:

The most tolerant developmental stage of infesting insects against cold treatment should be determined by this experiment.

2.2.1 Number of test fruit in each replicate

Table 7. Outline of the experimental procedure for Fundamental (Basic) Tests on *B. tryoni*

No. fruit / treatment / replicate	Variable
No. of treatments (including the Control)	11
No. of life stages	4
No. of replicates	3
No. of temperature treatments	3

The experiments consisted of 11 treatments per temperature including the untreated control. Fruit were selected at random from the infested batch for each replicated treatment and placed on mesh over a pupation medium of sand, damp vermiculite or damp hardwood sawdust in a plastic tray. The trays containing the infested fruit were covered with fine cloth to exclude contamination by *Drosophila* and reinfestation by *B. tryoni*.

2.2.1.1 Queensland fruit fly - *Bactrocera tryoni* - 1°C Tests

Fruit were infested as described earlier. The total number of fruit infested for the trials for each grape cultivar at 1°C varied depending on the method of infestation used and on the number of likely survivors in each untreated fruit (the Control fruit).

The fruit were held at $26 \pm 1^\circ\text{C}$ for the period of time required for the fruit flies to develop to the stage required for the experiment.

Temperatures were recorded on a "Squirrel" (Grant Instruments, Cambridge, England) data logger with an accuracy of $\pm 0.05^\circ\text{C}$ and a readout of $\pm 0.2^\circ\text{C}$. Five or six Type U mini thermistor probes were used, one or two to record air temperatures at various positions in the cold room, including the inlet and outlet air temperatures of the cooler. The remaining one to three thermistors were used to record fruit pulp temperatures at different positions in the experimental stacks containing the infested fruit.

Temperature recordings were automatically logged at 10, 15 or 30 minute intervals throughout the trial. The trial arrangements in the cold rooms are shown in the figure section.

The treatments consisted of 10 periods of cold exposure and one untreated control. The 10 cold exposures were: 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days at $1 \pm 0.5^\circ\text{C}$. Timing of exposure period began when the last probe (the probe in the warmest or slowest-to-cool fruit) in the fruit reached 1.4°C . The treatment conditions were held at $1 \pm 0.5^\circ\text{C}$ over the entire experimental period.

After exposure to the specified treatment, the box containing the fruit was removed to the controlled environment room containing the control fruit for collection of surviving stages as pupae (see figure section). The number of pupae emerging at each dose was compared with the number from the untreated controls to obtain the percentage responding to the treatment. The criterion for survival was the formation of an apparently normal puparium.

Replication: Three replicates were done for each of the three table grape cultivars. These replicates were conducted simultaneously - each replicate being based on adult fly age. Replicate One was on fruit infested by 3 week old fruit flies, Replicate Two was on fruit infested by 4 week old fruit flies and Replicate Three was on fruit infested by 5 week old fruit flies.

2.2.1.2 Queensland fruit fly - *Bactrocera tryoni* - 2°C and 3°C Tests

Fruit were infested as described earlier. In the 2°C and 3°C Basic Tests about 240 berries were used as Controls (per replicate, per insect life stage, per temperature treatment) and 75 berries as treated (per cold storage duration, per replicate, per insect life stage, per temperature treatment).

The fruit were held at $26 \pm 1^\circ\text{C}$ for the period of time required for the fruit flies to develop to the stage required for the experiment.

Temperatures were recorded on a "Squirrel" (Grant Instruments, Cambridge, England) data logger with an accuracy of $\pm 0.05^\circ\text{C}$ and a readout of $\pm 0.2^\circ\text{C}$. Up to 8 Type U mini thermistor probes were used, one or two to record air temperatures at various positions in the cold room, including the inlet and outlet air temperatures of the cooler. The remaining four to six thermistors were used to record fruit pulp temperatures at different positions in the experimental stacks containing the infested fruit.

Temperature recordings were automatically logged at 30 minute intervals throughout the trial. The trial arrangements in the cold rooms are shown in the figure section.

The treatments consisted of 18 periods of cold exposure and one untreated control. The 18 cold exposures were: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, and 17 days at $2^\circ\text{C} \pm 0.5^\circ\text{C}$ or $3^\circ\text{C} \pm 0.5^\circ\text{C}$. Timing of exposure period began when the $> 50\%$ of the probes in the fruit had reached 2.5°C or less (for the 2°C tests) or 3.5°C or less (for the 3°C tests). The treatment conditions were held at $2^\circ\text{C} \pm 0.5^\circ\text{C}$ or $3^\circ\text{C} \pm 0.5^\circ\text{C}$ over the entire experimental period.

After exposure to the specified treatment, the boxes containing the fruit were removed to the controlled environment room containing the control fruit for collection of surviving stages as pupae (see figure section). The number of pupae emerging at each dose was compared with the number from the untreated controls to obtain the percentage responding to the treatment. The criterion for survival was the formation of an apparently normal puparium.

Replication: Three replicates were done for each of the three table grape cultivars. These replicates were conducted simultaneously - each replicate being based on adult fly age. Replicate One was on fruit infested by 3 week old fruit flies, Replicate Two was on fruit infested by 4 week old fruit flies and Replicate Three was on fruit infested by 5 week old fruit flies.

2.2.2 Temperature and period of the treatment

2.2.2.1 Queensland fruit fly - *Bactrocera tryoni* - 1°C Tests

Temperature was $1^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Period of treatment was 0 days (Control), or 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 days. Treatment commenced when the temperature reading from the warmest temperature probe in the fruit registered 1.4°C .

2.2.2.2 Queensland fruit fly - *Bactrocera tryoni* - 2°C Tests

Temperature was $2^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Period of treatment was 0 days (Control), or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 days. Treatment commenced when the temperature reading from when >50% of the temperature probes in the fruit registered $<2.5^{\circ}\text{C}$.

2.2.2.3 Queensland fruit fly - *Bactrocera tryoni* - 3°C Tests

Temperature was $3^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Period of treatment was 0 days (Control), or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 days. Treatment commenced when the temperature reading from when >50% of the temperature probes in the fruit registered $<3.5^{\circ}\text{C}$.

2.2.3 Criteria for the determination of mortality and method of data analysis

2.2.3.1 Queensland fruit fly - *Bactrocera tryoni*

After exposure to the specified treatment, the trays containing the fruit were removed from 1°C , or 2°C or $3^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ treatments to the 26°C room with the control fruit for collection of surviving stages as pupae. Surviving insects were allowed to leave the fruit and pupate in the pupation medium. This medium was then sieved through wire mesh sieves at days 10, 17

and 21 after removal from the 1°C, or 2°C or 3°C \pm 0.5°C treatments. Surviving pupae and larvae were recovered from the sieves and counted. The number of pupae collected at each dose was compared with the number from the untreated controls to obtain the percentage responding to the treatment. The criterion for survival was the formation of an apparently normal puparium.

2.2.4 Storage period and storage conditions after treatment (Fruit holding container, prevention method of reinfestation, temperature, humidity, etc.)

2.2.4.1 Queensland fruit fly - *Bactrocera tryoni* - 1°C Tests

In the most tolerant stage trials plastic trays (520 mm x 340 mm x 190 mm high) were used to hold the infested fruit. Inside each tray was placed a mesh tray on which sat the infested fruit. This mesh tray was suspended over the pupation medium by wooden blocks. Each tray contained a layer of approximately 1.5 L of sand, dampened sawdust or dampened vermiculite at the bottom allowing emerging larvae to pupate. The trays containing the infested fruit were covered with fine cloth to exclude contamination by *Drosophila*. The fruit were held at 26°C. for the period of time required for surviving fruit flies to pupate.

2.2.4.1 Queensland fruit fly - *Bactrocera tryoni* - 2°C and 3°C Tests

In these Basic Tests infested (treated and control) grapes were placed into plastic “shopping” bags (380mm long x 300mm wide x 160mm deep not including the two handles measuring 150mm long) suspended on metal bars (see photos). Each bag held the fruit on 1L of damp vermiculite and the whole structure (metal bars and bags was covered with fine terylene cloth to allow ventilation but reduce contamination by *Drosophila* spp. Treated and control fruits were on separate racks. The treated fruit, on their stacks, were moved to the cold rooms for treatment and the control infested fruit were moved directly to the 26°C for incubation. After treatment the treated fruit, on their racks, were moved to the 26°C room for incubation of survivors.

This method of handling infested fruit allowed us to manage a great number of insects and fruit in the limited research space available.

2.2.5 Stacking conditions of the fruits in the cold treatment chamber (Size, capacity and materials of the fruit holding container, load factor t/m³ or kg.L, location of the infested fruits in the cold treatment chamber, existence of the non-infested filler fruits)

2.2.5.1 Queensland fruit fly - *Bactrocera tryoni*

See figure section

2.2.6 Measuring method of the temperature in the cold chamber and at the centre of the fruit during treatment, type, specifications and accuracy of measurement of the temperature recorders and sensors, intervals of temperature measuring

2.2.6.1 Queensland fruit fly - *Bactrocera tryoni*

The Gosford laboratory used the Squirrel meter/logger with mini thermistor probes for temperature sensing and recording. The meter/logger (type: SQ32-16U) is manufactured by Grant Industries Limited, Cambridge, England. It has a preset temperature specification range of -10°C to 40°C. The unit is controlled by a microprocessor which stores readings in binary form with an available memory capacity of 32,000 readings shared by up to 16 data-receiving channels. Temperature records can be transferred for storage and analysed onto an IBM-compatible personal computer via an RS-232 serial interface. The "checksum" feature allows the receiving computer to check that no errors in data transferral have occurred.

The mini thermistor (type U) sensor probes have a short response time with high electrical resistance which minimises the effect of contact resistance on plugs and sockets and allows long cables to be used without significant error. The longest cables used in these experiments were 5m long. Resistance is 2KOhms. Measurement resolution is 1 bit (0.4% span) ie. 0.2°C. Accuracy is 1 bit (0.4% span) with a maximum drift of 0.015°C/year. Fixed errors for additional cable length at 0°C is 0.0004°C/metre of cable length.

The numbers of pupae recovered from the control fruit were used to estimate the number of insects per fruit that were cold-treated. Pupal recoveries from the cold-treated fruit were compared with those obtained from the control fruit using probit analysis by GENSTAT (Alvey et al. 1983) to predict probit 9 (99.99683% mortality) dose-mortality. Data for the four life stages in each grape cultivar were pooled for this estimate. Comparison of pupal recoveries indicated which life stage would be most likely to survive storage at 1°C. A safety margin of 2 days at 1°C was added to the estimated probit 9 dose-mortality in line with procedures adopted for export submissions to Japan.

2.2.7 Calibration method of temperature recorder and sensors

2.2.7.1 Queensland fruit fly - *Bactrocera tryoni*

Squirrel meter/logger temperature sensors (mini thermistors) were connected to separate cables with both sensor and cables in each set labelled identically so that, for example, sensor number 1 was always used with cable number 1.

Sensors and cables were calibrated prior to the commencement of experiments by placing the sensors in a thermostatically-controlled water bath 30L capacity. The bath water was circulated using a small pump and plastic tubing which moved water at a rate of 2L/minute. This ensured that all positions within the bath were at the same temperature. An immersion thermometer certified as accurate was used to calibrate the sensor/cable units. The water bath temperatures used were 0°C (an ice/water slurry), 10°C and 20°C.

Six satisfactorily-calibrated sensor/cable units were then placed in water in the cold room for calibration of the cold room.

2.3 Applied (large scale) test:

The most tolerant developmental stage of the insect cold treatment should be killed completely by this experiment simulating commercial treatment.

The large scale trials were conducted in the following manner:

- (i) All fruit was received directly from the farms in plastic tubs and were pre-cooled in the cold rooms #1 to #5 through forced air cooling before being packed into export cartons for the large scale trials.
- (ii) A life history study was conducted before each series of trials to determine the rate of development of immature stages to be tested.
- (iii) From the data obtained the date when first instar stage was predominant was recorded and incubation of eggs for the trial to first instar stage was carried out at $26 \pm 1^\circ\text{C}$ and 60 - 65 % RH
- (iv) The number of fruit required to obtain more than 10,000 pupae was calculated and infested with 6 h old eggs and incubated to first instar.
- (v) The treated fruit was exposed to cold treatment for 12 days at $1^\circ\text{C} \pm 0.5^\circ\text{C}$, or 14 days at $2^\circ\text{C} \pm 0.5^\circ\text{C}$ or 14 days at $3^\circ\text{C} \pm 0.5^\circ\text{C}$ and then incubated at $26 \pm 1^\circ\text{C}$ and 60 - 65% RH for 3 weeks for emergence of any survivors.
- (vi) Untreated controls were also infested with each replicate. The pupae obtained from the untreated control was used to estimate the number of live first instar larvae exposed to the treatment.

- (vii) The cold exposure treatment was considered successful if no survivors were obtained after the incubation period.

2.3.1 General preparation of fruit

2.3.1.1 Queensland fruit fly - *Bactrocera tryoni*

Fruit was obtained from the sources given earlier. The fruit were grown free of pesticides and were of similar quality to export grade fruit.

Table grapes were cage infested as described earlier. Selection of fruits for treatment and control was done at random. The untreated controls were held in ventilated plastic boxes over sand and maintained in a controlled environment room at 26°C and 60-65% RH for development to pupae. The stacks of plastic boxes were covered with custom made terylene voile covers.

The fruit was packed into standard export cartons before being allowed to cooled down in the cold room.

The infested fruits were packed into unvented polythene-lined export following standard export practice. The cartons were then stacked in the cold rooms.

For the 1°C Tests each of the 3 replicated trials was set-up in the same cold room. For each of the 2°C and 3°C Tests each of the 3 replicated trials was set up in separate cold rooms. The layouts of the trials are shown in the figure section.

2.3.2 Number of test fruit in each replicate

2.3.2.1 Queensland fruit fly - *Bactrocera tryoni* - 1°C Tests

Table 8. Number of fruit infested for the Applied (Large Scale) Test

- *B. tryoni*

Table grape cultivar	Number of cage-infested fruit		Number of artificially infested fruit		Total number of fruit infested		Total
	Control	Treated	Control	Treated	Control	Treated	
Thompson's Seedless	19,461	80,284	0	0	19,461	80,284	99,745
Ruby Seedless	7,870	14,292	3,067	11,663	10,937	25,955	36,892
Flame Seedless	8,680	38,279	3,606	18,473	12,286	56,752	69,038

2.3.2.2 Queensland fruit fly - *Bactrocera tryoni* - 2°C Tests

Table 9. Number of fruit infested for the Applied (Large Scale) Test

- *B. tryoni*

Table grape cultivar	Number of artificially infested fruit		Total
	Control	Treated	
Thompson's Seedless	2,920	12,040	14,960
Red Globe	2,422	10,446	12,868
Crimson Seedless	3,350	19,400	22,750

2.3.2.3 Queensland fruit fly - *Bactrocera tryoni* - 3°C Tests

Table 10. Number of fruit infested for the Applied (Large Scale) Test

- *B. tryoni*

Table grape cultivar	Number of artificially infested fruit		Total
	Control	Treated	
Thompson's Seedless	3,746	13,000	16,746
Red Globe	3,722	12,028	15,750
Crimson Seedless	4,700	17,050	21,750

2.3.3 Temperature and period of the treatment

2.3.3.1 Queensland fruit fly - *Bactrocera tryoni*

12 days at 1°C± 0.5°C after warmest fruit had cooled to 1.4°C OR
 14 days at 2°C± 0.5°C after >50% of fruit had cooled to <2.5°C OR
 14 days at 3°C± 0.5°C after >50% of fruit had cooled to <3.5°C OR

2.3.4 Criteria for the determination of mortality and method of data analysis

2.3.4.1 Queensland fruit fly - *Bactrocera tryoni* – 1°C Tests

Treatment efficacy at 1°C was confirmed by infesting large numbers of grapes in a series of sub-trials starting in April, 1987 ('Thompson's Seedless') and ending in April, 1990 ('Flame Seedless' and 'Ruby Seedless') to produce three replicates of 10,000 or more insects in each cultivar treated at the most cold-tolerant life stage, with no survivors.

The infested fruits were packed into unvented polythene-lined export following standard export practice. The cartons were then stacked in the cold rooms.

Mini-thermistors monitored the core temperatures of one to three fruit in the centre of the stack. Temperature readings were taken automatically every 10, 15 or 30 minutes. Treatment commenced once the last of the mini thermistors (the grape berry that was the slowest to cool) registered 1.4°C.

After exposure to the specified treatment, the trays containing the fruit were removed from $1 \pm 0.5^{\circ}\text{C}$ to the 26°C room with the control fruit for collection of surviving stages as pupae. Surviving insects were allowed to leave the fruit and pupate in the pupation medium. This medium was then sieved through wire mesh sieves at days 10, 17 and 21 after removal from $1 \pm 0.5^{\circ}\text{C}$. Surviving pupae and larvae were recovered from the sieves and counted. The number of pupae collected at each dose was compared with the number from the untreated controls to obtain the percentage responding to the treatment. The criterion for survival was the formation of an apparently normal puparium.

2.3.4.2 Queensland fruit fly - *Bactrocera tryoni* – 2°C and 3°C Tests

Treatment efficacy at 2°C and 3°C were confirmed by infesting large numbers of grapes in a series of sub-trials starting in March, 2006 and ending in April, 2007 to produce three replicates of 10,000 or more insects in each cultivar treated at the most cold-tolerant life stage, with no survivors.

The infested fruits were packed into unvented polythene-lined export following standard export practice. The cartons were then stacked in the cold rooms.

Mini-thermistors monitored the core temperatures of eight fruit in various positions within the stack. Temperature readings were taken automatically every 30 minutes. Treatment commenced once >50% of the mini thermistors (the grape berry that was the slowest to cool) registered <2.5°C or <3.5°C.

After exposure to the specified treatment, the trays containing the fruit were removed from cold storage to the 26°C room with the control fruit for collection of surviving stages as pupae. Surviving insects were allowed to leave the fruit and pupate in the pupation medium. This medium was then sieved through wire mesh sieves at days 10, 17 and 21 after removal

from cold storage. Surviving pupae and larvae were recovered from the sieves and counted. The number of pupae collected at each dose was compared with the number from the untreated controls to obtain the percentage responding to the treatment. The criterion for survival was the formation of an apparently normal puparium.

2.3.5 Storage period and storage conditions after treatment (Fruit holding container, prevention method of reinfestation, temperature, humidity, etc.)

2.3.5.1 Queensland fruit fly - *Bactrocera tryoni*

In the Applied (Large Scale) Tests plastic trays (520 mm x 340 mm x 190 mm high) were used to hold the infested fruit after removal from cold storage treatments. Inside each tray was placed a mesh tray on which sat the infested fruit. This mesh tray was suspended over the pupation medium by wooden blocks. Each tray contained a layer of approximately 1.5 L of sand, dampened sawdust or dampened vermiculite at the bottom allowing emerging larvae to pupate. The trays containing the infested fruit were covered with fine cloth to exclude contamination by *Drosophila* and reinfestation by *B. tryoni*. The fruit were held at 26°C and 60 to 70% R.H. for the period of time required for the fruit flies to develop to the stage required for the experiment.

Infested fruit were held at 26°C for about 4 weeks. The pupation medium was sieved at 8 to 10 days after removal of the infested fruit from 1°C and then two more times after that at weekly intervals. Any surviving pupae were collected and counted.

2.3.6 Stacking conditions of the fruits in the cold treatment chamber (Size, capacity and materials of the fruit holding container, load factor t/m³ or kg.L, location of the infested fruits in the cold treatment chamber, existence of the non-infested filler fruits)

2.3.6.1 Queensland fruit fly - *Bactrocera tryoni*

The infested fruits were packed into unvented polythene-lined export cartons following standard export practice. The cartons were then stacked in the cold rooms (see figure section).

2.3.7 Measuring method of the temperature in the cold chamber and at the centre of the fruit during treatment, type, specifications and accuracy of measurement of the temperature recorders and sensors, intervals of temperature measuring

2.3.7.1 Queensland fruit fly - *Bactrocera tryoni*

The Gosford laboratory used the Squirrel meter/logger with mini thermistor probes for temperature sensing and recording. The meter/logger (type: SQ32-16U) is manufactured by Grant Industries Limited, Cambridge, England. It has a preset temperature specification range of -10°C to 40°C. The unit is controlled by a microprocessor which stores readings in binary form with an available memory capacity of 32,000 readings shared by up to 16 data-receiving channels. Temperature records can be transferred for storage and analysed onto an IBM-compatible personal computer via an RS-232 serial interface. The "checksum" feature allows the receiving computer to check that no errors in data transferral have occurred.

The mini thermistor (type U) sensor probes have a short response time with high electrical resistance which minimises the effect of contact resistance on plugs and sockets and allows long cables to be used without significant error. The longest cables used in these experiments were 5m long. Resistance is 2KOhms. Measurement resolution is 1 bit (0.4% span) ie. 0.2°C. Accuracy is 1 bit (0.4% span) with a maximum drift of 0.015°C/year. Fixed errors for additional cable length at 0°C is 0.0004°C/metre of cable length.

2.3.8 Calibration method of temperature recorder and sensors

2.3.8.1 Queensland fruit fly - *Bactrocera tryoni*

Squirrel meter/logger temperature sensors (mini thermistors) were connected to separate cables with both sensor and cables in each set labelled identically so that, for example, sensor number 1 was always used with cable number 1.

Sensors and cables were calibrated prior to the commencement of experiments by placing the sensors in a thermostatically-controlled water bath 30L capacity. The bath water was circulated using a small pump and plastic tubing which moved water at a rate of 2L/minute. This ensured that all positions within the bath were at the same temperature. An immersion thermometer certified as accurate was used to calibrate the sensor/cable units. The water bath temperatures used were 0°C (an ice/water slurry), 10°C and 20°C.

Six satisfactorily-calibrated sensor/cable units were then placed in water in the cold room for calibration of the cold room.

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

* * * * *

PART TWO – PHOTOS and DIAGRAMS

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

CONDUCTED AT

NEW SOUTH WALES AGRICULTURE,
GOSFORD, NSW. AUSTRALIA 2250

With the support of the

HORTICULTURE AUSTRALIA LIMITED
AUSTRALIAN TABLE GRAPE GROWERS ASSOCIATION AND
NEW SOUTH WALES DEPARTMENT OF PRIMARY INDUSTRIES

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Fig. 34 Infested, non cold-treated (Control) grapes after 7 days at 26°C. This tray, lined with 2mm mesh, is placed into a larger tray over damp vermiculite. Treated fruit are held in the same method.20

Fig. 35 Trays of fruit were covered with bags made of fine terylene which allowed air movement around the fruit but stopped contamination by other insects.20

Fig. 36 Vermiculite from pupation trays being sieved to inspect for surviving insects.21

Fig. 37 Surviving insects (pupae) being removed from vermiculite from non cold-treated (Control) grapes.21

Fig. 38 Surviving insects (pupae) being removed from vermiculite from non cold-treated (Control) grapes.21

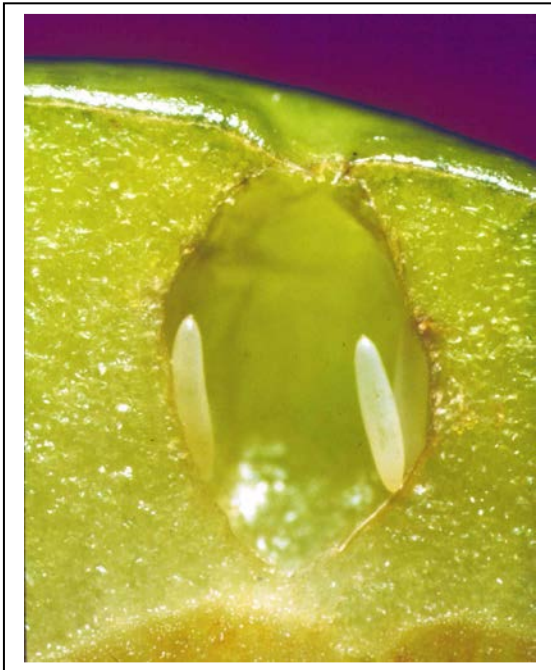


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Fig. 4 Adult flies must become sexually mature (takes about 5 to 7 days at 26°C) before they can lay eggs. The whole cycle from egg to egg takes, at 26°C, about 22 to 29 days.





Figs 5 and 6 External (above) and Internal (below) damage caused by Queensland fruit fly larvae and associated fungal pathogens.



Carton

**Weight with
fruit is 10 kg**

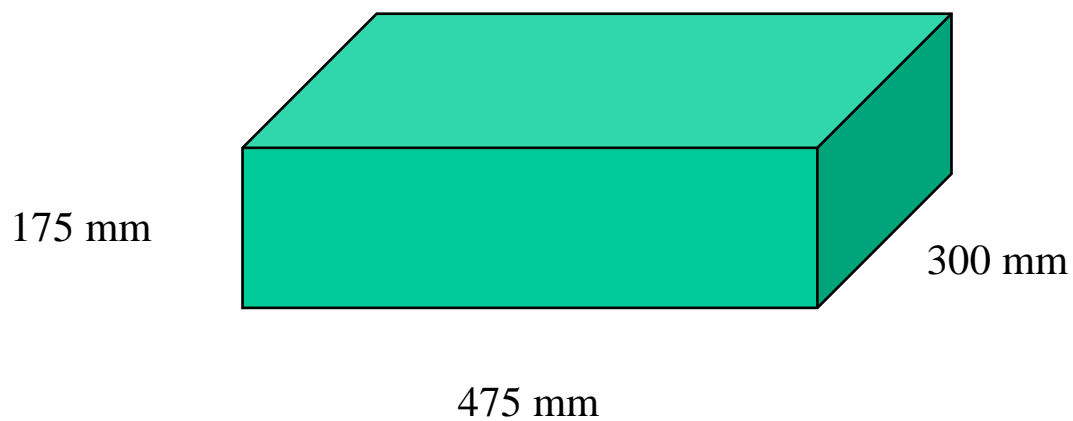
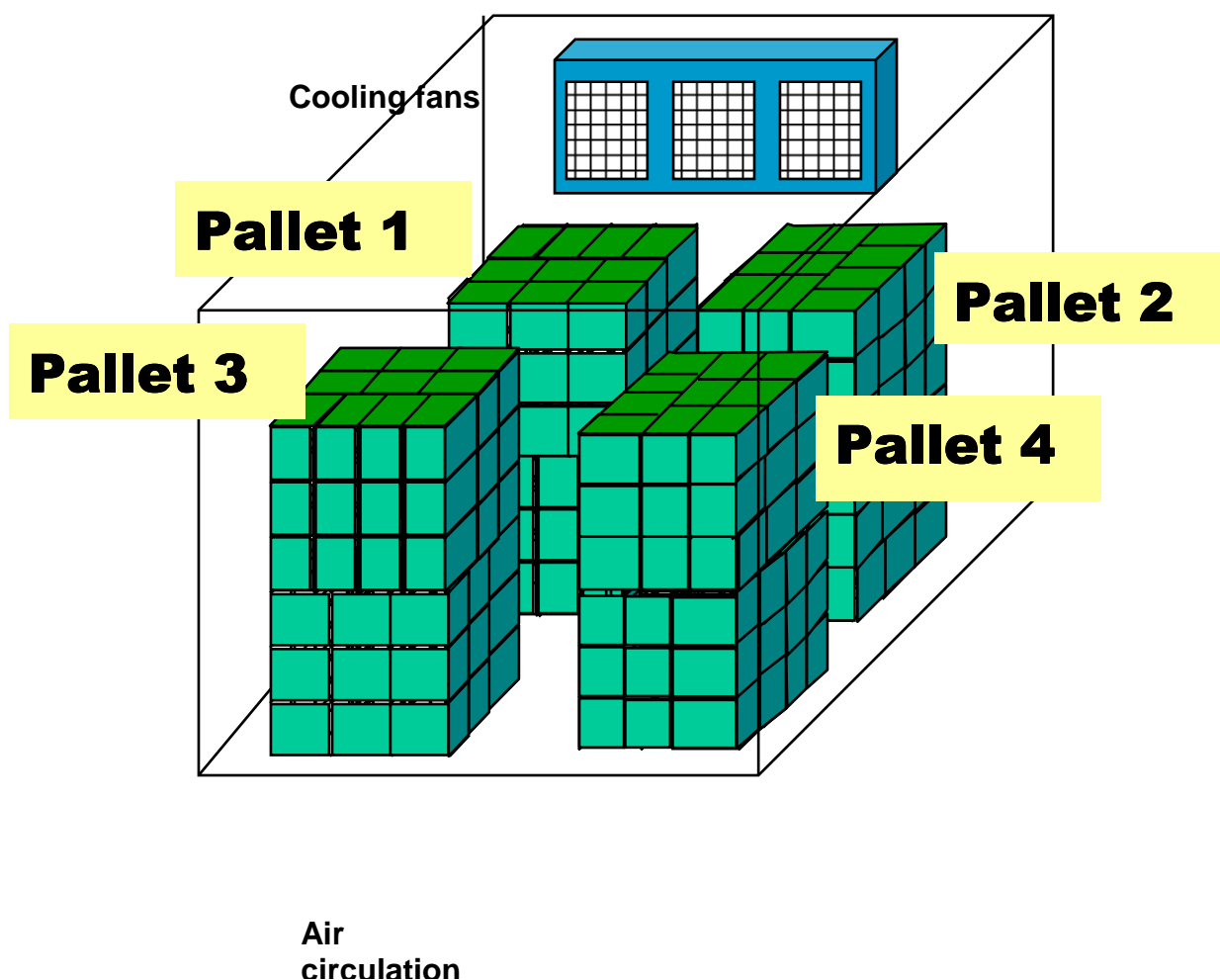


Fig. 7 Diagram of the fruit carton used in the Large Scale Trials showing its dimensions.



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.

Fig. 8 Only one cold room was available for tests at 1°C while three cold rooms were available for the 2°C and 3°C tests.

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.

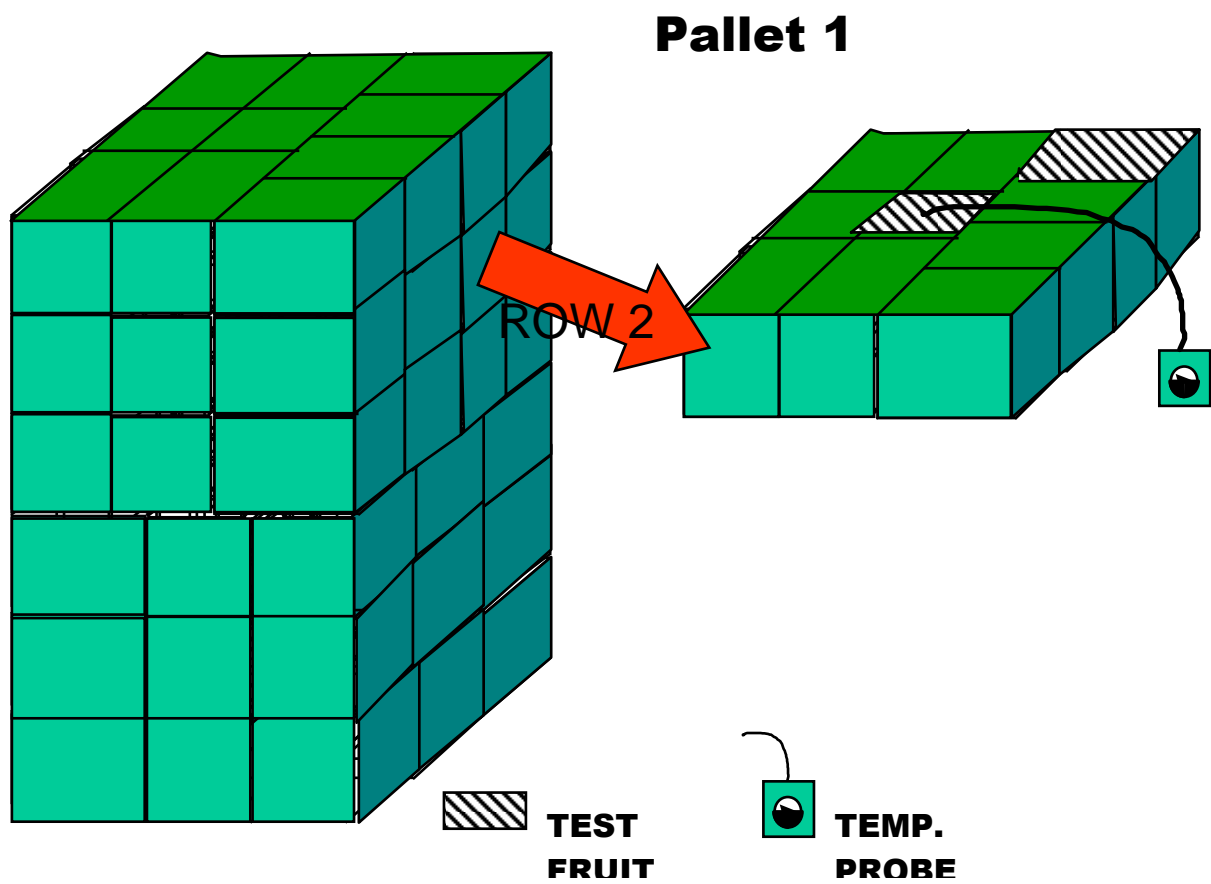


Fig 9 PALLET 1: Placement of infested (TEST) fruit and temperature data logger probes. Please refer to the Room Diagram for the pallet's location and alignment.

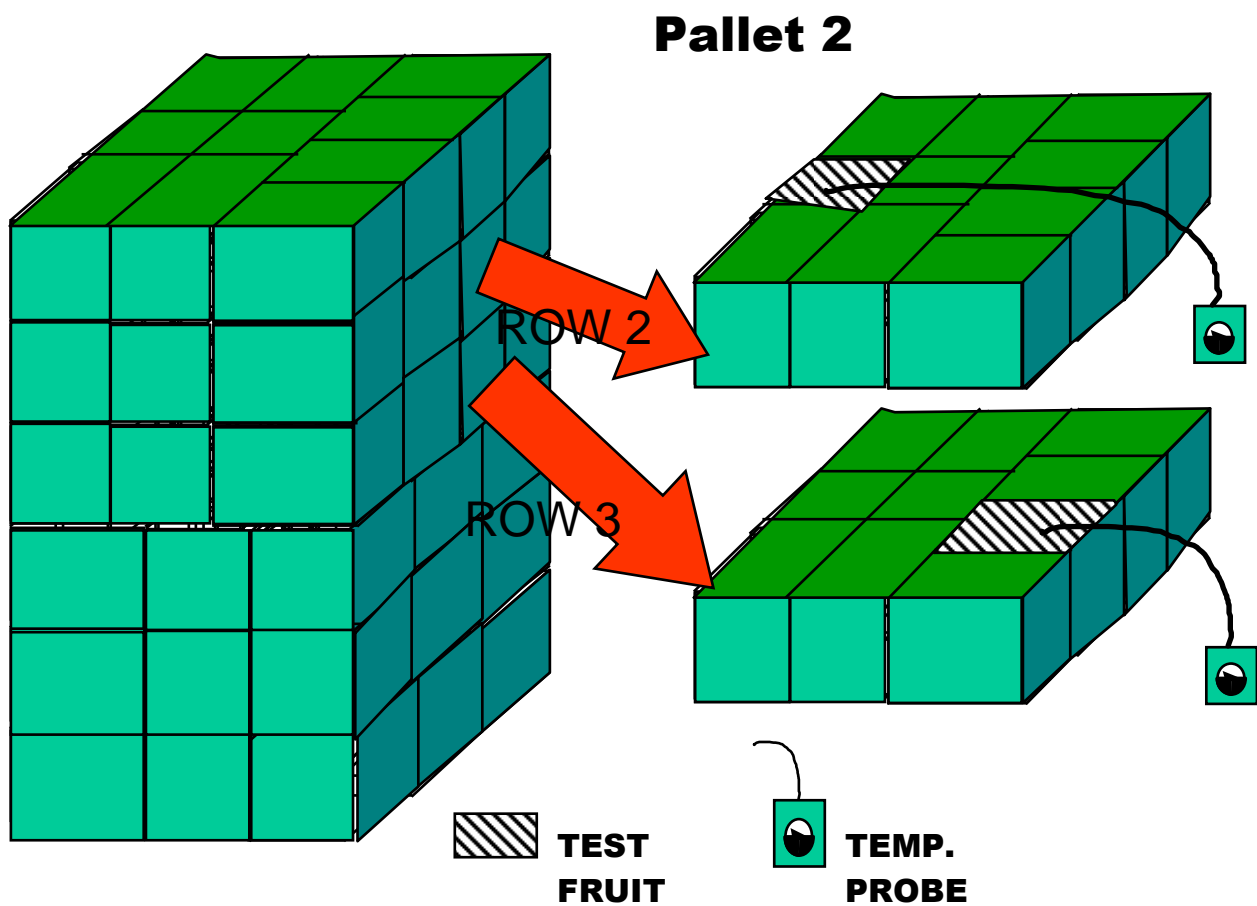


Fig 10 PALLET 2: Placement of infested (TEST) fruit and temperature data logger probes. Please refer to the Room Diagram for the pallet's location and alignment.

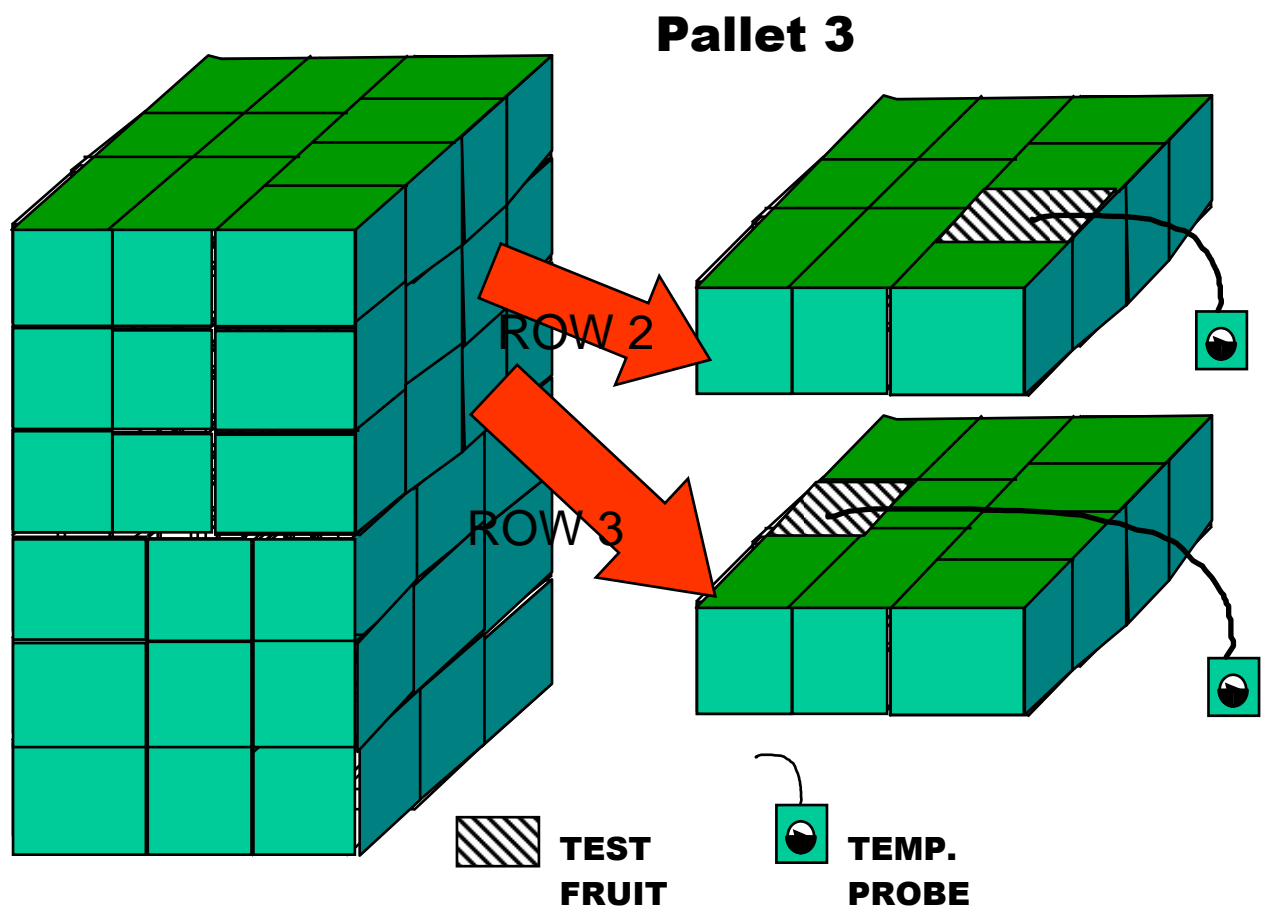


Fig 11 PALLET 3: Placement of infested (TEST) fruit and temperature data logger probes. Please refer to the Room Diagram for the pallet's location and alignment.

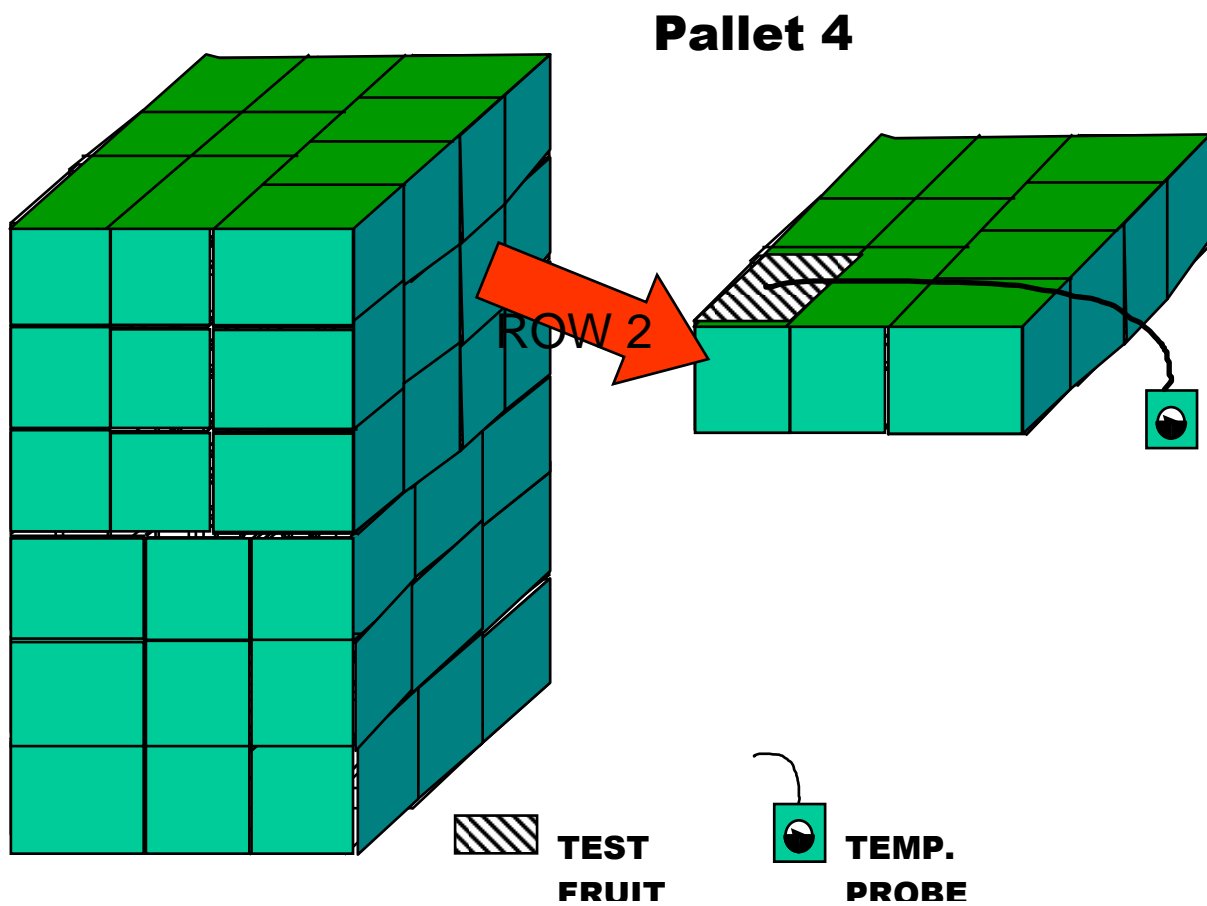


Fig 12 PALLET 4: Placement of infested (TEST) fruit and temperature data logger probes. Please refer to the Room Diagram for the pallet's location and alignment.

BASIC (MOST TOLERANT STAGE) TESTS AND LARGE SCALE TESTS CARRIED OUT AT 1°C



Fig. 13 In early trials fruit were simply placed on top of cages holding about 20,000 fruit flies (1:1 ratio male:female).

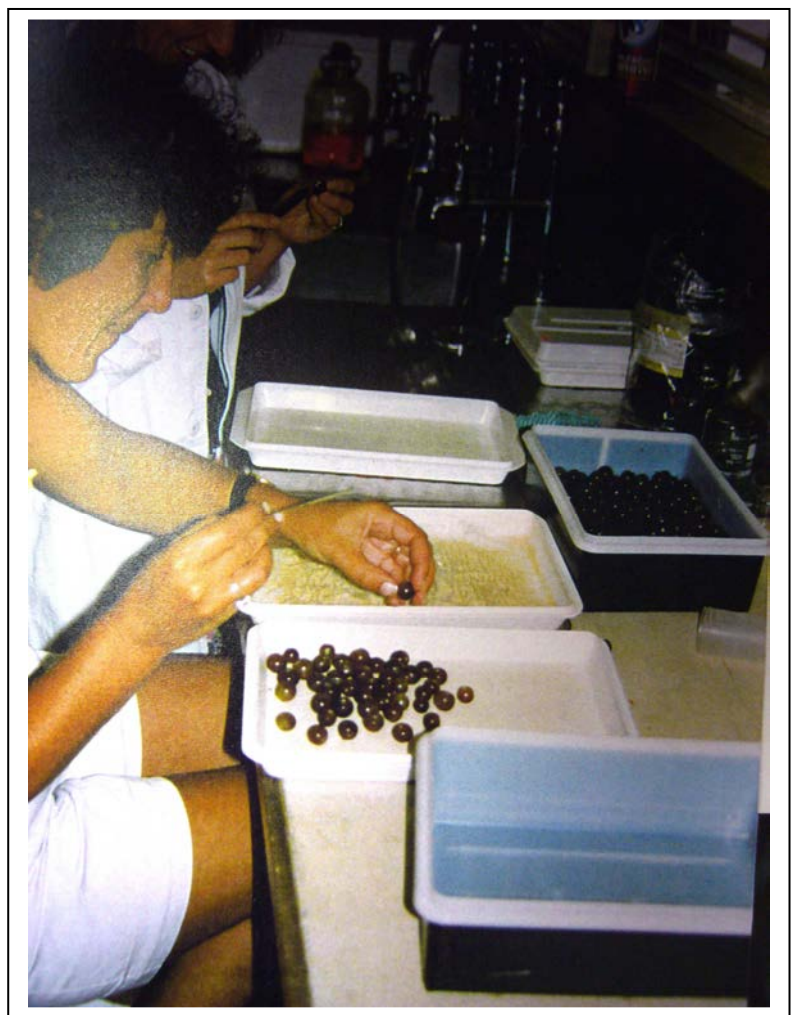


Fig. 14 Larval survival in cage-infested grapes (as above) was very low and variable so we changed the infestation method, after having determined that first instar larvae were the most cold-tolerant, to placing first instar larvae into grapes by small spatula.

BASIC (MOST TOLERANT STAGE) TESTS AND LARGE SCALE TESTS CARRIED OUT AT 1°C (Continued)



Fig. 15 For Basic (Most tolerant Stage) tests infested Test and Control grapes were placed in these trays at 1°C (Test fruit) or 26°C (Control fruit).

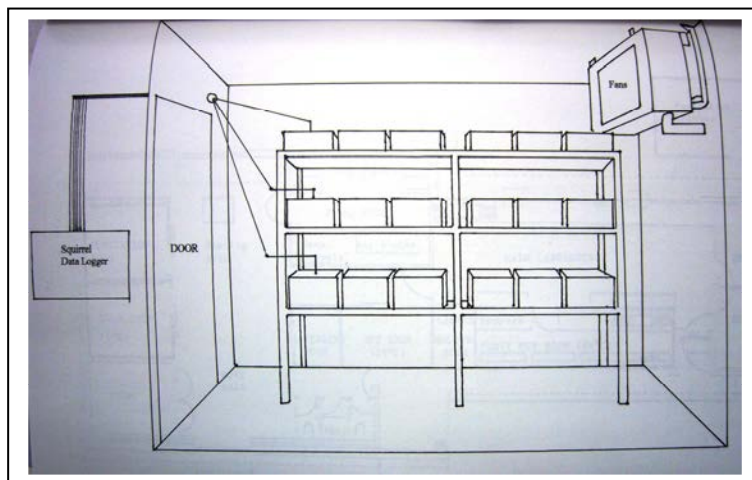


Fig. 16 (Above) Layout of 1°C cold room for Basic (Most Tolerant Stage) tests.



Fig. 17 Surviving insects pupated in sand which was then washed through sieve leaving pupae on top.



Fig. 18 Pupae were collected and counted and used to estimate treatment effect.

BASIC (MOST TOLERANT STAGE) TESTS AND LARGE SCALE TESTS CARRIED OUT AT 1°C (Continued)



Fig. 19 Squirrel data logger probes being calibrated in an ice/water slurry prior to treatment commencement.



Fig. 20 The Squirrel data logger.

BASIC (MOST TOLERANT STAGE) TESTS AND LARGE SCALE TESTS CARRIED OUT AT 2°C AND 3°C



Fig. 21 (Left) A block studded with 20 fine nails punctured the fruit to a depth of 2mm to facilitate egg laying by Queensland fruit fly adults.



Fig. 22 (Above) Fly cages for rearing QFF at Elizabeth McArthur Agricultural Institute. Eggs were harvested from these cages for use in the tests at 2°C and 3°C

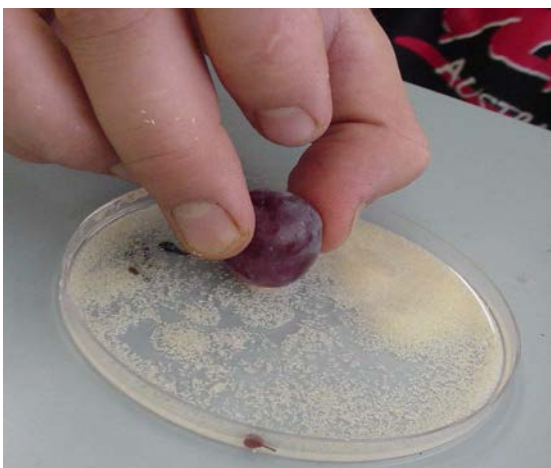


Fig. 23. (Above) Grapes being dipped in slurry of QFF eggs



Figs 24 and 25 . (Above) Grapes infested with QFF eggs

BASIC (MOST TOLERANT STAGE) TESTS AND LARGE SCALE TESTS CARRIED OUT AT 2°C AND 3°C (Continued)



Figs 26 and 27 . Grapes being dipped in slurry of QFF eggs

BASIC (MOST TOLERANT STAGE) TESTS AND LARGE SCALE TESTS CARRIED OUT AT 2°C AND 3°C (Continued)



Figs 28 and 29 (Left) The cold rooms used in the 2°C and 3°C Tests. Three rooms were available and tests, both Basic (Most Tolerant Stage) and Large Scale, were carried out in these rooms. Each room represented a separate replicate.



Fig. 30 (Left) The Squirrel Data Logger used in the 2°C and 3°C Tests. Data logger probes were placed into the centre of test fruit, at the centre of 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. We used several types of data loggers because we did not have enough of one type to monitor all the cartons of test fruit, especially when two citrus species/types were being treated at the same time.



BASIC (MOST TOLERANT STAGE) TESTS AND LARGE SCALE TESTS CARRIED OUT AT 2°C AND 3°C (Continued)



Fig. 31 Infested grapes being inspected for larval development.



Fig. 32 Infested grapes are placed into plastic bags with damp vermiculite (pupation medium) at either 2°C or 3°C for determination of the most cold-tolerant life stage.

BASIC (MOST TOLERANT STAGE) TESTS AND LARGE SCALE TESTS CARRIED OUT AT 2°C AND 3°C (Continued)



Fig. 33 (Left) Infested grapes prior to cold treatment. They are left at 26°C until eggs have hatched in first instar larvae. Then infested fruit is placed into export table grape cartons with filler fruit. After that data logger probes are inserted into fruit in the cartons and the cartons of fruit are placed in treatment at 2°C or 3°C. After treatment infested fruit are taken out of the carton and placed over damp vermiculite (the pupation medium) in plastic trays and held at 26°C until all insects surviving the treatment have pupated.



Fig. 34 Infested, non cold-treated (Control) grapes after 7 days at 26°C. This tray, lined with 2mm mesh, is placed into a larger tray over damp vermiculite. Treated fruit are held in the same method.



Fig. 35 Trays of fruit were covered with bags made of fine terylene which allowed air movement around the fruit but stopped contamination by other insects.

BASIC (MOST TOLERANT STAGE) TESTS AND LARGE SCALE TESTS CARRIED OUT AT 2°C AND 3°C (Continued)



Fig. 36 Vermiculite from pupation trays being sieved to inspect for surviving insects.



Fig. 37 Surviving insects (pupae) being removed from vermiculite from non cold-treated (Control) grapes.



Fig. 38 Surviving insects (pupae) being removed from vermiculite from non cold-treated (Control) grapes.

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

* * * * *

PART THREE – RESULTS OF THE EXPERIMENTS

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

CONDUCTED AT

NEW SOUTH WALES AGRICULTURE,
GOSFORD, NSW. AUSTRALIA 2250

With the support of the

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3 Results of the experiments

3.1 Fundamental and applied test:

3.1.1 Number of fruit, number of test insects, number of insects per fruit, number of survived, number of dead

3.1.1.1 Queensland fruit fly - *Bactrocera tryoni*

Eggs and first, second, and third instar larvae of *B. tryoni* dominated insect populations in the three grape cultivars on Days 1, 3, 5, and 7 after infestation respectively, in each of the three grape cultivars. On these days, between 75 and 100% of fruit flies infesting the grapes were at the nominated life stage.

Estimated treatment time for probit 9 dose-mortality ranged from 3.46 days at 1°C for *B. tryoni* eggs in 'Ruby Seedless' grapes to 9.64 days at 1°C for first instar *B. tryoni* larvae in 'Flame Seedless' grapes. Results showed that first instar larvae were consistently more cold-tolerant than the eggs and the other immature life stages in the three grape cultivars. It was decided, therefore to test for treatment efficacy on first instar larvae in the three grape cultivars following storage for 12 days at 1°C .

Treatment commenced at 8 to 12.7 hours after the infested fruit were placed in storage at 1°C .

Following treatment for 12 days at 1°C there were no survivors from a total of 39,928 first instar larvae in 'Thompson's Seedless' grapes treated in 17 sub-trials; from 47,341 first instar larvae in 9 sub-trials in 'Ruby Seedless' grapes, and from 66,895 first instar larvae treated in 13 sub-trials in 'Flame Seedless' grapes.

Table 1. Development of *B. tryoni* eggs and larvae in laboratory-infested table grapes – 1°C Tests

% Number of larvae at nominated larval instar															
Cultivar	'Thompson's Seedless'					'Ruby Seedless'					'Flame Seedless'				
Instar	Egg	1st	2nd	3rd	n ^B	Egg	1st	2nd	3rd	n ^B	Egg	1st	2nd	3rd	n ^B
Day ^A															
0	100	0	0	0	100	100	0	0	0	102	100	0	0	0	105
1	100	0	0	0	120	100	0	0	0	100	100	0	0	0	101
2	15	85	0	0	101	10	90	0	0	71	8	92	0	0	91
3	1	76	23	0	96	0	82	18	0	99	1	93	6	0	75
4	0	70	30	0	53	0	29	71	0	93	0	37	63	0	81
5	0	0	78	22	82	0	0	84	16	112	0	0	76	24	105
6	0	0	38	62	61	0	0	34	66	91	0	0	6	94	90
7	0	0	10	90	79	0	0	0	100	86	0	0	0	100	117
8	0	0	0	100	83	0	0	0	100	78	0	0	0	100	84

1st = First instar larvae; 2nd = Second instar larvae; 3rd = Third instar larvae

^A Fruit infested on Day 0 and stored at 26°C

^B Number of larvae examined

Table 2. Development of *B. tryoni* eggs and larvae in artificially-infested table grapes – 2°C and 3°C Tests

% Number of larvae at nominated larval instar															
Cultivar	'Thompson's Seedless'					'Red Globe'					'Crimson Seedless'				
Instar	Egg	1st	2nd	3rd	n ^B	Egg	1st	2nd	3rd	n ^B	Egg	1st	2nd	3rd	n ^B
Day ^A															
0	100	0	0	0	100	100	0	0	0	100	100	0	0	0	100
1	100	0	0	0	100	100	0	0	0	100	100	0	0	0	100
2	10	90	0	0	100	10	90	0	0	100	12	88	0	0	100
3	5	76	19	0	100	0	78	22	0	100	2	91	7	0	100
4	0	70	30	0	100	0	16	80	4	100	0	22	69	9	100
5	0	2	75	23	100	0	0	76	24	100	0	2	70	28	100
6	0	0	42	58	100	0	0	23	77	100	0	0	16	84	100
7	0	0	10	90	100	0	0	0	100	100	0	0	0	100	100
8	0	0	0	100	100	0	0	0	100	100	0	0	0	100	100

1st = First instar larvae; 2nd = Second instar larvae; 3rd = Third instar larvae

^A Fruit infested on Day 0 and stored at 26°C

^B Number of larvae examined

Table 3. Statistics for the Fundamental Tests (Determination of the Most Cold-Tolerant Insect Life stage) for ‘Thompson’s Seedless’ Table Grapes – 1°C Tests

Grape cvar	Insect life stage	Rep.	Start Date	Start Time	Finish Date	Finish Time	Data record interval (min.)	Hours to cool down
‘THOMPSON’S SEEDLESS’	EGG	1	24 Mar	11:48:00	20 Apr	7:48:00	30	12
		2	86	11:48:00	86	7:48:00	30	12
		3	24 Mar	11:48:00	20 Apr	7:48:00	30	12
			86		86			
			24 Mar		20 Apr			
			86		86			
‘THOMPSON’S SEEDLESS’	FIRST INSTAR	1	27 Mar	11:18:00	20 Apr	7:48:00	30	10
		2	86	11:18:00	86	7:48:00	30	10
		3	27 Mar	11:18:00	20 Apr	7:48:00	30	10
			86		86			
			27 Mar		20 Apr			
			86		86			
‘THOMPSON’S SEEDLESS’	SECOND INSTAR	1	29 Mar	11:18:00	20 Apr	7:48:00	30	8
		2	86	11:18:00	86	7:48:00	30	8
		3	29 Mar	11:18:00	20 Apr	7:48:00	30	8
			86		86			
			29 Mar		20 Apr			
			86		86			
‘THOMPSON’S SEEDLESS’	THIRD INSTAR	1	31 Mar	11:48:00	20 Apr	7:48:00	30	9
		2	86	11:48:00	86	7:48:00	30	9
		3	31 Mar	11:48:00	20 Apr	7:48:00	30	9
			86		86			
			31 Mar		20 Apr			
			86		86			

Grape cvar	Insect life stage	Rep.	% Time > 1.4°C	% Time = 1.4°C	% Time = 1.2°C	% Time = 1.0°C	% Time = 0.8°C	% Time = 0.6°C	% Time < 0.6°C
‘THOMPSON’S SEEDLESS’	EGG	1	0	1.1	69.6	29.3	0	0	0
		2	0	1.1	69.6	29.3	0	0	0
		3	0	1.1	69.6	29.3	0	0	0
‘THOMPSON’S SEEDLESS’	FIRST INSTAR	1	0	0.3	8.0	87.1	4.6	0	0
		2	0	0.3	8.0	87.1	4.6	0	0
		3	0	0.3	8.0	87.1	4.6	0	0
‘THOMPSON’S SEEDLESS’	SECOND INSTAR	1	0	7.1	63.4	29.1	0.4	0	0
		2	0	7.1	63.4	29.1	0.4	0	0
		3	0	7.1	63.4	29.1	0.4	0	0
‘THOMPSON’S SEEDLESS’	THIRD INSTAR	1	0	0.4	0.6	82.5	16.5	0	0
		2	0	0.4	0.6	82.5	16.5	0	0
		3	0	0.4	0.6	82.5	16.5	0	0

Table 4. Statistics for the Fundamental Tests (Determination of the Most Cold-Tolerant Insect Life stage) for 'Flame Seedless' (FS) and 'Ruby Seedless' (RS) Table Grapes – 1°C Tests

Grape cvar	Insect life stage	Rep.	Start Date	Start Time	Finish Date	Finish Time	Data record interval (min.)	Hours to cool down
FS and RS	EGG	1	18 Mar 88	9:06:00	6 Apr 88	14:22:00	10	8.3
		2	18 Mar 88	9:06:00	6 Apr 88	14:22:00	10	8.3
		3	18 Mar 88	9:06:00	6 Apr 88	14:22:00	10	8.3
FS and RS	FIRST INSTAR	1	21 Mar 88	8:36:00	6 Apr 88	14:22:00	10	12.7
		2	21 Mar 88	8:36:00	6 Apr 88	14:22:00	10	12.7
		3	21 Mar 88	8:36:00	6 Apr 88	14:22:00	10	12.7
FS and RS	SECOND INSTAR	1	23 Mar 88	9:06:00	6 Apr 88	14:22:00	10	8
		2	23 Mar 88	9:06:00	6 Apr 88	14:22:00	10	8
		3	23 Mar 88	9:06:00	6 Apr 88	14:22:00	10	8
FS and RS	THIRD INSTAR	1	25 Mar 88	8:36:00	6 Apr 88	14:22:00	10	8.8
		2	25 Mar 88	8:36:00	6 Apr 88	14:22:00	10	8.8
		3	25 Mar 88	8:36:00	6 Apr 88	14:22:00	10	8.8

Grape cvar	Insect life stage	Rep.	% Time > 1.4°C	% Time = 1.4°C	% Time = 1.2°C	% Time = 1.0°C	% Time = 0.8°C	% Time = 0.6°C	% Time < 0.6°C
FS and RS	EGG	1	0	0.2	15.4	82.5	1.9	0	0
		2	0	0.2	15.4	82.5	1.9	0	0
		3	0	0.2	15.4	82.5	1.9	0	0
FS and RS	FIRST INSTAR	1	0	6.5	9.9	82.4	1.4	0	0
		2	0	6.5	9.9	82.4	1.4	0	0
		3	0	6.5	9.9	82.4	1.4	0	0
FS and RS	SECOND INSTAR	1	0	0.2	75.6	24.1	0	0	0
		2	0	0.2	75.6	24.1	0	0	0
		3	0	0.2	75.6	24.1	0	0	0
FS and RS	THIRD INSTAR	1	0	0.3	1.5	45.2	52.1	0.9	0
		2	0	0.3	1.5	45.2	52.1	0.9	0
		3	0	0.3	1.5	45.2	52.1	0.9	0

Table 5. Statistics for the Fundamental Tests (Determination of the Most Cold-Tolerant Insect Life stage) for 'Thompson's Seedless' (TS), 'Red Globe' (RG) and 'Crimson Seedless' (CS) Table Grapes – 2°C Tests

Grape cvar	Insect life stage	Rep.	Start Date	Start Time	Finish Date	Finish Time	Data record interval (min.)	Hours to cool down
TS, RG and CS	EGG	1	9 Mar 05	9:30:00	26 Mar 05	10:30:00	30	10.5
		2	9 Mar 05	9:30:00	26 Mar 05	14:00:00	30	12.0
		3	9 Mar 05	9:30:00	26 Mar 05	14:30:00	30	12.5
TS, RG and CS	FIRST INSTAR	1	11 Mar 05	9:30:00	28 Mar 05	10:00:00	30	8.5
		2	11 Mar 05	9:30:00	28 Mar 05	12:30:00	30	10
		3	11 Mar 05	9:30:00	28 Mar 05	10:00:00	30	10
TS, RG and CS	SECOND INSTAR	1	13 Mar 05	9:30:00	30 Mar 05	9:30:00	30	10
		2	13 Mar 05	9:30:00	30 Mar 05	10:30:00	30	9
		3	13 Mar 05	9:30:00	30 Mar 05	10:30:00	30	10
TS, RG and CS	THIRD INSTAR	1	15 Mar 05	9:30:00	1 Apr 05	10:00:00	30	10
		2	15 Mar 05	9:30:00	1 Apr 05	12:00:00	30	10
		3	15 Mar 05	9:30:00	1 Apr 05	12:00:00	30	11

Grape cvar	Insect life stage	Rep.	% Time ≥ 2.5°C	% Time between 1.5°C and 2°C	% Time < 1.5°C
TS, RG and CS	EGG	1	0	100	0
		2	0	100	0
		3	0	100	0
TS, RG and CS	FIRST INSTAR	1	0	100	0
		2	0	100	0
		3	0	100	0
TS, RG and CS	SECOND INSTAR	1	0	100	0
		2	0	100	0
		3	0	100	0
TS, RG and CS	THIRD INSTAR	1	0	100	0
		2	0	100	0
		3	0	100	0

Table 6. Statistics for the Fundamental Tests (Determination of the Most Cold-Tolerant Insect Life stage) for 'Thompson's Seedless' (TS), 'Red Globe' (RG) and 'Crimson Seedless' (CS) Table Grapes – 3°C Tests

Grape cvar	Insect life stage	Rep.	Start Date	Start Time	Finish Date	Finish Time	Data record interval (min.)	Hours to cool down
TS, RG and CS	EGG	1	30 Mar 05	9:30:00	17 Apr 05	12:00:00	30	9
		2	30 Mar 05	9:30:00	17 Apr 05	14:00:00	30	9.5
		3	30 Mar 05	9:30:00	17 Apr 05	10:30:00	30	9
TS, RG and CS	FIRST INSTAR	1	1 Apr 05	9:30:00	19 Apr 05	10:00:00	30	9.5
		2	1 Apr 05	9:30:00	19 Apr 05	12:00:00	30	10
		3	1 Apr 05	9:30:00	19 Apr 05	12:00:00	30	10
TS, RG and CS	SECOND INSTAR	1	3 Apr 05	9:30:00	21 Apr 05	10:30:00	30	9
		2	3 Apr 05	9:30:00	21 Apr 05	10:00:00	30	9.5
		3	3 Apr 05	9:30:00	21 Apr 05	11:30:00	30	10
TS, RG and CS	THIRD INSTAR	1	5 Apr 05	9:30:00	23 Apr 05	14:00:00	30	9.5
		2	5 Apr 05	9:30:00	23 Apr 05	12:30:00	30	10
		3	5 Apr 05	9:30:00	23 Apr 05	13:00:00	30	9

Grape cvar	Insect life stage	Rep.	% Time ≥ 3.5°C	% Time between 2.5°C and 3°C	% Time < 2.5°C
TS, RG and CS	EGG	1	0	100	0
		2	0	100	0
		3	0	100	0
TS, RG and CS	FIRST INSTAR	1	0	100	0
		2	0	100	0
		3	0	100	0
TS, RG and CS	SECOND INSTAR	1	0	100	0
		2	0	100	0
		3	0	100	0
TS, RG and CS	THIRD INSTAR	1	0	100	0
		2	0	100	0
		3	0	100	0

Table 7. Survival of *B. tryoni* eggs and larvae in laboratory-infested ‘Thompson’s Seedless’ table grapes following storage at 1°C.

Number of survivors ^A (pupae) following storage at 1°C for 0 (Control), 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 days												
Thomps on’s Seedless	Replicate 1				Replicate 2				Replicate 3			
Instar	Egg	1st	2nd	3rd	Egg	1st	2nd	3rd	Egg	1st	2nd	3rd
Day												
0	39	144	80	19	91	182	128	89	52	121	119	115
1	32	111	29	6	88	121	61	41	49	98	93	51
2	54	33	31	7	52	51	38	32	16	55	31	19
3	0	15	3	2	6	16	5	3	5	20	10	2
4	0	3	0	1	0	2	2	1	1	7	2	1
5	0	3	1	0	0	2	1	1	0	3	0	0
6	0	1	0	0	0	1	0	0	0	1	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
Probit 5	2.21	1.15	0.80	0.84	1.60	1.06	0.84	0.88	1.37	1.52	1.14	0.63
Probit 9	3.54	5.27	5.15	5.91	4.25	5.17	4.92	5.11	4.51	6.26	4.71	3.94

^A No. of pupae per 100 fruit.

^B Fruit infested on Day 0 and stored at 26°C.

Table 8. Survival of *B. tryoni* eggs and larvae in laboratory-infested ‘Ruby Seedless’ table grapes following storage at 1°C.

Number of survivors ^A (pupae) following storage at 1°C for 0 (Control), 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 days												
Ruby Seedless	Replicate 1				Replicate 2				Replicate 3			
Instar	Egg	1st	2nd	3rd	Egg	1st	2nd	3rd	Egg	1st	2nd	3rd
Day												
0	31	71	49	51	80	91	62	85	58	49	78	62
1	25	61	10	13	63	65	41	29	31	35	21	18
2	2	23	4	5	21	29	15	11	18	20	8	6
3	0	8	2	1	3	9	3	2	1	10	2	2
4	1	7	1	1	1	6	1	0	1	5	1	1
5	0	4	0	0	0	2	0	0	0	2	0	0
6	0	1	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
Probit 5	0.50	1.46	0.35	0.39	1.10	1.22	0.97	0.49	0.88	1.47	0.41	0.44
Probit 9	3.46	6.53	3.63	3.57	4.30	5.84	4.56	3.60	4.67	6.96	3.58	3.70

^A No. of pupae per 100 fruit.

^B Fruit infested on Day 0 and stored at 26°C.

Table 9. Survival of *B. tryoni* eggs and larvae in laboratory-infested ‘Flame Seedless’ table grapes following storage at 1°C.

Number of survivors ^A (pupae) following storage at 1°C for 0 (Control), 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 days												
Flame Seedless	Replicate 1				Replicate 2				Replicate 3			
Instar	Egg	1st	2nd	3rd	Egg	1st	2nd	3rd	Egg	1st	2nd	3rd
Day												
0	285	125	141	48	201	195	210	153	175	215	206	181
1	240	82	89	34	188	173	129	108	101	188	116	99
2	40	63	79	9	51	106	82	31	56	151	91	17
3	10	38	4	4	10	36	11	5	16	48	10	8
4	2	36	2	2	3	28	3	3	6	31	3	2
5	0	15	0	3	1	15	1	1	0	9	0	1
6	0	5	2	2	0	3	1	2	1	5	1	0
7	0	2	2	1	0	1	1	1	0	1	1	1
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
Probit 5	1.02	1.93	1.26	1.15	1.26	1.86	1.09	0.99	1.03	2.01	1.08	0.71
Probit 9	3.67	9.64	5.82	6.74	4.12	7.41	5.33	4.84	5.48	7.30	5.44	4.17

^A No. of pupae per 100 fruit.

^B Fruit infested on Day 0 and stored at 26°C.

Table 10. Survival of *B. tryoni* eggs and larvae in laboratory-infested ‘Thompson’s Seedless’ table grapes following storage at 2°C.

Days in 2°C	No. fruit	Total survivors from 3 replicates				No. of survivors per fruit				% mortality			
		Eggs	1st Instar	2nd Instar	3rd Instar	Eggs	1st Instar	2nd Instar	3rd Instar	Eggs	1st Instar	2nd Instar	3rd Instar
0	240	1466	852	577	578	6.108333	3.55	2.404167	2.408333	0	0	0	0
1	75	264	184	117	113	3.52	2.453333	1.56	1.506667	42.37381	30.89202	35.11265	37.43945
2	75	97	114	69	73	1.293333	1.52	0.92	0.973333	78.82674	57.1831	61.7331	59.58478
3	75	70	55	34	36	0.933333	0.733333	0.453333	0.48	84.72033	79.34272	81.14385	80.0692
4	75	9	22	8	12	0.12	0.293333	0.106667	0.16	98.03547	91.73709	95.56326	93.3564
5	75	2	6	4	4	0.026667	0.08	0.053333	0.053333	99.56344	97.74648	97.78163	97.78547
6	75	1	5	2	2	0.013333	0.066667	0.026667	0.026667	99.78172	98.12206	98.89080	98.89272
7	75	0	3	0	1	0	0.04	0	0.013333	100	98.87324	100	99.44637
8	75	1	3	1	1	0.013333	0.04	0.013333	0.013333	99.78172	98.87324	99.44541	99.44637
9	75	0	2	0	1	0	0.026667	0	0.013333	100	99.24883	100	99.44637
10	75	0	1	0	1	0	0.013333	0	0.013333	100	99.62441	100	99.44637
11	75	0	1	0	0	0	0.013333	0	0	100	99.62441	100	100
12	75	0	1	0	0	0	0.013333	0	0	100	99.62441	100	100
13	75	0	0	0	0	0	0	0	0	100.00	100.00	100.00	100.00
14	75	0	0	0	0	0	0	0	0	100.00	100.00	100.00	100.00
15	75	0	0	0	0	0	0	0	0	100.00	100.00	100.00	100.00
16	75	0	0	0	0	0	0	0	0	100.00	100.00	100.00	100.00
17	75	0	0	0	0	0	0	0	0	100.00	100.00	100.00	100.00

Table 11. Survival of *B. tryoni* eggs and larvae in laboratory-infested ‘Red Globe’ table grapes following storage at 2°C.

Days in 2°C	No. fruit	Total survivors from 3 replicates				No. of survivors per fruit				% mortality			
		Eggs	1st Instar	2nd Instar	3rd Instar	Eggs	1st Instar	2nd Instar	3rd Instar	Eggs	1st Instar	2nd Instar	3rd Instar
0	240	2798	1623	2533	2367	11.65833	6.76250	10.55417	9.86250				
1	75	626	372	563	489	8.34667	4.96000	7.50667	6.52000	28.40598	26.65434	28.87487	33.89100
2	75	355	273	357	380	4.73333	3.64000	4.76000	5.06667	59.39956	46.17375	54.89934	48.62695
3	75	219	215	167	234	2.92000	2.86667	2.22667	3.12000	74.95353	57.60936	78.90249	68.36502
4	75	127	116	124	113	1.69333	1.54667	1.65333	1.50667	85.47534	77.12877	84.33479	84.72328
5	75	34	66	51	66	0.45333	0.88000	0.68000	0.88000	96.11151	86.98706	93.55705	91.07731
6	75	4	55	25	64	0.05333	0.73333	0.33333	0.85333	99.54253	89.15589	96.84169	91.34770
7	75	0	31	10	20	0	0.41333	0.13333	0.26667	100.00000	93.88787	98.73668	97.29615
8	75	0	9	4	7	0	0.12000	0.05333	0.09333	100.00000	98.22551	99.49467	99.05366
9	75	0	7	3	5	0	0.09333	0.04000	0.06667	100.00000	98.61984	99.62100	99.32404
10	75	0	4	1	2	0	0.05333	0.01333	0.02667	100.00000	99.21134	99.87367	99.72961
11	75	0	1	0	1	0	0.01333	0	0.01333	100.00000	99.80284	100	99.86481
12	75	0	1	0	0	0	0.01333	0	0	100.00000	99.80284	100	100
13	75	0	0	0	0	0	0	0	0	100	100	100	100
14	75	0	0	0	0	0	0	0	0	100	100	100	100
15	75	0	0	0	0	0	0	0	0	100	100	100	100
16	75	0	0	0	0	0	0	0	0	100	100	100	100
17	75	0	0	0	0	0	0	0	0	100	100	100	100

Table 12. Survival of *B. tryoni* eggs and larvae in laboratory-infested ‘Crimson Seedless’ table grapes following storage at 2°C.

Days in 2°C	No. fruit	Total survivors from 3 replicates				No. of survivors per fruit				% mortality			
		Eggs	1st Instar	2nd Instar	3rd Instar	Eggs	1st Instar	2nd Instar	3rd Instar	Eggs	1st Instar	2nd Instar	3rd Instar
0	240	825	662	702	573	3.43750	2.75833	2.92500	2.38750				
1	75	215	190	167	134	2.86667	2.53333	2.22667	1.78667	16.60605	8.15710	23.87463	25.16578
2	75	134	146	82	75	1.78667	1.94667	1.09333	1.00000	48.02423	29.42596	62.62109	58.11518
3	75	40	92	49	55	0.53333	1.22667	0.65333	0.73333	84.48486	55.52868	77.66383	69.28448
4	75	24	76	42	47	0.32000	1.01333	0.56000	0.62667	90.69091	63.26285	80.85470	73.75217
5	75	11	34	20	22	0.14667	0.45333	0.26667	0.29333	95.73332	83.56496	90.88318	87.71380
6	75	3	21	7	9	0.04000	0.28000	0.09333	0.12000	98.83636	89.84894	96.80913	94.97382
7	75	2	10	3	5	0.02667	0.13333	0.04000	0.06667	99.22423	95.16617	98.63248	97.20766
8	75	0	3	1	2	0	0.04000	0.01333	0.02667	100	98.54985	99.54417	98.88306
9	75	0	1	1	1	0	0.01333	0.01333	0.01333	100	99.51663	99.54417	99.44155
10	75	0	1	0	0	0	0.01333	0	0	100	99.51663	100	100
11	75	0	1	0	0	0	0.01333	0	0	100	99.51663	100	100
12	75	0	0	0	0	0	0	0	0	100	100	100	100
13	75	0	0	0	0	0	0	0	0	100	100	100	100
14	75	0	0	0	0	0	0	0	0	100	100	100	100
15	75	0	0	0	0	0	0	0	0	100	100	100	100
16	75	0	0	0	0	0	0	0	0	100	100	100	100
17	75	0	0	0	0	0	0	0	0	100	100	100	100

Table 13. Survival of *B. tryoni* eggs and larvae in laboratory-infested ‘Thompson’s Seedless’ table grapes following storage at 3°C.

Days in 3°C	No. fruit	Total survivors from 3 replicates				No. of survivors per fruit				% mortality			
		Eggs	1st Instar	2nd Instar	3rd Instar	Eggs	1st Instar	2nd Instar	3rd Instar	Eggs	1st Instar	2nd Instar	3rd Instar
0	240	1442	1002	1011	785	6.0083333	4.175	4.2125	3.2708333	0	0	0	0
1	75	262	201	191	141	3.4933333	2.68	2.5466667	1.88	41.85853	35.808383	39.545005	42.522293
2	75	100	136	106	93	1.3333333	1.8133333	1.4133333	1.24	77.808599	56.566866	66.44906	62.089172
3	75	56	72	66	46	0.7466667	0.96	0.88	0.6133333	87.572816	77.005988	79.109792	81.248408
4	75	9	35	33	14	0.12	0.4666667	0.44	0.1866667	98.002774	88.822355	89.554896	94.292994
5	75	3	16	15	8	0.04	0.2133333	0.2	0.1066667	99.334258	94.89022	95.252226	96.738854
6	75	1	6	2	3	0.0133333	0.08	0.0266667	0.04	99.778086	98.083832	99.366963	98.77707
7	75	0	4	1	1	0	0.0533333	0.0133333	0.0133333	100	98.722555	99.683482	99.592357
8	75	1	2	1	1	0.0133333	0.0266667	0.0133333	0.0133333	99.778086	99.361277	99.683482	99.592357
9	75	0	3	3	1	0	0.04	0.04	0.0133333	100	99.041916	99.050445	99.592357
10	75	0	1	1	1	0	0.0133333	0.0133333	0.0133333	100	99.680639	99.683482	99.592357
11	75	0	1	1	0	0	0.0133333	0.0133333	0	100	99.680639	99.683482	100
12	75	0	1	1	0	0	0.0133333	0.0133333	0	100	99.680639	99.683482	100
13	75	0	0	0	0	0	0	0	0	100	100	100	100
14	75	0	0	0	0	0	0	0	0	100	100	100	100
15	75	0	0	0	0	0	0	0	0	100	100	100	100
16	75	0	0	0	0	0	0	0	0	100	100	100	100
17	75	0	0	0	0	0	0	0	0	100	100	100	100

Table 14. Survival of *B. tryoni* eggs and larvae in laboratory-infested ‘Red Globe’ table grapes following storage at 3°C.

Days in 3°C	No. fruit	Total survivors from 3 replicates				No. of survivors per fruit				% mortality			
		Eggs	1st Instar	2nd Instar	3rd Instar	Eggs	1st Instar	2nd Instar	3rd Instar	Eggs	1st Instar	2nd Instar	3rd Instar
0	240	2421	2088	2426	2051	10.0875	8.7	10.108333	8.5458333	0	0	0	0
1	75	524	519	532	407	6.9866667	6.92	7.0933333	5.4266667	30.739364	20.45977	29.826876	36.499269
2	75	367	430	408	340	4.8933333	5.7333333	5.44	4.5333333	51.491119	34.099617	46.183017	46.952706
3	75	224	294	205	199	2.9866667	3.92	2.7333333	2.6533333	70.3924	54.942529	72.959604	68.951731
4	75	128	156	101	95	1.7066667	2.08	1.3466667	1.2666667	83.081371	76.091954	86.677659	85.177962
5	75	33	118	41	53	0.44	1.5733333	0.5466667	0.7066667	95.638166	81.915709	94.591921	91.730863
6	75	5	57	46	34	0.0666667	0.76	0.6133333	0.4533333	99.339116	91.264368	93.932399	94.695271
7	75	0	57	14	14	0	0.76	0.1866667	0.1866667	100	91.264368	98.153339	97.8157
8	75	0	27	9	6	0	0.36	0.12	0.08	100	95.862069	98.812861	99.063871
9	75	0	17	4	3	0	0.2266667	0.0533333	0.04	100	97.394636	99.472383	99.531936
10	75	0	6	2	1	0	0.08	0.0266667	0.0133333	100	99.08046	99.736191	99.843979
11	75	0	3	0	0	0	0.04	0	0	100	99.54023	100	100
12	75	0	1	0	0	0	0.0133333	0	0	100	99.846743	100	100
13	75	0	0	0	0	0	0	0	0	100	100	100	100
14	75	0	0	0	0	0	0	0	0	100	100	100	100
15	75	0	0	0	0	0	0	0	0	100.00	100.00	100.00	100.00
16	75	0	0	0	0	0	0	0	0	100.00	100.00	100.00	100.00
17	75	0	0	0	0	0	0	0	0	100.00	100.00	100.00	100.00

Table 15. Survival of *B. tryoni* eggs and larvae in laboratory-infested ‘Crimson Seedless’ table grapes following storage at 3°C.

Days in 3°C	No. fruit	Total survivors from 3 replicates				No. of survivors per fruit				% mortality			
		Eggs	1st Instar	2nd Instar	3rd Instar	Eggs	1st Instar	2nd Instar	3rd Instar	Eggs	1st Instar	2nd Instar	3rd Instar
0	240	864	874	852	778	3.6	3.6416667	3.55	3.2416667	0	0	0	0
1	75	229	258	217	204	3.0533333	3.44	2.8933333	2.72	15.185185	5.5377574	18.497653	16.092545
2	75	140	222	142	116	1.8666667	2.96	1.8933333	1.5466667	48.148148	18.718535	46.666667	52.287918
3	75	60	131	87	79	0.8	1.7466667	1.16	1.0533333	77.777778	52.036613	67.323944	67.506427
4	75	27	102	59	68	0.36	1.36	0.7866667	0.9066667	90	62.654462	77.840376	72.030848
5	75	12	49	28	25	0.16	0.6533333	0.3733333	0.3333333	95.555556	82.059497	89.483568	89.717224
6	75	2	28	10	11	0.0266667	0.3733333	0.1333333	0.1466667	99.259259	89.748284	96.244131	95.475578
7	75	1	18	5	3	0.0133333	0.24	0.0666667	0.04	99.62963	93.409611	98.122066	98.766067
8	75	0	7	2	1	0	0.0933333	0.0266667	0.0133333	100	97.437071	99.248826	99.588689
9	75	0	5	2	1	0	0.0666667	0.0266667	0.0133333	100	98.169336	99.248826	99.588689
10	75	0	2	1	0	0	0.0266667	0.0133333	0	100	99.267735	99.624413	100
11	75	0	3	0	0	0	0.04	0	0	100	98.901602	100	100
12	75	0	1	0	0	0	0.0133333	0	0	100	99.633867	100	100
13	75	0	0	0	0	0	0	0	0	100	100	100	100
14	75	0	0	0	0	0	0	0	0	100	100	100	100
15	75	0	0	0	0	0	0	0	0	100.00	100.00	100.00	100.00
16	75	0	0	0	0	0	0	0	0	100.00	100.00	100.00	100.00
17	75	0	0	0	0	0	0	0	0	100.00	100.00	100.00	100.00

Table 15a. Comparison of the number of days exposure at 2 & 3°C required to kill 50% (LD₅₀) and 99% (LD₉₉) of the four immature life stages of Queensland fruit fly (Qfly), *Bactrocera tryoni* Froggatt, in 3 table grapes cultivars. The analysis is based on three replicate trials for each life stage.

Temperature, Cultivar, and Life stage treated	Days	95% confidence intervals		Days	95% confidence intervals	
	LD ₅₀	<u>Lower</u>	<u>Upper</u>	LD ₉₉	<u>Lower</u>	<u>Upper</u>
2°C						
<i>Red Globe</i>						
<i>Eggs</i>	2.339	2.248	2.429	7.407	7.136	7.703
1 st instar larvae	3.267	3.132	3.401	10.347	9.955	10.773
2 nd instar larvae	2.490	2.391	2.587	7.885	7.606	8.188
3 rd instar larvae	2.833	2.723	2.942	8.972	8.659	9.312
<i>Crimson Seedless</i>						
<i>Eggs</i>	2.062	1.917	2.206	7.117	6.679	7.622
1 st instar larvae	3.134	2.928	3.335	10.814	10.174	11.557
2 nd instar larvae	2.207	2.049	2.362	7.616	7.155	8.146
3 rd instar larvae	2.495	2.315	2.673	8.610	8.070	9.233
<i>Thompson Seedless</i>						
<i>Eggs</i>	1.653	1.510	1.790	5.506	5.177	5.890
1 st instar larvae	2.270	2.063	2.470	7.560	7.093	8.100
2 nd instar larvae	2.028	1.828	2.226	6.755	6.243	7.351
3 rd instar larvae	2.126	1.918	2.332	7.082	6.567	7.679
3°C						
<i>Red Globe</i>						
<i>Eggs</i>	2.533	2.440	2.626	7.614	7.336	7.916
1 st instar larvae	3.570	3.445	3.694	10.731	10.376	11.115
2 nd instar larvae	2.701	2.602	2.799	8.117	7.843	8.414
3 rd instar larvae	2.780	2.675	2.884	8.354	8.058	8.676
<i>Crimson Seedless</i>						
<i>Eggs</i>	2.128	2.008	2.249	6.913	6.533	7.342
1 st instar larvae	3.333	3.169	3.494	10.826	10.303	11.417
2 nd instar larvae	2.529	2.394	2.664	8.216	7.797	8.687
3 rd instar larvae	2.530	2.392	2.668	8.219	7.785	8.707
<i>Thompson Seedless</i>						
<i>Eggs</i>	1.685	1.550	1.817	5.673	5.335	6.061
1 st instar larvae	2.449	2.253	2.640	8.246	7.768	8.792
2 nd instar larvae	2.246	2.064	2.425	7.563	7.116	8.073
3 rd instar larvae	2.133	1.949	2.316	7.182	6.706	7.725

Table 16. Summary of trials to confirm efficacy of 12 days at 1°C against first instar larvae *B. tryoni* in ‘Thompson’s Seedless’ table grapes.

(C= Cage infested - fruit placed on cage of flies; A= Artificially infested - grapes impregnated with first instar larvae)

Replicate	Number of Control fruit	Number of insects/fruit	Number of treated fruit	Estimated number of insects treated	Number of surviving pupae	Infestation method	% No. of insects at first instar
1	400	0.45	3000	1350	0	C	81
2	400	1.80	3300	5990	0	C	72
3	400	0.30	3600	1080	0	C	92
4	500	0.83	5930	4922**	0	C	86
5	500	0.68	6491	4414**	0	C	75
6	500	1.07	6321	6763**	0	C	69
7	2651	0.03	7954	239	0	C	82
8	1884	0.03	5651	170	0	C	81
9	2325	0.04	6975	279	0	C	90
10	2445	0.33	7338	2422	0	C	82
11	900	0.52	1634	854	0	C	85
12	624	0.56	3430	1921	0	C	75
13	1083	0.08	2841	227	0	C	74
14	1380	0.05	2959	148	0	C	78
15	1469	0.07	2860	200	0	C	76
16	1000	0.91	5000	4550	0	C	68
17	1000	0.86	5000	4300	0	C	69
TOTALS	19461		80284	39829	0		

** The temperature profiles of these three Replicate Trials are detailed in the Appendix

Table 17. Summary of trials to confirm efficacy of 12 days at 1°C against first instar larvae *B. tryoni* in ‘Ruby Seedless’ table grapes.

(C= Cage infested - fruit placed on cage of flies; A= Artificially infested - grapes impregnated with first instar larvae)

Replicate	Number of Control fruit	Number of insects/fruit	Number of treated fruit	Estimated number of insects treated	Number of surviving pupae	Infestation method	% No. of insects at first instar
1	1493	0.14	2400	336	0	C	69
2	1613	0.10	3600	360	0	C	75
3	1474	0.10	3000	300	0	C	78
4	1792	0.14	2775	389	0	C	79
5	1498	0.21	2517	529	0	C	78
6	747	4.21	2994	12605**	0	A	100
7	807	2.59	3117	8073	0	A	100
8	928	4.02	3215	12924**	0	A	100
9	585	5.06	2337	11825**	0	A	100
TOTALS	10937		25955	47341	0		

** The temperature profiles of these three Replicate Trials are detailed in the Appendix

Table 18. Summary of trials to confirm efficacy of 12 days at 1°C against first instar larvae B. tryoni in ‘Flame Seedless’ table grapes.

(C= Cage infested - fruit placed on cage of flies; A= Artificially infested - grapes impregnated with first instar larvae)

Replicate	Number of Control fruit	Number of insects/fruit	Number of treated fruit	Estimated number of insects treated	Number of surviving pupae	Infestation method	% No. of insects at first instar
1	500	0.83	4570	3793	0	C	80
2	500	0.45	4450	2003	0	C	82
3	500	0.82	4600	3772	0	C	71
4	1985	0.06	6025	362	0	C	79
5	1674	0.12	6325	759	0	C	75
6	1516	0.11	5636	620	0	C	72
7	2005	0.10	6673	667	0	C	89
8	435	3.11	1686	5243	0	A	100
9	801	2.34	3198	7483	0	A	100
10	870	3.57	1589	5673	0	A	100
11	500	2.96	4000	11840**	0	A	100
12	500	3.11	4000	12440**	0	A	100
13	500	3.06	4000	12240**	0	A	100
TOTALS	12286		56752	66895	0		

** The temperature profiles of these three Replicate Trials are detailed in the Appendix

Table 19. Survival of insects in untreated (Control) fruit – Red Globes Large Scale Trial @ 3°C Replicate 1

4000 red Globes were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 972 fruit (CONTROL) were randomly chosen and placed in 12 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 3028 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 3°C disinfestation treatment.				
LARGE SCALE TRIAL AT 3°C RED GLOBES REPLICATE 1 – Infested 12 April 2006				
Sample unit (81 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	24 April 2006	01 May 2006		
1	254	168	422	5.21
2	62	107	169	2.09
3	89	150	239	2.95
4	127	152	279	3.44
5	88	147	235	2.90
6	56	148	204	2.52
7	46	109	155	1.91
8	115	97	212	2.62
9	68	109	177	2.19
10	27	119	146	1.80
11	38	116	154	1.90
12	34	103	137	1.69

Table 20 Survival of insects in untreated (Control) fruit – Red Globes Large Scale Trial @ 3°C Replicate 2

<p>3000 red Globes were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 1000 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 2000 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1st instar larval stage for the 3°C disinfestation treatment.</p>				
LARGE SCALE TRIAL AT 3°C RED GLOBES REPLICATE 1 – Infested 22 February 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	05 March 2007	12 March 2007		
1	1635	18	1653	6.61
2	1400	38	1438	5.75
3	1807	18	1825	7.30
4	1778	28	1806	7.22

Table 21. Survival of insects in untreated (Control) fruit – Red Globes Large Scale Trial @ 3°C Replicate 3

5000 red Globes were manually infested by puncturing the fruit and dipping in slurry of QFF eggs. 1000 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 4000 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 3°C disinfestation treatment.				
LARGE SCALE TRIAL AT 3°C RED GLOBES REPLICATE 1 – Infested 02 March 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	12 march 2007	19 March 2007		
1	1047	52	1099	4.40
2	585	38	623	2.49
3	790	85	875	3.50
4	970	128	1098	4.39

Table 22. Survival of insects in untreated (Control) fruit – Red Globes Large Scale Trial @ 3°C Replicate 4

3750 red Globes were manually infested by puncturing the fruit and dipping in slurry of QFF eggs. 750 fruit (CONTROL) were randomly chosen and placed in 3 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 3000 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 3°C disinfestation treatment.				
LARGE SCALE TRIAL AT 3°C RED GLOBES REPLICATE 1 – Infested 08 March 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	19 march 2007	26 march 2007		
1	540	57	597	2.39
2	760	8	768	3.07
3	508	7	515	2.06

Table 23. Survival of insects in untreated (Control) fruit – Crimson Seedless Large Scale Trial @ 3°C Replicate 1

8000 Crimson Seedless were manually infested by puncturing the fruit and dipping in slurry of QFF eggs. 1950 fruit (CONTROL) were randomly chosen and placed in 13 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 6050 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 3°C disinfestation treatment.				
LARGE SCALE TRIAL AT 3°C CRIMSON SEEDLESS REPLICATE 1 – Infested 11 April 2006				
Sample unit (150 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	24 April 2006	01 May 2006		
1	21	5	26	0.17
2	17	3	20	0.13
3	12	1	13	0.09
4	10	5	15	0.1
5	8	3	11	0.07
6	3	0	3	0.02
7	14	3	17	0.17
8	1	1	2	0.01
9	10	1	11	0.07
10	11	9	20	0.13
11	8	0	8	0.05
12	5	1	6	0.04
13	17	3	20	0.13

Table 24. Survival of insects in untreated (Control) fruit – Crimson Seedless Large Scale Trial @ 3°C Replicate 3

5000 Crimson Seedless were manually infested by puncturing the fruit and dipping in slurry of QFF eggs. 1000 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 4000 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 3°C disinfestation treatment.				
LARGE SCALE TRIAL AT 3°C CRIMSON SEEDLESS REPLICATE 2 – Infested 22 February 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	05 March 2007	12 march 2007		
1	1457	44	1501	6.00
2	1730	65	1795	7.18
3	1710	24	1734	6.94
4	1800	114	1914	7.66

Table 25. Survival of insects in untreated (Control) fruit – Crimson Seedless Large Scale Trial @ 3°C Replicate 3

5000 Crimson Seedless were manually infested by puncturing the fruit and dipping in slurry of QFF eggs. 1000 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 4000 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 3°C disinfestation treatment.				
LARGE SCALE TRIAL AT 3°C CRIMSON SEEDLESS REPLICATE 3 – Infested 02 March 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	12 March 2007	19 march 2007		
1	624	498	1122	4.49
2	449	540	989	3.96
3	496	304	800	3.20
4	1096	470	1566	6.26

Table 26. Survival of insects in untreated (Control) fruit – Crimson Seedless Large Scale Trial @ 3°C Replicate 4

3750 Crimson Seedless were manually infested by puncturing the fruit and dipping in slurry of QFF eggs. 750 fruit (CONTROL) were randomly chosen and placed in 3 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 3000 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 3°C disinfestation treatment.				
LARGE SCALE TRIAL AT 3°C CRIMSON SEEDLESS REPLICATE 4 – Infested 08 March 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	19 March 2007	26 March 2007		
1	1578	30	1608	6.43
2	1509	12	1521	6.08
3	3014	4	3018	12.07

Table 27. Survival of insects in untreated (Control) fruit – Thompson Seedless Large Scale Trial @ 3°C Replicate 1

5996 Thompson Seedless were manually infested by puncturing the fruit and dipping in slurry of QFF eggs. 996 fruit (CONTROL) were randomly chosen and placed in 6 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 5000 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 3°C disinfestation treatment.				
LARGE SCALE TRIAL AT 3°C THOMPSON SEEDLESS REPLICATE 1 – Infested 18 May 2006				
Sample unit (166 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	29 May 2006	05 June 2006		
1	729	7	736	4.43
2	1351	5	1356	8.17
3	799	5	804	4.84
4	726	4	730	4.40
5	854	5	859	5.17
6	1663	7	1670	10.06

Table 28. Survival of insects in untreated (Control) fruit – Thompson Seedless Large Scale Trial @ 3°C Replicate 2

5000 Thompson Seedless were manually infested by puncturing the fruit and dipping in slurry of QFF eggs. 1000 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 4000 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 3°C disinfestation treatment.				
LARGE SCALE TRIAL AT 3°C THOMPSON SEEDLESS REPLICATE 2 – Infested 22 February 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	05 March 2007	12 March 2007		
1	3925	10	3935	15.74
2	3740	10	3750	15
3	4240	11	4251	17
4	3866	10	3876	15.5

Table 29. Survival of insects in untreated (Control) fruit – Thompson Seedless Large Scale Trial @ 3°C Replicate 3

3000 Thompson Seedless were manually infested by puncturing the fruit and dipping in slurry of QFF eggs. 1000 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 2000 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 3°C disinfestation treatment.				
LARGE SCALE TRIAL AT 3°C THOMPSON SEEDLESS REPLICATE 3 – Infested 02 March 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	12 March 2007	19 March 2007		
1	3180	0	3180	12.72
2	2150	0	2150	8.6
3	2257	0	2257	9.03
4	1750	0	1750	7.00

Table 30. Survival of insects in untreated (Control) fruit – Thompson Seedless Large Scale Trial @ 3°C Replicate 4

2750 Thompson Seedless were manually infested by puncturing the fruit and dipping in slurry of QFF eggs. 750 fruit (CONTROL) were randomly chosen and placed in 3 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 2000 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 3°C disinfestation treatment.				
LARGE SCALE TRIAL AT 3°C THOMPSON SEEDLESS REPLICATE 4 – Infested 08 March 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	19 March 2007	26 March 2007		
1	1578	30	1608	6.4
2	1509	12	1521	6.08
3	3014	4	3018	12.07

Table 31. Survival of insects in untreated (Control) fruit – Red Globes Large Scale Trial @ 2°C Replicate 1

3750 Red Globes were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 450 fruit (CONTROL) were randomly chosen and placed in 3 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 3300 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 2°C disinfestation treatment.				
LARGE SCALE TRIAL AT 2°C RED GLOBES REPLICATE 1 – Infested 08 March 2006				
Sample unit (150 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	15 March 2006	22 March 2006		
1	507	39	546	3.64
2	844	182	1026	6.84
3	726	133	859	5.73

Table 32. Survival of insects in untreated (Control) fruit – Red Globes Large Scale Trial @ 2°C Replicate 2

<p>2618 Red Globes were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 522 fruit (CONTROL) were randomly chosen and placed in 6 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 2096 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1st instar larval stage for the 2°C disinfestation treatment.</p>				
LARGE SCALE TRIAL AT 2°C RED GLOBES REPLICATE 2 – Infested 08 June 2006				
Sample unit (87 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	19 June 2006	26 June 2006		
1	32	4	36	0.41
2	60	4	64	0.74
3	13	6	19	0.22
4	0	4	4	0.05
5	7	8	15	0.17
6	31	9	40	0.5

Table 33. Survival of insects in untreated (Control) fruit – Red Globes Large Scale Trial @ 2°C Replicate 3

<p>5000 Red Globes were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 1000 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 2096 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1st instar larval stage for the 2°C disinfestation treatment.</p>				
LARGE SCALE TRIAL AT 2°C RED GLOBES REPLICATE 3 – Infested 15 March 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	27 March 2007	03 April 2007		
1	839	8	847	3.39
2	581	5	586	2.34
3	535	5	540	2.16
4	550	7	557	2.28

Table 34. Survival of insects in untreated (Control) fruit – Red Globes Large Scale Trial @ 2°C Replicate 4

1500 Red Globes were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 450 fruit (CONTROL) were randomly chosen and placed in 3 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 1050 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 2°C disinfestation treatment.				
LARGE SCALE TRIAL AT 2°C RED GLOBES REPLICATE 4 – Infested 22 March 2007				
Sample unit (150 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	03 April 2007	10 April 2007		
1	3167	231	3398	22.65
2	3653	31	3684	24.56
3	3375	32	3407	22.71

Table 35. Survival of insects in untreated (Control) fruit – Crimson Seedless Large Scale Trial @ 2°C Replicate 1

9750 Crimson Seedless were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 750 fruit (CONTROL) were randomly chosen and placed in 3 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 9000 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 2°C disinfestation treatment.				
LARGE SCALE TRIAL AT 2°C CRIMSON SEEDLESS REPLICATE 1 – Infested 08 March 2006				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	15 March 2006	22 March 2006		
1	195	12	207	0.83
2	148	12	160	0.64
3	93	4	97	0.39

Table 36. Survival of insects in untreated (Control) fruit – Crimson Seedless Large Scale Trial @ 2°C Replicate 2

3000 Crimson Seedless were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 600 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 st instar larval stage for the 2°C disinfestation treatment.				
LARGE SCALE TRIAL AT 2°C CRIMSON SEEDLESS REPLICATE 2 – Infested 08 June 2006				
Sample unit (150 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	19 June 2006	26 June 2006		
1	620	69	689	4.59
2	461	38	499	3.33
3	406	52	458	3.05
4	281	45	326	2.17

Table 37. Survival of insects in untreated (Control) fruit – Crimson Seedless Large Scale Trial @ 2°C Replicate 3

5000 Crimson Seedless were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 1000 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 4000 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 2°C disinfestation treatment.				
LARGE SCALE TRIAL AT 2°C CRIMSON SEEDLESS REPLICATE 3 – Infested 15 march 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	27 march 2007	03 April 2007		
1	1381	10	1391	5.56
2	1280	11	1291	5.16
3	1265	13	1278	5.11
4	1376	12	1388	5.55

Table 38. Survival of insects in untreated (Control) fruit – Crimson Seedless Large Scale Trial @ 2°C Replicate 4

5000 Crimson Seedless were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 1000 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 4000 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 2°C disinfestation treatment.				
LARGE SCALE TRIAL AT 2°C CRIMSON SEEDLESS REPLICATE 4 – Infested 22 march 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	03 April 2007	10 April 2007		
1	924	39	963	3.85
2	728	5	733	2.93
3	726	16	742	2.97
4	885	14	899	3.60

Table 39. Survival of insects in untreated (Control) fruit – Thompson Seedless Large Scale Trial @ 2°C Replicate 1

6000 Thompson Seedless were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 600 fruit (CONTROL) were randomly chosen and placed in 3 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 5400 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 2°C disinfestation treatment.				
LARGE SCALE TRIAL AT 2°C THOMPSON SEEDLESS REPLICATE 1 – Infested 08 March 2006				
Sample unit (200 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	15 March 2006	22 March 2006		
1	279	60	339	1.70
2	174	38	212	1.06
3	311	42	353	1.77

Table 40. Survival of insects in untreated (Control) fruit – Thompson Seedless Large Scale Trial @ 2°C Replicate 2

2848 Thompson Seedless were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 570 fruit (CONTROL) were randomly chosen and placed in 3 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 2278 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 2°C disinfestation treatment.				
LARGE SCALE TRIAL AT 2°C THOMPSON SEEDLESS REPLICATE 2 – Infested 08 June 2006				
Sample unit (190 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	19 June 2007	26 June 2006		
1	1700	0	1700	8.95
2	1024	0	1024	5.40
3	489	0	489	2.57

Table 41. Survival of insects in untreated (Control) fruit – Thompson Seedless Large Scale Trial @ 2°C Replicate 3

2987 Thompson Seedless were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 750 fruit (CONTROL) were randomly chosen and placed in 3 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 2237 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 2°C disinfestation treatment.				
LARGE SCALE TRIAL AT 2°C THOMPSON SEEDLESS REPLICATE 3 – Infested 15 March 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	27 march 2007	03 April 2007		
1	1927	0	1927	7.71
2	2177	0	2177	8.71
3	1396	3	1399	5.60

Table 42. Survival of insects in untreated (Control) fruit – Thompson Seedless Large Scale Trial @ 2°C Replicate 4

3125 Thompson Seedless were manually infested by puncturing the fruit and dipping in a slurry of QFF eggs. 1000 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 2125 fruit (TREATED) were packed into mesh lined trays and allowed to develop to 1 st instar larval stage for the 2°C disinfestation treatment.				
LARGE SCALE TRIAL AT 2°C THOMPSON SEEDLESS REPLICATE 4 – 22 MARCH 2007				
Sample unit (250 fruit/tray)	No. pupae (sieve 1)	No. pupae (sieve 2)	Total pupae	Mean pupae/fruit (rounded to 2 dec places)
Sieve date	03 April 2007	10 April 2007		
1	54	4	58	0.23
2	268	26	294	1.18
3	146	29	175	0.70
4	162	23	185	0.74

3.1.2 Data from the temperature measurements in the cold chamber and at the centre of the fruit during treatment

3.1.2.1 Queensland fruit fly - *Bactrocera tryoni*

The records of the temperatures from the cold treatment trials for each replicate treatment are summarised in the tables and figures that follow. The data show that the required temperatures of $1 \pm 0.5^{\circ}\text{C}$ were maintained throughout the trials. The data loggers were calibrated before and after each trial and the summary tables for calibration are also given. These show that the records of temperatures were accurate throughout the trials.

3.1.3 Calibration data of temperature recorder and sensors in each replicate

3.1.3.1 Queensland fruit fly - *Bactrocera tryoni*

The records of the temperatures from the data logger calibrations for each replicate treatment are summarised in the tables and figures that follow. The data show that the data logger temperature probes tested true throughout the trials.

Temperature 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:

Table 43. Calibration for data loggers for large scale cold disinfestation trial

LOGGER KV0529008								
DATE / TIME	CH 1	CH 2	CH 3	CH 4	CH 5	CH 6	CH 7	CH 8
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

Table 44. Calibration for data loggers for large scale cold disinfestation trial

LOGGER KV0624006								
DATE / TIME	CH 1	CH 2	CH 3	CH 4	CH 5	CH 6	CH 7	CH 8
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

Table 45. Calibration for data loggers for large scale cold disinfestation trial

LOGGER KV0624007								
DATE / TIME	CH 1	CH 2	CH 3	CH 4	CH 5	CH 6	CH 7	CH 8
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

Table 46. Calibration for data loggers for large scale cold disinfestation trial

LOGGER	CH 1	CH 2	CH 3	CH 4	CH 5	CH 6	CH 7	CH 8
KV0445002								
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 47. Data logger statistics for large scale trials at 3°C of table grapes (Red Globes) replicate 1.

Table Grapes Large scale trial at 3°C Replicate 1 (Red Globes)								
LOGGER KV0529008	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8
Total treatment time(hrs)	374.0	374.0	374.0	374.0	374.0	374.0	374.0	374.0
Average temp (°C)	3.2	3.2	3.3	3.2	3.3	3.2	3.2	3.4
no. readings above 3.5°C	3.0	12.0	0.0	0.0	41.0	40.0	0.0	6.0
no. readings below 2.5°C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total number of readings	749.0	749.0	749.0	749.0	749.0	749.0	749.0	749.0
Time > 3.5°C (hrs)	1.5	6.0	0.0	0.0	20.5	20.0	0.0	3.0
Time < 2.5°C (hours)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% time > 3.5°C	0.4	1.6	0.0	0.0	5.5	5.3	0.0	0.8
% time < 2.5°C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% time within 2.5°C - 3.5°C	99.6	98.4	100.0	100.0	94.5	94.7	100.0	99.2

Table 48. Data logger statistics for large scale trials at 3°C of table grapes (Crimson seedless) replicate 1.

Table Grapes Large scale trial at 3°C Replicate 1 (Crimson Seedless)								
LOGGER KV0624006	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8
Total treatment time(hrs)	357.5	357.5	357.5	357.5	357.5	357.5	357.5	357.5
Average temp (°C)	3.2	3.1	3.3	3.4	3.1	3.3	2.8	3.2
no. readings above 3.5°C	2.0	0.0	16.0	21.0	0.0	18.0	0.0	4.0
no. readings below 2.5°C	0.0	0.0	0.0	0.0	0.0	0.0	6.0	0.0
Total number of readings	715.0	715.0	715.0	715.0	715.0	715.0	715.0	715.0
Time > 3.5°C (hrs)	1.0	0.0	8.0	10.5	0.0	9.0	0.0	2.0
Time < 2.5°C (hours)	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0
% time > 3.5°C	0.3	0.0	2.2	2.9	0.0	2.5	0.0	0.6
% time < 2.5°C	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0
% time within 2.5°C - 3.5°C	99.7	100.0	97.8	97.1	100.0	97.5	99.2	99.4

Table 49. Data logger statistics for large scale trials at 3°C of table grapes (Thompson seedless) replicate 1.

Table Grapes Large scale trial at 3°C Replicate 1 (Thompson Seedless)								
LOGGER KV0624007	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8
Total treatment time(hrs)	362.0	362.0	362.0	362.0	362.0	362.0	362.0	362.0
Average temp (°C)	3.1	3.3	3.2	3.1	3.1	3.2	3.1	3.2
no. readings above 3.5°C	0	7	0	0	1	0	3	1
no. readings below 2.5°C	0	0	0	0	0	0	0	0
Total number of readings	725	725	725	725	725	725	725	725
Time > 3.5°C (hrs)	0	3.5	0	0	0.5	0	1.5	0.5
Time < 2.5°C (hours)	0	0	0	0	0	0	0	0
% time > 3.5°C	0.0	1.0	0.0	0.0	0.1	0.0	0.4	0.1
% time < 2.5°C	0	0	0	0	0	0	0	0
% time within 2.5°C - 3.5°C	100.0	99.0	100.0	100.0	99.9	100.0	99.6	99.9

Table 50. Data logger statistics for large scale trials at 3°C of table grapes (3 varieties)

Table Grapes Large scale trial at 3°C Replicate 2 (3 varieties)								
LOGGER # KV0624007	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8
Total treatment time(hrs)	358.5	358.5	358.5	358.5	358.5	358.5	358.5	358.5
Average temp (°C)	2.9	2.8	2.8	2.8	3.1	3.0	2.8	3.0
no. readings above 3.5°C	2	3	4	3	3	0	0	0
no. readings below 2.5°C	0	0	0	0	0	0	26	0
Total number of readings	718	718	718	718	718	718	718	718
Time > 3.5°C (hrs)	1	1.5	2	1.5	1.5	0	0	0
Time < 2.5°C (hours)	0	0	0	0	0	0	13	0
% time > 3.5°C	0.3	0.4	0.6	0.4	0.4	0.0	0.0	0.0
% time < 2.5°C	0.0	0.0	0.0	0.0	0.0	0.0	3.6	0.0
% time within 2.5°C - 3.5°C	99.7	99.6	99.4	99.6	99.6	100.0	96.4	100.0

Table 51. Data logger statistics for large scale trials at 3°C of table grapes (3 varieties)

Table Grapes Large scale trial at 3°C Replicate 3 (3 varieties)						
LOGGER # KV0624006	CH1	CH2	CH3	CH4	CH5	CH6
Total treatment time(hrs)	353.5	353.5	353.5	353.5	353.5	353.5
Average temp (°C)	3.2	3.1	3.3	3.2	3.2	3.1
no. readings above 3.5°C	2	2	2	3	3	0
no. readings below 2.5°C	0	0	0	0	0	0
Total number of readings	708	708	708	708	708	708
Time > 3.5°C (hrs)	1	1	1	1.5	1.5	0
Time < 2.5°C (hours)	0	0	0	0	0	0
% time > 3.5°C	0.3	0.3	0.3	0.4	0.4	0.0
% time < 2.5°C	0	0	0	0	0	0
% time within 2.5°C - 3.5°C	99.7	99.7	99.7	99.6	99.6	100.0

Table 52. Data logger statistics for large scale trials at 3°C of table grapes (3 varieties)

Table Grapes Large scale trial at 3°C Replicate 4 (3 varieties)								
LOGGER # KV0624007	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8
Total treatment time (hrs)	357.5	357.5	357.5	357.5	357.5	357.5	357.5	357.5
Average temp (°C)	2.9	2.8	2.8	2.8	3.1	3.0	2.8	3.0
no. readings above 3.5°C	0	2	3	3	2	0	0	0
no. readings below 2.5°C	0	0	0	0	0	0	26	0
Total number of readings	716	716	716	716	716	716	716	716
Time > 3.5°C (hrs)	0	1	1.5	1.5	1	0	0	0
Time < 2.5°C (hours)	0	0	0	0	0	0	13	0
% time > 3.5°C	0.0	0.3	0.4	0.4	0.3	0.0	0.0	0.0
% time < 2.5°C	0.0	0.0	0.0	0.0	0.0	0.0	3.6	0.0
% time within 2.5°C - 3.5°C	100.0	99.7	99.6	99.6	99.7	100.0	96.4	100.0

Table 53. Data logger statistics for large scale trials at 2°C of table grapes (3 varieties)

Table Grapes Large scale trial at 2°C Replicate 1 (3 varieties)								
LOGGER # KV0624007	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8
Total treatment time(hrs)	311.0	311.0	311.0	311.0	311.0	311.0	311.0	311.0
Average temp (°C)	2.7	2.7	2.8	1.9	2.7	2.1	1.9	2.1
no. readings above 2.5°C	623.0	599.0	623.0	33.0	623.0	2.0	2.0	0.0
no. readings below 1.5°C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total number of readings	623.0	623.0	623.0	623.0	623.0	623.0	623.0	623.0
Time > 2.5°C (hrs)	311.0	299.5	311.0	16.5	311.0	1.0	1.0	0.0
Time < 1.5°C (hours)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% time > 2.5°C	100.0	96.3	100.0	5.3	100.0	0.3	0.3	0.0
% time < 1.5°C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% time within 1.5°C - 2.5°C	0.0	3.7	0.0	94.7	0.0	99.7	99.7	100.0

Table 54. Data logger statistics for large scale trials at 2°C of table grapes (3 varieties)

Table Grapes Large scale trial at 2°C Replicate 2 (3 varieties)								
LOGGER # KV0529008	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8
Total treatment time(hrs)	348.5	348.5	348.5	348.5	348.5	348.5	348.5	348.5
Average temp (°C)	2.0	2.0	2.3	2.2	2.2	2.2	2.1	2.4
no. readings above 2.5°C	21.0	22.0	46.0	14.0	5.0	21.0	62.0	55.0
no. readings below 1.5°C	3.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Total number of readings	698.0	698.0	698.0	698.0	698.0	698.0	698.0	698.0
Time > 2.5°C (hrs)	10.5	11.0	23.0	7.0	2.5	10.5	31.0	27.5
Time < 1.5°C (hours)	1.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0
% time > 2.5°C	3.0	3.2	6.6	2.0	0.7	3.0	8.9	7.9
% time < 1.5°C	0.4	0.1	0.0	0.0	0.0	0.0	0.0	0.0
% time within 1.5°C - 2.5°C	96.6	96.7	93.4	98.0	99.3	97.0	91.1	92.1

Table 55. Data logger statistics for large scale trials at 2°C of table grapes (3 varieties)

Table Grapes Large scale trial at 2°C Replicate 3 (3 varieties)								
LOGGER # KV0445002	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8
Total treatment time(hrs)	352.5	352.5	352.5	352.5	352.5	352.5	352.5	352.5
Average temp (°C)	1.4	1.3	1.5	1.3	1.8	1.7	1.7	1.4
no. readings above 2.5°C	4.0	0.0	3.0	1.0	0.0	1.0	1.0	0.0
no. readings below 1.5°C	614.0	686.0	323.0	664.0	13.0	33.0	39.0	425.0
Total number of readings	706.0	706.0	706.0	706.0	706.0	706.0	706.0	706.0
Time > 2.5°C (hrs)	2.0	0.0	1.5	0.5	0.0	0.5	0.5	0.0
Time < 1.5°C (hours)	307.0	343.0	161.5	332.0	6.5	16.5	19.5	212.5
% time > 2.5°C	0.6	0.0	0.4	0.1	0.0	0.1	0.1	0.0
% time < 1.5°C	87.1	97.3	45.8	94.2	1.8	4.7	5.5	60.3
% time within 1.5°C - 2.5°C	12.3	2.7	53.8	5.7	98.2	95.2	94.3	39.7

Table 56. Data logger statistics for large scale trials at 2°C of table grapes (3 varieties)

Table Grapes Large scale trial at 2°C Replicate 4 (3 varieties)								
LOGGER # KV0624008	CH 1	CH 2	CH 3	CH 4	CH 5	CH 6	CH 7	CH 8
Total treatment time(hrs)	358.5	358.5	358.5	358.5	358.5	358.5	358.5	358.5
Average temp (°C)	1.6	1.9	2.2	1.9	1.9	1.9	1.9	2.0
no. readings above 2.5°C	0.0	5.0	3.0	3.0	0.0	3.0	0.0	2.0
no. readings below 1.5°C	41.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total number of readings	718.0	718.0	718.0	718.0	718.0	718.0	718.0	718.0
Time > 2.5°C (hrs)	0.0	2.5	1.5	1.5	0.0	1.5	0.0	1.0
Time < 1.5°C (hours)	20.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
time > 2.5°C	0.0	0.7	0.4	0.4	0.0	0.4	0.0	0.3
% time < 1.5°C	5.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% time within 1.5°C - 2.5°C	94.3	99.3	99.6	99.6	100.0	99.6	100.0	99.7

3.2 Fruit injury test:

No tests were done on assessing the tolerance of table grapes to storage at 1°C. Information on the tolerance of table grapes to cold storage is presented in the Appendix.

4 Other information

Pictures and figures of the cold treatment chamber, fruit holding container, stacking pattern, temperature recorder, etc will facilitate better understanding of the report.

4.1 Queensland fruit fly - *Bactrocera tryoni*

See figure section

5 References

Anon 1988: GENSTAT 5 : Reference Manual. Rothamstead Experimental Station. Oxford University Press. New York.

Finney, D. J. 1971 Probit Analysis, 3rd ed. Cambridge University Press, London.