

Heat disinfestation of capsicums for export to New Zealand and interstate

**Horticulture Australia Limited (HAL)
Project Number VG04006**

Final Report (October 2007)

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**Horticulture and Forestry Science
Department of Primary Industries and Fisheries**



Know-how for Horticulture™



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Funding Sources

Horticulture Australia Limited (HAL)
National Vegetable Levy
Queensland Government - Department of Primary Industries & Fisheries

Date of Report

December 2007

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1 MEDIA SUMMARY

Due to the presence of fruit flies in the main tropical and sub-tropical horticultural production areas of Australia, trade restrictions are in place both for interstate and overseas trade for many fruit fly host commodities. Postharvest treatments are often required in order to overcome these quarantine barriers.

One of the more economical quarantine treatments is the use of postharvest insecticides. These chemical dips and sprays are used to control fruit fly in a wide variety of commodities for interstate trade and to gain access to New Zealand markets. However, the use of these chemical treatments is currently under review by regulatory bodies, and their use may be severely restricted or lost in the near future. One alternative to chemical treatments is the use of postharvest heat treatments which have been developed for a range of commodities and pests worldwide. The main advantage of heat treatments is that they are residue free and, as such, can be used by both conventional and organic producers. One disadvantage is that not all crops tolerate the high temperatures required to provide effective control against insect infestation.

The aim of this project was to develop a non-chemical heat disinfestation treatment for capsicums against fruit flies. Experimental methodology was based on that required by New Zealand Ministry of Agriculture and Forestry. Physiological research to determine the effects of heat treatment on capsicum fruit quality was also undertaken.

Research was undertaken on standard heat treatments, combined heat plus cold treatments and heat with low oxygen treatments against fruit fly in capsicums. Unfortunately, predicted doses that would be efficacious against Australian species of fruit fly caused unacceptable levels of fruit damage.

Further research is required to investigate treatments to control fruit fly in capsicums. One option may be the use of irradiation.

2 TECHNICAL SUMMARY

Many horticultural products are hosts for fruit flies (family Tephritidae), which are often considered high-risk quarantine pests by regulatory authorities. The presence of fruit flies in the main tropical and sub-tropical production areas of Australia, including Queensland, results in the imposition of quarantine barriers for many fruit fly host commodities, including capsicums (*Capsicum annuum*). These quarantine barriers greatly impede trade both within Australia and to overseas markets that are free of these pests. Postharvest disinfestation treatments are often required in order to overcome these quarantine barriers.

One of the more economical quarantine treatments is the use of postharvest insecticides. These chemical dips and sprays are used to control fruit fly in a wide variety of commodities for interstate trade and to gain access to New Zealand markets. However, the use of these chemical treatments is currently under review by the Australian Pesticides and Veterinary Medicine Authority (APVMA) and their use may be severely restricted or lost in the near future. An alternative non-chemical postharvest treatment will be required to maintain both domestic and New Zealand market access for capsicums if chemical treatments are restricted or lost. One alternative may be the use of postharvest heat treatments. Heat treatments have previously been developed for capsicum by American and Japanese researchers.

The aim of this project was to develop a non-chemical heat disinfestation treatment for capsicums against Australian fruit fly species. Experimental methodology was based on that required by New Zealand Ministry of Agriculture and Forestry. Physiological research to determine the effects of heat treatment on capsicum fruit quality was also undertaken.

The initial focus of this project was the use of vapour heat treatment. However, predicted doses that would be efficacious against fruit fly caused unacceptable levels of fruit damage. In an attempt to improve the efficacy of the treatment and maintain fruit quality a combination treatment using vapour heat treatment and cold treatment was investigated. The use of this combined treatment did not achieve the required insect mortality and also resulted in unacceptable fruit damage. A third treatment option using vapour heat treatment with low oxygen was examined. Again, predicted doses that would be efficacious against fruit fly caused unacceptable levels of fruit damage. Fruit injury recorded with the use of low oxygen include skin pitting, severe fruit softening and diffuse grey discolouration of the skin. An atypical slightly off smelling odour was evident from fruit treated under low oxygen.

Further research is required to investigate treatments to control fruit fly in capsicums. One option may be the use of irradiation.

3 INTRODUCTION

One of the more economical quarantine treatments for fruit fly is the use of postharvest insecticide dips or sprays. These chemical dips and sprays are used to control fruit fly in a wide variety of commodities for interstate trade and to gain access to New Zealand markets. However, the use of these chemical treatments is currently under review by the Australian Pesticides and Veterinary Medicine Authority (APVMA) and their use may be severely restricted or lost in the near future. If alternative treatments are not in place, exports of fruit fly host commodities to New Zealand may be severely constrained. Additionally, there is currently a strong consumer preference for products which receive minimal or no treatment with insecticides. The development of non-chemical postharvest treatments would also reduce the use of chemicals in the production process, improved health and safety for workers in packing sheds and lower chemical residues in product reaching the consumer. It is therefore necessary to develop effective, preferably non-chemical, alternative quarantine treatments.

One alternative to postharvest chemical treatments is the use of heat treatments. The main advantage of heat treatments is that they are residue free and, as such, can be used by both conventional and organic producers. The main disadvantage with heat treatments is that they may cause physiological damage to some commodities.

Successful heat treatments have previously been developed for capsicum. The United States Department of Agriculture Plant Protection and Quarantine Treatment Manual (APHIS 2002-2006), lists an approved heat treatment for bell pepper (*Capsicum annuum*) capsicum. This treatment is for importation of bell pepper from areas with Mediterranean fruit fly (*Ceratitis capitata*), Oriental fruit fly (*Bactrocera dorsalis*) and Melon fly (*B. cucurbitae*). The treatment involves heating the core temperature of the fruit to 112°F (44.4°C) for 8.75 hours, and then immediately cooling the fruit. An important note in the APHIS Treatment Schedule for bell pepper states “Commodities should be exposed at 112°F to determine tolerance to the treatment before commercial shipments are attempted”.

Japanese researchers have also developed a treatment against Oriental fruit fly in capsicum (Sugimoto et al. 1983). Sugimoto investigated a range of temperatures and found that the only efficacious non-damaging treatment was 43.4±0.4°C for 3 hours. Raising the air temperature or extending holding periods resulted in injuries such as skin pitting and skin malformation. Sugimoto noted that there was a large variation between the treatment developed in Japan and the APHIS treatment. Possible suggestions for this difference included differences in fruit cultivars, growing conditions, harvest season, fruit fly species and geographical distribution.

The aim of this project was to develop a heat disinfestation treatment for capsicums (*Capsicum annuum*) (Family: Solanaceae) against fruit flies. Experimental methodology was based on the requirements established in the New Zealand Ministry of Agriculture and Forestry (NZ MAF) Standard 155.02.03 –“*Specification for the Determination of Fruit Fly Disinfestation Treatment Efficacy*” (Anon 2001). Physiological research to determine the effects of heat treatment on capsicum fruit quality was also undertaken.

4 REQUIREMENTS FOR NEW ZEALAND MARKET ACCESS

One of the first requirements in the NZ MAF Standard is to develop a list of fruit flies which have been recorded as attacking the commodity. In the case of capsicums the following fruit fly species are all recorded infesting capsicums in field situations in Australia (Hancock et al. 2000):

Ceratitis capitata (Wiedemann) (Diptera: Tephritidae)
Bactrocera jarvisi (Tryon) (Diptera: Tephritidae)
Bactrocera tryoni (Froggatt) (Diptera: Tephritidae)
Bactrocera neohumeralis (Hardy) (Diptera: Tephritidae)
Bactrocera cucumis (French) (Diptera: Tephritidae)
Bactrocera bryoniae (Tryon) (Diptera: Tephritidae)
Bactrocera cacuminata (Hering) (Diptera: Tephritidae)
Dirioxa pornia (Walker) (Diptera: Trypetinae)

The second requirement in the New Zealand Standard is to determine which of the above species are considered quarantine pests and subject to phytosanitary restrictions. *Bactrocera bryoniae* and *B. cacuminata* records for capsicum are listed in the Hancock publication as “probable error” and “confirmation required” respectively and are not considered to be potential quarantine pests. *Dirioxa pornia* has only been recorded as infesting ripe or damaged capsicums and is also not considered to be a potential quarantine pest by New Zealand.

The third requirement is to undertake comparative testing of all immature stages of fruit fly species of quarantine importance. These experiments are *in vitro* tests that expose eggs and larvae to a range of doses in hot water. This research was completed in a previous Horticulture Australia project HG96019 (Corcoran et al. 2003). Mature eggs (60% development), first instars and third instars of *B. jarvisi* were significantly more tolerant to hot water immersion (46°C) than any other stage of any other species tested (*Ceratitis capitata*, *Bactrocera tryoni*, *B. neohumeralis* and *B. cucumis*). As such, any heat treatment for capsicums to New Zealand needs to be developed against *B. jarvisi*.

The fourth stage of testing is to determine the most tolerant lifestage in the commodity. *In vivo* dose mortality trials are undertaken against the two most tolerant lifestages identified from *in vitro* testing.

The final stage of testing requires large scale trials to be undertaken against the most tolerant stage of the most tolerant species.

5 MATERIALS AND METHODS

To further validate the results from previous *in vitro* studies on heat tolerance and to enable our research to have a broader application, trials on all in-fruit lifestages of *B. jarvisi* (mature eggs, first instars, second instars and third instars) were undertaken. The most tolerant stage (*in vivo*) was then subjected to a range of doses to predict an effective treatment dose in the commodity.

Although not a technical requirement of the New Zealand Standard, research evaluating the most appropriate method of applying heat to minimise physiological damage to fruit was also undertaken.

5.1 Equipment

5.1.1 Vapour Heat Treatment System

Trials were conducted in Sanshu vapour heat treatment systems (EHK-1000-B and EHK-1000-D Models) produced by Sanshu Sangyo, Kagoshima, Japan. Specifications of the vapour heat treatment system are listed below:

- METHOD: Direct control of humidity
 - Forced air circulation system
 - PERFORMANCE CAPABILITY*:
 - Temperature range : 10 ~ 60°C
 - Humidity range : 50 ~ 95% RH
 - Temperature range of errors : $\pm 0.1^\circ\text{C}$
 - Temperature distribution : $\pm 0.4^\circ\text{C}$
 - Humidity range of errors : ± 0.1 deg
 - Humidity distribution : $\pm 3\%$ RH
 - Duration of temperature rise : 30 - 60°C within 30 minutes (ambient temp: 20°C)
- *The above mentioned performance capability occurs only when the chamber is empty, and 92% RH.

Fruit and chamber temperatures were monitored using platinum resistance probes calibrated to $\pm 0.1^\circ\text{C}$. Fruit probes were inserted into the fruit stem with the tip of the probe located in the core of the seed mass.

5.1.2 Low Oxygen Heat Treatment Unit

Trials were conducted in an experimental prototype treatment unit developed by DPI&F staff (Jordan unpublished). The system consists of the chamber with its mechanical and electrical fittings, and the measurement and control system. The chamber, of external dimensions 2m X 2m X 1m, was fabricated using 75mm insulated panels of the type used in the fabrication of commercial cool rooms. This consists of expanded polystyrene foam between painted steel sheets on each side. Two front doors provide access to the product treatment space.

A backward curve open scroll centrifugal fan circulates air through the chamber at a nominal 2m/sec velocity through the product crates. The fan is driven by an externally mounted three phase motor attached to the fan wheel by a shaft entering the chamber through a Teflon gas tight seal. The air from the fan passes into a plenum at the top of the chamber, through a perforated plate, and vertically through the treatment chamber. Fruit to be treated is placed in plastic crates or stainless steel trays depending on the volume of fruit to be treated. Crates are constructed from rigid plastic with external dimensions 580mm x 385mm x 166mm and stacked in two columns to a maximum of six crates high. Stainless steel trays with external dimensions 500mm x 305mm x 70mm slide into two stainless steel cabinets (with four drawers each) with a front opening door.

Air heating is performed by 2.2 KW W-shape finned heater mounted upstream of the fan, and controlled by an electronic proportional controller.

Temperatures are measured using Class A thin film 100 ohm RTDs (2.2mm \times 2.3mm element) in a 3 mm diameter \times 150mm long sheath. Initial accuracy is $\pm 0.15^\circ\text{C}$ and after calibration in water against a NATA certified standard thermometer, accuracy is better than $\pm 0.1^\circ\text{C}$. Humidity is measured using RTD wet and dry bulb sensors placed in the downstream air above the product crates or trays.

The system is fully automatic with the software for measurement and control developed by DPI&F on a Citect® SCADA development system platform. The system controls and measures temperature, relative humidity and treatment times, as well as printing and logging results.

Gaseous nitrogen for the system is provided from a commercial pressurised liquid container (PLC) containing 540L of liquid nitrogen. The liquid nitrogen after passing through a vaporiser coil, is delivered as gaseous nitrogen at ambient temperature, at a controlled flow rate of up to 200L min⁻¹. The nitrogen supply is delivered to the treatment chamber at two points at each end of the chamber. Concentration of oxygen in the chamber was measured at two points in the chamber and remote from the nitrogen entry points.

Samples for determination of oxygen concentration were removed by a sampling pump connected to solenoid valve controlled sampling tubes. The pump and solenoid valves were activated by the control system with the valve being opened sequentially to enable analysis samples to be withdrawn. Chamber atmosphere was sampled at three minute intervals from effluent gas flow using a GE Panametrics Thermoparamagnetic model XMO2 oxygen analyser. The oxygen analyser was calibrated at 0% oxygen using nitrogen. Span calibration was performed against air.

When undertaking trials the following procedure was followed. Fruit was loaded into the chamber and the treatment unit sealed. To reduce oxygen levels the treatment unit was flushed with nitrogen (≥ 150 L/min) until the chamber oxygen concentration was reduced to 0.5%. Reducing oxygen levels in this manner is referred to in the remainder of the report as a “flushdown” period. Unless otherwise stated the flushdown period used in this project was approximately 30 minutes.

After the flushdown, a 30 minute holding period at ambient temperature was initiated. The aim of the holding period is to allow the oxygen levels in the fruit to reach equilibrium with chamber oxygen levels. Previous trials have shown that the holding period can increase efficacy of treatments (Leach unpublished). To maintain an oxygen level of 0.5% the flow rate of nitrogen into the chamber was continuously adjusted and typically ranged between 80 to 50 L/min during the holding period.

Following the holding period, the heat treatment was initiated. Heating rates and humidity levels in the treatment chamber are described for each trial (e.g. 25°C to 44°C in 1 hour, 95% RH). To maintain an oxygen level of 0.5% the flow rate of nitrogen into the chamber was continuously adjusted and typically ranged between 50 to 30 L/min during the heat treatment.

5.2 Efficacy Trials

5.2.1 Fruit Fly Colonies

Laboratory colonies of *B. jarvisi* were established and maintained at the Department of Primary Industries and Fisheries laboratories in Cairns and Indooroopilly, Queensland.

Fruit fly adults were held in 650mm × 650mm × 650mm aluminium framed cages covered on the sides and top with nylon mesh (2mm aperture) with approximately 15 000 flies per cage. Flies were held at 26 ± 2°C and 70 or 75 ± 5% RH with natural daylight supplemented with fluorescent lighting. Adult flies were provided water, sugar, and autolyzed brewers yeast from emergence.

Bactrocera jarvisi were cultured using a carrot-based semi-artificial diet using the method described by Heather and Corcoran (1985), except that eggs were collected from adults using a plastic pin-holed collection cup, rather than a hollowed apple as the oviposition receptacle. The collection cup was coated internally and externally with orange juice before being placed into the adult cage. The collected eggs were then suspended in an agar solution.

5.2.2 Fruit

Organic green capsicums were used in the entomology trials. Varieties included Bombardier, Fortress and Helix.

5.2.3 Fruit Holding

Control fruit were held under standard conditions of temperature and humidity (approximately 26-27°C and 70-75%RH) while test fruit were being treated. After treatment, control fruit and treated fruit were placed on gauze topped plastic containers to allow surviving insects to develop and to allow excess liquid from fruit breakdown to drain away. The containers were held in larger crispers or cages containing sawdust as a pupation medium. Control and treated fruit were held under standard conditions of temperature and humidity and surviving pupae were collected.

5.2.4 Most Tolerant Stage Testing (*in vivo*)

5.2.4.1 Artificial infesting methods

Most tolerant stage trials were performed by artificially infesting the test fruit and allowing the insects to develop under controlled conditions. Mature eggs of *B. jarvisi* were treated at 22-24 hours old, first instars at 50 hours, second instars at four days and third instars at six days in capsicums.

To infest the capsicums a square wedge of flesh was removed from the side of the fruit. This allowed the immature stages to be placed in the central cavity of the fruit. After infestation the wedge of flesh was put back in place and sealed with wax and waterproof tape.

While artificial infesting provides a known number of insects at the correct lifestage it is labour intensive and time consuming. As such, it is not possible to conduct all the infestation on the day of treatment. Infestation for the larval stages was conducted approximately 24 prior to treatment while mature eggs were added on the day of treatment.

For mature eggs and first instars (added as eggs 24 hours prior to treatment) collections of eggs were made by placing plastic egg cups smeared with orange juice into cage's holding gravid females for approximately 1 hour. Eggs were then washed out using tap water and collected under mild suction by filtration through a 9cm Buchner funnel containing black filter paper or by being suspended in an agar solution and pipetted onto black filter paper. The filter papers carrying the eggs were then placed on cellulose sponge saturated with water. The filter paper was cut into pieces, each containing the required number of eggs. To infest the fruit, eggs were placed in the central cavity of the fruit with the filter paper touching part of the fruit to prevent desiccation.

For second and third instar infestations, larvae were collected from carrot media that had been infested with eggs approximately three and five days earlier. Larvae from the media were placed into water and counted under a dissection microscope. Once counted the larvae were drained of water using 1 ply tissue. The tissue was then inverted so that the larvae were visible and then placed in the central cavity of the fruit. As stated previously, the infestation of second and third instar larvae was conducted approximately 24 hours prior to treatment. By holding the fruit for 24 hours the larvae developed in the fruit to second and third instars at the time of treatment.

5.2.4.2 Most tolerant stage testing

Three replicates treating all life stages of *B. jarvisi* in capsicums were performed to determine the most tolerant stage to heat. One hundred second and third instars were counted and placed separately into single test fruit, with 400 insects (4 fruit) treated at each dose in each replicate. Two hundred eggs were counted and placed into test fruit to treat eggs and first instars, with 800 insects (4 fruit) treated at each dose in each replicate. Higher numbers of eggs and first instars were used to account for the fact that egg hatch may be less than 100%. Fruit infested with all stages of *B. jarvisi* were treated simultaneously in the vapour heat treatment system.

The treatment chamber was programmed to ramp from 30°C to 46°C over 1 hour, with relative humidity set above 90% for the duration of the treatment. Test fruit for a given dose containing each life stage, were removed from the chamber after being treated for 20, 40, 60, 80, 100 and 120 minutes once their core temperatures had reached 45°C in replicate 1; and removed from the chamber after being treated for 40, 60, 80, 100, 120 and 150 minutes once their core temperatures had reached 45°C in replicate 2 and 3. Fruit were forced air cooled to a core temperature of 30°C in a cold room set at 20°C immediately after being removed from the vapour heat treatment system.

In later trials the effect of vapour heat treatment with low oxygen treatment was investigated. To confirm that the most tolerant stage to combined heat and low oxygen was not different to the most tolerant stage to heat, two trials treating all in-fruit immature stages were performed. Fruit were artificially infested as above and treated simultaneously in the vapour heat treatment system with low oxygen. In the first trial the chamber of the vapour heat treatment system with low oxygen was programmed as follows; hold at ambient temperature over 1 hour with nitrogen introduced to flush chamber to ~0.5% oxygen (O₂) and hold with relative humidity set at 92%; ramp from 30°C to 46°C over 1 hour with relative humidity set at 92%.

In the second trial the chamber was programmed as follows; ramp from 26°C to 27°C over 1 hour with nitrogen introduced to flush chamber to ~0.5% (O₂) and hold with relative humidity set at 92%; ramp from 27°C to 46°C over 30 minutes with relative humidity set at 92%. In both trials test fruit for a given dose containing each life stage, were removed from the chamber once their core temperatures reached 40, 41, 42, 43, 44 and 45°C. Once the chamber had been opened to remove the 40°C sample the oxygen levels were returned to normal in the chamber and nitrogen was not reintroduced. Fruit were forced air cooled to 30°C in a cold room set at 20°C immediately after being removed from the treatment chamber.

In all the above five trials, additional fruit were infested with each immature larval stage and were sampled at the time of treatment to confirm that the correct larval stage was being treated.

5.2.5 Preliminary Trials

Preliminary trials were undertaken using cage infested fruit and testing the most tolerant life stage of *B. jarvisi* as determined above (shown to be mature eggs - Results 6.1.1)

5.2.5.1 Cage infesting of fruit

Cage infesting of fruit involved damaging the fruit (30 pinholes per fruit) to assist in obtaining an increased and even distribution of insects within each fruit and a more uniform infestation level across all fruit. Damaged capsicums were placed in cages of laboratory cultured flies containing approximately 15 000 adults (1:1 sex ratio) and allowing the females flies to infest the fruit for 30 min to 90 min depending on the observed interest of flies in oviposition. Fruit were held under standard

conditions of temperature and humidity (approximately 26-27°C and 70-75%RH) before treatment for insects to develop to the required lifestage.

Samples from each cage of infested fruit were kept as control fruit. The estimated number of treated insects was calculated by the following formula: (Number of treated fruit/ Number of control fruit) x the number of pupae recovered from control fruit.

5.2.5.2 Vapour heat treatment

Five trials tested the effects of vapour heat against *B. jarvisi* mature eggs in capsicums. These trials were based on the parameters in the United States Department of Agriculture Plant Protection and Quarantine Treatment Manual (APHIS 2002-2006) which lists an approved vapour heat treatment for bell pepper. This treatment holds the fruit temperature at 112°F (44.4°C) for 8.75 hours, and then fruit must be cooled immediately. In the vapour heat trials conducted against *B. jarvisi* temperatures of 44°C and 45°C were used but for shorter time periods than the APHIS schedule due to concerns about fruit quality. A summary of the five trials is outlined in Table 1.

Table 1. Summary of vapour heat treatment trials.

| Trial number | Vapour heat system chamber program | Dose (Fruit core temperature / holding time) | Cooling method (immediately after removal from chamber) |
|---------------------|--|---|---|
| 1 | Ramp from 30°C to 46°C over 1 hour Relative humidity set at >95% | 45°C / 1 hour 45°C / 1.5 hours 45°C / 2 hours 45°C / 2.5 hours | Forced air cooled to 30°C core temperature in a 20°C cold room |
| 2 | Ramp from 30°C to 45°C* over 1 hour Relative humidity set at >95% | 44°C / 3 hours 44°C / 3.5 hours 44°C / 4 hours | Forced air cooled to 30°C core temperature in a 20°C cold room |
| 3 | Ramp from 30°C to 43°C over 1 hour with relative humidity set at 50% Ramp from 43°C to 46°C over 30 minutes with relative humidity set at 50% Hold at 46°C* and increase relative humidity to 92% | 45°C / 1 hour 45°C / 1.5 hours 45°C / 2 hours 45°C / 2.5 hours | Forced air cooled to 30°C core temperature in a 20°C cold room |
| 4 | Ramp from 30°C to 43°C over 20 minutes with relative humidity set at 50% Ramp from 43°C to 45.5°C over 10 minutes with relative humidity set at 50% Hold at 45.5°C and increase relative humidity to 92% | 45°C / 1 hour 45°C / 1.5 hours 45°C / 2 hours 45°C / 2.5 hours | Forced air cooled to 30°C core temperature in a 20°C cold room |
| 5 | Ramp from 30°C to 43°C over 30 minutes with relative humidity set at 50% Ramp from 43°C to 46°C over 20 minutes with relative humidity set at 50% Hold at 46°C* and increase relative humidity to 92% | 45°C / 3 hours | <i>Treatment 1.</i> Forced air cooled to 30°C core temperature in a 20°C cold room <i>Treatment 2.</i> Forced air cooled to 8°C core temperature in a 7°C cold room <i>Treatment 3.</i> Shower cooled to 30°C core temperature using shower in treatment chamber |

*When probed fruit had reached the required core temperature the chamber temperature was dropped by 0.5°C (by 0.1°C in intervals of 5 minutes).

Based on the results of the above entomology trials and associated fruit quality studies (Section 6.2.1.1), further trials were conducted testing a lower temperature vapour heat treatment combined with a cold treatment.

5.2.5.3 Vapour heat treatment plus cold treatment

Three trials tested the effects of vapour heat treatment plus cold treatment against *B. jarvisi* mature eggs in capsicums. The trial parameters for heat treatment were based on Japanese research which developed a successful heat treatment against Oriental fruit fly in green pepper (Sugimoto et al. 1983). This treatment holds the fruit core temperature at 43°C for 3 hours. In the following trials we tested 43°C core temperature for 3 hours combined with various cold treatments with the aim of increasing insect mortality. A summary of the three trials is outlined in Table 2.

Table 2. Summary of vapour heat plus cold treatment trials.

| Trial number | Vapour heat system chamber program | Dose (Fruit core temperature / holding time) | Cooling method and cold treatment |
|--------------|--|--|--|
| 1 | Ramp from 30°C to 44°C* over 20 minutes Relative humidity set at >95% | 43°C / 3 hours | Forced air cooled to 7°C core temperature in a 6°C cold room. When core temperature of fruit had reached 7°C the cold room set point was increased to 6.5°C. <i>Treatment 1.</i> Fruit removed from cold room when core temperature had reached 7°C <i>Treatment 2.</i> Fruit removed from cold room 1 day after core temperature had reached 7°C. <i>Treatment 3.</i> Fruit removed from cold room 2 days after core temperature had reached 7°C. |
| 2 | Ramp from 30°C to 44°C* over 20 minutes** Relative humidity set at ~90% | 43°C / 3 hours | Forced air cooled to 3°C core temperature in a 2°C cold room. When core temperature of fruit had reached 3°C the cold room set point was increased to 2.5°C. <i>Treatment 1.</i> Fruit removed from cold room when core temperature had reached 3°C <i>Treatment 2.</i> Fruit removed from cold room 1 day after core temperature had reached 3°C. <i>Treatment 3.</i> Fruit removed from cold room 2 days after core temperature had reached 3°C. |
| 3 | Ramp from 30°C to 43.5°C over 20 minutes Relative humidity set at 90% | 43°C / 3 hours | <i>Treatment 1.</i> No forced air cooling The following samples were forced air cooled to 3°C core temperature in a 2°C cold room. When core temperature of fruit had reached 3°C the cold room set point was increased to 2.5°C. <i>Treatment 2.</i> Fruit removed from cold room when core temperature had reached 3°C. <i>Treatment 3.</i> Fruit removed from cold room 1 day after core temperature had reached 3°C. <i>Treatment 4.</i> Fruit removed from cold room 2 days after core temperature had reached 3°C. |

* When probed fruit had reached the required core temperature the chamber temperature was dropped by 0.5°C (by 0.1°C in intervals of 5 minutes).

Based on the results of the above entomology trials and associated fruit quality studies (Section 6.2.1.2), further trials were conducted testing vapour heat treatment with low oxygen.

5.2.5.4 Vapour heat treatment with low oxygen treatment

Two trials tested the effects of low oxygen vapour heat treatment against *B. jarvisi* mature eggs (~22 hours) in capsicums. A summary of the two trials is outlined in Table 3.

Table 3. Summary of vapour heat with low oxygen treatment.

| Trial number | Low oxygen heat treatment unit chamber program | Dose (Fruit core temperature / holding time) | Cooling method (immediately after removal from chamber) |
|---------------------|---|---|---|
| 1 | Following the flushdown and holding period the temperature was ramped from 27°C to 45°C over 30 minutes while maintaining low oxygen levels Relative humidity set at 92% | 44°C / 0 minutes | Forced air cooled to 8°C core temperature in a 7°C cold room. |
| 2 | Treatment 1: vapour heat only Ramp from 25°C to 26°C over 1 minute Ramp from 26°C to 27°C over 1 hour Ramp from 27°C to 41°C over 30 minutes Relative humidity set at 92% Treatment 2: Following the flushdown and holding period the temperature was ramped from 27°C to 41°C over 30 minutes while maintaining low oxygen levels Relative humidity set at 92% | 40°C / 4 hours | Forced air cooled to 30°C core temperature in a 20°C cold room. |

5.2.6 Data Analysis

In the initial experiments that were conducted to compare the heat tolerance of immature stages, insect mortality for each of several treatment doses was determined. In such experiments, the resulting percentage mortality typically follows a sigmoid curve increasing from zero mortality at low doses to 100% mortality at high doses. In fitting a dose-response model to this data, it is necessary to determine a linearising transformation (f) which will give an equation of the form

$$Y = f(p) = a + b X \text{ where } p \text{ is the proportion mortality and } X \text{ is the dose}$$

Since it is not possible to determine the correct tolerance distribution (and hence linearising transformation) prior to analysis, the data, corrected for control mortality, were fitted to six dose-response models: probit, logit, complementary log-log, each with and without log transformation of the explanatory variable (temperature or time) using the computer program GenStat 9 (GenStat 2006). These models are regularly used to linearise and interpret dose-response data (Chew 1994; Robertson et al. 1994; Throne et al. 1995).

- probit - this transformation is based on the proportions of the normal curve and if the distribution of tolerances is normal the probit transformed response will be linearly related

to the dose stimulus. The probit transformation of the mortality proportion (p) cannot be expressed as a simple mathematical relationship, only as the indefinite integral:

$$p = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{z_p} e^{-\frac{1}{2}u^2} du = \Phi(z_p), \text{ and symbolically, } z_p = \Phi^{-1}(p)$$

where $\Phi(z)$ is the cumulative probability of the standard normal distribution (mean zero and standard deviation one) and z_p is the probit transform of p.

- logit - this transformation is appropriate where the distribution of tolerances follow the logistic distribution; the linearising transformation for the mortality proportion, (p) is:

$$L(p) = \text{logit}(p) = \ln [p/(1-p)]$$

- complementary log-log (CLL) is appropriate if the distribution of tolerances follow an extreme value distribution; the linearising transformation for the mortality proportion, (p) is:

$$\text{CLL}(p) = \ln [-\ln(1-p)]$$

Linearization of these tolerance distributions may be improved by logarithmic transformation of the doses as, for example, in a probit transformation when the tolerance distribution is log-normal (Finney 1971).

The goodness of fit of the data to each of these models was determined by examination of the fitted curve, the residual deviance and the width of the LD₉₉ fiducial limits. Since the main area of interest lies in the fit of the upper portion of the curve, discrimination between models was done using goodness of fit statistics (such as residual deviance) and width of the fiducial limits at LD₉₉ to supplement visual examination.

5.3 Fruit Quality Trials

Quality assessments were carried out by experienced assessors using quality characteristics most likely to be affected by the treatments and which are critical for product acceptability.

- (i) External appearance. A visual measure of general acceptability (GA) was made using the hedonic scale (1=dislike extremely, 9=like extremely).
- (ii) External injury. Skin pitting lesions were rated for size using the 0-5 scale (0=nil, 1=to 1mm ϕ ; 2=to 2mm ϕ ; 3=to 3mm ϕ ; 4=to 4mm ϕ ; 5= > 4mm ϕ). External injury coverage was calculated as the percentage of skin affected by pitting using the 0-5 scale (0=nil, 1=to 5%; 2=to 25%; 3=to 50%; 4=to 75%; 5= to 100%).
- (iii) Firmness. Fruit firmness was determined as the ability of the flesh to yield to hand pressure using the 0-5 scale (0=hard with no give; 1=firm and springs back with moderate hand pressure; 2=rubbery; 3=beginning to soften and deforms to moderate hand pressure; 4=soft and deforms to slight hand pressure; 5=very soft and almost liquid to touch).
- (iv) Internal injury. Fruit were sliced into halves from stem to blossom end rated for any visible internal injuries. Internal appearance of the fruit halves by examination of the seed space and surrounding tissues was made using the 1-9 scale (1=dislike extremely, 9=like extremely).

5.3.1.1 Fruit

Unless otherwise stated, medium sized green capsicums were sourced from two commercial growers in the Burdekin region and one commercial grower on the Atherton Tablelands. Fruit were harvested by hand in the morning and packed into cartons without undergoing postharvest treatments. Cartons remained in the packing shed at ambient conditions before despatch by road transport to Cairns. Fruit were treated within 24 hours of harvest.

It was also notable from trials on green capsicums that fruit showing advancement in colour past breaker stage suffered higher levels of flesh injury than fruit without any stage of colour development. Where possible fruit with advancement in colour were removed during sorting and not used in trials.

5.3.1.2 Vapour heat treatment

Four trials testing the effects of vapour heat on fruit quality of capsicums were undertaken and are summarised in Table 4.

Table 4. Summary of VHT temperature conditions, treatment dose, cooling method and storage of heat treated green capsicums.

| Trial Number | Vapour heat system chamber program | Dose (Fruit core temperature / holding time) | Cooling Method | Storage |
|---------------------|--|---|---|---|
| 1 | Ramp from 30°C to 46°C over 1 hour. Hold at 46°C. Relative humidity set at 95% | 45°C for 3 hours | Forced air cooled to 30°C core temperature in a 20°C cold room | 7°C for 5 days / 95% relative humidity |
| 2 | Ramp from 30°C to 45°C over 1 hour. Hold at 45°C. Relative humidity set at 95% | 44°C for 4 hours | Forced air cooled to 30°C core temperature in a 20°C cold room | 7°C for 5 days / 95% relative humidity |
| 3 | Ramp from 30°C to 44°C over 1 hour. Hold at 44°C. Chamber temperature decreased by 0.5°C at fruit core temperature of 43°C. Relative humidity set at 95% | 43°C for 3 hours | Forced air cooled to 30°C core temperature in a 20°C cold room. | 7°C for 5 days / 95% relative humidity |
| 4 | Ramped from 30°C to 44°C over 20 minutes. Hold at 44°C. Relative humidity set at 95%. | 43°C for 3 hours | <p>Treatment 1. Forced air cooled to 30°C core temperature in a 20°C cold room.</p> <p>Treatment 2. Forced air cooled to 30°C core temperature in a 20°C cold room.</p> <p>Treatment 3. Water cooled to 30°C core temperature.</p> <p>Treatment 4. Water cooled to 30°C core temperature.</p> | <p>Treatment 1. 7°C for 5 days 95% relative humidity</p> <p>Treatment 2. 20°C for 12 hours + 7°C for 4.5 days.</p> <p>Treatment 3. 7°C for 5 days 95% relative humidity</p> <p>Treatment 4. 20°C for 12 hours + 7°C for 4.5 days.</p> |

5.3.1.3 Vapour heat treatment plus cold treatment

Three trials testing the effects of vapour heat and cold treatment on fruit quality of capsicums were undertaken and are summarised in Table 5.

Table 5. VHT temperature conditions, treatment dose, revised cooling method and storage of heat treated green capsicums.

| Trial Number | Vapour heat system chamber program | Dose (Fruit core temperature / holding time) | Cooling Method | Storage |
|---------------------|--|---|---|---|
| 5 | Ramp from 30°C to 44°C over 1 hour. Hold at 44°C. Relative humidity set at 95%. | 43°C for 3 hours 43°C for 4 hours | Forced air cooled to 8°C core temperature in a 7°C cold room | 7°C for 5 days Relative humidity 95% |
| 6 | Ramp from 30°C to 44°C over 30 minutes. Relative humidity set at 90%. Chamber temperature decreased by 0.5°C when fruit core temperature of 43°C reached. Increased back to 44°C after 20 minutes when fruit core temperatures started to drop back to 43°C. | 43°C for 3 hours 43°C for 4 hours | Forced air cooled to 8°C core temperature in a 7°C cold room | 3°C for 2 days, then 7°C for 3 days. Relative humidity 95% |
| 7 | Ramp from 30°C to 44°C over 30 minutes with relative humidity set at 50%. Chamber temperature decreased by 0.5°C when fruit core temperature of 43°C. Relative humidity set at 90%. | 43°C for 4 hours | Forced air cooled to 4°C core temperature in a 3°C cold room. | 3°C for 2 days, then 7°C for 3 days. Relative humidity 95% |

5.3.1.4 Low Oxygen Heat Treatment

Five trials testing the effects of low oxygen heat treatment on fruit quality of capsicums were undertaken and are summarised in Table 6.

Table 6. Low oxygen + VHT temperature conditions, treatment dose, cooling method and storage of heat treated green capsicums.

| Trial Number | Vapour heat system chamber program | Dose (Fruit core temperature / holding time) | Cooling Method | Storage |
|---------------------|--|---|---|--|
| 8 | Flushdown in 1 hour. 1 hour holding period at ambient temperature. Ramp from 25°C to 44°C over 1 hour. Hold at 44°C. Relative humidity set at 90%. | 43°C for 1 hour | Forced air cooled to 30°C core temperature in a 20°C cold room. | 7°C for 5 days / 95% relative humidity |
| 9 | Flushdown in 1 hour. 1 hour holding period at ambient temperature. Ramp from 25°C to 43°C over 1 hour. Hold at 44°C. Relative humidity set at 90%. | 42°C for 1 hour | Treatment 1. Forced air cooled to 8°C core temperature in a 7°C cold room Treatment 2. Forced air cooled to 30°C core temperature in a 20°C cold room. Treatment 3. Forced air cooled to 30°C core temperature in a 20°C cold room | Treatment 1. 7°C for 5 days / 92% relative humidity. Treatment 2. 10°C for 5 days / 92% relative humidity. Treatment 3. 7°C for 5 days / 92% relative humidity |
| 10 | Flushdown in 1 hour. 1 hour holding period at ambient temperature. Ramp from 25°C to 45°C over 1 hour. Hold at 45°C. Relative humidity set at 90%. | 44°C for 0 minutes | Treatment 1. Forced air cooled to 8°C core temperature in a 7°C cold room Treatment 2. Forced air cooled to 30°C core temperature in a 20°C cold room. Treatment 3. Forced air cooled to 30°C core temperature in a 20°C cold room | Treatment 1. 7°C for 5 days / 92% relative humidity. Treatment 2. 10°C for 5 days / 92% relative humidity. Treatment 3. 7°C for 5 days / 92% relative humidity |
| 11 | Flushdown in 1 hour. 1 hour holding period at ambient temperature. Ramp from 25°C to 42°C over 1 hour. Hold at 42°C. Relative humidity set at 90%. | 41°C for 4 hours | Treatment 1. Forced air cooled to 8°C core temperature in a 7°C cold room Treatment 2. Open tray shelf cooled to 30°C core temperature in a 20°C cold room. | Treatment 1. 7°C for 5 days / 92% relative humidity. Treatment 2. 7°C for 5 days / 92% relative humidity |
| 12 | Flushdown in 1 hour. 1 hour holding period at ambient temperature. Ramp from 25°C to 40°C in 30 minutes. Hold at 40°C. Relative humidity set at 90%. | 39°C for 6 hours | Treatment 1. Forced air cooled to 30°C core temperature in a 20°C cold room Treatment 2. Forced air cooled to 30°C core temperature in a 20°C cold room. Treatment 3. Forced air cooled to 8°C core temperature in a 7°C cold room | Treatment 1. 7°C for 5 days / 92% relative humidity. Treatment 2. 12 hours at 20°C, then 7°C for 4.5 days / 92% relative humidity Treatment 3. 7°C for 5 days / 92% relative humidity |

6 RESULTS

6.1 Efficacy Trials

6.1.1 Most Tolerant Stage Testing

6.1.1.1 Vapour heat treatment

Dose response models were fitted to the mortality data for all immature life stages of *B. jarvisi*. Based on the criteria described in data analysis, the complementary log-log model, without a log transformation of dose, was selected as the model that was most appropriate for this data. GenStat analysis showed that independent response lines were appropriate (a significant interaction between dose and stage, $F_{3,20}=6.72$ $p=0.003$) indicating differences between the stages were not uniform across all doses. Based on the non-overlap of the LD₉₉ fiducial limits, it is clear that mature eggs were significantly more tolerant than all other stages at this point (Whiting and Hoy 1997; Soderstrom et al. 1996) (Table 7).

Table 7. LD₉₉ and fiducial limits based on independent response lines (*B. jarvisi* in capsicum against vapour heat treatments).

| Life stage | LD ₉₉ (mins) | Fiducial limits (95%) |
|---------------|-------------------------|-----------------------|
| Mature Egg | 352 | 251 - 792 |
| First Instar | 171 | 150 - 209 |
| Second Instar | 160 | 142 - 192 |
| Third Instar | 162 | 143 - 194 |

6.1.1.2 Vapour heat treatment with low oxygen

As the run up conditions for each of the replicates differed, each was examined separately. In both cases, a dose response model was fitted to the mortality data (as with the vapour heat treatment only data). However, for most of the stages the slopes of the regressions were not significant. Though comparisons across the stages were not possible using dose-response curves and LD₉₉ estimates, it is evident that at the higher temperatures mature eggs are more tolerant than the other stages (Table 8 and Table 9).

Table 8. Survival of *B. jarvisi* immature stages in capsicum treated with vapour heat plus controlled atmosphere. (Trial 1.)

| Stage | Dose | Number of insects treated | Number of surviving pupae | Corrected* Mortality (%) |
|-------------|---------|---------------------------|---------------------------|--------------------------|
| Mature Eggs | control | 800 | 82 | - |
| | 40°C | 800 | 162 | 0 |
| | 41°C | 800 | 140 | 0 |
| | 42°C | 800 | 122 | 0 |
| | 43°C | 800 | 64 | 22.0 |
| | 44°C | 800 | 134 | 0 |
| | 45°C | 800 | 58 | 29.3 |
| L1 | control | 800 | 75 | - |
| | 40°C | 800 | 118 | 0 |
| | 41°C | 800 | 89 | 0 |
| | 42°C | 800 | 106 | 0 |
| | 43°C | 800 | 86 | 0 |
| | 44°C | 800 | 30 | 60.0 |
| | 45°C | 800 | 3 | 96.0 |
| L2 | control | 400 | 164 | - |
| | 40°C | 400 | 232 | 0 |
| | 41°C | 400 | 209 | 0 |
| | 42°C | 400 | 197 | 0 |
| | 43°C | 400 | 204 | 0 |
| | 44°C | 400 | 104 | 36.6 |
| | 45°C | 400 | 44 | 73.2 |
| L3 | control | 400 | 342 | - |
| | 40°C | 400 | 220 | 35.7 |
| | 41°C | 400 | 238 | 30.4 |
| | 42°C | 400 | 195 | 43.0 |
| | 43°C | 400 | 197 | 42.4 |
| | 44°C | 400 | 63 | 81.6 |
| | 45°C | 400 | 9 | 97.4 |

* using Abbott's correction for control mortality

Table 9. Survival of *B. jarvisi* immature stages in capsicum treated with vapour heat plus controlled atmosphere. (Trial 2.)

| Stage | Dose | Number of insects treated | Number of surviving pupae | Corrected* Mortality (%) |
|-------|---------|---------------------------|---------------------------|--------------------------|
| Eggs | control | 800 | 178 | - |
| | 40°C | 800 | 133 | 25.3 |
| | 41°C | 800 | 143 | 19.7 |
| | 42°C | 800 | 109 | 38.8 |
| | 43°C | 800 | 188 | 0 |
| | 44°C | 800 | 188 | 0 |
| | 45°C | 800 | 151 | 15.2 |
| L1 | control | 800 | 184 | - |
| | 40°C | 800 | 115 | 37.5 |
| | 41°C | 800 | 48 | 73.9 |
| | 42°C | 800 | 108 | 41.3 |
| | 43°C | 800 | 129 | 30.0 |
| | 44°C | 600 | 32 | 76.8 |
| | 45°C | 1000 | 73 | 68.3 |
| L2 | control | 400 | 140 | - |
| | 40°C | 400 | 143 | 0 |
| | 41°C | 400 | 144 | 0 |
| | 42°C | 400 | 112 | 20.0 |
| | 43°C | 400 | 166 | 0 |
| | 44°C | 400 | 61 | 56.4 |
| | 45°C | 400 | 4 | 97.1 |
| L3 | control | 400 | 281 | - |
| | 40°C | 400 | 189 | 32.7 |
| | 41°C | 400 | 134 | 52.3 |
| | 42°C | 400 | 198 | 29.5 |
| | 43°C | 400 | 175 | 37.7 |
| | 44°C | 500 | 175 | 50.2 |
| | 45°C | 300 | 30 | 85.8 |

* using Abbott's correction for control mortality

6.1.2 Preliminary Trials

6.1.2.1 Vapour heat treatment

Trial 1 treating *B. jarvisi* mature eggs in capsicums with vapour heat treatment is summarised in Table 10. A dose of 45°C core temperature for 2.5 hours, achieved 99.72% mortality which is sufficient for domestic market access ($\geq 99.6\%$) but well below the requirements of the majority of international markets ($\geq 99.99\%$ at the 95% confidence level).

Table 10. Survival of *B. jarvisi* mature eggs in capsicum treated with vapour heat at 45°C at a range of times.

| Dose | Cooling method | Number of fruit Control: Treated | Number of insects in control | Estimated number of insects in treated | Number of pupae surviving treatment | Mortality (%) | True Mortality (\geq) (95% confidence) |
|------------------|---------------------------|----------------------------------|------------------------------|--|-------------------------------------|---------------|--|
| 45°C / 1 hour | Forced air cooled to 30°C | 20:20 | 1 075 | 1 075 | 392 | 63.5 | * |
| 45°C / 1.5 hours | as above | 20:20 | 1 075 | 1 075 | 122 | 88.7 | 86.1000 |
| 45°C / 2 hours | as above | 20:20 | 1 075 | 1 075 | 2 | 99.8 | 99.4144 |
| 45°C / 2.5 hours | as above | 20:20 | 1 075 | 1 075 | 0 | 100 | 99.7213 |

*unable to compute a 95% confidence limit

Trial 2 treating *B. jarvisi* mature eggs in capsicums with vapour heat treatment at 44 °C is summarised in Table 11. The maximum mortality achieved in this trial (74.4%) was not sufficient to meet domestic or international requirements.

Table 11. Survival of *B. jarvisi* mature eggs in capsicum treated with vapour heat at 44°C at a range of times.

| Dose | Cooling method | Number of fruit Control: Treated | Number of insects in control | Estimated number of insects in treated | Number of pupae surviving treatment | Mortality (%) | True Mortality (\geq) (95% confidence) |
|------------------|---------------------------|----------------------------------|------------------------------|--|-------------------------------------|---------------|--|
| 44°C / 3 hours | Forced air cooled to 30°C | 33:33 | 5 060 | 5 060 | 3 022 | 40.3 | * |
| 44°C / 3.5 hours | as above | 33:33 | 5 060 | 5 060 | 1 488 | 70.6 | * |
| 44°C / 4 hours | as above | 33:33 | 5 060 | 5 060 | 1 293 | 74.4 | * |

*unable to compute a 95% confidence limit

Trial 3 treating *B. jarvisi* mature eggs in capsicums with vapour heat treatment at 45 °C but with different ramping conditions is summarised in Table 12. The maximum mortality achieved in this trial (75.2%) was not sufficient to meet domestic or international requirements.

Table 12. Survival of *B. jarvisi* mature eggs in capsicum treated with vapour heat at 45°C at a range of times.

| Dose | Cooling method | Number of fruit Control: Treated | Number of insects in control | Estimated number of insects in treated | Number of pupae surviving treatment | Mortality (%) | True Mortality (\geq) (95% confidence) |
|------------------|---------------------------|----------------------------------|------------------------------|--|-------------------------------------|---------------|--|
| 45°C / 1 hour | Forced air cooled to 30°C | 22:22 | 4 945 | 4 945 | 6 603 | 0 | * |
| 45°C / 1.5 hours | as above | 22:22 | 4 945 | 4 945 | 4 286 | 13.3 | * |
| 45°C / 2 hours | as above | 22:21 | 4 945 | 4 720 | 3 231 | 31.5 | * |
| 45°C / 2.5 hours | as above | 22:23 | 4 945 | 5 170 | 1 283 | 75.2 | * |

*unable to compute a 95% confidence limit

Trial 4 treating *B. jarvisi* mature eggs in capsicums with vapour heat treatment at 45 °C and further variations of the ramping conditions is summarised in Table 13. The maximum mortality achieved in this trial (99.3%) was not sufficient to meet domestic or international requirements.

Table 13. Survival of *B. jarvisi* mature eggs in capsicum treated with vapour heat at 45°C at a range of times.

| Dose | Cooling method | Number of fruit Control: Treated | Number of insects in control | Estimated number of insects in treated | Number of pupae surviving treatment | Mortality (%) | True Mortality (≥) (95% confidence) |
|------------------|---------------------------|----------------------------------|------------------------------|--|-------------------------------------|---------------|-------------------------------------|
| 45°C / 1 hour | Forced air cooled to 30°C | 33:33 | 20 380 | 20 380 | 323 | 98.4 | * |
| 45°C / 1.5 hours | as above | 33:33 | 20 380 | 20 380 | 530 | 97.4 | * |
| 45°C / 2 hours | as above | 33:33 | 20 380 | 20 380 | 210 | 99.0 | * |
| 45°C / 2.5 hours | as above | 33:33 | 20 380 | 20 380 | 145 | 99.3 | * |

*unable to compute a 95% confidence limit

Trial 5 treating *B. jarvisi* mature eggs in capsicums with vapour heat treatment is summarised in Table 14. Mortality in each of the three treatments (99.59 – 99.89%) was sufficient for domestic requirements but was failed to meet international requirements.

Table 14. Survival of *B. jarvisi* mature eggs in capsicum treated with vapour heat at 45°C for 3 hours and cooled by a range of methods.

| Dose | Cooling method | Number of fruit Control: Treated | Number of insects in control | Estimated number of insects in treated | Number of pupae surviving treatment | Mortality (%) | True Mortality (≥) (95% confidence) |
|----------------|---------------------------|----------------------------------|------------------------------|--|-------------------------------------|---------------|-------------------------------------|
| 45°C / 3 hours | Forced air cooled to 30°C | 30:30 | 15 491 | 15 491 | 50 | 99.68 | 99.5915 |
| 45°C / 3 hours | Forced air cooled to 8°C | 30:30 | 15 491 | 15 491 | 19 | 99.88 | 99.8200 |
| 45°C / 3 hours | Shower cooled to 30°C | 30:30 | 15 491 | 15 491 | 10 | 99.94 | 99.8905 |

6.1.2.2 Vapour heat treatment plus cold treatment

Trial 1 treating *B. jarvisi* mature eggs in capsicums with vapour heat treatment plus cold treatment is summarised in Table 15. The maximum mortality achieved in this trial (96.6%) was not sufficient to meet domestic or international requirements.

Table 15. Survival of *B. jarvisi* mature eggs in capsicum treated with vapour heat at 43°C for 3 hours followed by a range of cold treatments.

| Dose | Cold treatment | Number of fruit Control: Treated | Number of insects in control | Estimated number of insects in treated | Number of pupae surviving treatment | Mortality (%) | True Mortality (≥) (95% confidence) |
|----------------|---|----------------------------------|------------------------------|--|-------------------------------------|---------------|-------------------------------------|
| 43°C / 3 hours | Forced air cooled to 7°C, then sample removed | 30:30 | 10 848 | 10 848 | 3 189 | 70.6 | * |
| 43°C / 3 hours | Forced air cooled to 7°C, and held for 1 day | 30:30 | 10 848 | 10 848 | 612 | 94.4 | * |
| 43°C / 3 hours | Forced air cooled to 7°C, and held for 2 days | 30:30 | 10 848 | 10 848 | 367 | 96.6 | * |

*unable to compute a 95% confidence limit

Trial 2 treating *B. jarvisi* mature eggs in capsicums with vapour heat treatment plus cold treatment is summarised in Table 16. Cooling the fruit to 3°C resulted in higher mortality (78.39 - 98.97%) than cooling to 7°C but was not sufficient to meet domestic or international requirements.

Table 16. Survival of *B. jarvisi* mature eggs in capsicum treated with vapour heat at 43°C for 3 hours followed by a range of cold treatments.

| Dose | Cold treatment | Number of fruit Control: Treated | Number of insects in control | Estimated number of insects in treated | Number of pupae surviving treatment | Mortality (%) | True Mortality (≥) (95% confidence) |
|----------------|---|----------------------------------|------------------------------|--|-------------------------------------|---------------|-------------------------------------|
| 43°C / 3 hours | Forced air cooled to 3°C, then sample removed | 26:26 | 461 | 461 | 83 | 82.0 | 78.3898 |
| 43°C / 3 hours | Forced air cooled to 3°C, and held for 1 day | 26:26 | 461 | 461 | 19 | 95.9 | 93.9523 |
| 43°C / 3 hours | Forced air cooled to 3°C, and held for 2 days | 26:26 | 461 | 461 | 1 | 99.8 | 98.9711 |

Trial 3 treating *B. jarvisi* mature eggs in capsicums with vapour heat treatment plus cold treatment is summarised in Table 17. The maximum mortality achieved in this trial (99.2%) was not sufficient to meet domestic or international requirements.

Table 17. Survival of *B. jarvisi* mature eggs in capsicum treated with vapour heat at 43°C for 3 hours followed by a range of cold treatments.

| Dose | Cold treatment | Number of fruit Control: Treated | Number of insects in control | Estimated number of insects in treated | Number of pupae surviving treatment | Mortality (%) | True Mortality (\geq) (95% confidence) |
|----------------|---|----------------------------------|------------------------------|--|-------------------------------------|---------------|--|
| 43°C / 3 hours | No forced air cooling | 34:34 | 41 406 | 41 406 | 544 | 98.7 | * |
| 43°C / 3 hours | Forced air cooled to 3°C, then sample removed | 34:34 | 41 406 | 41 406 | 1 282 | 96.9 | * |
| 43°C / 3 hours | Forced air cooled to 3°C, and held for 1 day | 34:34 | 41 406 | 41 406 | 751 | 98.2 | * |
| 43°C / 3 hours | Forced air cooled to 3°C, and held for 2 days | 34:34 | 41 406 | 41 406 | 318 | 99.2 | * |

*unable to compute a 95% confidence limit

6.1.2.3 Vapour heat treatment with low oxygen treatment

Trial 1 treating *B. jarvisi* mature eggs in capsicums with vapour heat treatment with low oxygen treatment is summarised in Table 18. The mortality achieved in this trial (78.85%) was not sufficient to meet domestic or international requirements.

Table 18. Survival of *B. jarvisi* mature eggs in capsicum treated with vapour heat at 44°C with low oxygen.

| Dose | Cold treatment | Number of fruit Control: Treated | Number of insects in control | Estimated number of insects in treated | Number of pupae surviving treatment | Mortality (%) | True Mortality (\geq) (95% confidence) |
|------------------|---|----------------------------------|------------------------------|--|-------------------------------------|---------------|--|
| 44°C / 0 minutes | Forced air cooled to 8°C, then sample removed | 12:60 | 63 | 315 | 53 | 83.2 | 78.8479 |

Trial 2, comparing the efficacy of vapour heat treatment and vapour heat treatment with low oxygen is summarised in Table 19. Incorporating low oxygen as part of the treatment dramatically increased the mortality recorded (98.8% compared to 38.9%) but was not sufficient to meet domestic or international requirements.

Table 19. Survival of *B. jarvisi* and *B. tryoni* mature eggs in capsicum treated with vapour heat at 40°C for 4 hours with and without low oxygen treatment.

| Dose | Cooling method | Number of fruit Control: Treated | Number of insects in control | Estimated number of insects in treated | Number of pupae surviving treatment | Mortality (%) | True Mortality (\geq) (95% confidence) |
|---------------------------------|---------------------------|----------------------------------|------------------------------|--|-------------------------------------|---------------|--|
| 40°C / 4 hours | Forced air cooled to 30°C | 24:24 | 14 637 | 14 637 | 8 950 | 38.9 | * |
| 40°C / 4 hours under low oxygen | Forced air cooled to 30°C | 24:24 | 14 637 | 14 637 | 181 | 98.8 | * |

*unable to compute a 95% confidence limit

6.2 Fruit Quality Trials

6.2.1.1 Vapour heat treatment

Fruit treated to a core temperature of 45°C for 3 hours were rated to have a mean general acceptability of 4.1 compared to the highest possible rating of 9 for untreated fruit (Table 20). Skin pitting was recorded in treated fruit only and averaged >2mm² and up to 20% coverage of the skin. It should be noted that immediately after treatment capsicums generally showed no signs of heat injury with external pitting on the skin appearing approximately 3 days after treatment. Untreated fruit remained firm at assessment (mean rating 1.0) whereas heat treated fruit (mean rating 1.8) were rubbery by feel. Internal appearance was approximately one rating unit lower in treated fruit (mean rating 7.9) than untreated fruit (mean rating 9.0).

Table 20. Fruit injury and quality characteristics of untreated and vapour heat treated fruit held at a core temperature of 45°C for 3 hours prior to air cooling to 30°C and storage at 7°C (Trial 1)

| Fruit quality | Untreated | 45°C for 3 hours |
|----------------------------|------------------|-------------------------|
| | | |
| External assessment | | |
| General appearance | 9.0 | 4.1 |
| Pitting – size | 0.0 | 2.2 |
| - coverage | 0.0 | 1.5 |
| Firmness | 1.0 | 1.8 |
| | | |
| Internal assessment | | |
| General appearance | 9.0 | 7.9 |
| Injury | 0.0 | 0.1 |

Heat treatment at 1°C lower core temperature for 1 hour longer duration (44°C for 4 hours) resulted in a higher general acceptability (mean rating 5.2) than fruit treated in Trial 1 (Table 21). However it was still much lower than untreated fruit (mean rating of 5.2 compared to 8.9). Skin pitting, although smaller in size (mean rating 1.5) and reduced coverage (mean rating 1.1) compared to treated fruit in Trial 1, was still deemed to be of unacceptable quality.

Table 21. Fruit injury and quality characteristics of untreated and vapour heat treated fruit held at a core temperature of 44°C for 4 hours prior to air cooling to 30°C and storage at 7°C (Trial 2)

| Fruit quality | Untreated | 44°C for 4 hours |
|----------------------------|------------------|-------------------------|
| | | |
| External assessment | | |
| General appearance | 8.9 | 5.2 |
| Pitting – size | 0.0 | 1.5 |
| - coverage | 0.0 | 1.1 |
| Firmness | 1.0 | 1.5 |
| | | |
| Internal assessment | | |
| General appearance | 9.0 | 8.8 |
| Injury | 0.0 | 0.1 |

Vapour heat treated capsicums to a core temperature of 43°C for 3 hours had a greater general acceptability rating (mean rating 6.0) (Table 22) than fruit treated at 44°C for 4 hours. Skin pitting size (mean rating 0.9) and coverage (mean rating 0.9) was also reduced compared to fruit in the previous trial.

Table 22. Fruit injury and quality characteristics of untreated and vapour heat treated fruit held at a core temperature of 43°C for 3 hours prior to air cooling to 30°C and storage at 7°C for 5 days (Trial 3)

| Fruit quality | Untreated | 43°C for 3 hours |
|----------------------------|------------------|-------------------------|
| | | |
| External assessment | | |
| General appearance | 8.0 | 6.0 |
| Pitting – size | 0.0 | 0.9 |
| - coverage | 0.0 | 0.9 |
| Firmness | 1.4 | 1.6 |
| | | |
| Internal assessment | | |
| General appearance | 8.8 | 8.5 |
| Injury | 0.0 | 0.0 |

Reducing the chamber ramp up period from 1 hour to 20 minutes for fruit treated at a core temperature of 43°C for 3 hours resulted in a further reduction in general acceptability across all treatments (Table 23). Slight improvements to general acceptability were achieved when fruit were transferred immediately to 7°C for both water and air cooling treatments, however only very slight differences in pitting size and coverage were seen.

Table 23. Fruit injury and quality characteristics of untreated and vapour heat treated fruit held at a core temperature of 43°C for 3 hours prior to cooling. (Trial 4)

| Fruit quality | Untreated | FAC 30°C No delay | FAC 30°C 12h delay | WC 8°C No delay | WC 8°C 12h delay |
|----------------------------|------------------|------------------------------|-------------------------------|----------------------------|-----------------------------|
| External assessment | | | | | |
| General appearance | 8.4 | 5.5 | 5.0 | 5.8 | 5.2 |
| Pitting - size | 0.1 | 1.6 | 1.6 | 1.5 | 1.6 |
| - coverage | 0.0 | 1.3 | 1.6 | 1.3 | 1.6 |
| Firmness | 1.2 | 1.2 | 1.1 | 1.0 | 1.3 |
| Internal assessment | | | | | |
| General appearance | 8.8 | 8.6 | 8.8 | 8.6 | 8.7 |
| Injury | 0.0 | 0.1 | 0.1 | 0.2 | 0.2 |

FAC = forced air cooling

WC = water cooling

6.2.1.2 Vapour heat treatment plus cold treatment

Vapour heat treatment of green capsicums to a core temperature of 43°C for 3 and 4 hours prior to forced air cooling to 8°C resulted in the highest general acceptability recorded in any trial (mean rating 6.7, 6.7 respectively) (Table 24). Pitting size and coverage rates were similar across 3 and 4 hour treatments at 43°C. Heat treatment did not have any notable effect on internal quality in this trial.

Table 24. Fruit injury and quality characteristics of untreated and vapour heat treated fruit held at a core temperature of 43°C for 3 and 4 hours prior to forced air cooling to 8°C and storage at 7°C for 5 days. (Trial 5)

| Fruit quality | Untreated | 43°C for 3 hours | 43°C for 4 hours |
|----------------------------|------------------|-----------------------------|-----------------------------|
| External assessment | | | |
| General appearance | 8.9 | 6.7 | 6.7 |
| Pitting – size | 0.0 | 0.5 | 0.5 |
| - coverage | 0.0 | 0.5 | 0.5 |
| Firmness | 1.1 | 1.3 | 1.4 |
| Internal assessment | | | |
| General appearance | 8.9 | 9.0 | 8.8 |
| Injury | 0.0 | 0.0 | 0.0 |

Heat treatment at 43°C for 3 and 4 hours, followed by 2 days cool storage at 3°C and 3 days at 7°C resulted in a low general acceptability rating (mean rating 4.6, 4.7) (Table 25). Moderate skin pitting was recorded with average size (mean rating 1.9, 1.8) and coverage (mean rating 2.3, 2.0) in treated fruit. Untreated fruit showed a slight level of skin pitting. It should be noted that fruit was purchased towards the end of the commercial capsicum season.

Table 25. Fruit injury and quality characteristics of untreated and vapour heat treated fruit held at a core temperature of 43°C for 3 and 4 hours prior to forced air cooling to 8°C and storage at 3°C for 2 days then 7°C for 3 days. (Trial 6)

| Fruit quality | Untreated | 43°C for 3 hours | 43°C for 4 hours |
|----------------------------|------------------|-------------------------|-------------------------|
| | | | |
| External assessment | | | |
| General appearance | 8.2 | 4.6 | 4.7 |
| Pitting – size | 0.6 | 1.9 | 1.8 |
| - coverage | 0.4 | 2.3 | 2.0 |
| Firmness | 1.4 | 1.5 | 1.4 |
| | | | |
| Internal assessment | | | |
| General appearance | 8.9 | 9.0 | 9.0 |
| Injury | 0.1 | 0.0 | 0.0 |

Heat treatment of capsicums at 43°C for 3 and 4 hours with an initial period of low relative humidity (approximately 50%) resulted in a further reduction in general acceptability despite an improvement in overall quality of untreated fruit compared to the previous trial (Table 26). Heat treatment again resulted in unacceptable skin pitting in both treatments examined in this trial.

Table 26. Fruit injury and quality characteristics of untreated and vapour heat treated fruit held at a core temperature of 43°C for 3 and 4 hours prior to forced air cooling to 8°C and storage at 3°C for 2 days then 7°C for 3 days (50% RH inside chamber until 43°C core temperature followed by 90% RH for remainder of treatment). (Trial 7)

| Fruit quality | Untreated | 43°C for 3 hours | 43°C for 4 hours |
|----------------------------|------------------|-------------------------|-------------------------|
| | | | |
| External assessment | | | |
| General appearance | 9.0 | 4.4 | 4.6 |
| Pitting – size | 0.0 | 1.4 | 1.5 |
| - coverage | 0.0 | 2.2 | 1.9 |
| Firmness | 1.5 | 1.5 | 1.5 |
| | | | |
| Internal assessment | | | |
| General appearance | 9.0 | 8.9 | 8.8 |
| Injury | 0.0 | 0.0 | 0.0 |

6.2.1.3 Vapour heat treatment plus controlled atmosphere

Initial fruit quality assessments on green capsicums treated in the low oxygen heat treatment system (Trial 8 & 9) were observed but not recorded to as heat damage was very severe and fruit were not of acceptable quality.

Low oxygen heat treatment at 44°C for 0 minutes caused unacceptable skin pitting in all treatment groups (Table 27). Pitting sizes and coverage rates were similar across all treatment groups within this trial. Treated fruit also scored a low general acceptability (mean rating 3.2 – 3.9) compared to untreated fruit (mean rating 8.2). Similar incidence of diffuse grey discoloration of the skin was recorded in all three treatment groups. An atypical slightly off smelling odour was evident from fruit treated under low oxygen.

Table 27. Fruit injury and quality characteristics of untreated and low oxygen + vapour heat treated fruit reaching a core temperature of 44°C then forced air cooled and stored (Trial 10)

| Fruit quality | Untreated | FAC 8°C Stored at 7°C for 5 days | FAC 30°C Stored at 10°C for 5 days | FAC 30°C Stored at 7°C |
|-------------------------------|------------------|---|---|-----------------------------------|
| | | | | |
| External assessment | | | | |
| General appearance | 8.2 | 3.9 | 3.2 | 3.3 |
| Pitting - size | 0.0 | 1.5 | 1.6 | 1.3 |
| - coverage | 0.0 | 2.0 | 1.9 | 2.0 |
| Skin greying incidence (%) | 0.0 | 11.1 | 13.8 | 8.3 |
| Firmness | 1.3 | 1.8 | 2.2 | 1.8 |
| | | | | |
| Internal assessment | | | | |
| General appearance | 8.9 | 7.6 | 7.9 | 7.7 |
| Injury | 0.0 | 0.4 | 0.5 | 0.6 |

FAC = forced air cooling

Heat treatment with low oxygen to a lower fruit core temperature and longer time (41°C for 4 hours) than previous trial resulted in a higher general acceptability for both forced air and shelf cooled fruit (mean rating 6.2, 5.9) (Table 28). Skin pitting size and coverage rates, at similar levels for both cooling methods, were at lower levels than previous (Trial 10). Skin greying was only present on treated fruit and was rated at similar incidences for both cooling types. Untreated fruit (mean rating 1.1) were firmer than both treatment groups (mean rating 1.7, 1.8).

Table 28. Fruit injury and quality characteristics of untreated and low oxygen + vapour heat treated fruit held at a core temperature of 41°C for 4 hours prior to cooling to 30°C. (Trial 11)

| Fruit quality | Untreated | Forced air cooling | Shelf cooling |
|----------------------------|------------------|---------------------------|----------------------|
| | | | |
| External assessment | | | |
| General appearance | 8.5 | 6.1 | 5.9 |
| Pitting – size | 0.0 | 1.0 | 1.1 |
| - coverage | 0.0 | 0.9 | 0.9 |
| Skin greying incidence (%) | 0.0 | 24.4 | 26.6 |
| Firmness | 1.1 | 1.7 | 1.8 |
| | | | |
| Internal assessment | | | |
| General appearance | 8.9 | 8.2 | 8.2 |
| Injury | 0.0 | 0.3 | 0.5 |

Heat treatment with low oxygen for a longer duration of 6 hours at 39°C core temperature caused severe fruit injury at each cooling and storage regime tested (Trial 12) (Table 29). General acceptability rating was extremely low for treated fruit (mean rating 3.5 – 3.8) compared to untreated fruit (mean rating 8.4). Skin pitting was similar in size and coverage across all treatments. Skin greying incidence of treated fruit ranged between 47% and 67%. Treated fruit were greater than 1 rating unit softer in firmness than untreated fruit.

Table 29. Fruit injury and quality characteristics of untreated and low oxygen + vapour heat treated fruit held at a core temperature of 39°C for 6 hours prior to forced air cooling. (Trial 12)

| Fruit quality | Untreated | FAC 30°C No delay | FAC 30°C 12h delay | FAC 8°C No delay |
|----------------------------|------------------|--------------------------|---------------------------|-------------------------|
| | | | | |
| External assessment | | | | |
| General appearance | 8.4 | 3.6 | 3.5 | 3.8 |
| Pitting - size | 0.1 | 1.4 | 1.3 | 1.6 |
| - coverage | 0.0 | 1.2 | 1.1 | 1.2 |
| Skin greying incidence (%) | 0.0 | 66.7 | 55.6 | 47.2 |
| Firmness | 1.12 | 2.3 | 2.2 | 2.4 |
| | | | | |
| Internal assessment | | | | |
| General appearance | 8.8 | 6.9 | 6.4 | 6.9 |
| Injury | 0.0 | 0.5 | 0.5 | 0.6 |

FAC = forced air cooling

7 DISCUSSION

The aim of this project was to determine if a heat treatment could be successfully developed to control Australian fruit fly species in capsicum. The first step was to undertake most tolerant stage trials against *B. jarvisi* which previous projects (Corcoran *et al.* 2003) had identified as the most heat tolerant pest fruit fly species infesting capsicum. Dose mortality studies using vapour heat treatment found that mature eggs were significantly more tolerant than all other stages. These results conform with previous research carried out by this project team which has shown that in most cases mature eggs are the most tolerant stage to heat with in-fruit testing, for example, *B. cucumis* eggs in zucchini (Corcoran *et al.* 1993); *B. cucumis* eggs in zucchini, button squash, cucumber, rockmelon, honeydew, and watermelon (Hall *et al.* 2004); and *B. tryoni* eggs in mango (Heather *et al.* 1997) and tomato (Hall *et al.* 2004). Once the most tolerant stage was identified, trials were undertaken to evaluate the treatments developed by US (APHIS) and Japanese researchers (Sugimoto *et al.* 1983).

Efficacy and fruit quality trials were run concurrently using a range of temperatures (44 and 45°C), humidity's (50 - 95%), ramping rates (20 - 60 minutes) and holding periods (1- 4 hours). The most promising treatment in terms of efficacy was a treatment of 45°C for 2.5 with 95% humidity throughout the treatment which recorded no survivors from 1075 treated insects. This equates to 99.72% mortality at the 95% confidence level which is sufficient to meet current domestic quarantine requirements ($\geq 99.6\%$). While it was a positive result, high levels of damage were recorded in fruit quality trials (45°C for 3 hours, 95% humidity). The major problem was skin pitting which averaged $\geq 2\text{mm}^2$ and up to 20% coverage of the skin (See Appendix 1). The use of lower temperatures for longer time periods (e.g. 44°C for 4 hours, 43°C for 3 hours) reduced the symptoms but not to commercially acceptable limits. Additionally, lower temperatures corresponded with lower mortality rates (e.g. $< 75\%$).

The results clearly show that treatments developed overseas are not applicable to Australian conditions. Possible suggestions for this difference include fruit cultivars, growing conditions, harvest season, fruit fly species and geographical distribution (Sugimoto *et al.* 1983). Another important point that should be noted is that during the course of this trial the APHIS approved vapour heat treatment was rescinded due to the presence of the exotic fruit fly species *B. latifrons* in Hawaii. Trials comparing the heat tolerance (*in vitro*) of *B. latifrons* to *C. capitata*, *B. dorsalis* and *B. cucurbitae* showed that *B. latifrons* was significantly more heat tolerant than the other species tested (Jang *et al.* 1999). As capsicums are a recorded host to *B. latifrons* the treatment has been suspended until further efficacy trials can be undertaken. This leaves irradiation as the only non-chemical treatment currently approved for export of Hawaiian capsicums to mainland USA. Small scale, preliminary trials on the tolerance of Australian grown capsicum varieties has been undertaken with very positive results recorded (See Appendix 2). However, irradiation is not currently permitted under Standard 1.5.3 of the Food Standards Australia New Zealand (FSANZ) Act. As such, further research may be required to develop a submission to FSANZ if irradiation is to be used to control Australian fruit fly species in capsicums.

In an attempt to improve the efficacy of the treatment and reduce fruit damage a series of trials examining cooling regimes were undertaken. After treatment at 45°C for 3 hours, fruit was forced air cooled (30°C and 8°C) or water cooled (30°C). Mortality rates for all three cooling regimes were similar ($>99\%$ mortality) but did not meet domestic requirements.

Similarly, a treatment of 43°C for 3 hours followed by forced air cooling to 7°C was unsuccessful. Using storage periods of 0 (removed immediately once temperature reached 7°C), 24 and 48 hours at 7°C resulted in 70.6, 94.4 and 96.6% mortality respectively. This is well below the efficacy required for current domestic requirements. Fruit quality trials using 43°C for 3 and 4 hours followed by forced air cooling and storage at 7°C for 5 days recorded reduced damage levels compared to standard

vapour heat treatment but were still commercially unacceptable. The general appearance of the capsicums was reduced and skin pitting was recorded.

A treatment of 43°C for 3 hours followed by forced air cooling to 3°C was also unsuccessful. Using storage periods of 0 (removed immediately once temperature reached 3°C), 24 and 48 hours at 3°C resulted in 78.39, 93.95 and 98.97% mortality respectively. This is well below the efficacy required for current domestic requirements.

As none of the above treatments were successful, the use of vapour heat treatment with low oxygen (hypoxia) was investigated. Hypoxia has been utilised successfully in conjunction with vapour heat treatments to enhance treatment mortality against fruit flies and other insects (Yocum & Denlinger 1994, Soderstrom *et al.* 1996, Nevan and Drake 2000, Leach *et al.* 2005). Hypoxia during heat treatment may prevent conditioning in the insects due to suppression of some aerobic components of the heat shock response.

Before large scale trials were undertaken, most tolerant stage testing using vapour heat and low oxygen was undertaken. Two trials were conducted and mature eggs of *B. jarvisi* were arithmetically more tolerant than first, second and third instars. Large scale trials were then undertaken using a range of treatments. After reducing oxygen levels within the treatment chamber to approximately 0.5%, fruit were heated to 44°C then forced air cooled to 8°C. A total of 53 insects out of an estimated 315 treated insects survived the treatment. This represents a mortality of 78.85% which is below the efficacy required for current domestic requirements. Fruit quality trials using an identical heat treatment and a range of cooling regimes resulted in skin pitting, poor general acceptance, greying of the skin and an atypical slightly off smelling odour.

A second series of trials using lower temperatures and longer times periods was undertaken. A treatment of 40°C for 4 hours under low oxygen resulted in 98.8% mortality. A comparative trial using identical heating and cooling regimes but with standard vapour heat treatment only resulted in 38.9% mortality. As such, it is clear that the use of low oxygen did enhance mortality. However, the use of low oxygen resulted in the most severe fruit damage of all treatments examined.

8 CONCLUSIONS

In spite of testing a wide range of vapour heat treatments, vapour heat plus cold treatments and vapour heat with low oxygen a non-damaging treatment for capsicums which would meet domestic or international quarantine requirements for fruit fly could not be found.

9 TECHNOLOGY TRANSFER

During the course of this project, discussions have occurred with the Principal Physiologist, Senior Entomologists and researchers in the Market Access Team within the DPI&F and also with international scientists who have researched vapour heat and low oxygen treatments against fruit fly to gain knowledge and directions for this project.

Progress on this project has been reported in milestone reports to Horticulture Australia. Kim James (Portfolio Manager – Biosecurity & Market Access Research & Development, Horticulture Australia) and Kate Dunn (Vegetable Industry Development Officer, Growcom) also visited researchers at the DPI&F Cairns laboratory in 2006.

An article ‘New research turning up the heat on fruit fly’ was published in Volume 2.1, July/August 2006 edition of Vegetables Australia, which is produced by AUSVEG. This article discussed successful heat treatments developed by DPI&F for mangoes, tomatoes, rockmelon, honeydew, watermelon, zucchini and button squash and included the current capsicum project with the aim of developing a non-chemical heat treatment.

Results of this project have been communicated to industry stakeholders at several forums. Presentations on project progress overview of results to the HAL Working Group on Market Access Research & Development in early December. Project results were also presented at a Growcom and DPI&F forum for tomato and capsicum growers held in Bowen, Queensland in April 2007.

10 RECOMMENDATIONS

Insecticide postharvest treatments are currently under review and their use may be severely restricted or lost in the near future. Research is required to investigate other treatment options that may be suitable for fruit and vegetables that are susceptible to damage from heat treatments.

One alternative may be the use of irradiation. Preliminary fruit physiology studies indicate that capsicums will tolerate irradiation treatment at doses required for control of fruit fly.

11 ACKNOWLEDGEMENTS

This project was facilitated by HAL in partnership with AUSVEG and was funded by the National Vegetable Levy. The Australian Government provides matched funding for all HAL's R&D activities.

The contribution from Entomology, Physiology and Biometry staff from the Department of Primary Industry and Fisheries at Cairns and Indooroopilly laboratories (listed on the inside cover) is greatly appreciated.

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13 APPENDIX 1:

Skin pitting coverage in vapour heat treated green capsicums after 5 days storage at 7°C.



Fig 1. Rating 1 (to 5% coverage)



Fig 2. Rating 3 (to 50% coverage)



Fig 3. Rating 5 (to 100% coverage)

Skin pitting size in vapour heat treated green capsicums after 5 days storage at 7°C.



Fig 4. 1 mm pitting size



Fig 5. ≤ 3 mm pitting size



Fig 6. >5 mm pitting size

14 APPENDIX 2:

Fruit quality of ‘Red’ and ‘Green’ capsicum following Cobalt-60 irradiation.

Materials and methods

Green and red capsicums (*Capsicum annuum*) were obtained from the Sydney markets from separate commercial growers. Fruit were held in cold storage for an unspecified period at Sydney markets before being delivered to the Australian Nuclear Science and Technology Organisation (ANSTO) in Sydney.

One box each of green and red capsicum were treated at 250, 400, 500 and 600 Gray (Gy) respectively. One box each of green and red capsicum were left untreated as controls. After treatment, all fruit were air freighted to the DPI&F laboratory in Cairns, Queensland. Fruit were held for 5 days at 7°C prior to assessment. Assessment criteria were similar to methods described in section 5.3.

Results

Fruit quality indicators of ‘Green’ and ‘Red’ capsicums following irradiation with Cobalt-60 is shown in Table 1.

‘Green’ Capsicums

External appearance rated high with 7.9 – 8.0 general acceptability relating to like very much. External injury rating was very low in all treatments and control fruit with between 4 and 12 % incidence. There was some skin shrivelling observed possibly due to age and postharvest handling and storage conditions. External rots were also of low incidence and low severity across all treatments. There were not any considerable differences in firmness between untreated and treated capsicums.

‘Red’ capsicums

Red capsicums had greater rots in both untreated and treated samples than ‘green’ capsicums. External appearance was lesser than green capsicums and scores a general acceptability rating of 6.2 to 6.6. External injury of untreated and 400Gy treated red capsicums scored the lowest rating of 0.3, only slightly lower than all other treatments which all scored a rating of 0.4. A moderate incidence of skin shrivelling (untreated 16%, treated 20-24%) that was recorded was most likely due to the fruit condition at treatment and not a direct result of irradiation. The firmness of red capsicums at assessment did not differ greatly between treatments (mean rating 2.0 – 2.1). However it was close to 1 rating unit softer than the green capsicums. The incidence of external rots was much greater than green capsicums at assessment.

Table 1 Means and standard errors of the fruit quality properties of capsicum following irradiation

| Type | Dose (Gy) | Fruit quality property (mean ± SE)* | | | | | |
|-------|-----------|-------------------------------------|----------------------------|---------------------|--------------------------|-------------------|---------------------|
| | | External Appearance | External injury (severity) | External injury (%) | External rots (severity) | External rots (%) | Hand firmness (0-5) |
| Green | 0 | 7.96±0.09 | 0.08±0.05 | 8 | 0 | 0 | 1.20±0.08 |
| | 250 | 7.92±0.09 | 0.04±0.04 | 4 | 0 | 0 | 1.12±0.07 |
| | 400 | 8.00±0.10 | 0.08±0.05 | 8 | 0.04±0.04 | 4 | 1.12±0.07 |
| | 500 | 7.92±0.09 | 0.08±0.05 | 8 | 0.04±0.04 | 4 | 1.16±0.07 |
| | 600 | 8.00±0.10 | 0.12±0.07 | 12 | 0 | 0 | 1.16±0.07 |
| Red | 0 | 6.56±0.30 | 0.28±0.14 | 16 | 0.56±0.16 | 36 | 2.12±0.12 |
| | 250 | 6.60±0.33 | 0.44±0.16 | 24 | 0.44±0.15 | 28 | 1.96±0.09 |
| | 400 | 6.20±0.33 | 0.28±0.12 | 20 | 0.44±0.15 | 28 | 2.12±0.09 |
| | 500 | 6.16±0.35 | 0.36±0.15 | 20 | 0.40±0.15 | 24 | 2.00±0.12 |
| | 600 | 6.24±0.35 | 0.40±0.15 | 24 | 0.48±0.15 | 32 | 2.04±0.11 |

* The eating quality of the treated fruit was not evaluated as irradiation is not yet an approved treatment for capsicum in Australia.

Discussion

Treatment as high as 600 Gy did not result in any loss of quality to either ‘Green’ or ‘Red’ capsicums after 5 days storage at 7°C. As such, irradiation may be an effective disinfestation alternative to heat sensitive commodities such as capsicum. However, the use of irradiation on capsicums is not currently permitted under Standard 1.5.3 of the Food Standards Australia New Zealand (FSANZ) Act. As such, further research using export quality fruit under controlled handling conditions will be required to develop a submission to FSANZ if irradiation is to be used to control Australian fruit fly species in capsicums.

Funding

Funding for this trial was provided by the International Atomic Energy Agency and ANSTO. Fruit was donated by Jacqui Allison of Harrowsmiths International, Brisbane, Queensland. Fruit quality assessments were conducted by John Cavallaro, DPI&F.