Know-how for Horticulture™

Heat disinfestation of vegetables for access to interstate and New Zealand markets

Liz Hall
QLD Department of Primary Industries and Fisheries

Project Number: VX99038

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Heat disinfestation of vegetables for export to New Zealand and Interstate

Horticulture Australia Project Number VX99038

Final Report (April 2004)

Elizabeth Hall *et al*

Agency for Food and Fibre Sciences
Department of Primary Industries and Fisheries

Horticulture Australia

Queensland Fruit & Vegetable Growers

Queensland Government
Department of Primary Industries and Fisheries

Heat disinfestation of vegetables for export to New Zealand and Interstate

Horticulture Australia Project Number VX99038

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TABLE OF CONTENTS

1 MEDIA SUMMARY

Many horticultural products are hosts for fruit flies, 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 to the movement of fresh produce. 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.

Currently there is a heavy reliance on the use of chemical treatments to meet postharvest quarantine requirements for interstate trade and export to New Zealand. However, the use of chemical treatments is under review by regulatory bodies and there is currently a strong consumer preference for products which receive minimal or no treatment with synthetic chemicals. It is likely that the availability of chemical treatments for disinfestation will be severely restricted within the next few years when, if an alternative treatment is not in place, all exports of fruit fly host commodities to New Zealand will be severely constrained. It is therefore necessary to develop effective, preferably non-chemical, alternative quarantine treatments. Development of non-chemical postharvest treatments also leads to a reduction in 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.

The cucurbit and tomato industries are at present heavily reliant on the use of chemical insecticides for postharvest disinfestation for export of fresh product to New Zealand and for interstate trade (Victoria, South Australia, Western Australia and Tasmania). The aim of the project was to develop new disinfestation treatments against fruit flies which would meet the quarantine requirements for both New Zealand and interstate trade.

This research involved specific disinfestation experimentation, large scale treatment confirmatory trials and evaluation of the effects of heat treatments on fruit quality and shelf life. This project has resulted in the development of non-chemical, non-damaging postharvest heat treatments against fruit flies for the following commodities

- Zucchini
- Button squash
- Rockmelon
- Honeydew
- Watermelon
- Tomato

The project outcomes will ensure the maintenance of Australia's ~$4 million annual cucurbit and $5.7 million annual tomato export markets to New Zealand and the $20 million interstate market for tomatoes if the current chemical treatments are not available in the future.

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 to the movement of fresh produce. 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.

Currently there is a heavy reliance on the use of chemical treatments in postharvest quarantine. This includes dips and sprays with the insecticide dimethoate (Rogor) or fumigation with methyl bromide, which has been identified as an ozone depleter. The use of chemical treatments is under review by regulatory bodies such as the APVMA (Australian Pesticides and Veterinary Medicine Authority) [previously National Registration Authority (NRA)] and the Codex Alimentarius Commission and there is currently a strong consumer preference for products which receive minimal or no treatment with synthetic chemicals. It is likely that the availability of chemical treatments for disinfestation will be severely restricted within the next few years when, if an alternative treatment is not in place, all exports to New Zealand will be severely constrained. It is therefore necessary to develop effective, preferably non-chemical, alternative quarantine treatments. The development of non-chemical postharvest treatments also leads to a reduction in 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.

The cucurbit and tomato industries are heavily reliant on the use of chemical insecticides for postharvest disinfestation for export of fresh product to New Zealand and interstate (Victoria, South Australia, Western Australia and Tasmania). This project aimed to develop new disinfestation treatments against fruit flies which is essential for the maintenance of Australia's ~$4 million annual cucurbit and $5.7 million annual tomato export markets to New Zealand (2002/2003 data: Office of Economic and Statistical Research, Queensland Treasury) and the $20 million interstate market for tomatoes. The loss of chemical treatments before suitable replacement treatments are developed and approved would be catastrophic for the vegetable industry in general.

Experimental methodology was based on that required by New Zealand Ministry of Agriculture and Forestry (MAF). This methodology is set out in MAF Biosecurity Authority Standard 155.02.03 – 'Specification for the Determination of Fruit Fly Disinfestation Treatment Efficacy'. Physiological research to determine the most appropriate method of applying heat to minimise loss of fruit quality was also undertaken.

This project has resulted in the development of non-chemical, non-damaging postharvest heat treatments against fruit flies for the following commodities

- Zucchini
- Button squash
- Rockmelon
- Honeydew
- Watermelon
- Tomato

While the outcomes of this research aim to meet the quarantine requirements of New Zealand they also meet the requirements of Australian states, providing non-chemical treatments for interstate trade. This research will lead to the development of commercial treatment protocols negotiated through Biosecurity Australia for export to New Zealand and through the Domestic Quarantine and Market Access Working Group for interstate trade.

3 INTRODUCTION

The cucurbit and tomato industries are heavily reliant on the use of chemicals for postharvest fruit fly quarantine disinfestation treatments for export of fresh product to New Zealand and interstate (Victoria, South Australia, Western Australia and Tasmania). These treatments are based on dips and sprays with the insecticide dimethoate (Rogor) or fumigation with methyl bromide. Dimethoate is currently under review by the Codex Alimentarius Commission and the Australian Pesticides and Veterinary Medicines Authority (APVMA) [Previously National Registration Authority (NRA)] and methyl bromide has been identified as an ozone depleter and its use is being phased out. The use of both chemicals is expected to be severely restricted within the next few years when, if an alternative treatment is not in place, export of fruit fly host commodities to New Zealand will be severely constrained. Australia exports ~$4M worth of cucurbits and $5.7M worth of tomatoes to New Zealand annually (2002/03 data: Office of Economic and Statistical Research, Queensland Treasury). The interstate market for tomatoes is valued at $20M. The loss of chemical treatments before suitable replacement treatments are developed and approved would be catastrophic for the vegetable industry in general.

It is therefore necessary to develop effective, preferably non-chemical, alternative quarantine treatments. There is also a strong preference by consumers for fresh produce that receives minimal or no treatment with synthetic chemicals. The development of non-chemical postharvest treatments also leads to a reduction in 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.

Postharvest quarantine heat treatments are widely used and accepted world-wide for a range of commodities and pests and have the advantage of being residue free. The use of hot air to disinfest fresh produce of fruit flies was first reported in Australia by Weddell (1931) and in the USA by Mason and McBride (1933). Precise control of temperature and humidity was difficult at that time, leading to significant fruit damage in many cases. As a result, the use of this treatment process lapsed, as chemical fumigants became available (Balock and Lindgren 1951). With the advent of microprocessor technology, heat treatment systems with precise control of temperature and humidity became available and successful disinfestation treatments began to be developed (Sugimoto et al. 1983; Armstrong et al.1989; Mangan and Ingle 1992; Corcoran et al. 1993; Sharp 1993). Computerised hot air treatment systems now allow programming and precise control of all treatment parameters to the extent that sensitive products can be conditioned or acclimated as part of the treatment process so minimising damage (Paull and Chen 1990). Successful hot air treatments have been developed for some members of the plant family Cucurbitaceae, for example zucchini (Corcoran et al. 1993) and netted melon (Iwata et al. 1990). The zucchini treatment developed in Australia against *Bactrocera cucumis* (Corcoran et al. 1993) did not specifically follow the New Zealand Ministry of Agriculture and Forestry (NZ MAF) procedures, as these were not available at the time. Successful hot air treatments have also been developed for some members of the plant family Solanaceae, for example capsicum (Sugimoto et al. 1983) and eggplant (Furasawa et al. 1984).

The aim of this project was to develop a new disinfestation treatment against fruit flies using heat which meets the phytosanitary requirements of the NZ MAF and thus leads to the development of a commercial treatment protocol to allow the continued export of Australian produce to New Zealand and within Australia. New Zealand MAF has a standard that describes the procedure for developing a disinfestation treatment against fruit flies. This methodology is set out in MAF Biosecurity Authority Standard 155.02.03 – 'Specification for the Determination of Fruit Fly Disinfestation Treatment Efficacy' (Anon 2001). The research reported here aimed to meet the technical requirements for the development of a treatment protocol for zucchini and button squash (*Cucurbita pepo* L.); cucumber (*Cucumis sativus* L.); rockmelon and honeydew (*Cucumis melo* L.);

watermelon (*Citrullus lanatus* (Thunb.) Matsumura & Nakia) and tomato (*Solanum lycopersicum* L.). Research to determine the most appropriate method of applying heat to minimise physiological damage to fruit was also undertaken.

4 MATERIALS AND METHODS

Experimental methodology was based on that required by New Zealand MAF (Anon 2001). This standard requires comparative testing of all immature stages of fruit fly species of quarantine importance capable of infesting the commodity for which the treatment is to be developed. These experiments are carried out '*in vitro*' using exposed insects. This research was completed in Horticulture Australia project HG96019 (Corcoran et al. 2003). The two most tolerant species/stages are determined and then used in subsequent (in-fruit) testing.

To confirm the most tolerant stage of the most tolerant species '*in vivo*' tests were conducted in each commodity against the two most tolerant life stages/species identified from the *in vitro* testing. Each commodity was infested and the fruit fly immatures allowed to develop under controlled conditions so as to be at the most tolerant life stages at the time of treatment. Extra fruit were infested and sampled at the time of treatment to confirm that the correct larval stage was being treated. Infested fruit were treated at varying temperatures/times and dose response curves were derived for each life stage in each commodity. Data analysis followed standard quantal response/bioassay procedures.

Research to determine the most appropriate method of applying heat to minimise physiological damage to fruit was also undertaken. The range of treatments available and the effects on fruit quality were determined.

The species and insect stage, which was identified as being the most tolerant *in vivo*, was then subjected to a range of doses to predict an effective treatment dose in the commodity. The predicted treatment dose was then confirmed in large scale tests on the most tolerant stage, treating more than 30 000 insects over three replicated confirmatory tests.

All trials were performed in a Sanshu vapour heat treatment system (Model No. EHK-1000-B, Sanshu Sangyo, Kagoshima, Japan). Fruit and chamber temperatures were monitored using platinum resistance probes calibrated to 0.1°C. Fruit probes were inserted into the fruit with the tip of the probe located in the centre of the fruit. Relative humidity was set above 90% for the duration of the treatments.

4.1 Entomology

4.1.1 In vitro testing

4.1.1.1 Cucurbits

Bactrocera cucumis is the only fruit fly of quarantine importance in Australia that has been recorded infesting zucchini, button squash, cucumber, rockmelon and watermelon in field situations (Hancock et al. 2000). Both *B. cucumis* and *Dirioxa pornia* (Walker) (Diptera: Trypetinae) are recorded infesting cucumbers in field situations (Hancock et al. 2000) though *D. pornia* has only been recorded as infesting ripe or damaged cucumbers and is not considered to be a potential quarantine pest based on the requirements set out by NZ MAF (Anon 2001). There are no known records of fruit fly infesting honeydew in field situations in Australia. Honeydew and rockmelon are the same species (*Cucumis melo),* and since *B. cucumis* is the only fruit fly of quarantine

importance in Australia that is recorded infesting rockmelons in field situations we performed our research for honeydew on *B. cucumis*.

Previous *in vitro* studies using exposed insects compared all stages of *B. cucumis* at 46°C (Corcoran et al. 2003). These studies found that non-feeding third instars, third instars and mature eggs were the three most tolerant stages to heat of this species (Table 1). Mature eggs were treated when they had completed 60% of their development (oviposition was considered 0% development; egg hatch was considered 100% development). Sixty percent development has been identified as a point of high heat tolerance for *B. cucumis* embryos (Corcoran 2002). Since non-feeding thirds are not present in fruit, in-fruit testing was performed on mature eggs and third instars in zucchini, button squash, cucumber, rockmelon, honeydew and watermelon to determine the most tolerant stage.

Table 1. Response to heat of exposed *B. cucumis* immatures treated with hot water immersion at 46°C (Corcoran et al. 2003) based on the complementary log-log model.

Species	Stage	LD_{99} [min] (95% fiducial limits)
Bactrocera cucumis	Young Eggs (2 hours old)	0.95 *(0.91-1.00)*
	Mature Eggs (60% development)	4.37 *(4.16-4.63)*
	First Instars	4.01 *(3.78-4.30)*
	Second Instars	3.49 *(3.15-4.08)*
	Third Instars	5.96 *(5.73-6.26)*
	Non-Feeding Third Instars	6.61 *(6.44-6.81)*

4.1.1.2 Tomato

Ceratitis capitata, B. tryoni, B. neohumeralis, B. cucumis, B. kraussi, B. musae, B. bryoniae and *B. cacuminata* are all recorded infesting tomatoes in field situations (Hancock et al. 2000). All of these species, except for *B. bryoniae* and *B. cacuminata* are considered to be potential quarantine pests based on the NZ MAF Standard (Anon 2001).

Previous *in vitro* studies using exposed insects compared all stages of the quarantine pest species infesting tomatoes at 44°C (except *B. cucumis*). Corcoran et al. (2003) showed that life stages varied in their tolerance to hot water immersion. Mature eggs and first instars were consistently significantly more tolerant than other stages when treated at 44°C based on the non-overlap of fiducial limits (Corcoran et al. 2003). Mature eggs were treated when they had completed 60% of their development (oviposition was considered 0% development; egg hatch was considered 100% development). Sixty percent development has been identified as a point of high heat tolerance for *Bactrocera* spp embryos (Corcoran 2002). When mature eggs and first instars from all the quarantine pest species were compared, *C. capitata* and *B. tryoni* were the species with the arithmetically greatest LD_{99} values (though not significantly greater than some of the other species) (Table 2).

Although no data is available on the tolerance of *B. cucumis* at 44° C, data at 46° C shows that *B. cucumis* is less tolerant to heat than *C. capitata* and *B. tryoni* (Table 3). *Ceratitis capitata* was more tolerant than *B. tryoni,* however, *B. tryoni* was used in further testing as it is endemic to Queensland whereas *C. capitata* only occurs in Western Australia. Mature eggs (60% developed) and first instars were the two most tolerant stages of *B. tryoni* at 44°C and were used in further testing.

Table 2. Comparison of fruit fly species response of exposed mature eggs and first instars to hot water immersion at 44°C (Corcoran et al. 2003) using the complementary log-log model.

Species	Stage: Mature eggs LD_{99} [min] (95% fiducial limits)	Stage: First instars LD_{99} [min] (95% fiducial limits)
Bactrocera musae	40.29 *(37.27-44.63)*	39.61 *(35.80-45.42)*
Bactrocera kraussi	40.57 *(38.75-42.78)*	24.84 *(22.09-29.20)*
Bactrocera neohumeralis	47.22 *(44.09-51.61)*	36.41 *(33.04-42.13)*
Bactrocera tryoni	52.96 *(48.85-58.58)*	40.78 *(38.58-43.69)*
Ceratitis capitata	56.55 *(50.66-65.96)*	60.76 *(56.58-66.30)*

Table 3. Response to heat of exposed *B. cucumis, B. tryoni and C. capitata* immatures treated with hot water immersion at 46°C (Corcoran et al. 2003) based on the complementary log-log model.

Species	Stage	LD_{99} [min] (95% fiducial limits)
Bactrocera cucumis	Young Eggs (2 hours old)	0.95 (0.91-1.00)
	Mature Eggs (60% development)	4.37 *(4.16-4.63)*
	First Instars	4.01 *(3.78-4.30)*
	Second Instars	3.49 *(3.15-4.08)*
	Third Instars	5.96 *(5.73-6.26)*
	Non-Feeding Third Instars	6.61 *(6.44-6.81)*
Bactrocera tryoni	Young Eggs (2 hours old)	2.51 *(2.15-3.13)*
	Mature Eggs (60% development)	7.69 *(7.18-8.39)*
	First Instars	11.58 *(10.63-12.99)*
	Second Instars	5.24 *(4.59-6.33)*
	Third Instars	9.95 *(9.30-10.83)*
	Non-Feeding Third Instars	8.87 *(8.47-9.42)*
Ceratitis capitata	Young Eggs (2 hours old)	3.75 *(3.42-4.22)*
	Mature Eggs (60% development)	9.86 *(9.17-10.75)*
	First Instars	20.49 *(18.31-23.78)*
	Second Instars	14.19 *(13.24-15.43)*
	Third Instars	19.43 *(18.34-20.79)*
	Non-Feeding Third Instars	10.00 *(9.64-10.44)*

4.1.2 Fruit fly colonies

Laboratory colonies of *Bactrocera cucumis* (French) (Cucumber fly) and *Bactrocera tryoni* (Froggatt) (Queensland fruit fly) were required to perform this research. Colonies of *B. cucumis* and *B. tryoni* were established and maintained at the Department of Primary Industries and Fisheries laboratories in Cairns and Indooroopilly, Queensland.

Fruit fly adults were held in 65x65x65cm 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. Both species were provided water, sugar, and autolyzed brewers yeast from emergence.

Bactrocera tryoni was 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

collection cup, punctured using a pin 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. *Bactrocera cucumis* was cultured using a similar method but using a pumpkin-based semi-artificial diet as described by Swaine et al. (1978).

4.1.3 Most tolerant stage testing (in vivo)

4.1.3.1 Artificial infesting methods

Insect development studies were performed by artificially infesting the test fruit and allowing the insects to develop under controlled conditions. Periodic samples were taken to determine the development time required to enable treatment of the correct life stages.

In trials against eggs, *B. cucumis* eggs were collected by placing a hollow zucchini dome punctured using a pin into the cage holding gravid females for a period of 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. The filter papers carrying the eggs were placed on cellulose sponge saturated with water. The filter paper was cut into pieces, each containing the required number of eggs to infest each piece of fruit. *Bactrocera tryoni* eggs were collected and handled similarly, however eggs were collected by placing artificial plastic egging cups punctured using a pin and smeared with orange juice into the cage holding gravid females for approximately 1 hour.

For zucchini, button squash and cucumber to be infested with *B. cucumis* a cork borer was used to cut a cylindrical hole sideways all the way through the fruit. The end of the removed section (approximately 10mm in height) was cut off and placed back into one end of the hole and sealed with paraffin wax to create a well in the fruit. The cut black filter papers containing the eggs were placed into the fruit so that the length of the filter paper was touching the flesh of the fruit. The infested fruit was held overnight for the eggs to develop to the required age for treatment. To ensure the eggs did not dry out a fine mist of water using a water spray bottle was used to wet the infested eggs.

For third instar infestation treatments *B. cucumis* eggs were collected in zucchini domes and placed on pumpkin media to develop. The fruit were prepared as for eggs, and infesting also occurred on the day prior to treatment. Larvae from the media were placed into water and counted by pipetting into petri dishes. Once counted the larvae were drained of water using 1 ply tissue, the tissue inverted so that the larvae were visible and this end was placed first into the fruit. The infested fruit was held overnight for larvae to develop in fruit to third instars.

On treatment day fruit containing eggs and larvae were prepared in the same way. Before treatment a cork borer was used to cut wads from spare fruit. The cork borer size was slightly larger than used on the previous day to ensure a good fit. The end of the wad (approximately 10mm in height) was placed in the top of the fruit and sealed with wax. For heat treatments waterproof tape was also used over the waxed sections of the fruit for extra security.

For rockmelon and honeydew to be infested with *B. cucumis* the method used was similar, however instead of using a cork borer a circular sliced section of flesh was cut from the fruit, and some flesh was removed from the fruit before adding eggs and larvae. Before treatment the slice was waxed and taped into place. For watermelon to be infested with *B. cucumis* the method used was again similar, however a square wedged section of flesh was cut out of the fruit, and some flesh was removed from the fruit before adding eggs and larvae. Before treatment the wedge was waxed and taped into place.

For tomatoes to be infested with *B. tryoni*, a square wedged section of flesh was cut out of the fruit, and some flesh removed from the fruit before adding the eggs. The day prior to treatment eggs were counted on the black filter paper and placed into the fruit so that the length of the filter paper was touching the flesh of the fruit. To ensure the eggs did not dry out a fine mist of water using a water spray bottle was used to wet the infested eggs. For first instar treatments *B. tryoni* eggs were collected and placed into the fruit to develop. Before treatment the wedge was waxed and taped into place.

Extra fruit were infested to contain the larval stage being tested. These extra fruit were sampled at the time of treatment and the life stages present in the fruit recorded to confirm that the correct larval stage was being treated. Control fruit were held under standard conditions of temperature and humidity (approximately 26-27°C and 70-75%RH) while treated fruit were being treated. After treatment, control fruit and treated fruit were placed on gauzed plastic crispers to allow surviving insects to develop and to allow excess liquid from fruit breakdown to drain away. The crispers were held in larger crispers with gauzed lids containing sawdust as a pupation medium. Control and treated fruit were held under standard conditions of temperature and humidity and surviving pupae were collected.

4.1.3.2 Zucchini

Insect development studies of *B. cucumis* demonstrated that eggs reached 60% development (mature eggs) at 16 hours, and that third instars were to be treated at 4 days in zucchini. Three replicates treating mature eggs and third instars were performed to determine the most tolerant stage. Fifty insects were counted and placed into each test fruit, with 500 insects (10 fruit) treated at each dose in each replicate. Organic zucchinis artificially infested with mature eggs and third instars of *B. cucumis* were treated simultaneously in the vapour heat treatment system. The chamber of the vapour heat treatment system was programmed to ramp from 25°C to 49°C over 1:08 hours. Test fruit for a given dose containing each life stage, were removed from the chamber once their core temperatures reached 43, 44, 45, 46, 47 and 48°C, being shower cooled to 30°C immediately after being removed from the vapour heat treatment system.

4.1.3.3 Button squash

Insect development studies of *B. cucumis* demonstrated that eggs reached 60% development (mature eggs) at 16 hours, and that third instars were to be treated at 4 days in button squash. Three replicates treating mature eggs and third instars were performed to determine the most tolerant stage. Fifty insects were counted and placed into each test fruit, with 500 insects (10 fruit) treated at each dose in each replicate. Organic button squash artificially infested with mature eggs and third instars of *B. cucumis* were treated simultaneously in the vapour heat treatment system. The chamber of the vapour heat treatment system was programmed to ramp from 30°C to 46.5°C over 2 hours. Test fruit for a given dose containing each life stage, were removed from the chamber after being treated for 0, 2.5, 5, 7.5, 10 and 15 minutes once their core temperatures had reached 45°C. The fruit was shower cooled to 30°C immediately after being removed from the vapour heat treatment system.

4.1.3.4 Cucumber

Insect development studies of *B. cucumis* demonstrated that eggs reached 60% development (mature eggs) at 16 hours, and that third instars were to be treated at 5 days in cucumber. Three replicates treating mature eggs and third instars were performed to determine the most tolerant stage. Fifty insects were counted and placed into each test fruit, with 500 insects (10 fruit) treated at each dose in each replicate. Organic cucumbers artificially infested with mature eggs and third

instars of *B. cucumis* were treated simultaneously in the vapour heat treatment system. The chamber of the vapour heat treatment system was programmed to ramp from 25°C to 46°C over 1 hour. Test fruit for a given dose containing each life stage, were removed from the chamber once their core temperatures reached 37, 39, 41, 42, 43, 44 and 45°C, being shower cooled to 30°C immediately after being removed from the vapour heat treatment system.

4.1.3.5 Rockmelon

Insect development studies of *B. cucumis* demonstrated that eggs reached 60% development (mature eggs) at 16 hours, and that third instars were to be treated at 5 days in rockmelon. Three replicates treating mature eggs and third instars were performed to determine the most tolerant stage. Two hundred insects were counted and placed into each test fruit, with 600 insects (3 fruit) treated at each dose in each replicate. Organic rockmelons artificially infested with mature eggs and third instars of *B. cucumis* were treated simultaneously in the vapour heat treatment system. The chamber of the vapour heat treatment system was programmed to ramp from 25°C to 46°C over 1 hour. Test fruit for a given dose containing each life stage, were removed from the chamber once their core temperatures reached 37, 39, 41, 43, 44 and 45°C, being shower cooled to 30°C immediately after being removed from the vapour heat treatment system.

4.1.3.6 Honeydew

Insect development studies of *B. cucumis* demonstrated that eggs reached 60% development (mature eggs) at 16 hours, and that third instars were to be treated at 5 days in honeydew. Three replicates treating mature eggs and third instars were performed to determine the most tolerant stage. Two hundred insects were counted and placed into each test fruit, with 600 insects (3 fruit) treated at each dose in each replicate. Organic honeydews artificially infested with mature eggs and third instars of *B. cucumis* were treated simultaneously in the vapour heat treatment system. The chamber of the vapour heat treatment system was programmed to ramp from 25°C to 46°C over 1 hour. Test fruit for a given dose containing each life stage, were removed from the chamber once their core temperatures reached 37, 39, 41, 42, 43, 44 and 45°C, being shower cooled to 30°C immediately after being removed from the vapour heat treatment system.

4.1.3.7 Watermelon

Insect development studies of *B. cucumis* demonstrated that eggs reached 60% development (mature eggs) at 15-16 hours, and that third instars were to be treated at 5 days in watermelon. Three replicates treating mature eggs and third instars were performed to determine the most tolerant stage. Three hundred insects were counted and placed into each test fruit, with 600 insects (2 fruit) treated at each dose in each replicate. Organic watermelons artificially infested with mature eggs and third instars of *B. cucumis* were treated simultaneously in the vapour heat treatment system. The chamber of the vapour heat treatment system was programmed to ramp from 25°C to 46°C over 1 hour. Test fruit for a given dose containing each life stage, were removed from the chamber once their core temperatures reached 35, 37, 39, 40, 41 and 42°C, being shower cooled to 33°C immediately after being removed from the vapour heat treatment system. In the first replicate the ambient water temperature was 31-32°C, therefore the fruit was cooled to 33°C core temperature. In subsequent replicates fruit were cooled to a core temperature of 33°C to ensure consistency in methods.

4.1.3.8 *Tomato*

Insect development studies of *B. tryoni* demonstrated that eggs reached 60% development (mature eggs) at 24 hours, and that first instars were to be treated at 52 hours in tomatoes. Four replicates treating mature eggs and first instars were performed to determine the most tolerant stage. One hundred insects were counted and placed into each test fruit, with 500 insects (5 fruit) treated at each dose in each replicate. Organic tomatoes artificially infested with mature eggs and first instars of *B. tryoni* were treated simultaneously in the vapour heat treatment system. The chamber of the vapour heat treatment system was programmed to ramp from 25°C to 45°C over 1 hour. Test fruit for a given dose containing each life stage, were removed from the chamber after being treated for 0, 5, 10, 15, 20 and 30 minutes once their core temperatures had reached 44°C. The fruit was shower cooled to 30°C immediately after being removed from the vapour heat treatment system.

4.1.4 Preliminary trials

Our original aim was to develop a generic treatment for cucurbits (Family: Cucurbitaceae) against *B. cucumis*. Unfortunately due to preliminary fruit quality testing results it became apparent that a generic dose for all cucurbits would not be possible. We therefore aimed to develop two treatments, one for cucurbit vegetables (zucchini, button squash and cucumber) and one for cucurbit fruits (rockmelon, honeydew and watermelon).

4.1.4.1 Cage infesting of fruit

Insect development studies were performed by cage infesting the test fruit and allowing the insects to develop under controlled conditions. Periodic samples were taken to determine the development time required to enable treatment of the correct life stage.

Cage infesting of fruit involves pin holing each fruit to assist in obtaining an increased and even distribution of insects within each fruit and more uniform infestation level across all fruit. Fruit were placed in cages of laboratory cultured flies containing approximately 15 000 adults and the females allowed to oviposit eggs for a time so as not to over or under infest the test fruit. Samples from each cage of the infested fruits were kept as control fruit to estimate the number of insects treated.

4.1.4.2 Cucurbit vegetables

Zucchini was chosen as the test fruit for further trials to develop a generic treatment, as the fruit circumference was generally smaller than that of button squash and cucumber. The time for zucchini to heat to the required core temperature would be less than that of fruit with a larger fruit circumference giving an overall shorter heat time.

In initial trials, zucchini were artificially infested with *B. cucumis* mature eggs (60% developed) at an age of 16 hours and subjected to a vapour heat treatment. The fruit were heated until the core temperature of the fruit was 45°C and samples of fruit were removed from the chamber after the core temperature of the fruit had been at 45°C for 10, 15, 20, 25, 30 and 35 minutes. Fruit were removed from the vapour heat treatment system at the designated times and shower cooled immediately to 30°C. The vapour heat treatment system was programmed to ramp from 30°C to 46°C over 2 hours. Fifty insects were counted and placed into each test fruit, with 500 insects treated at each dose. After treatment, control fruit and treated fruit were held under standard conditions of temperature and humidity and surviving pupae were collected (as described in *Most tolerant stage testing (in vivo),* page 10).

Based on the results of this initial trial, a dose of 45°C for 35 minutes was chosen for further tests using higher numbers of fruit and insects. Zucchinis were cage infested and treated to a core temperature of 45°C for 35 minutes and shower cooled immediately to 30°C. The vapour heat treatment system was programmed to ramp from 30°C to 46°C over 2 hours.

Based on the results from the above trial, an additional trial was run using the same vapour heat treatment system parameters as above, treating zucchini to confirm that a core temperature of 45°C for 40 minutes would be an adequate dose.

4.1.4.3 Cucurbit fruits

Rockmelon was chosen as the test fruit for further trials to develop a generic treatment, as the fruit circumference was generally equal to or smaller than that of honeydew and watermelon. There are also no known records of fruit fly infesting honeydew in field situations in Australia. The time for rockmelon to heat to the required core temperature would be less than that of fruit with a larger fruit circumference giving an overall shorter heat time.

Bactrocera cucumis mature eggs (60% developed) at an age of 16 hours were tested in rockmelon trials to determine a confirmatory dose. Initially large numbers of insects were treated with a vapour heat treatment where the core temperature of the fruit was 45°C and treatment times were 0, 5, 10, 15 and 20 minutes. Fruit were shower cooled to 30°C immediately after being removed from the vapour heat treatment system. The vapour heat treatment system was programmed to ramp from 30°C to 46°C over 1 hour.

Based on the results of the above trial and fruit quality studies, further trials were conducted testing large numbers of insects with a vapour heat treatment where the core temperature of the fruit was 44°C and treatment times were 0, 15, 30, 45 and 60 minutes. Fruit were shower cooled to 30°C immediately after being removed from the vapour heat treatment system. The vapour heat treatment system was programmed to ramp from 30°C to 45°C over 1 hour. After treatment, control fruit and treated fruit were held under standard conditions of temperature and humidity and surviving pupae were collected (as described in *Most tolerant stage testing (in vivo),* page 10).

4.1.4.4 Tomato

Bactrocera tryoni mature eggs (60% developed) at an age of 24 hours were tested in trials to determine a confirmatory dose. The dose initially used in the preliminary trials was a fruit core temperature of 44°C for 90 minutes. However, this dose failed with survivors recovered from the treated fruit. Treatment time was then increased to 120 minutes at 44°C and again survivors were recovered. Therefore it was decided to increase the treatment core temperature to 45°C.

Treatments at a core temperature of 45°C for 60, 75 and 90 minutes, were tested with the vapour heat treatment system programmed to ramp from 30°C to 46°C over 2 hours. Fruit were cooled to 35°C immediately after treatment. After treatment, control fruit and treated fruit were held under standard conditions of temperature and humidity and surviving pupae were collected (as described in *Most tolerant stage testing (in vivo),* page 10).

4.1.5 Confirmatory testing

4.1.5.1 Cucurbit vegetables

In an attempt to confirm a generic cucurbit vegetable treatment, *B. cucumis* mature eggs (60% developed) at an age of 16 hours were tested in zucchinis. Each fruit was pin holed 5 times, placed in cages of laboratory cultured *B. cucumis* and the females were allowed to oviposit eggs for an average over the three trials of 12 minutes. Samples from each cage of the infested fruit were kept as control fruit to estimate the number of insects treated. In these trials the ratio of control to treated fruit was 1:5. Three replicated trials were carried out testing a confirmatory dose of 45°C core temperature for 40 minutes. The vapour heat treatment system was programmed to ramp from 30°C to 46°C over 2 hours. Once fruit were treated they were immediately shower cooled to 30°C. After treatment, control and treated fruit were held under standard conditions of temperature and humidity and surviving pupae were collected (as described in *Most tolerant stage testing (in vivo),* page 10). The number of pupae recovered from the controls was used to estimate the number of insects treated (i.e. Estimated number treated = 5*Number of pupae recovered from the controls).

An additional infestation experiment was carried in conjunction with the confirmatory trials to determine the inherent variability in the cage infestation procedure used for estimating the number of insects treated. One cage of infested fruit was separated into two groups, a simulated control group and a simulated treated fruit group as above. No treatment was applied to either group. Both groups of fruit were held as above so that pupae could be recovered and counted. Actual numbers from the simulated treated group were determined by counting recovered pupae. The number of pupae recovered from the simulated control group was used to calculate an estimate of the number treated. Comparison of the actual count and the estimated number treated provides an indication of the inherent variability in the number of eggs oviposited during cage infestation. This check on cage infested fruit variability was also performed during the confirmatory trials for cucurbit fruits and tomatoes.

4.1.5.2 Cucurbit fruits

In an attempt to confirm a generic cucurbit fruit treatment, *B. cucumis* mature eggs (60% developed) at an age of 16 hours were tested in organic rockmelons. Each fruit was pin holed 50 times, placed in cages of laboratory cultured *B. cucumis* and the females were allowed to oviposit eggs for an average over the three trials of 47 minutes. Samples from each cage of the infested fruit were kept as control fruit to estimate the number of insects treated. In these trials the ratio of control to treated fruit was 1:3. Three trials were carried out testing a confirmatory dose of 44°C core temperature for 0 minutes. The vapour heat treatment system was programmed to ramp from 30°C to 45°C over 1 hour. Once fruit reached a core temperature of 44°C they were immediately shower cooled to 35°C. After treatment, control and treated fruit were held under standard conditions of temperature and humidity and surviving pupae were collected (as described in *Most tolerant stage testing (in vivo),* page 10). The number of pupae recovered from the controls was used to estimate the number of insects treated (i.e. Estimated number treated = 3*Number of pupae recovered from the controls).

4.1.5.3 Tomato

In an attempt to confirm a treatment for tomatoes, *B. tryoni* mature eggs (60% developed) at an age of 24 hours were tested in organic tomatoes. Each fruit was pin holed 10 times, placed in cages of laboratory cultured *B. tryoni* and the females were allowed to oviposit eggs for an average over the three trials of 32 minutes. Samples from each cage of the infested fruit were kept as control fruit to estimate the number of insects treated. In these trials the ratio of control to treated fruit was 1:5.

Three trials were carried out testing a confirmatory dose of 45°C core temperature for 90 minutes. The vapour heat treatment system was programmed to ramp from 30°C to 46°C over 2 hours. Once fruit were treated they were immediately shower cooled to 35°C. After treatment, control and treated fruit were held under standard conditions of temperature and humidity and surviving pupae were collected (as described in *Most tolerant stage testing (in vivo),* page 10). The number of pupae recovered from the controls was used to estimate the number of insects treated (i.e. Estimated number treated = 5*Number of pupae recovered from the controls).

4.1.6 Data Analysis

In 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 where p is the proportion mortality and X 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 6 (GenStat 2002). 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:

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

Linearisation 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 LD_{99} 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 LD_{99} to supplement visual examination.

4.2 Fruit Studies

Disinfestation treatments which satisfy regulatory requirements for access to a market with quarantine restrictions, need only demonstrate that the required level of efficacy has been achieved with the particular pest. However, commercial acceptability of a treatment requires that the treatment does not induce quality loss. Consequently, the evaluation of quality effect of treatment are a key component of any treatment development.

The work described here was performed within the development cycle of the insect mortality studies, except for the studies of tomato quality, which were completed within other projects (Jordan and Cavallaro 2000, Jordan et al 2000, Jordan 2003).

4.2.1 Cucurbit vegetables

A single generic treatment based on insect efficacy trials was tested on zucchini, button squash and cucumber for evaluation of fruit quality following a period of storage. The treatment consisted of a 2 hour ramp of air temperature from 25°C to 46°C until a core temperature of the slowest monitored probe fruit attained a temperature of 45°C for 40 minutes.

4.2.1.1 Zucchini

Fruit

Zucchini (green skin, unspecified cultivar) were harvested from 3 commercial growers in the production area of Ayr, North Queensland in July 2002 and were held at 10°C in the packing shed before road transport to Cairns in air conditioning the same day. Upon arrival at the Cairns laboratories, fruit were sorted for uniformity of size and shape, with any blemished or disease fruit removed. Fruit were stored overnight at ambient temperature prior to heat treatment.

Treatment

Eighty fruit of weight range (148-250g) from each grower were randomly assigned to two groups for storage for 7 and 14 days after treatment. Fruit from each group was then randomly allocated to groups of 30 for heat treatment and 10 as untreated controls. Untreated fruit were held at ambient temperature for the duration of the treatment.

Fruit were treated in an experimental Sanshu Vapour Heat Treatment System (Model No. EHK-1000-B) within 48 hours from time of harvest. Fruit temperatures were measured using platinum resistance probes, calibrated to 0.1°C and inserted into the blossom end of the fruit. The tip of the probe was located halfway along the fruit near the centre of the placental tissue. Air temperature in the chamber was programmed to run up linearly from 25°C to 46°C over 2 hours. Relative humidity was maintained above 90% for the duration of the treatment.

When the lowest temperature being monitored had reached 45°C, treatment was continued for a further 40 minutes. Cooling was achieved using a flood spray of ambient temperature water until the centre temperature of the fruit had stabilised near 30°C.

After treatment, fruit were removed and stored at 10°C and >90% relative humidity for 7 and 14 days.

Quality assessment

(i) External appearance. A visual measure of general acceptability (GA) was made using the 1-9 scale (1=dislike extremely, 9=like extremely). Skin colour was measured at 3 sites on the skin surface, using a Minolta Colormeter model CR300 fitted with an 8mm orifice and a 0° observer. Data was collected as L'a'b' units and converted to chroma and hue (McGuire, 1992).

(ii) External rots. The severity of external rots was visually assessed and calculated as percentage of coverage using the 0-5 scale (0=nil; 1=<1 cm^2; 2=<2cm^2; 3=<25%; 4=25-50%; 5=>50%). The incidence of rots was calculated as the percentage of fruit with a rating of 2 or greater.

(iii) External injury. Skin pitting, manifested as sunken areas on the skin was visually assessed using the 0-5 scale (0=nil; 5=very severe). The incidence of pitting was calculated as the percentage of fruit with a rating of 2 or greater.

(iv) Fruit firmness was measured using a Chatillon digital force gauge fitted with a 12mm spherical probe. Measurements were taken at the skin surface and were recorded as the Newton (N) force required to displace 2mm on the fruit surface.

(v) Internal injury. Fruit were sliced into halves from stem to blossom end rated for any visible internal injuries. Fruit halves were also inspected for the presence of internal rots.

(vi) Eating quality. One half portion from each fruit previously cut was sliced into 1cm thick pieces, bulked and mixed thoroughly. Samples for tasting were cooked in a 900W microwave oven on 'high' setting for 2 minutes, presented hot to a panel of 10 members and rated for general acceptability of flavour using the hedonic scale of 1=dislike extremely, 9=like extremely.

(vii) Chemical analysis. A portion of each composite sample was finely ground for determination of total acidity by titration to pH 8.1 with 0.1N NaOH and expressed as % citric acid (Schott Gerate Model Titroline 96 autotitrator). A few drops of juice were measured for total soluble solids using an Atago Model 3T refractometer.

4.2.1.2 Button squash

Fruit

Button squash (gold skinned, unspecified cultivar) were harvested from 3 commercial growers in the production area of Ayr, North Queensland in July 2002 and were held at 10°C in the packing shed before road transport to Cairns in air conditioning the same day. Upon arrival at the Cairns laboratories, fruit were sorted for uniformity of size and shape, with any blemished or disease fruit removed. Fruit were stored overnight at ambient temperature prior to heat treatment.

Treatment

Eighty fruit of weight range (63-88g) from each grower were randomly assigned to two groups for storage for 7 and 14 days after treatment. Fruit from each group was then randomly allocated to groups of 30 for heat treatment and 10 as untreated controls. Untreated fruit were held at ambient temperature for the duration of the treatment.

Fruit were treated in an experimental Sanshu Vapour Heat Treatment System (Model No. EHK-1000-B) approximately 36 hours from time of harvest. Fruit temperatures were measured using platinum resistance probes, as described previously, and inserted into the side of the fruit with the tip located near the centre of the placental tissue. Air temperature in the chamber was programmed

to run up linearly from 25°C to 46°C over 2 hours. Relative humidity was maintained above 90% for the duration of the treatment.

When the lowest temperature being monitored had reached 45°C, treatment was continued for a further 40 minutes. Fruit were cooled using a flood spray of ambient temperature water until the centre temperature of the fruit had stabilised at 30°C.

After treatment, fruit were removed and stored at 10°C and >90% relative humidity for 7 and 14 days.

Quality assessment

(i) External appearance. A visual measure of general acceptability was made using the 1-9 scale (1=dislike extremely, 9=like extremely). Skin colour was measured at 3 sites on the skin surface, using a Minolta Colormeter model CR300 fitted with an 8mm orifice and a 0° observer. Data was collected as L'a'b' units and converted to chroma and hue (McGuire, 1992).

(ii) External rots. The severity of external rots was visually assessed and calculated as percentage of area affected using the 0-5 scale (0=nil; 1=<1 cm^2; 2=<2cm^2; 3=<25%; 4=25-50%; 5=>50%). The incidence of rots was calculated as the percentage of fruit with a rating of 2 or greater.

(iii) External injury. Skin pitting, manifested as sunken areas on the skin was visually assessed using the 0-5 scale (0=nil; 5= very severe). The incidence of pitting was calculated as the percentage of fruit with a rating of 2 or greater.

(iv) Fruit firmness was measured using a Chatillon digital force gauge fitted with a 12mm spherical probe. Measurements were taken at the skin surface and were recorded as the Newton force required to displace 2mm on the fruit surface.

(v) Internal injury. Fruit were sliced into halves from stem to blossom end and rated for internal injury. The incidence of placental tissue breakdown was calculated as the percentage of fruit with a severity rating of 2 or greater.

(vi) Eating quality. One half portion from each fruit previously cut was further cut into quarters, bulked and mixed thoroughly. Samples for tasting were cooked on high for 2 minutes in a 900W microwave oven, presented hot to a panel of 10 members and rated for general acceptability of flavour using the hedonic scale of 1=dislike extremely, 9=like extremely.

(vii) Chemical analysis. A portion of each composite sample was finely ground for determination of total acidity by titration to pH 8.1 with 0.1N NaOH and expressed as % citric acid (Schott Gerate Model Titroline 96 autotitrator). A few drops of juice of each composite sample was measured for total soluble solids using an Atago Model 3T refractometer.

4.2.1.3 Cucumber

Fruit

Cucumbers (green slicing type, cultivar unspecified) were harvested from 3 commercial growers in the production area of Gumlu, North Queensland in July 2003 and were held at ambient temperature in the packing shed before road transport to Cairns in air conditioning the same day. Fruit were stored overnight at ambient temperature in the laboratory then sorted for uniformity of size and shape, with any blemished or disease fruit removed.

Treatment
Forty fruit of weight range (378-504g) were selected from each grower and randomly assigned into two groups, 30 for heat treatment and 10 as untreated controls. Untreated fruit were held at ambient temperature for the duration of the treatment.

Fruit were treated in an experimental Sanshu Vapour Heat Treatment System (Model No. EHK-1000-B) within 48 hours from harvest. Fruit temperatures were measured using platinum resistance probes, calibrated to 0.1°C and inserted into the blossom end of the fruit. The tip of the probe was located halfway along the fruit near the centre of the placental tissue. Air temperature in the chamber was programmed to run up linearly from 25°C to 46°C over 2 hours. Relative humidity was maintained above 90% for the duration of the treatment.

When the lowest temperature being monitored had reached 45°C, treatment was continued for a further 40 minutes. Treated fruit were cooled with a flood spray of ambient temperature water until the centre temperature of the fruit had stabilised near 30°C.

After treatment, fruit were removed and stored at 11°C and 90-95% relative humidity for 7 days.

Quality assessment

(i) External appearance. A visual measure of general acceptability was made using the 1-9 scale (1=dislike extremely, 9=like extremely). Factors influencing general acceptability score included yellowing on the skin and loss of glossy appearance to the skin. Skin colour was measured at 3 sites on the skin surface, using a Minolta Colormeter model CR300 fitted with an 8mm orifice and a 0° observer. Data was collected as L'a'b' units and converted to chroma and hue (McGuire, 1992).

(ii) External rots. The severity of external rots was visually assessed and calculated as percentage of coverage using the 0-5 scale (0=nil; 1=<1 cm^2; 2=<2cm^2; 3=<25%; 4=25-50%; 5=>50%). The incidence of rots was calculated as the percentage of fruit with a rating of 2 or greater.

(iii) External injury. Sunken cavities on the skin were visually assessed using the 0-5 scale (0=nil; 5=very severe). The incidence of skin shrivelling was calculated as the percentage of fruit with a rating of 2 or greater.

(iv) Fruit firmness was measured using a Chatillon digital force gauge fitted with a 12mm spherical probe. Measurements were taken at the skin surface and were recorded as the Newton force required to displace 2mm on the fruit surface.

(v) Internal injury. Fruit were sliced into halves from stem to blossom end and rated for internal injury using the 0-5 scale (0=nil; 5=very severe). The incidence of internal injury symptoms such as internal cavities and translucent flesh was calculated as the percentage of fruit with a severity rating of 2 or greater.

(vi) Chemical analysis. A portion of each composite sample was finely ground for determination of total acidity by titration to pH 8.1 with 0.1N NaOH and expressed as % citric acid (Schott Gerate Model Titroline 96 autotitrator). A few drops of juice of each composite sample was measured for total soluble solids using an Atago Model 3T refractometer.

4.2.2 Cucurbit fruits

A generic treatment was developed for honeydew, rockmelon and watermelon. The treatment consists of a 1 hour ramp of air temperature from 30 to 45°C until a core temperature of the slowest monitored probe fruit has attained a temperature of 44°C. The evaluation of fruit quality using this treatment has been tested on "seedless" watermelon. Prior to development of the final generic treatment, fruit quality testing of honeydew and rockmelon was undertaken at 44°C core temperature for 30 minutes to allow for uncertainty in insect responses. It is expected that a further reduction in time will have no detrimental affects and possibly some improvements to fruit quality of honeydew and rockmelon.

4.2.2.1 Rockmelon

Fruit

Rockmelon (round netted type, cultivar unspecified) were harvested from 3 commercial growers in the production area of Ayr, North Queensland in October 2002 and were held at ambient temperature in the packing shed before road transport to Cairns in air conditioning the same day. Upon arrival at the Cairns laboratories fruit were held at 20°C overnight and sorted for uniformity of size, shape and freedom of disease or visible defects the following morning.

Treatment

Twenty-five fruit of weight range (880-1170g) from each grower were randomly assigned into groups of 15 for heat treatment and 10 as untreated controls. Untreated fruit were held at ambient temperature for the duration of the treatment.

Fruit were treated in an experimental Sanshu Vapour Heat Treatment System (Model No. EHK-1000-B) within 36 hours from harvest. Fruit temperatures were measured using the same platinum resistance probes described previously and inserted into the side of the fruit with the tip of the probe located near the centre of the seed cavity. Air temperature in the chamber was programmed to run up linearly from 30°C to 45°C over 1 hour. Relative humidity was maintained above 90% for the duration of the treatment.

When the lowest temperature being monitored had reached 44°C for 30 minutes, fruit were immediately cooled with a flood spray of ambient temperature water until the centre temperature of the fruit had stabilised near 30°C.

After treatment, fruit were removed and stored at 10°C and 80-85% relative humidity for 7 days.

Quality Assessment

(i) External appearance. A visual measure of general acceptability was made using the 1-9 scale (1=dislike extremely, 9=like extremely). Skin colour was measured at 3 sites on the skin surface, using a Minolta Colormeter model CR300 fitted with an 8mm orifice and a 0° observer. Data was collected as L'a'b' units and converted to chroma and hue (McGuire, 1992).

(ii) Internal injury. Fruit were sliced into halves from stem to blossom end and rated for internal injury using the 0-5 scale (0=nil; 5=very severe). Internal colour was measured using the Minolta Colormeter previously described at 3 points on the pulp of the cut fruit halves.

(iii) Fruit firmness was measured using a Chatillon digital force gauge fitted with a 12mm spherical probe. Two measurements were taken at the skin surface approximately 90° apart on each fruit and were recorded as the Newton force required to displace 2mm on the fruit surface.

(iv) Eating quality. Fruit halves were prepared into random samples as previously described and were presented to a panel of 10 members and rated for general acceptability of flavour using the hedonic scale (1=dislike extremely, 9=like extremely).

(v) Chemical analysis. A portion of each composite sample was finely ground and a few drops were used to measure for total soluble solids using an Atago Model 3T refractometer.

4.2.2.2 Honeydew

Fruit

Honeydew melon (green flesh, cultivar unspecified) were harvested from 3 commercial growers in the production area of Ayr, North Queensland in October 2002 and were held at ambient temperature in the packing shed before road transport to Cairns in air conditioning the same day. Upon arrival at the Cairns laboratories fruit were held at 20°C overnight and sorted for uniformity of size, shape and freedom of disease or visible defects the following morning.

Treatment

Twenty-five fruit of weight range (1050-1350g) from each grower were randomly assigned into groups of 15 for heat treatment 10 as untreated controls. Untreated fruit were held at ambient temperature for the duration of the treatment.

Fruit were treated in an experimental Sanshu Vapour Heat Treatment System (Model No. EHK-1000-B) within 36 hours from harvest. Fruit temperatures were measured using the same platinum resistance probes described previously and inserted into the side of the fruit with the tip of the probe located near the centre of the seed cavity. Air temperature in the chamber was programmed to run up linearly from 30°C to 45°C over 1 hour. Relative humidity was maintained above 90% for the duration of the treatment.

When the lowest temperature being monitored had reached 44°C for 30 minutes, fruit were immediately cooled with a flood spray of ambient temperature water until the centre temperature of the fruit had stabilised near 30°C.

After treatment, fruit were removed and stored at 10°C and 80-85% relative humidity for 7 days.

Quality assessment

(i) External appearance. A visual measure of general acceptability was made using the 1-9 scale (1=dislike extremely, 9=like extremely). Skin colour was measured at 3 sites on the skin surface, using a Minolta Colormeter model CR300 fitted with an 8mm orifice and a 0° observer. Data was collected as L'a'b' units and converted to chroma and hue (McGuire, 1992).

(ii) External injury. Skin pitting, characterised by small sunken lesions on the skin was visually assessed using the 0-5 scale (0=nil, 5=very severe). The incidence of pitting was calculated as the percentage of fruit with a rating of 2 or greater.

(iii) Internal injury. Fruit were sliced into halves from stem to blossom end and rated for internal injury using the 0-5 scale (0=nil; 5=very severe). The incidence of jellyness in the seed cavity was calculated as the percentage of fruit with a rating of 2 or greater.

(iv) Internal colour. Pulp colour was measured using the Minolta Colormeter previously described at 3 points on the pulp of the cut fruit halves.

(v) Eating quality. One half portion from each fruit previously cut was peeled with seeds removed. A transverse slice from each half was cut into 2cm cubes which were bulked and mixed thoroughly. Random samples were presented to a panel of 10 members and rated for general acceptability of flavour using the hedonic scale (1=dislike extremely, 9=like extremely).

(vi) Chemical analysis. A portion of each composite sample was finely ground and a few drops were used to measure for total soluble solids using an Atago Model 3T refractometer.

4.2.2.3 Watermelon

Fruit

Watermelons (small round seedless type, cultivar unspecified) were harvested from 3 commercial growers in North Queensland, two from the Ingham region and the other from Cairns in October 2003. Fruit harvested from Ingham were held in the transport shed at ambient temperature overnight prior to road transport at ambient temperature to Cairns. Fruit from Cairns were transported at ambient temperature on the day of harvest.

Treatment

Due to the large size of the fruit and the relatively small treatment chamber size, only minimal numbers of fruit could be treated at any time. Three separate vapour heat treatments on single grower lines were performed concurrently over a two day period to enable sufficient fruit to be treated.

Fruit were randomly assigned into groups of 10 for heat treatment and 10 as untreated controls from selected weight ranges from each grower.
Grower 1 weight range - (4219-4620g)
Grower 2 weight range - (6015-7017g)
Grower 3 weight range - (6706-7612g)

Untreated fruit were held at ambient temperature for the duration of the treatment.

Fruit were treated in an experimental Sanshu Vapour Heat Treatment System (Model No. EHK-1000-B) within 48 hours from harvest. Fruit temperatures were measured using the platinum resistance probes described previously and inserted into the side of the fruit with the tip of the probe located near the centre. Air temperature in the chamber was programmed to run up linearly from 30°C to 45°C over 1 hour. Relative humidity was maintained above 90% for the duration of the treatment.

When the lowest temperature being monitored had reached 44°C, fruit were immediately cooled with a flood spray of ambient temperature water until the centre temperature of the fruit had stabilised near 30°C.

After treatment, fruit were removed and stored at 10°C and 80-85% relative humidity for 7 days.

Quality Assessment

(i) External appearance. A visual measure of skin colour was made using the 0-5 scale (0=fully green; 1=tinge yellow, 2=25% of surface area with some yellowing, 5= 100% yellowing). Skin colour was measured at 3 sites on the skin surface, using a Minolta Colormeter model CR300 fitted with an 8mm orifice and a 0° observer. Data was collected as L'a'b' units and converted to chroma and hue (McGuire, 1992).

(ii) Internal appearance. Fruit were sliced into halves from stem to blossom end and rated for pulp colour using the 0-5 scale (0=fully white; 1=tinge red, 2=25% of surface area red, 5= 100% red). Internal colour was measured using the Minolta Colormeter previously described at 3 points on the pulp of the cut fruit halves.

(iii) Internal firmness. Pulp firmness was measured using the Chatillon digital force gauge described previously. Two measurements at either end of the fruit were taken on the pulp, avoiding any natural fissures in the flesh. Firmness was recorded as the Newton force required to displace 2mm on the pulp surface.

(iv) Eating quality. Fruit halves were prepared into random samples as previously described and were presented to a panel of 10 members and rated for general acceptability of flavour using the hedonic scale (1=dislike extremely, 9=like extremely).

(v) Chemical analysis. A portion of each composite sample was finely ground and a few drops were used to measure for total soluble solids as previously described.

4.2.3 Data Analysis

The fruit injury and quality characteristics of the fruit were analysed in GenStat 6 (GenStat 2002) using a 1-way analysis of variance without blocking (except in the case of watermelons where the data was blocked on heat treatment unit). Comparison of treatment means was done using Fisher's Protected Least Significant Difference test.

5 RESULTS

5.1 Entomology studies

5.1.1 Most tolerant stage testing

5.1.1.1 Zucchini

Dose response models were fitted to the mortality data for both mature eggs and third instars of *B. cucumis*. Based on the criteria described in data analysis, the complementary log-log (CLL) model, without a log transformation of dose, was selected as the model that was most appropriate for this data. GenStat analysis showed that parallel response lines were appropriate (a non significant interaction between dose and stage, $F_{1,8}=1.31$ $p=0.285$) indicating that the relative differences between the stages were maintained across all response levels. Mature eggs had the higher LD_{99} and calculation of the relative potency showed that they were significantly more tolerant than third instars ($p<0.05$) (Table 4).

Table 4. LD_{99} and fiducial limits based on parallel dose response lines (*B. cucumis* in zucchini).

Fruit type	Life stage	LD_{99} (°C)	Fiducial limits (95%)
Zucchini	Mature Egg	46.56	46.12-47.20
	Third Instar	45.45	45.06-46.00

5.1.1.2 Button squash

Dose response models were fitted to the mortality data for both mature eggs and third instars of *B. cucumis*. Based on the criteria described in data analysis, the complementary log-log (CLL) model, without a log transformation of dose, was selected as the model that was most appropriate for this data. GenStat analysis showed that independent dose response lines for each stage were appropriate (a significant interaction between the dose and stage, $F_{1,8}$=19.66 p=0.002) indicating differences between stages are not uniform across all responses. Based on the non-overlap of the LD_{99} fiducial limits, it is clear that mature eggs were significantly more tolerant than third instars at this point (Whiting and Hoy 1997; Soderstrom et al. 1996) (Table 5).

Table 5. LD_{99} and fiducial limits based on independent dose response lines (*B. cucumis* in button squash).

Fruit type	Life stage	LD_{99} (min)	Fiducial limits (95%)
Button Squash	Mature Egg	25.50	23.21-28.56
	Third Instar	7.47	6.73-8.47

5.1.1.3 Cucumber

Dose response models were fitted to the mortality data for both mature eggs and third instars of *B. cucumis*. Based on the criteria described in data analysis, the complementary log-log (CLL) model, without a log transformation of dose, was selected as the model that was most appropriate for this data. GenStat analysis showed that independent dose response lines for each stage were appropriate (a significant interaction between the dose and stage, $F_{1,10}$=8.20 p=0.017) indicating differences between stages are not uniform across all responses. Based on the non-overlap of the LD_{99} fiducial limits, it is clear that mature eggs were significantly more tolerant than third instars at this point (Whiting and Hoy 1997; Soderstrom et al. 1996) (Table 6).

Table 6. LD_{99} and fiducial limits based on independent dose response lines (*B. cucumis* in cucumber).

Fruit type	Life stage	LD_{99} (°C)	Fiducial limits (95%)
Cucumber	Mature Egg	45.09	44.21-46.52
	Third Instar	42.39	41.85-43.19

5.1.1.4 Rockmelon

Dose response models were fitted to the mortality data for both mature eggs and third instars of *B. cucumis*. Based on the criteria described in data analysis, the complementary log-log (CLL) model, without a log transformation of dose, was selected as the model that was most appropriate for this data. Statistically, there was no significant difference between the two stages at any dose (i.e. neither slope, $F_{1,8}$=4.15 p=0.076, nor intercept, $F_{1,8}$=0.21 p=0.662, for the two dose-response lines was significantly different). At LD_{99} mature eggs were, however, arithmetically more tolerant than third instars though there was considerable overlap of the fiducial limits. Further rockmelon trials testing heat treatments were therefore performed on mature eggs (Table 7).

Table 7. LD99 and fiducial limits based on parallel dose response lines (*B. cucumis* in rockmelon).

Fruit type	Life stage	LD_{99} (°C)	Fiducial limits (95%)
Rockmelon	Mature Egg	43.70	42.90-44.83
	Third Instar	43.51	42.72-44.63

5.1.1.5 Honeydew

Dose response models were fitted to the mortality data for both mature eggs and third instars of *B. cucumis*. Based on the criteria described in data analysis, the complementary log-log (CLL) model, without a log transformation of dose, was selected as the model that was most appropriate for this data. Statistically, there was no significant difference between the two stages at any dose (i.e. neither slope, $F_{1,10}$=0.01 p=0.935, nor intercept, $F_{1,10}$=0.36 p=0.564, for the two dose-response lines was significantly different). At LD_{99} mature eggs were, however, arithmetically more tolerant than third instars though there was considerable overlap of the fiducial limits (Table 8).

Table 8. LD_{99} and fiducial limits based on parallel dose response lines (*B. cucumis* in honeydew).

Fruit type	Life stage	LD_{99} (°C)	Fiducial limits (95%)
Honeydew	Mature Egg	42.58	41.91-43.47
	Third Instar	42.34	41.69-43.21

5.1.1.6 Watermelon

Dose response models were fitted to the mortality data for both mature eggs and third instars of *B. cucumis*. Based on the criteria described in data analysis, the complementary log-log (CLL) model, without a log transformation of dose, was selected as the model that was most appropriate for this data. GenStat analysis showed that parallel response lines were appropriate (a non significant interaction between dose and stage, $F_{1,8}$=0.02 p=0.885) indicating that the relative differences between the stages are maintained across all response levels. Mature eggs again had the higher LD_{99} and calculation of the relative potency showed that they were significantly more tolerant than third instars ($p<0.05$) (Table 9).

Table 9. LD_{99} and fiducial limits based on parallel dose response lines (*B. cucumis* in watermelon).

Fruit type	Life stage	LD_{99} (°C)	Fiducial limits (95%)§
Watermelon	Mature Egg	43.33	42.57-44.35
	Third Instar	42.31	41.63-43.21

§ Note that although the fiducial limits overlap mature eggs are significantly more tolerant than third instars as indicated by both the relative potency and F test for differences in intercept ($F_{1,8}$=6.62, p=0.033). Non-overlap of the fiducial limits is sometimes considered equivalent to a test of significance at the 1% level (Whiting and Hoy 1997), however, overlap of the limits does not necessarily imply non significant differences.

5.1.1.7 Tomato

Dose response models were fitted to the mortality data for both mature eggs and first instars of *B. tryoni*. Based on the criteria described in data analysis, the complementary log-log (CLL) model, without a log transformation of dose, was selected as the model that was most appropriate for this data. Statistically, there was no significant difference between the two stages at any dose (i.e. neither slope, $F_{1,8}$=3.64 p=0.093, nor intercept, $F_{1,8}$=3.98 p=0.081, for the two dose-response lines was significantly different). At LD_{99} mature eggs were, however, arithmetically more tolerant than first instars though there was considerable overlap of the fiducial limits (Table 10).

Table 10. LD_{99} and fiducial limits based on parallel dose response lines (*B. tryoni* in tomato).

Fruit type	Life stage	LD_{99} (min)	Fiducial limits (95%)
Tomato	Mature Egg	37.43	32.01-45.93
	First Instar	34.11	29.23-41.71

5.1.2 Preliminary trials

5.1.2.1 Cucurbit vegetables

Zucchini treated at a core temperature for 45°C for a range of times showed that a treatment time of 35 minutes may be suitable as a confirmatory dose (Table 11).

Table 11. Survival of *B. cucumis* mature eggs in zucchini after treatment at 45°C for a range of times (500 insects treated at each point).

Dose	Number of survivors Pupal numbers	Corrected mortality (%)
Control	436	(87.2)
45°C/10min	217	50.2
45°C/15min	137	68.6
45°C/20min	24	94.5
45°C/25min	1	99.8
45°C/30min	0	100
45°C/35min	0	100

A trial using cage infested fruit treated zucchinis to a core temperature of 45°C for 35 minutes. A total of 47 505 insects were treated and although no pupae were recovered from this trial, one dead larva was obtained from the pupation medium. Based on this result the dose for the confirmatory trials was increased to 40 minutes.

An additional trial (45°C for 40 minutes) was undertaken before proceeding to the confirmatory trials. It resulted in zero survivors from an estimated 8 735 treated insects in zucchini. From these results a dose of 45°C for 40 minutes was chosen as the final confirmatory dose.

5.1.2.2 Cucurbit fruits

Due to the size of these larger cucurbits, the heating time to reach a set core temperature was greater than that of the cucurbit vegetables and slight deterioration in fruit quality occurred using the confirmatory dose suitable for the cucurbit vegetables. It was expected that the dose required to disinfest melons would be a lower temperature and/or time treatment due to the longer heating profiles for these fruits.

A trial using cage infested fruit treated rockmelons to a core temperature of 45°C for 0, 5, 10, 15 and 20 minutes. Zero survivors were obtained from all treatment times at 45°C, treating an estimated 17 499 insects at each dose.

Additional trials using a lower temperature were undertaken treating rockmelons to a core temperature of 44°C for 0, 15, 30, 45 and 60 minutes. Zero survivors were obtained from all

treatment times at 44°C, treating an estimated 11 235 insects at each dose. A dose of 44°C for 0 minutes was chosen as the final confirmatory dose.

5.1.2.3 Tomato

The initial confirmatory dose of a fruit core temperature of 44°C for 90 minutes was insufficient with 40 survivors obtained from an estimated number treated of 13 047 insects. Tomatoes were then treated at an increased dose of a fruit core temperature of 44°C for 120 minutes, however this trial also resulted in insect survival with four survivors from an estimated number treated of 12 087 insects.

To obtain complete kill of the insects in tomatoes it was decided to increase the fruit core temperature to 45°C and treat for 60 minutes. Several confirmatory trials were performed with these treatment parameters. A large number of insects were treated, however two trials failed as pupae were recovered from treated fruit. In the first failed trial one survivor was obtained from an estimated number treated of 14 475 insects and in the second failed trial three pupae were recovered from an estimated number treated of 16 185 insects.

Three trials were performed treating tomatoes at a core temperature of 45°C for an increased time of 75 minutes, however the third trial failed with one pupae recovered from treated fruit. Again the treatment time was increased treating the fruit to a core temperature of 45°C for 90 minutes. This was chosen as the final confirmatory dose.

5.1.3 Confirmatory testing

5.1.3.1 Cucurbit vegetables

A treatment at a core temperature of 45°C for 40 minutes was found to be an effective dose with zero survivors from a total of 109 480 *B. cucumis* insects (mature eggs) treated in 3 replicates in zucchini (Table 12). This represents a true mortality of greater than ≥99.9973% (95% confidence) (Couey and Chew 1986). From fruit quality trials undertaken this heat treatment can be used to disinfest zucchini and button squash but resulted in unacceptable damage to cucumber.

Table 12. Confirmatory trials treating *B. cucumis* eggs in zucchini at a core temperature of 45°C for 40 minutes.

Fruit	Stage/ replicate	Average weight of trial fruit	Number of fruit Control: Treated	Number of insects in controls	Estimated number of insects treated	Number of pupae surviving treatment	Mortality (%)	True mortality (≥) (95% confidence)
Zucchini	Egg 1	140g	30:150	7 524	37 620	0	100	99.9920
Zucchini	Egg 2	169g	30:150	5 705	28 525	0	100	99.9895
Zucchini	Egg 3	165g	30:150	8 667	43 335	0	100	99.9931
TOTALS	**EGG**	**158g**	**90:450**	**21 896**	**109 480**	**0**	**100**	**99.9973**

Comparison of estimated and actual numbers of pupae resulting from cage infestation showed variation (Table 13). Over the three replicates the ratio of simulated control numbers: simulated treated numbers varied between 1:3.64 and 1:4.83, where 1:5 was expected. The difference between the actual numbers of pupae and the estimated numbers were not significantly different to zero (t_2=-2.19, p=0.160, 95% confidence interval –5 398, 1 756).

Estimating treated numbers in the confirmatory trials using the average ratio of simulated control numbers: simulated treated numbers as in Table 13 (1:3.96), the estimated number treated is 86 708. Hence a conservative estimate of the true mortality percentage is ≥99.9965% (95% confidence).

Table 13. Zucchini infestation experiment: Comparison of the actual number of insects treated compared with the estimated number treated.

Stage/ replicate	Number of fruit Simulated control: Simulated treated	Number of insects in simulated control	Number of insects in simulated treated	Estimated number of insects treated based on simulated control numbers	Ratio Simulated control pupae: Simulated treated pupae
Egg 1	6:30	1 771	6 451	8 855	1:3.64
Egg 2	6:30	2 401	9 127	12 005	1:3.80
Egg 3	6:30	1 058	5 109	5 290	1:4.83
TOTALS	**18:90**	**5 230**	**20 687**	**26 150**	**1:3.96**

5.1.3.2 Cucurbit fruits

A treatment at a core temperature of 44°C for 0 minutes was found to be an effective dose with zero survivors from a total of 234 429 *B. cucumis* insects (mature eggs) treated in 3 replicates in rockmelon (Table 14). This represents a true mortality of ≥99.9987% (95% confidence) (Couey and Chew 1986). From fruit quality trials undertaken this heat treatment can be used as a generic treatment to disinfest rockmelon, honeydew and watermelon.

Table 14. Confirmatory trials treating *B. cucumis* eggs in rockmelon at a core temperature of 44°C for 0 minutes.

Fruit/ variety	Stage/ replicate	Average weight of treated fruit	Number of fruit Control: Treated	Number of insects in controls	Estimated number of insects treated	Number of pupae surviving treatment	Mortality (%)	True mortality (≥) (95% confidence)
Rockmelon (orange flesh with netted skin)	Egg 1	2 471g	9:27	3 245	9 735	0	100	99.9692
	Egg 2	1 550g	16:48	32 178	96 534	0	100	99.9969
	Egg 3	1 602g	12:36	42 720	128 160	0	100	99.9977
TOTALS	**EGG**	**1 874g**	**37:111**	**78 143**	**234 429**	**0**	**100**	**99.9987**

Comparison of estimated and actual numbers of pupae resulting from cage infestation showed variation (Table 15). Over the three replicates the ratio of simulated control numbers: simulated treated numbers varied between 1:1.25 and 1:2.89, where 1:3 was expected. The difference between the actual numbers of pupae and the estimated numbers were not significantly different to zero (t_2=-1.82, p=0.210, 95% confidence interval –26 124, 10 575).

Estimating treated numbers in the confirmatory trials using the average ratio of simulated control numbers: simulated treated numbers as in Table 15 (1:2.20), the estimated number treated is 171 915. Hence a conservative estimate of the true mortality percentage is ≥99.9983% (95% confidence).

Table 15. Rockmelon infestation experiment: Comparison of the actual number of insects treated compared with the estimated number treated.

Stage/ replicate	Number of fruit Simulated control: Simulated treated	Number of insects in simulated control	Number of insects in simulated treated	Estimated number of insects treated based on simulated control numbers	Ratio Simulated control pupae: Simulated treated pupae
Egg 1	3:9	3 213	4 013	9 639	1:1.25
Egg 2	4:12	10 563	15 692	31 689	1:1.49
Egg 3	4:12	15 454	44 662	46 362	1:2.89
TOTALS	**11:33**	**29 230**	**64 367**	**87 690**	**1:2.20**

5.1.3.3 Tomato

A treatment at a core temperature of 45°C for 90 minutes was found to be an effective dose with zero survivors from a total of 55 005 *B. tryoni* insects (mature eggs) treated in 3 replicates in tomato (Table 16). This represents a true mortality of ≥99.9946% (95% confidence) (Couey and Chew 1986).

Table 16. Confirmatory trials treating *B. tryoni* eggs in tomatoes at a core temperature of 45°C for 90 minutes.

Fruit/ variety	Stage/ replicate	Average weight of trial fruit	Number of fruit Control: Treated	Number of insects in controls	Estimated number of insects treated	Number of pupae surviving treatment	Mortality (%)	True mortality (≥) (95% confidence)
Tomato Variety: Rosemary	Egg 1	120g	30:150	2 825	14 125	0	100	99.9788
	Egg 2	143g	30:150	4 408	22 040	0	100	99.9864
	Egg 3	160g	30:150	3 768	18 840	0	100	99.9841
TOTALS	**EGG**	**141g**	**90:450**	**11 001**	**55 005**	**0**	**100**	**99.9946**

Comparison of estimated and actual numbers of pupae resulting from cage infestation showed variation (Table 17). Over the three replicates the ratio of simulated control numbers: simulated treated numbers varied between 1:2.32 and 1:6.25, where 1:5 was expected. The difference between the actual numbers of pupae and the estimated numbers were not significantly different to zero (t_2=-0.47, p=0.685, 95% confidence interval –7 297, 5 863).

Estimating treated numbers in the confirmatory trials using the average ratio of simulated control numbers: simulated treated numbers as in Table 17 (1:4.22), the estimated number treated is 46 424. Hence a conservative estimate of the true mortality percentage is ≥99.9935% (95% confidence).

Table 17. Tomato infestation experiment: Comparison of the actual number of insects treated compared with the estimated number treated.

Stage/ replicate	Number of fruit Simulated control: Simulated treated	Number of insects in simulated control	Number of insects in simulated treated	Estimated number of insects treated based on simulated control numbers	Ratio Simulated control pupae: Simulated treated pupae
Egg 1	6:30	533	3 330	2 665	1:6.25
Egg 2	6:30	815	5 030	4 075	1:617
Egg 3	6:30	1 408	3 269	7 040	1:2.32
TOTALS	**18:90**	**2 756**	**11 629**	**13 780**	**1:4.22**

5.2 Fruit Studies

5.2.1 Cucurbit vegetables

5.2.1.1 Zucchini

Fruit injury and quality characteristics of zucchini following vapour heat treatment are shown in Table 18. Except for one variable, p values from the ANOVAs were greater than 0.05 indicating that the differences between the untreated and treated fruit were not statistically significant.

No external injuries were recorded on the skin of untreated and treated fruit after 7 days storage. Very slight pitting on the skin surface was evident after 14 days storage on untreated (severity 0.5, incidence 13.3%) and treated fruit (severity 0.36, incidence 8.9%) at the end of its marketable life.

External rots were only recorded after 14 days storage with similar levels of severity in untreated (0.53) and treated fruit (0.38). The incidence of external rots was slightly higher in untreated fruit (16.7%) than treated fruit (12.2%).

There were no significant differences in weight loss between untreated and treated fruit after 7 and 14 days, however there was an increase in weight loss with increasing storage time. Fruit firmness was not affected by heat treatment after 7 days (untreated 14.4N, treated 13.8N), remained similar in untreated fruit (14.4N) and increased slightly in treated fruit (16.3N) after 14 days storage.

Skin colour was unaffected by heat treatment with no significant differences between untreated and treated fruit after 7 and 14 days storage (Table 18). External appearance ratings were very similar for untreated (7.3) and treated fruit (7.2) after 7 days and both decreased to similar levels after 14 days storage (untreated 5.9, treated 5.9).

There were no differences in brix levels (or total soluble solids) as a result of heat treatment, however there was a slight but statistically significant reduction in titratable acidity at 7 days storage (p=0.036). The slight reduction at 14 days storage was not significant.

Table 18. Fruit injury and quality characteristics of vapour heat treated zucchini after 7 and 14 days storage.

	7 days storage		14 days storage	
	Untreated	**Treated**	**Untreated**	**Treated**
External Injury				
Pitting - severity	0	0	0.5	0.36
- incidence (%)	0	0	13.3	8.9
External rots - severity	0	0	0.53	0.38
- incidence (%)	0	0	16.7	12.2
Quality Attributes				
Weight loss (%)	6.9	7.2	12.9	11.5
Firmness (N)	14.4	13.8	14.4	16.3
External appearance (GA)	7.3	7.2	5.9	5.9
Skin Colour - Hue (°)	123.8	122.2	123.7	123.3
- Chroma	20.5	23.4	18.4	20.7
Eating Quality	5.1	5.6	5.9	6.0
Brix (°Brix)	6.0	5.8	5.9	5.9
Acid (% citric acid)	0.21	0.17	0.20	0.18

5.2.1.2 Button squash

Fruit injury and quality characteristics of button squash following vapour heat treatment are shown in Table 19. Again, except for one variable, p values from the ANOVAs were greater than 0.05 indicating the differences between the treated and untreated fruit were not statistically significant.

The severity of external injury symptoms such as pitting on the skin surface was very slight after 7 days storage (untreated 0.1, treated 0.03) and remained similar after14 days (untreated 0.33, treated 0.22). Treated fruit recorded a lower incidence of injury symptoms (3.3%) than untreated fruit (6.6%) after 14 days storage. External rots were not present after 7 days and were only recorded in 3.3% of untreated fruit (severity, 0.2) and 2.2% of treated fruit (severity 0.14) at 14 days storage.

Weight loss after 7 and 14 days was slightly higher in untreated fruit (7 days 9.5%, 14 days 12.6%) than in treated fruit (7 days 5.7%, 14 days 7.8%). External appearance ratings were similar for untreated (7.4) and treated fruit (7.5) after 7 days, but declined towards dislike slightly for both untreated (4.6) and treated fruit (4.9) after 14 days.

There were no significant differences in the eating quality between untreated and treated "Gold" button squash after 7 and 14 days storage. The differences in the level of brix between untreated and treated fruit were not significant, however there was again a slight but statistically significant reduction in titratable acidity between untreated and treated fruit after 7 and 14 days storage (p=0.023 and 0.007 respectively).

Table 19. Fruit injury and quality characteristics of vapour heat treated button squash after 7 and 14 days storage.

	7 days storage		14 days storage	
	Untreated	**Treated**	**Untreated**	**Treated**
External Injury				
Pitting - severity	0.1	0.03	0.33	0.22
- incidence (%)	0	0	6.6	3.3
External rots - severity	0	0	0.2	0.14
- incidence (%)	0	0	3.3	2.2
Quality Attributes				
% Weight loss	9.5	5.7	12.6	7.8
Firmness (N)	4.61	5.12	-	-
External appearance (GA)	7.4	7.5	4.6	4.9
Skin Colour - Hue (°)	-	-	45.6	45.4
- Chroma	-	-	0.65	0.65
Eating Quality	5.7	6.0	5.5	5.7
Brix (°Brix)	5.5	5.5	5.6	5.5
Acid (% citric acid)	0.21	0.19	0.21	0.19

5.2.1.3 Cucumber

Fruit injury and quality characteristics of cucumber following vapour heat treatment are shown in Table 20.

Heat treatment resulted in the formation of sunken cavities at the skin surface with an incidence of 26.7% in the treated fruit. No external injury symptoms were recorded in untreated fruit. External appearance (GA) was significantly decreased as a result of heat treatment with an overall rating of 4.1 for the treated fruit (dislike slightly) compared to 7.0 for untreated fruit (like moderately) (p=0.008).

Internal injuries, cavity formation and translucence (water soaked areas) were significantly increased as a result of heat treatment with 43.3% of fruit having a rating of 'slight' or above compared to none in the untreated fruit.

Weight loss was only slightly increased in treated fruit (2.7%) compared with untreated fruit (2.3%). Fruit firmness was slightly reduced by heat treatment (22.4N) compared with untreated fruit (25.6N). Areas of sunken cavities on the skin were avoided to reduce biasing firmness result. Both indicators of skin colour showed significant differences (p=0.012, hue; and p=0.014, chroma) between the treated and untreated fruit indicating a slight increase in intensity and a shift towards the yellow end of the colour spectrum.

Table 20. Fruit injury and quality characteristics of vapour heat treated "green slicing"cucumber after 7 days storage.

	7 days storage	
	Untreated	**Treated**
External Injury		
Sunken cavities - severity	0	0.7
- incidence (%)	0	26.7
Internal Injury		
Combined Injury - severity	0	1.2
- incidence (%)	0	43.3
Internal cavity - severity	0	0.7
- incidence (%)	0	22.2
Translucence - severity	0	0.6
- incidence (%)	0	23.3
Quality Attributes		
Weight loss (%)	2.3	2.7
Firmness (N)	25.6	22.4
External appearance (GA)	7.0	4.1
Skin Colour - Hue (°)	127.3	122.0
- Chroma	18.0	26.3
Brix (° Brix)	4.0	4.0
Acid (% citric acid)	0.09	0.09

5.2.2 Cucurbit fruits

5.2.2.1 Rockmelon

Fruit injury and quality characteristics of rockmelon following vapour heat treatment are shown in Table 21. Except for one variable, p values from the ANOVAs were greater than 0.05 indicating that the differences between the untreated and treated fruit were not statistically significant.

There were no external or internal injuries recorded in either untreated or treated fruit. External appearance was, however, significantly affected by heat treatment (p=0.009) though there was only a very slight difference in general acceptability between untreated (8.0) and treated fruit (7.8). Skin colour, as expressed by hue angle (untreated 73.3, treated 72.8) and chroma values (untreated 35.7, treated 36.7) was not greatly affected by heat treatment.

Heat treatment did not have a major effect on fruit firmness with only a very slight but non-significant decrease in firmness in treated fruit (22.1) compared to untreated fruit (24.1). Brix levels were unaffected by the treatment (untreated 9.8, treated 9.8).

Table 21. Quality characteristics of vapour heat treated rockmelon after 7 days storage.

	7 days storage	
	Untreated	**Treated**
Quality Attributes		
Weight loss (%)	2.0	2.8
External appearance (GA)	8.0	7.8
Skin Colour - Hue (°)	85.0	85.4
- Chroma	35.3	33.9
Internal Colour – Hue (°)	73.3	72.8
- Chroma	35.7	36.7
Firmness (N)	24.1	22.1
Eating Quality	5.3	5.7
Brix (°Brix)	9.8	9.8

5.2.2.2 Honeydew

Fruit injury and quality characteristics of honeydew melon following vapour heat treatment are shown in Table 22.

Heat treatment did not cause any significant increase in external or internal fruit injury. Skin pitting was very slight with low severity in untreated (0.03) and treated fruit (0.04). External appearance rated high after storage and did not differ significantly between untreated (7.7) and treated fruit (7.6).

Jellyness in the seed cavity was unaffected by heat treatment with similar severity between untreated (0.1) and treated fruit (0.1) recorded.

Pulp colour did not alter significantly as a result of treatment as shown by internal hue angle and chroma values of untreated (hue angle 114.5, chroma 24.6) and treated fruit (hue angle 114.3, chroma 24.8). Brix levels were unaffected by heat treatment, however a slight increase in eating quality was seen (untreated 6.1, treated 6.7).

Table 22. Fruit injury and quality characteristics of vapour heat treated green fleshed honeydew melon after 7 days storage.

	7 days storage	
	Untreated	**Treated**
External Injury		
Pitting - severity	0.03	0.04
- incidence (%)	0	0
Internal Injury		
Jellyness - severity	0.1	0.1
- incidence (%)	0	0
Quality Attributes		
Weight loss (%)	0.5	0.5
External appearance (GA)	7.7	7.6
Skin Colour - Hue (°)	103.7	103.1
- Chroma	20.6	24.7
Internal Colour – Hue (°)	114.5	114.3
- Chroma	24.6	24.8
Eating Quality	6.1	6.7
Brix	10.2	10.2

5.2.2.3 Watermelon

Fruit injury and quality characteristics of watermelon following vapour heat treatment are shown in Table 23. Except for one variable, p values from the ANOVAs were greater than 0.05 indicating that the differences between the untreated and treated fruit were not statistically significant.

There were no external or internal injuries as a result of heat treatment in any fruit from the three concurrent vapour heat treatments. Observed skin colour was not affected by heat treatment (untreated 0.05, treated 0.07). However, the differences in hue angle though only slightly lower in treated fruit (121.8) than untreated fruit (123.8) were significant (p=0.002). Chroma values of the pulp did not significantly differ between untreated and treated fruit.

Internal firmness readings were slightly lower in untreated fruit (4.2) than treated fruit (4.9) but are not of statistical significance. There were no differences in eating quality between untreated (6.7) and treated fruit. Brix was not affected as a result of heat treatment. (untreated 14.3, treated 14.4).

Table 23. Quality characteristics of vapour heat treated seedless watermelon after 8 days storage.

	8 days storage	
	Untreated	**Treated**
Quality Attributes		
Weight loss (%)	0.4	0.5
Skin Colour - Observed	0.05	0.07
- Hue (°)	123.8	121.8
- Chroma	27.1	29.5
Internal Colour - Observed	5	5
- Hue (°)	35.7	37.0
- Chroma	30.0	29.2
Internal Firmness (N)	4.2	4.9
Eating Quality	6.7	6.7
Brix (°Brix)	14.3	14.4

6 DISCUSSION

6.1 Entomology Studies

The research reported here aimed to meet specific technical requirements of New Zealand MAF and so form the basis for the development of export protocols for zucchini, button squash, rockmelon, honeydew, watermelon and tomato from Eastern Australia to New Zealand based on heat to replace the existing treatment using chemicals. The use of chemical treatments is under review by regulatory bodies such as the APVMA (Australian Pesticides and Veterinary Medicine Authority) and the Codex Alimentarius Commission and the use of chemicals for postharvest disinfestation treatments is likely to be phased out in the next few years. Treatments based on heat are likely to have long term suitability and are more environmentally and socially acceptable. Development of non-chemical postharvest treatments will result in a reduction in 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.

The New Zealand MAF Biosecurity Authority Standard 155.02.03 – "Specification for the Determination of Fruit Fly Disinfestation Treatment Efficacy" states that countries wishing to export fruit fly host commodities to New Zealand must comply with three technical requirements. The first technical requirement is a list of all fruit fly species and their host records occurring in the country of origin and recommendations as to which species require treatment in specific commodities. This requirement was met in the HRDC-DPI Project HG645 (Published as Hancock et al. 2000). The second technical requirement in the MAF Standard is to test the heat tolerance of all economic pest species. This research was conducted against naked insects in hot water immersion tests in a static hot water bath. This work was completed in HAL-DPI Project HG96019 (Corcoran et al. 2003).

This current project addressed the third technical requirement of the MAF Standard which relates to specific steps in the development of an effective disinfestation treatment for particular commodities. As required by the Standard the two most tolerant species/stages were tested in fruit to determine the most tolerant life stage under these conditions. The most tolerant life stage was then used in subsequent fruit testing to predict the confirmatory dose and confirmatory trials were then undertaken to confirm an effective dose. *Bactrocera cucumis* was the species of concern for the cucurbit commodities and *B. tryoni* was the species tested in tomatoes.

6.1.1 Most tolerant stage testing

The most tolerant immature stages of *B. cucumis* from previous *in vitro* hot water dipping data were non-feeding third instars, followed by third instars then mature eggs. Third instars and mature eggs were tested in fruit in this project and mature eggs were arithmetically or statistically more tolerant than third instars in the cucurbit fruits tested. The most tolerant immature stages of *B. tryoni* from previous *in vitro* hot water dipping data were mature eggs followed by first instars. Mature eggs and first instars were tested in tomatoes in this project and mature eggs were arithmetically more tolerant than first instars.

Previous research carried out by this project team has shown that in most cases mature eggs are the most tolerant stage to heat with in-fruit testing eg *B. cucumis* eggs in zucchini (Corcoran et al. 1993), and *B. tryoni* eggs in mango (Heather et al. 1997). It is not surprising that the order of heat tolerance between stages differed between the hot water immersion tests of naked insects and the in-fruit studies. Hot water immersion tests against naked insects allows precise control of insect age and number, treatment temperature and duration which enables accurate comparisons to be made between species and stages. However the manner of heat application is significantly different when

insects are inside fruit. The rate of heat transfer to an insect will occur much more slowly when the insect is inside the fruit in a heated air chamber than when it is immersed naked in a static hot water bath. Insects exposed in a static water bath experience an instant heat shock on immersion, since the surface temperature of the insects effectively equals the water temperature. By contrast, insects treated in fruit in a hot air treatment system experience heat shock gradually as heat must be conducted through the fruit tissue to the site of insect infestation. (Corcoran et al. 2002). Therefore the fruit acts as a buffer, and tends to damp out the temperature gradient (Corcoran et al. 2002). Furthermore, the insect stages may be at different depths when treated in a fruit and therefore experience different heating regimes. For example, eggs will always be situated near the surface but larvae may move deeper into the fruit. Heating of fruit produces physiological changes in that fruit and in some cases the release of metabolites that may act as insect stressors, such as the release of gaseous CO_2 from heated mangoes (Mitcham and McDonald 1993, R.A. Jordan DPI&F, personal communication). Our experience in treatment of insects in both environments suggests that hot water immersion is a good predictor of intrinsic differences in heat tolerance between species but not between stages, for the reasons outlined above (Corcoran et al. 2002).

6.1.2 Infestation experiments

Our extensive experience in disinfestation research with a wide range of commodities has lead us to conclude that uniformity of oviposition is not possible using the cage infestation method. However, alternative methods can lead to other inaccuracies. For example, natural infestation requires the collection of infested fruit from the field. Under these conditions insects are in their natural environment but it is impossible to estimate the life stages present. The inaccuracies resulting from this fact and the amount of fruit that may need to be treated would be unacceptable. Another method, artificial infestation, involves inserting eggs or larvae into the fruit. Numbers and life stages present can be accurately determined but the insects are not in their normal natural position within the fruit and the fruit and insects may be damaged during the process. Another major problem with artificially infesting fruit is that it is labour intensive and time consuming especially in large scale research such as confirmatory trials. Cage infestation of fruit with laboratory reared flies results in the natural placement of eggs within the fruit, good control of life stages and some control of insect numbers to ensure that the required numbers are treated for confirmatory scale trials. Cage infestation also allows for reasonably accurate estimation of insect numbers, which may not be sufficient for dose response trials but is sufficient for large scale confirmatory testing.

6.1.3 Confirmatory testing

While the New Zealand Standard does not prescribe the number of insects that must be tested in the large scale confirmatory trials, historically no survivors from > 30 000 tested - equivalent to 99.99% mortality at the 95% confidence level (Couey and Chew 1986) has been accepted by New Zealand MAF prior to the development of this standard and is required by other countries including Japan (Anon 1996).

For the reasons outlined in Sections 4.1.4.2 and 4.1.4.3, zucchini was chosen as a representative cucurbit vegetable and rockmelon was chosen as a representative cucurbit fruit for these confirmatory trials. Confirmatory trials in zucchini, rockmelon and tomato were carried out according to the New Zealand Standard requirements and results with all three commodities showed a level of efficacy (at 95% confidence level) which exceeded the New Zealand Standard requirements (zucchini ≥ 99.9965%, rockmelons ≥99.9983%, tomato ≥99.9935%).

A treatment core temperature of 45°C for 40 minutes provided the required level of efficacy for *B. cucumis* in zucchini and is recommended for acceptance as a generic treatment for cucurbit vegetables. A treatment which brought the core temperature to 44°C with no holding period was

effective for *B. cucumis* in rockmelons and is recommended for acceptance as a generic treatment for cucurbit fruits. A treatment core temperature of 45°C for 90 minutes was effective for *B. tryoni* in tomatoes.

6.1.4 Fruit Studies

Fruit studies on all six cucurbit commodities (vegetables- zucchini, button squash and green cucumbers; fruits- rockmelon, honeydew watermelon) were undertaken to determine the effects of treatments on fruit quality.

6.1.4.1 Cucurbit vegetables

The treatment effective for *B. cucumis* (2 hour ramp of air temperature from 25°C to 46°C until the slowest monitored probe fruit has attained a core temperature of 45°C for 40 minutes), caused minimal detrimental quality changes for zucchini and button squash. However, for the green cucumber type tested, this treatment resulted in external pitting injury and large areas of internal injury, and consequently would not be commercially acceptable for this commodity.

6.1.4.2 Cucurbit fruits

The treatment effective for *B. cucumis* (1hour ramp of air temperature from 30 to 45°C until the core temperature of all fruit have equilibrated at 44°C) did not result in quality loss in rockmelon, honeydew and watermelon.

6.1.4.3 Tomato

The impact of quarantine heat treatment has been extensively studied in other projects (HG97019 'Preliminary investigation of simplified heat treatment systems for disinfestations of vegetables for New Zealand' and VG98136 'Heat treatment of tomatoes for New Zealand – commercial prototype development'). A range of treatment conditions have been investigated in this work which occurred prior to the final determination of the treatment conditions of the required efficacy level for treatment of *B. tryoni*. Also investigated was the impact of pre-treatment conditioning on injury development.

The earliest work investigated 44°C centre temperature for 90 minutes, 45°C centre temperature for 45 minutes, as well as the widely used treatment for *B. tryoni* of 47°C for 15 minutes. The treatment at 44°C produced low internal and external injury levels, slightly lower than the treatment at 45°C for 45 minutes. Treatment at 47°C for 15 minutes while being more severe, resulted in only moderate injury levels. The use of pre-treatment conditioning (38°C for 16 hours) reduced the injury but is probably commercially impractical. In all of the work, fruit at least at 'breaker' ripeness stage were necessary for successful treatment.

Treatments performed in the commercial prototype equipment investigated the 45°C for 45 minutes conditions on 'gourmet' cultivars 'Mercedes', 'Petula' and 'Isabella', but actually subjected the fruit to a period of approximately 5 hours at 45-46°C while the fruit equilibrated to 45°C for 60 minutes. The fruit from this treatment were commercially assessed and found to be of acceptable quality. Experimental assessment also found minimal impact on the fruit.

While the effective treatment condition (45°C centre temperature for 90 minutes) was not specifically tested in this current work, there is evidence to provide confidence that provided tomatoes are at 'breaker' stage, there will be minimal reduction of fruit quality.

7 CONCLUSIONS

These trials meet the requirements of the New Zealand Standard for determination of treatment efficacy for all commodities tested with the exception of green cucumbers. The confirmed treatments produced fruit of acceptable commercial quality. These outcomes will be used as the basis for the development of an export protocol for zucchini, button squash, watermelon, rockmelon and honeydew and tomato to New Zealand using hot air as the disinfestation treatment.

The project outcomes are also highly relevant to interstate market access within Australia because they provide a non-chemical alternative to the currently used insecticide postharvest treatments which are likely to be restricted in the near future pending the outcomes of APVMA reviews of dimethoate and fenthion. Furthermore, these heat treatments have the potential to be extended to other Australian produced commodities and to other export markets. Successful hot air treatments have already been developed overseas for fruit of the plant family Solanaceae eg. Capsicum against oriental fruit fly (*Dacus dorsalis*) (Sugimoto et al. 1983) and eggplant against melon fly (*Dacus cucurbitae*) (Furasawa et al. 1984). Future research on Australian fruit fly species could also be completed on capsicum and eggplant. Controlled atmosphere heat treatments may be a solution for cucumber and other fruits which are found to be susceptible to damage from standard heat treatments.

8 TECHNOLOGY TRANSFER

Progress on this project has been reported in milestone reports to Horticulture Australia. A project update was presented by Elizabeth Hall at the QFVG Growing for Profit Day, Gympie, November 2000, and a written summary was published in the QFVG Ltd Growing for Profit Day Program. Results were presented at other industry meetings by former project leader Dr Robert Corcoran, prior to his departure from DPI&F.

A detailed report to meet New Zealand MAF Standard requirements will be prepared and submitted to Biosecurity Australia for negotiation of an export protocol to New Zealand. Results will also be submitted to the Domestic Quarantine and Market Access Working Group for approval for commercial treatment protocols to be developed under the ICA system for interstate market access.

9 RECOMMENDATIONS

- Results will be presented to New Zealand quarantine authorities through Biosecurity Australia for approval as quarantine export protocols for cucurbits and tomatoes.
- Results will be presented to interstate quarantine authorities (now called Domestic Quarantine and Market Access Working Group) for approval and development of ICA-protocols for cucurbits and tomatoes.
- In both interstate and export market access situations approvals will be sought for these results to be accepted as generic treatments for cucurbit vegetables, cucurbit fruit, and tomatoes respectively.
- Once approvals have been obtained, a grower and industry targeted communication plan should be developed to promote heat treatments as an alternative to current chemical treatments (essential if dimethoate use is restricted in the near future). This will be incorporated into a new DPI&F proposed project on heat treatments for capsicums if Horticulture Australia funding is approved.
- Controlled atmosphere heat treatments should be further investigated for commodities which do not retain acceptable commercial quality with the treatments studied in this project.

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