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Postharvest Biology and Technology 28 (2003) 123–133

Postharvest Biology and Technology

www.elsevier.com/locate/postharvbio

Low temperature conditioning before cold disinfestation improves 'Hass' avocado fruit quality

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Received 7 February 2002; accepted 25 August 2002

Abstract

The potential for low temperature conditioning (LTC) treatments, either alone or in combination with hot water treatment (HWT), to improve the quality of 'Hass' avocado (*Persea americana* Mill.) fruit following cold disinfestation of Queensland fruit flies, was investigated. Avocado fruit were held at 4–8 °C for 3–4 days then disinfested for 16 days at 1 °C before ripening at 16 °C. In a second experiment, fruit were placed in water at 41–42 °C for 15–25 min, either with or without LTC, then disinfested and ripened. In both experiments, LTC at 4 °C for 4 days or at 6–8 °C for 3–4 days increased the percentage of fruit with acceptable external appearance (less than 5% of the fruit with discrete dark patches on the skin, and skin spotting combined) after disinfestation from 0 to 100% due to the effective elimination of discrete patches on the skin. Disinfestation alone increased body rots and diffuse flesh discolouration severity in ripe fruit, while LTC before disinfestation reduced severity of these disorders to similar levels as those in non-disinfested, non-stored fruit. LTC before disinfestation reduced discrete patches severity and improved flesh quality more than HWT. Combined treatments of HWT and LTC before disinfestation were no more beneficial compared with LTC alone. Conditioning of fruit at 6 °C for 3 days followed by disinfestation resulted in no survivors from 50 748 third instars of Queensland fruit fly (*Bactrocera tryoni* Froggatt). LTC efficacy was verified commercially by conditioning fruit at 6 °C for 3 days followed by disinfestation and airfreight to New Zealand. External fruit appearance and internal ripe fruit quality after disinfestation were found to be high. These results show that LTC before cold disinfestation effectively eliminates skin damage and improves flesh quality of ripe 'Hass' avocado fruit, with no negative effect on Queensland fruit fly disinfestation efficacy.

Keywords: Avocado; Cold; Conditioning; Disinfestation; Hot water; Quality; Chilling injury; Disease; Queensland fruit fly (*Bactrocera tryoni*)

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0925-5214/02/$ - see front matter.
PII: S0925-5214(02)00175-8

1. Introduction

Disinfestation treatments to minimise the risk of insect pests in horticultural produce are required for fruit entry into many markets. Previous disinfestation treatments have been based on chemical fumigation and dips. However, many of these treatments have been withdrawn, and safer alternatives, mainly using physical methods, are being developed. These have focussed on heat, cold, irradiation and controlled atmospheres, either alone or as combinations.

Avocado fruit from many production areas containing insect pests require a disinfestation treatment against fruit flies if sold outside these areas. Cold disinfestation of 1 °C for 16 days is an accepted treatment for many fruit fly species (Jessup, 1994) and is the approved quarantine treatment for 'Hass' avocados exported to New Zealand. However, skin damage ('chilling injury') often develops during storage at 1 °C (Hofman et al., 1998).

Much of the recent research into reducing external chilling injury of avocado has focussed on using hot air (Sanxter et al., 1994; Woolf et al., 1995; Florissen et al., 1996) and hot water (Woolf, 1997; Grové et al., 2000). Recently, Hofman et al. (2002) examined the use of hot water treatments (HWTs) to reduce skin damage caused by cold disinfestation of Australian 'Hass' avocados. Although results were promising, external damage could not be minimised to commercially acceptable levels.

Woolf et al. (2002) recently examined the use of low temperature conditioning (LTC) treatments to reduce external chilling symptoms of 'Hass' avocados. Fruit were exposed to a range of LTC treatments from 4 to 15 °C for 1–5 days, followed by 3 or 4 weeks at 0 °C. LTC at 6–8 °C for 3–5 days was the most effective treatment for reducing external chilling injury.

Here, we examine the potential for LTC to provide sufficient chilling tolerance for 'Hass' avocados to be of acceptable commercial quality after subsequent cold disinfestation (1 °C for 16 days). These treatments were compared with optimum HWTs (as found by Hofman et al. (2002)), and combined HWT and LTCs to determine the potential for synergistic effects of these treatments on fruit quality after disinfestation.

Since Chen et al. (1991) have shown that moderate cold temperatures can protect insects against subsequent severe cold treatments, it is possible that LTC might also protect insects from the quarantine treatment. Therefore, we also examined the efficacy of the combined LTC and cold disinfestation treatments, and undertook outturn assessment of commercially disinfested fruit following export.

2. Materials and methods

2.1. Fruit quality trials: experimental

2.1.1. Fruit harvest and handling

'Hass' avocado fruit (*Persea americana* Mill.) (average mass 280 g) were harvested from three separate orchard blocks (each block containing about 100 trees being a replicate) on the same commercial orchard near Bundaberg (25°15′ S, 152°30′ E) on 29 April 1999 ('early season'; dry matter = 21.6%) for experiment 1, and 28 May (early-mid season; dry matter = 23.1%) for experiment 2. Equal numbers of fruit were hand-picked from the same side of each of five (experiment 1) or ten (experiment 2) visually similar trees per block, to provide 20 fruit for each of the three replicates. The fruit were placed in single layer trays and transported to the laboratory. The percentage dry matter of the flesh at harvest was determined by combining representative flesh samples of six fruit from each block, and drying at 65 °C to a constant mass.

2.1.2. Experiment 1

Within 5 h of harvest, fruit were dipped in 0.55 ml l^{-1} Sportak® (a.i. prochloraz; 0.05% v/v) for 30 s for disease control, dried, and within 2 h given a LTC treatment of either 4, 6 or 8 °C for either 3 or 4 days. The fruit were then held at a fruit core temperature of 1 °C for 16 days. At the end of the disinfestation treatment, the fruit were treated with 80 mg kg^{-1} ethylene for 2.5 days at 16 °C, then ripened at 16 °C to simulate recommended ripening conditions in New Zealand. Several

controls were used: not conditioned or disinfested (non-treated); conditioned at 4 or 8 °C but not disinfested; and not conditioned but disinfested. All controls were ethylene treated and ripened at 16 °C.

2.1.3. Experiment 2

Within 5 h of harvest, fruit were dipped in 0.55 ml l^{-1} Sportak® and given a LTC treatment of 6 or 8 °C for 3 days then disinfested as above. Other fruit were placed into a hot water bath either at 41 °C for 20 or 25 min, or at 42 °C for 15 min (treatments found by Hofman et al. (2002) to be most effective for maintaining external fruit quality). Approximately 4 h after HWT, the fruit were dipped in Sportak® as above, dried, and 2 h later given a LTC treatment of 6 or 8 °C for 3 days, then disinfested and ethylene treated and ripened as in experiment 1. Several controls were used: no conditioning or disinfestation (non-treated); conditioned at 6 °C for 3 days or 46 °C for 20 min but not disinfested; and not conditioned but disinfested. All controls were ethylene treated and ripened at 16 °C.

The HWTs were applied using a 400 l stainless steel insulated tank, as described by Hofman et al. (2002).

2.1.4. Fruit quality

Fruit quality assessments were done as described by White et al. (2001). External fruit quality was assessed at removal from ethylene treatment (3 days after removal from disinfestation) by recording the percentage of skin area with discrete, dark to black patches (called discrete patches). Skin spotting (mostly caused by damage to the raised nodules on the skin) was rated as the percentage of the nodules with black colour. Fruit firmness was assessed daily by gentle hand pressure. The days to ripe was determined as the number of days from harvest or from removal from 1 °C to reach a hand pressure corresponding to a firmness of about 4 N (fruit skin not removed), as measured by an Instron Universal Testing Machine (Instron, High Wycombe, UK) model 1122, fitted with an 8 mm hemispherical probe (probe penetration 2 mm).

At the ripe stage, skin colour was assessed as the percentage of the skin surface area with dark purple to black colour. Fruit were then cut longitudinally and peeled, and rated for the severity of body rots (caused mainly by *Colletotrichum* spp), stem end rots (caused mainly by *Dothiorella* spp) and the internal disorders of diffuse flesh discolouration and vascular browning, as the percentage of the flesh volume affected. For disease identification, skin and flesh samples were taken from the advancing margin of representative lesions and incubated on potato dextrose agar with and without streptomycin (approx 0.5%) at 25 °C for 7–10 days.

Fruit were rated as having acceptable external appearance 3 days after removal from 1 °C if there was less than 5% discrete patches and skin spotting combined. When ripe, fruit were considered to have acceptable external appearance if more than 60% of the skin had dark purple to black colour, while fruit were considered to have acceptable internal or flesh quality if there was less than 5% of the flesh with rots or internal disorders combined.

2.1.5. Experimental design and statistical analysis

Twenty fruit were used from each of the 3 treatment replicates. Results were analysed with Genstat 4.1 (4th edition) using general analysis of variance with the orchard block as the block. The percentage ratings data were angular transformed before analysis. For clarity, only the back-transformed means are presented in the tables. Where required, the data are plotted on an angular scale for graphs. The protected least significant difference (LSD) procedure at $P = 0.05$ was used to test for differences in treatment means. Only significant differences at $P = 0.05$ are discussed, unless stated otherwise.

2.2. Insect mortality trials: experimental

Certified organically-grown 'Hass' avocados were weighed, then sorted into batches such that the weight range in any treatment did not exceed 70 g (mean of about 260 g). At the softening stage (firmness of 3–4 where 0 = firm, and 5 = eating soft; White et al. 2001), fruit were infested with

eggs of Queensland fruit fly [*Bactrocera tryoni* (Froggatt)] by puncturing 20 times with a fine pin, then cage infesting for 30 min with a minimum of 10 000 adult flies. Infested fruit were held at 26 °C for 6 days so that third instars were present at the time of treatment, since Jessup (1994) had shown this to be the most cold tolerant stage. One fifth of infested fruit was randomly allocated to a control that remained untreated. The number of puparia recovered from the control was used to estimate the number of insects treated.

Infested fruit were held at 6 °C for 3 days (the most effective LTC treatment), followed by the approved disinfestation treatment (16 days at 1 °C). Air temperature and fruit temperatures adjacent to the seed were measured using RTD probes (Grant thermistor CS-UU-V10-0; Grant, Cambridge, UK) and recorded using Squirrel data loggers (Eltek 1000 Series Squirrel Data Logger; Eltek, Cambridge, UK) at 30 min intervals during treatment. Some fruit was withheld and dissected at the time of treatment to check the state of development of the insects in the treated batch.

After treatment, both treated and control fruit were held over cloth-covered drip trays which retained the fruit and larvae and allowed excess liquid to drain away. Trays were held in cages over clean, sieved sawdust as a pupation medium at 26 °C and 70% relative humidity. The sawdust was sieved twice during the development period to recover surviving pupae.

2.2.1. Experimental design and statistical analysis

This experiment consisted of 3 replicates with about 200–240 fruit and about 12 000–23 000 insects. The fruit for each replicate were obtained from 3 different farms. The true survival proportion was calculated based on the number of insects treated and the number surviving (Couey and Chew, 1986).

2.3. Fruit quality trials: commercial out-turn

Fruit (average mass 270–300 g) from the same commercial orchard as in experiments 1 and 2 were harvested and packed in the standard commercial manner, and low temperature conditioned for 3 days at 6 °C in a commercial coolstore on site. Fruit was then transported by refrigerated truck to Harrowsmiths International, Brisbane (about 3 h), where the New Zealand Ministry of Agriculture and Forestry (NZ MAF)-approved low temperature disinfestation treatment was applied (fruit core temperature of 1 °C for 16 days). The consignment was then air-freighted to Auckland, New Zealand, where it was held under ambient conditions for 2 days during NZ MAF inspection and clearance, then a further 2 days at 15 °C at a distribution centre. The top layer of two pallets was removed for assessment. (The top layer was selected since this position is most likely to be exposed to the coldest temperatures during the disinfestation treatment.)

On arrival at the Mt Albert Research Centre, Auckland (27 days after harvest), external appearance of the fruit was assessed. Ethylene treatment (100 mg kg^{-1} at 20 °C for 2 days) was applied to half the fruit (120 fruit), and fruit were then ripened at either 15 or 20 °C. Three replicates (trays containing about 20 fruit each) were used for each treatment. Fruit quality assessments were carried out using the same criteria to that described above.

3. Results

3.1. Fruit quality trials: experimental

3.1.1. Experiment 1

Discrete patches were present only in disinfested fruit (Table 1). Disinfestation without LTC resulted in high discrete patches severity, while all LTC treatments reduced discrete patches severity to negligible levels. Severity of discrete patches after 4 °C for 3 days plus disinfestation was higher than the other LTC treatments, but there was no significant difference between 4 °C for 4 days and 6–8 °C for 3–4 days.

Skin spotting 3 days after disinfestation increased with LTC and disinfestation compared with disinfestation alone, but severity was not higher than that in non-disinfested fruit (Table 1). LTC without disinfestation increased the days to ripe compared with no LTC or disinfestation. Also, LTC and disinfestation reduced the days to

Table 1
Experiment 1. The percentage area of the skin of 'Hass' avocado fruit with discrete dark to black patches, and with skin spotting (damage to the raised nodules) at removal from ethylene (3 days after disinfestation), the number of days after harvest (for no disinfestation) or after removal from disinfestation to reach the eating soft stage, the percentage of the skin of the ripe fruit with dark purple to black colour, and the percentage of the flesh volume of ripe fruit with diffuse flesh discolouration either without or following low temperature conditioning at 4–8 °C for 3 or 4 days, or disinfestation (1 °C for 16 days)

Conditioning treatment	% of skin affected 3 days after removal		Days to ripe	% of ripe skin area with dark colour	% of ripe flesh volume with diffuse discolouration
	Discrete patches	Skin spotting			
No disinfestation (no storage, ripened at 16 °C)					
None	0.0 a	2.7 e	11.0 a	6.5 a	0.0 a
4 °C, 4 days	0.0 a	2.1 cd	17.4 d	14.1 b	0.1 a
8 °C, 4 days	0.0 a	1.5 ab	17.1 d	9.7 ab	0.0 a
Disinfestation (1 °C for 16 days, ripened at 16 °C)					
None	74.4 d	1.2 a	13.3 c	57.4 d	3.2 b
4 °C, 3 days	2.4 c	2.8 e	13.0 c	49.0 cd	0.1 a
4 °C, 4 days	0.2 b	2.1 cd	12.9 c	56.0 c	0.1 a
6 °C, 3 days	0.2 b	2.1 cd	13.1 c	46.9 c	0.0 a
6 °C, 4 days	0.1 ab	2.6 cde	13.0 c	54.8 cd	0.2 a
8 °C, 3 days	0.2 b	2.0 bc	13.2 c	51.8 cd	0.1 a
8 °C, 4 days	0.2 ab	2.0 bc	12.2 b	50.6 cd	0.1 a

The percentage data have been angular transformed. However, for clarity, only the back-transformed means are presented. Means followed by the same letter in each column are not significantly different at $P = 0.05$ ($n = 60$).

ripe compared with LTC alone, but the days to ripe was still higher than in fruit receiving no treatment.

The percentage of the skin of ripe fruit with dark purple to black colour increased following disinfestation compared with no disinfestation, while LTC before disinfestation resulted in similar skin colour as with disinfestation alone (Table 1).

The diffuse discolouration severity in ripe fruit increased following disinfestation alone compared to no disinfestation, while all LTC treatments reduced diffuse discolouration severity to similar levels as in non-disinfested fruit (Table 1). Body rots severity in ripe fruit was slightly higher in LTC, non-disinfested fruit compared with non-treated fruit (Fig. 1). Disinfestation alone increased body rots severity compared to no disinfestation, but treatment at 4 or 8 °C for 4 days, or 6 °C for 3–4 days before disinfestation reduced body rots to levels similar to that of non-disinfested LTC fruit.

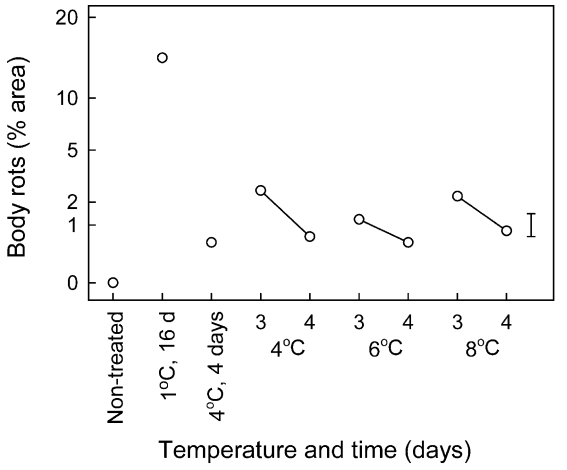

Fig. 1. The percentage of the flesh volume of ripe 'Hass' avocado fruit with body rots following either ripening without any additional treatment (non-treated), or disinfesting at 1 °C for 16 days without low temperature conditioning (LTC), or LTC at 4 °C for 4 days without subsequent disinfestation, or LTC at 4–8 °C for 3–4 days, then disinfesting at 1 °C for 16 days (experiment 1). The vertical bar indicates significant difference of means at LSD of $P = 0.05$.

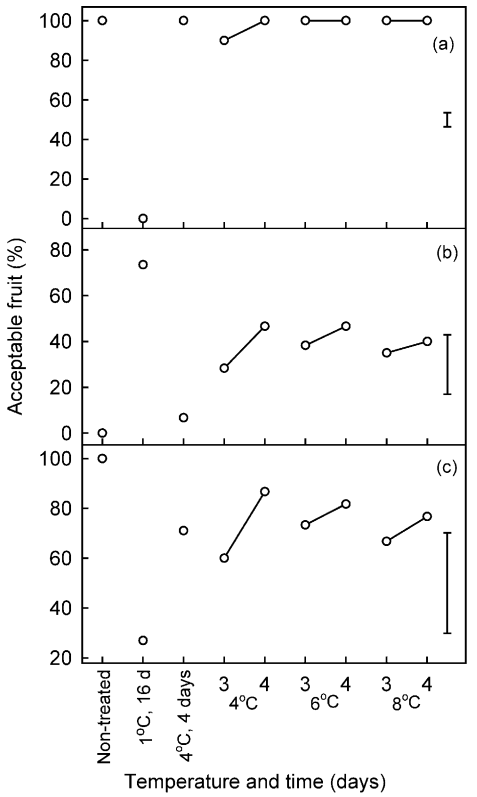

Fig. 2. The percentage of 'Hass' avocado fruit with (a) acceptable external appearance (less than 5% of the skin area with discrete patches and skin spotting combined) at removal from ethylene (3 days after removal from disinfestation), (b) acceptable external appearance when ripe (at least 60% of the skin with dark purple to black colour), and (c) acceptable internal appearance when ripe (less than 5% of the flesh volume with rots or flesh disorders combined) following either ripening without any additional treatment (non-treated), or disinfesting at 1 °C for 16 days without low temperature conditioning (LTC), or LTC at 4 °C for 4 days without subsequent disinfestation, or LTC at 4–8 °C for 3–4 days, then disinfesting at 1 °C for 16 days (experiment 1). The vertical bar indicates significant difference of means at LSD of $P = 0.05$.

At the ripe stage, all non-disinfested treatments had less than 7% externally acceptable fruit (greater than 60% skin colouration) (Fig. 2). Disinfestation alone resulted in 73% of the fruit with acceptable external quality, while LTC before disinfestation reduced this to 28–46%. External quality of fruit among the LTC treatments was similar following disinfestation. The incidence of internally acceptable fruit declined from 100% in non-disinfested fruit to 27% after disinfestation alone (Fig. 2). However, LTC at 4 °C for 4 days and 6–8 °C for 3–4 days before disinfestation resulted in higher incidence of acceptable fruit than with disinfestation alone, and was similar to that in the non-disinfestation treatments.

3.1.2. Experiment 2

Discrete patches were absent in all non-disinfested treatments (Table 2). Its severity was reduced by both LTC and HWT before disinfestation compared with disinfestation alone, but only LTC reduced severity to levels similar to the non-disinfested treatments. There was no consistent treatment effect on skin spotting.

Disinfestation increased the percentage of ripe fruit skin with purple/black colour compared to no disinfestation, while most LTC and HWT treatments reduced the percentage dark skin compared with disinfestation alone (Table 2). Body rots severity again increased with disinfestation alone. All LTC and HWTs reduced body rots severity, but only LTC treatments, either alone or in combination with HWT, reduced severity to levels similar to those without disinfestation. Combined treatments were not better than LTC alone before disinfestation.

Disinfestation generally increased diffuse discolouration compared with no disinfestation (Table 2). Conditioning before disinfestation did not significantly increase diffuse discolouration severity compared with disinfestation alone, except with 42 °C for 15 min, then 6 °C for 3 days. Also, diffuse discolouration severity after disinfestation increased with increasing HWT temperature or duration in all cases, except in combination with 8 °C for 3 days.

Disinfestation alone resulted in only 1% of fruit having acceptable external appearance 3 days after removal from disinfestation (Table 3). All LTC treatments, either alone or in combination with HWT, resulted in 100% of the fruit having acceptable external quality, while HWT alone before disinfestation resulted in 60–82% lower fruit acceptability.

Disinfestation alone resulted in the highest incidence of externally acceptable fruit when ripe (Table 3). In most instances, pre-treatment before

Table 2
Experiment 2. The percentage area of the skin of 'Hass' avocado fruit with discrete patches and with skin spotting (damage to the raised nodules) at removal from ethylene (3 days after disinfestation), the percentage of the ripe fruit skin with dark purple/black colour, and the percentage of the flesh volume of ripe fruit with body rots or diffuse flesh discolouration, either without or following low temperature conditioning at 4–8 °C for 3 or 4 days, hot water treatment at 41 or 42 °C for 15–25 min, or disinfestation (1 °C for 16 days)

Conditioning treatment	% of skin affected 3 days after removal		% of ripe skin area with dark colour	% of ripe flesh volume with	
	Discrete patches	Skin spotting		body rots	diffuse discolouration
No disinfestation (no storage, ripened at 16 °C)					
None	0.0 a	0.1 def	2.9 a	0.1 a	0.2 ab
6 °C, 3 days	0.0 a	2.0 g	9.5 b	0.2 ab	0.1 a
41 °C, 20 min	0.0 a	0.0 a	26.0 c	0.8 c	0.7 abcd
Disinfestation (1 °C for 16 days, ripened at 16 °C)					
None	43.4 d	0.3 bcd	71.4 j	25.6 g	1.2 cdef
6 °C, 3 days	0.0 a	0.2 ab	48.9 def	0.8 bc	1.6 def
8 °C, 3 days	0.0 a	0.3 bcde	44.0 de	0.2 abc	0.5 abc
41 °C, 20 min	4.8 c	0.1 a	31.6 c	9.7 e	1.0 bcde
41 °C, 25 min	3.6 b	0.2 abc	27.1 c	4.8 d	1.8 defg
42 °C, 15 min	3.2 b	0.2 abc	40.9 d	9.0 e	2.8 fg
41 °C, 20 min, 6 °C	0.0 a	0.6 ef	56.9 fghi	0.2 abc	0.1 a
41 °C, 25 min, 6 °C	0.0 a	0.4 cdef	59.9 ghi	0.2 abc	1.3 cdef
42 °C, 15 min, 6 °C	0.0 a	0.7 f	56.3 fgh	0.2 ab	3.5 g
41 °C, 20 min, 8 °C	0.0 a	0.2 abc	65.6 ij	0.1 a	1.0 bcde
41 °C, 25 min, 8 °C	0.0 a	0.4 cdef	52.1 efg	0.1 a	2.4 efg
42 °C, 15 min, 8 °C	0.0 a	0.3 bcd	62.1 hi	0.1 a	2.4 efg

The percentage data have been angular transformed. However, for clarity, only the back-transformed means are presented. Means followed by the same letter in each column are not significantly different at $P = 0.05$ ($n = 60$).

disinfestation reduced the incidence compared to disinfestation alone, while the combined HWT and LTC treatments often produced more externally acceptable fruit than either HWT of LTC alone before disinfestation.

Disinfestation alone reduced the incidence of internally acceptable ripe fruit compared with no disinfestation. Two of the HWTs alone did not increase the incidence of internally acceptable fruit after disinfestation. However, all LTC treatments except one, either alone or combined with HWT, significantly improved acceptability compared with disinfestation alone, and in most cases resulted in similar incidence as non-disinfested fruit.

The severity of stem end rots and vascular browning was low in both experiments, and not affected by the treatments.

3.2. Insect mortality trials: experimental

When 'Hass' avocados containing third instars of Queensland fruit fly were treated with LTC followed by disinfestation, no survivors were recovered from an estimated 50 748 treated insects (Table 4). This represents a true mortality of 99.9941% at the 0.95 confidence level.

3.3. Fruit quality trials: commercial out-turn

On arrival in New Zealand, the green, unripe fruit had highly acceptable external appearance. The entire fruit sample assessed had less than 5% of the fruit surface with discrete patches or skin spotting (Table 5).

Internal quality of ripe fruit was very good with over 80% of fruit in all ripening scenarios having

Table 3
Experiment 2. The percentage of 'Hass' avocado fruit with acceptable external appearance at removal from ethylene (3 days after disinfestation) and at the ripe stage, and the percentage of the ripe fruit with acceptable internal appearance, either after conditioning at 4–8 °C for 3 or 4 days, hot water treatment at 41 or 42 °C for 15–25 min, or disinfestation (1 °C for 16 days)

Conditioning treatment	Acceptable fruit (%)		
	External appearance		Internal appearance at ripe[c]
	3 days after removal[a]	At ripe[b]	
No disinfestation (no storage, ripened at 16 °C)			
None	100 d	5 a	83 g
6 °C, 3 days	100 d	12 ab	70 efg
41 °C, 20 min	100 d	24 abc	69 efg
Disinfestation (1 °C for 16 days, ripened at 16 °C)			
None	1 a	75 h	6 a
6 °C, 3 days	100 d	33 bcde	56 def
8 °C, 3 days	100 d	25 abcd	75 fg
41 °C, 20 min	60 b	13 ab	21 ab
41 °C, 25 min	72 bc	8 a	41 bcd
42 °C, 15 min	82 c	35 cde	26 abc
41 °C, 20 min, 6 °C	100 d	46 ef	81 g
41 °C, 25 min, 6 °C	100 d	58 fgh	48 cde
42 °C, 15 min, 6 °C	100 d	45 def	46 cde
41 °C, 20 min, 8 °C	100 d	61 fgh	67 efg
41 °C, 25 min, 8 °C	100 d	50 efg	35 bcd
42 °C, 15 min, 8 °C	100 d	69 gh	51 def

[a] Based on less than 5% of the skin surface with discrete patches and skin spotting.
[b] Based on more than 60% of the skin with dark purple to black colour.
[c] Based on less than 5% of the flesh volume with rots or flesh disorders combined. Means followed by the same letter in each column are not significantly different at $P = 0.05$ ($n = 60$).

less than 5% of flesh affected by rots or disorders combined (Table 5). Less than 0.5% of fruit ripened at 20 °C had unacceptable internal disorders severity. However, at 15 °C there were more disorders, particularly in fruit ripened without ethylene, where up to 27% of fruit were affected by diffuse discolouration. At 20 °C, fruit ripened with ethylene were of similar quality to fruit ripened without ethylene.

The time to ripe was about 5 days for fruit ripened at 20 °C, and 9 days for fruit ripened at 15 °C. Ethylene treatment reduced the days to ripe by about 2 days at 15 °C, but there was no effect of this treatment on ripening rate at 20 °C.

Table 4
Insect mortality

Trial (replication)	No. fruit treated	No. insects treated	No. pupae recovered
1	204	14 832	0
2	202	12 440	0
3	240	23 476	0
Total	646	50 748	0

Efficacy of low temperature conditioning (3 days at 6 °C) followed by quarantine treatment (16 days at 1 °C) against third instars of Queensland fruit fly (*Bactrocera tryoni*) in 'Hass' avocados.

Table 5
Commercial out-turn. The percentage of 'Hass' avocado fruit with acceptable internal quality when ripe, the number of days for the fruit to reach the ripe stage after arrival at the laboratory, and the percentage of ripe fruit with any level of rots (body and stem end rots combined) or diffuse flesh discolouration

Ripening temperature	Ethylene	Days to ripe[a]	Acceptable quality (%)[b]	Internal disorders (%)[c]	
				Rots	Diffuse discolouration
20 °C	No	5.5±0.1	100 b	0 a	0 a
	Yes	5.1±0.1	99 b	0 a	0 a
15 °C	No	9.1±0.2	83 a	7 a	27 b
	Yes	6.9±0.2	94 ab	3 a	10 a

[a] Means±S.E.M.
[b] Based on less than 5% of the flesh volume with rots or flesh disorders combined.
[c] The percentage data are angular transformed for statistical purposes and only the back-transformed means presented. Ethylene treatment carried out at 20 °C for 2 days with 100 mg kg^{-1} ethylene.
Means followed by the same letter in each column are not significantly different at $P = 0.05$.

4. Discussion

The results of this study show that LTC prior to cold disinfestation of 'Hass' avocado can effectively eliminate skin damage and improve internal quality. Woolf et al. (2002) also found that LTC prevented skin damage, manifested as discrete dark patches on the skin, after 3 weeks at 0 °C. This suggests that LTC can provide very effective protection against damage during short duration storage at very low temperatures.

Li et al. (1989) reported similar responses in litchi, where pre-treatment of fruits at 5 °C for 5 days improved subsequent storage behaviour at 1 °C and increased the shelf life of fruits after storage. Other reports on LTC in fruit are limited, but numerous studies have shown that heat treatment before cold storage of a range of fruit can reduce damage on removal from storage (Lurie, 1998). Considerable research has been conducted on LTC or cold acclimation in whole plants or other plant tissues, mainly in relation to withstanding winter chill conditions (Kushad and Yelenosky, 1987; Hamman et al., 1996; Palonen et al., 2000). In many cases, acclimation can reduce lesion development on plant tissues (Ciardi et al., 1997) similar to the skin damage noted in the present study.

LTC treatments were more effective at improving internal and external quality of cold disinfested 'Hass' avocado fruit than HWTs in the present study, and when compared with the HWT results of Hofman et al. (2002). In addition, the results with LTC alone and when combined with HWT (experiment 2), suggests that LTC alone is more effective in improving fruit quality after disinfestation, and that there is little benefit in the combined treatments.

Woolf et al. (2002) found the optimal LTC treatments were 6–8 °C for 3–5 days, and the results here confirm this. We also found that 4 °C could be as effective as 6–8 °C if longer conditioning periods are used, as found by Woolf et al. (2002). As with heat treatments (Woolf et al., 1995), the LTC response is time and temperature dependant, since discrete patches and rots were more severe with 3 days at 4 °C and 3 days at 8 °C than longer durations at these temperatures. This suggests that conditioning development was slower at 4 °C because of the lower temperatures, while the responses at 8 °C was slower because the temperature was above the optimum for rapid conditioning development. The best temperature identified here was 6 °C, which is close to the 5 °C used by Li et al. (1989) with litchi. However, temperatures between 4 and 8 °C for 4 days provided similar results in the present study, indicating some flexibility in the temperatures and durations that can be used.

External appearance of fruit 3 days after removal from disinfestation was improved by LTC as discrete patches were effectively eliminated. It is

likely that this reduction in discrete patches contributed to the often lower external acceptability in ripe fruit after LTC and disinfestation compared with disinfestation without LTC. The discrete patches, often difficult to differentiate from the typical purple–black skin colour of ripe 'Hass' fruit, increased the dark colour of the ripe fruit. However, ripe skin colour is influenced by ripening temperatures. Relatively low ripening temperatures of 15–16 °C are recommended for 'Hass' avocado to reduce rots in ripe fruit (Hopkirk et al., 1994), but these temperatures can reduce the purple–black skin colour on ripe fruit (Hofman, unpublished data). However, the results of the commercial out-turn experiment suggest that ripening at 15 °C does not reduce rots incidence after LTC and disinfestation, so ripening of these fruit at 20 °C would improve skin colour and external acceptability, with no negative impact on internal quality.

The reduced rots severity in LTC, disinfested fruit was also observed by Woolf et al. (2002) and with HWT by Hofman et al. (2002). This response may be associated with less discrete patches with LTC or HWT, and improved ability of the skin to retard disease development. In addition, antioxidants reduce decay from *C. gloeosporioides* in avocados by reducing the decline in antifungal dienes (Prusky and Keen, 1993), and antioxidants have been shown to increase during LTC of rice seedlings (Li MeiRu et al., 1996). This positive effect of LTC on rots in ripe fruit was the major factor in the significantly improved internal quality of ripe fruit after LTC and disinfestation.

Previous research has shown that insects can develop tolerance to temperature extremes by milder hot or cold treatments (Kelty and Lee, 1999). This response could potentially render disinfestation treatments ineffective when combined with conditioning treatments designed to reduce fruit damage. However, the present study indicates that the LTC treatment of 6 °C for 3 days did not reduce the efficacy of the cold disinfestation treatment of 1 °C for 16 days. This, and the fact that disinfestation at 1 °C for 16 days is effective for most fruit fly species, suggests that this combined LTC and disinfestation treatment is an effective quarantine treatment for 'Hass' avocados. Conditioning responses can vary with growing conditions (Peynado, 1982). However, the commercial outturn results, and trials with fruit from other commercial orchards (Hofman; unpublished data) have confirmed that it is effective for 'Hass' fruit grown under different conditions.

Thus, our results confirm that LTC can effectively eliminate skin damage of 'Hass' avocado caused by low temperatures, and that LTC can be used to provide an effective commercial low temperature disinfestation treatment with acceptable external and internal quality of ripe fruit. However, the benefits of LTC need to be confirmed under local conditions.

Acknowledgements

We thank R. Simpson for supply of fruit and valuable practical help, Elizabeth Pike for technical assistance in the insect studies and the Department of Primary Industries, Queensland, and Goodward Farms for financial support for this project.

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