

The Influence of Breaks in Storage Temperature on ‘Cripps Pink’ (Pink Lady™) Apple Physiology and Quality

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Abstract. The maintenance of optimal storage conditions for fresh produce is rarely achieved in commercial cool chains. The impact of deviations for short time periods from these optimal storage conditions on fruit quality has not been thoroughly investigated. In this study, ‘Cripps Pink’ apples (*Malus domestica*) stored at 0 °C in air were exposed to periods at 20 °C (for 1, 3, and 6 days) to simulate breaks in the cool chain. The influence of harvest maturity, storage time before exposure, length of exposure, and multiple exposures to 20 °C on fruit physiology during and after the exposures was monitored through 27 laboratory-based scenario simulations. Preclimacteric apples exposed to 20 °C hastened climacteric development, whereas postclimacteric apples were induced to produce ethylene at ≈1.5 times the normal on return to cool storage at 0 °C irrespective of the fruit harvest maturity or timing, length, and number of exposures to 20 °C. The observed increase in ethylene production did not increase rates of reduction of either fruit stiffness (a measure of flesh texture) or background color measurement (hue angle). This research suggests that fluctuations in temperature have a greater effect in terms of changes in quality for preclimacteric apples than postclimacteric fruit. The reasons why changes in fruit stiffness and background hue angle in postclimacteric fruit did not respond to increases in ethylene production require further investigation.

The effects of temperature and gas atmosphere on fresh produce physiology and quality have been extensively studied to the point where optimal storage conditions for most products are widely available (Anon., 2006; Gross et al., 2004). However, in the commercial environment, temperature variations occur as a result of logistic constraints (e.g., limited access to power at the location of the container storage unit), mechanical refrigeration breakdown, or management decisions (e.g., choice of nonrefrigerated

vehicles over refrigerated vehicles). Another common temperature break scenario arises through the refrigerated storage of fruit in bins immediately after harvest. At a later time, the fruit are removed from the cool store, graded, and packed before shipping in a refrigerated environment to markets. Because the packing facility is often not refrigerated, the fruit is warmed and then re-cooled.

The use of controlled breaks in cool storage, either before (DeLong et al., 2004) or during storage (Watkins et al., 2000), has previously been investigated primarily as a means to minimize chilling injury development and hence extend the product storage life. This body of work provides evidence of increased ethylene production (Liu, 1986; Zhou et al., 2001) as a result of exposure to ambient temperatures (10 to 25 °C) for short time periods. Unintentional breaks in refrigeration differ from the application of inten-

tionally applied cool storage breaks, because they tend to be sporadic and unpredictable in terms of magnitude (time and temperature). How these uncontrolled breaks in storage conditions affect product physiology and quality on return to refrigerated storage conditions has rarely been investigated.

The postharvest behavior of apples is well documented. Apples ripen with a typical climacteric ethylene and respiratory pattern during postharvest storage (Jobling and McGlasson, 1995; Larrigaudiere et al., 1997), whereas fruit quality changes progress for some time during the postclimacteric phase (Johnston et al., 2001a). Key quality changes for apples are changes in firmness (from hard to soft) and background color (from green to yellow). There is significant experimental evidence that both of these quality attributes are ethylene-sensitive (Golding et al., 2005; Saftner et al., 2003).

This study aimed to assess the effects of exposing apples stored at 0 °C in air to short-term periods at 20 °C on subsequent fruit physiology and quality both during the time of the exposure and on return to cool storage at 0 °C. This investigation was based around assessing fruit response to what would be considered the most extreme (yet still likely) breaks in the cool chain (3 d at 20 °C). This approach was taken to detect changes in the extreme situation with the assumption that if no significant changes occur in this scenario, then breaks of a lesser magnitude (in time or temperature) could also be assumed to have no effect. The influence of harvest maturity, time in storage before exposure, length of exposure to 20 °C, and multiple exposures to 20 °C on the responses of the fruit were also studied. ‘Cripps Pink’ (‘Pink Lady™’) apples were chosen as the cultivar in which to investigate these effects.

Materials and Methods

Fruit samples. In 2003, ‘Cripps Pink’ apples were sourced from a commercial orchard in Hawkes Bay, New Zealand. Fruit were harvested on two separate harvest dates (9 and 22 Apr.) and transported to Palmerston North on the day after harvest. In 2004, ‘Cripps Pink’ were harvested on 1 May from a commercial orchard in Batlow, NSW, Australia, stored for 3 d at 0 °C in air at Batlow, and then transported nonrefrigerated to North Ryde, Sydney (a journey of 1 d). For all harvests, fruit were preclimacteric at the time of harvest as determined by low rates of respiration and ethylene production.

Temperature treatments. The 2003 experiment was designed to simulate possible commercial practices whereby, after a period of cool storage at 0 °C in air, fruit were exposed to a break in temperature control (e.g., to simulate repacking or loading onto a ship) and then followed by a small cool storage and shelf life period (Table 1; Fig. 1). This simulated break (B = break) was 3 d (subscript = 3d) at 20 °C, with each treatment that experienced a break having an equivalent control (C = control) treatment of 3 d remaining

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Table 1. Range of variable temperature storage treatments used in the experimental work.²

Harvest	Treatment	Initial storage time	Length of break	Storage after break	Shelf life at 20 °C
2003	C ₀	0 mo	None	3 weeks	2 weeks
	C ₂	2 mo	None	3 weeks	2 weeks
	C ₄	4 mo	None	3 weeks	2 weeks
	C ₆	6 mo	None	3 weeks	2 weeks
	B _{0,3d}	0 mo	3 d	3 weeks	2 weeks
	B _{2,3d}	2 mo	3 d	3 weeks	2 weeks
	B _{4,3d}	4 mo	3 d	3 weeks	2 weeks
	B _{6,3d}	6 mo	3 d	3 weeks	2 weeks
2004	C	5.5 mo	None	None	None
	B _{2,1d}	2 mo	1 d	3.5 mo	None
	B _{2,3d}	2 mo	3 d	3.5 mo	None
	B _{2,6d}	2 mo	6 d	3.5 mo	None
	2B _{2,1,2}	2 mo	3 d + 3 d	1 mo + 2 mo	None
	2B _{2,2,1}	2 mo	3 d + 3 d	2 mo + 1 mo	None
	3B	2 mo	3 d + 3 d + 3 d	1 mo + 1 mo + 1 mo	None

²Graphical depictions of these treatments are represented in Figure 1.

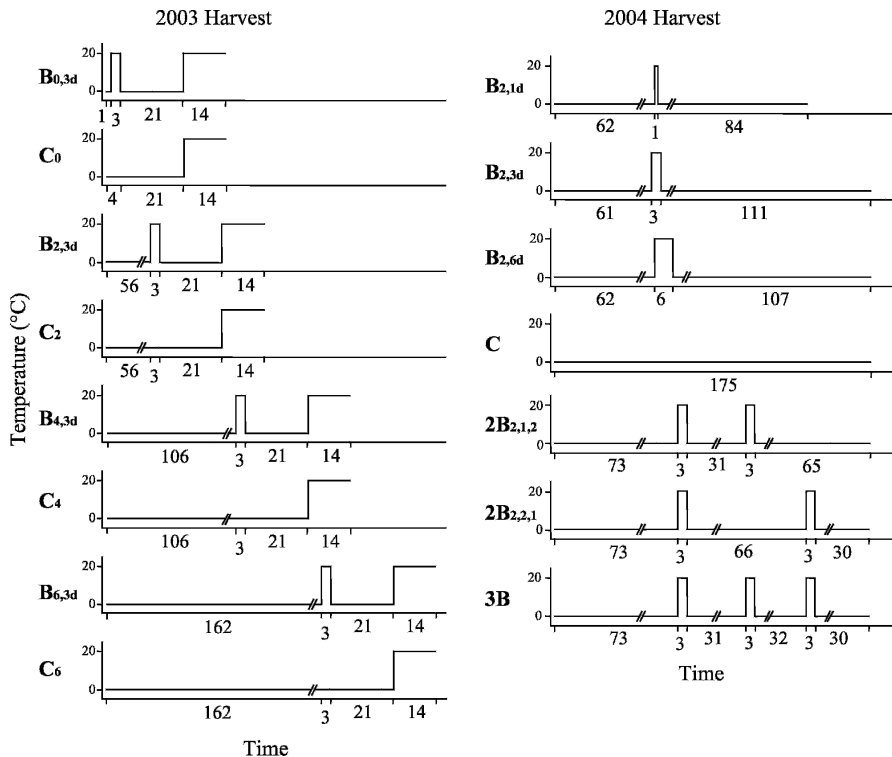


Fig. 1. Temperature treatments applied to apples from two 2003 harvests (Hawkes Bay, New Zealand) and the 2004 harvest (Batlow, NSW, Australia). The time scale is shown in days and is not drawn to scale. Details of these same treatments are provided in Table 1.

at 0 °C. The apples were then subjected to 3 weeks storage at 0 °C followed by 2 weeks at 20 °C to simulate shipment and retail handling. The influence of harvest maturity and time in storage before exposure (0, 2, 4, or 6 months; subscripts = 0, 2, 4, 6, respectively) were investigated. Each treatment was represented by 20 fruit for which respiration rate and ethylene production measurements were made. All fruit were stored on fiberboard trays in telescopic corrugated cardboard boxes as conducted commercially in New Zealand and Australia.

In 2004, the influences of the length (1, 3, or 6 d) of the break in temperature control and multiple breaks in temperature control were investigated (Table 1; Fig. 1). The apples

were stored at 0 °C in sealed 60-L barrels. Unhumidified air was supplied to the barrels at 400 mL·min⁻¹ in a flow through system. The physiological status of 10 fruit and the quality of 30 fruit were measured nondestructively for each treatment.

In both years, the temperature changes were achieved by physically moving fruit from a room at one prescribed temperature to another. Temperature change was aided by spreading the apples along a tabletop and using a household fan to force air past them. By this method, the temperature of the apples was raised from 0 to 20 °C in ≈4 h.

Physiological status. Ethylene production and respiration rate of individual fruit (20 per measurement in 2003, 10 per measurement in

2004) were assessed simultaneously at regular intervals throughout storage. Fruit were enclosed in an airtight container (1 L) and gas samples (2 × 1 mL) were taken at the time of closure and after a measured time of ≈1.5 h. One sample was analyzed for carbon dioxide concentration with the other used for ethylene concentration (as described below). Rates of ethylene production and respiration (CO₂ production) were calculated with respect to the apple mass, volume of the container, and time between sampling and expressed as recommended by Banks et al. (1995).

In 2003, CO₂ concentration in the gas samples was analyzed using a miniature infrared carbon dioxide transducer (Analytical Development, Hoddesdon, UK) with oxygen-free nitrogen as a carrier gas and a Hewlett Packard Integrator (model 3396A; Hewlett Packard, Singapore) with area under the peak used as an indicator of concentration. In 2004, CO₂ concentration was analyzed with a Gow-Mac (Gow-Mac, Bethlehem, PA) gas chromatograph (series 580) fitted with a CTR1 column (Alltech, Grace Davidson Discovery Sciences, Deerfield, IL) and a Riken Denshi chart recorder (model SP-G6P; Tokyo) with helium as a carrier gas. Peak height was used as an indicator of concentration. In both years, the equipment was calibrated with external CO₂ standards (certified as β-standard by B.O.C. Gases (Auckland, New Zealand), New Zealand or Australia, respectively) and linearity of output to sample concentration and volume was validated.

In 2003, ethylene concentration was analyzed using a gas chromatograph (Varian 3400, Palo Alto, CA) fitted with a flame ionization detector and a mesh alumina column with nitrogen as a carrier gas. Data were analyzed with a Hewlett Packard Integrator (model 3396A). In 2004, ethylene concentration was analyzed with a Shimadzu gas chromatograph (model GC-17A; Kyoto, Japan) fitted with a GS-Q column (J and W Scientific, Folsom, CA) and using helium as the carrier gas; data were analyzed with a Shimadzu integrator (model C-R7A). In both years, the equipment was calibrated with external ethylene standards (certified as β-standard by B.O.C. Gases) with 101 μL·L⁻¹ and 11.2 μL·L⁻¹ standards used in 2003 and 2004, respectively.

Quality assessment. In 2004 only, an acoustic firmness measurement of each apple was conducted with an acoustic firmness sensor (Aweta, Nootdorp, The Netherlands) at the treatment temperature (0 or 20 °C). The average of three measurements per fruit was taken to be the apple stiffness (10⁶Hz²/kg^{2/3}). Thirty fruit were measured at each sampling time.

A Minolta model CR-400 (Konica Minolta Sensing, Osaka, Japan) chroma meter calibrated with a white color standard (Commission Internationale de l'Éclairage units of Y = 92.9, x = 0.3134, y = 0.3196 using an illuminant C light source) was used to measure the background color of fruit from the 2004 harvest. Because the 'Cripps Pink'

cultivar is a bicolored fruit, at the onset of the experiment, the “greenest patch on the apple shoulder” on each fruit was identified by eye and marked. This location was measured on each successive occasion at the treatment temperature (0 or 20 °C). Fruit that recorded a hue angle of less than 90° at the onset of the experiment were considered not to have any significant green region and removed from calculation of the treatment average.

Data transformation. To remove some of the fruit to fruit variability, the measured attributes at each time were compared with the attribute of the same fruit before exposure to 20 °C. When possible, respiration rate and ethylene production data were normalized to those measured immediately before temperature exposure (for those treatments with an exposure) or that measured at the equivalent time (for the control treatments). A value equal to 1.0 indicates no change in physiological status of that fruit, whereas values above or below 1.0 indicate a more rapid or slower metabolism, respectively. Hue angle and stiffness are reported as changes from the time before first exposure to a break in temperature control. Because hue angle and stiffness both reduce during storage of ‘Cripps Pink’ apples, the parameter change values are expected to become increasingly more negative with time.

Data analysis. A one-way analysis of variance (Minitab v13.31; Minitab, State College, PA) was conducted at key points identified from the graphs constructed. When the variance of data were not homogeneous as assessed by Levene’s test ($P < 0.05$, Minitab v13.31; Minitab), values were then transformed using log with base of 10 to regain homogeneity. Significant differences between treatments and significant departure from physiological status before exposure (log-normalized respiration rate/ethylene production $\neq 0$) were determined with the use of Fisher’s least significant difference (LSD) method ($P < 0.05$, Minitab v13.31; Minitab).

The rates of loss of hue angle and stiffness were calculated for individual apples between defined time periods using linear regression (Excel v9; Microsoft Corp., Redmond, WA). Comparison of rates of loss of stiffness and hue angle between treatments was also conducted using a one-way analysis of variance (Minitab v13.31; Minitab) and Fisher’s LSD ($P < 0.05$, Minitab v13.31; Minitab).

Results and Discussion

No substantial differences in the response of the two 2003 harvests were observed (data not shown), suggesting that harvest maturity (of preclimacteric fruit) did not influence the observed behavior. Consequently, only the data for the late harvest of 2003 and the 2004 harvest are presented.

Respiration rate. On initial exposure to 20 °C, the respiration rate increased ≈ 5 times over that at 0 °C (Figs. 2A and 3A). When returned to 0 °C, the respiration rate of the apples then declined to be similar to apples when stored constantly at 0 °C. During the

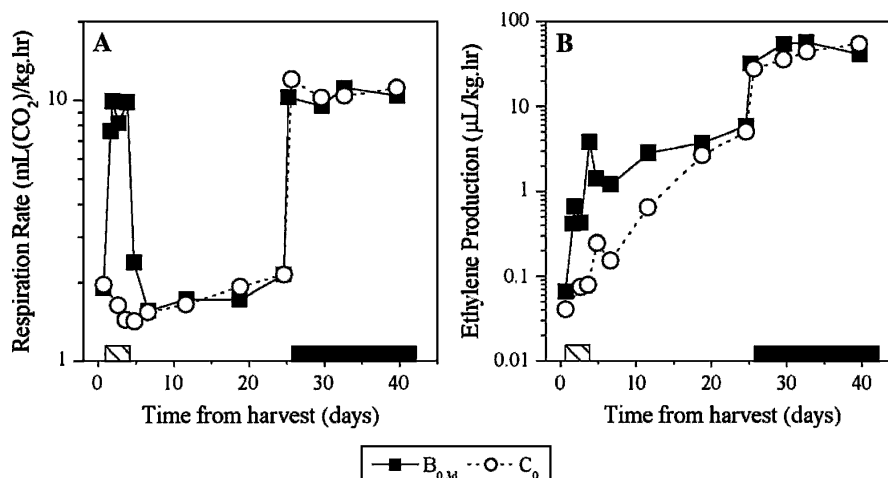


Fig. 2. The effect of a temperature break ($B_{0,3d}$, 3 d at 20 °C, solid square symbols) in comparison with control fruit (C_0 , 3 d at 0 °C, open circle symbols) on ‘Cripps Pink’ apple (A) respiration rate and (B) ethylene production after 0 months storage at 0 °C for late harvest fruit from the 2003 harvest. After the exposure of 3 d at 20 °C, fruit were returned to 0 °C for 21 d followed by 14 d at 20 °C. Hatched bars along the x-axis represent time of exposure to 20 °C for treatment $B_{0,3d}$, whereas solid bars represent time at 20 °C for both treatments.

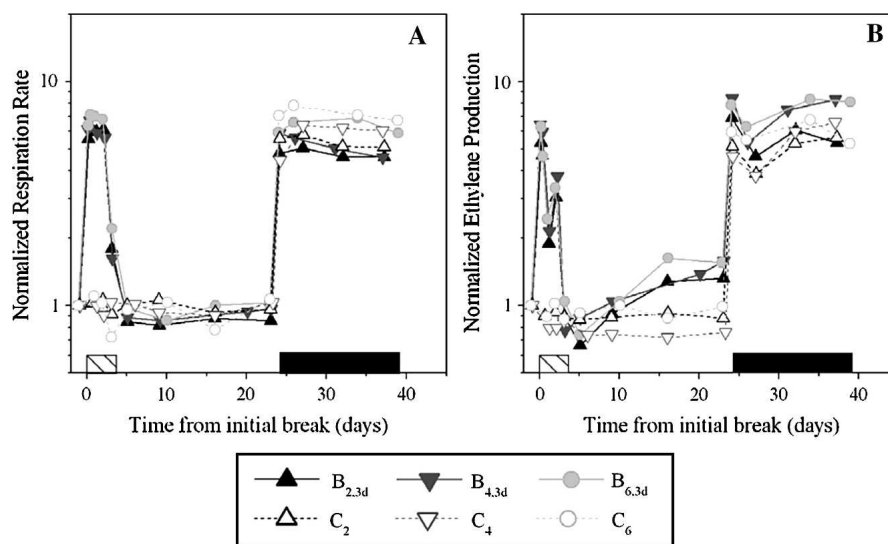


Fig. 3. The effect of a temperature break (B, 3 d at 20 °C, solid symbols and lines) in comparison with control fruit (C, 3 d at 0 °C, open symbols and dashed lines) on ‘Cripps Pink’ apple normalized (A) respiration rate and (B) ethylene production after 2 (upward triangle symbols), 4 (downward triangle symbols), and 6 (circle symbols) months storage at 0 °C. Data presented are for the late 2003 harvest only. After break treatments were exposed to 3 d at 20 °C, apples were returned to 0 °C for 21 d followed by 14 d at 20 °C. Hatched bars along the x-axis represent time of exposure to 20 °C for treatments B, whereas solid bars represent time at 20 °C for both treatments.

simulated shelf life period (2 weeks at 20 °C), the respiration rates of all treatments were again six to eight times greater than at 0 °C (Fig. 3A). The respiration rates on return from a temperature break (for treatments $B_{2,3d}$, $B_{4,3d}$, and $B_{6,3d}$) were not substantially different from those before the temperature break (normalized respiration rate = 1) or fruit not exposed to the break (treatments C_2 , C_4 , and C_6 ; Fig. 3A). The timing of exposure, length of exposure, harvest maturity, or multiple exposures to 20 °C had no influence on this behavior (data not shown).

Ethylene production. At the time of harvest, fruit were in a preclimacteric state as evidenced by the low rate of ethylene production ($\approx 0.04 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$; Fig. 2B). Ethyl-

ene production increased dramatically in the first 25 d of cool storage at 0 °C, reaching production rates of $5 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (treatment C_0 ; Fig. 2B). Ethylene production of apples maintained at 0 °C peaked (i.e., reached the climacteric) at 30 to 50 d of cool storage (East, 2006) at a value of $\approx 12 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$.

Physiological status at the time of the exposure to a break in temperature control had an effect on the response of ‘Cripps Pink’ apple ethylene production. Those apples exposed to raised temperature soon after harvest (treatment $B_{0,3d}$; Fig. 2B) and still in a preclimacteric state displayed a different behavior to fruit that had been stored for sufficient time to develop a climacteric response (treatments $B_{2,3d}$, $B_{4,3d}$, and $B_{6,3d}$; Fig. 3B).

Fruit exposed to 20 °C after 0 months storage (treatment B_{0,3d}) displayed an ethylene production increase that was maintained in the subsequent 21 d at 0 °C. As time during simulated transport increased, ethylene production differences between treatments B_{0,3d} and C₀ decreased with ethylene production of control fruit increasing to a level equal to that of the fruit exposed to the break. It would appear that a period at 20 °C for preclimacteric ‘Cripps Pink’ apples advances the start of the climacteric peak. During the subsequent shelf life period, neither respiration rate nor ethylene production was substantially influenced by previous temperature exposure (Fig. 2A–B).

In previous studies, delays before cooling ‘Royal Gala’ and ‘Cox’s Orange Pippin’ apples have led to increased internal ethylene concentrations (Johnston et al., 2005). In this study, a 3-d exposure to 20 °C after zero months at 0 °C advanced the maturity of the ‘Cripps Pink’ apples toward climacteric development, providing further evidence that a delay in the cooling of apples after harvest advances the development of the ethylene production climacteric peak. If the apple cultivar is sensitive to ethylene, this reduction of preclimacteric storage time may result in a reduced storability of the fruit as a result of more rapid softening during storage (Johnston et al., 2005).

Apples stored for 2, 4, and 6 months all showed similar patterns of respiration rate and ethylene production on exposure to 20 °C for 3 d and during subsequent cool storage and shelf life periods (Fig. 3). Compared with the rate of ethylene production before the temperature change, ethylene production was five to seven times higher on the first day of raised temperature, reducing to two to four times higher on the next day and increasing to four to six times higher on the third day at 20 °C (Fig. 3B). Two days after return to cool storage (at 5 d), fruit stored for 2 months before the temperature cycle initially produced ethylene at a rate less than that before the break, but the rates for fruit stored for 4 or 6 months were not different from their control treatments (Table 2). All postclimacteric apples exposed for 3 d at 20 °C and returned to 0 °C eventually showed increased ethylene production at a level ≈ 1.5 times that of the prebreak ethylene production (at 18 d) and those treatments not exposed to a break in temperature control (Fig. 3B; Table 2).

On initiation of the shelf life period at 20 °C (at 24 d after the break), fruit that had previously been exposed to 20 °C continued to produce ethylene at a higher rate than those fruit stored under constant temperature conditions (Fig. 3B; Table 2). This difference between treatment groups decreased during the shelf life period and resulted in both break and control fruit treatments eventually producing ethylene at the same rate after 2 weeks at 20 °C (at 36 d; Table 2).

The length of exposure to 20 °C influenced the pattern of ethylene production of ‘Cripps Pink’ apples on return to cool storage (Fig. 4A). Fruit that were exposed to 1 d at

Table 2. Comparison of Cripps Pink apple normalized ethylene production for treatments exposed to 3 d at 20 °C (B) after variable times in storage at 0 °C to apples not exposed to a break in temperature control (C).^z

Late harvest Time after initial exposure to 20 °C	Log-normalized ethylene production											
	5 d (0 °C) ^y		18 d (0 °C)		24 d (20 °C)		36 d (20 °C)					
		n		n		n		n				
B _{2,3d}	-0.188	b*	20	0.099	ab	19	0.825	a	17	0.710	a	19
C ₂	-0.064	a	19	-0.038	c	19	0.703	bc	18	0.738	a	18
B _{4,3d}	-0.136	ab*	18	0.051	bc	18	0.860	a	17	0.823	a	18
C ₄	-0.133	ab*	18	-0.142	d*	17	0.665	c	18	0.792	a	17
B _{6,3d}	-0.087	a	13	0.166	a*	16	0.851	a	17	0.760	a	15
C ₆	-0.048	a	20	-0.029	c	17	0.790	ab	19	0.725	a	18
Least significant difference _{0.05}	0.096		0.102		0.105		NS					

^zSubscripts 2, 4, and 6 refer to 2, 4, and 6 mo storage at 0 °C before exposure, respectively. After exposure, fruit were returned to 0 °C for 21 d followed by 14 d at 20 °C. Data points are extracted from those presented in Figure 3A.

^yDifferent letters in columns indicate significant differences between treatments ($P < 0.05$).

*Significant differences from initial physiological status (0) for fruit measured at 0 °C.

NS = no significant differences.

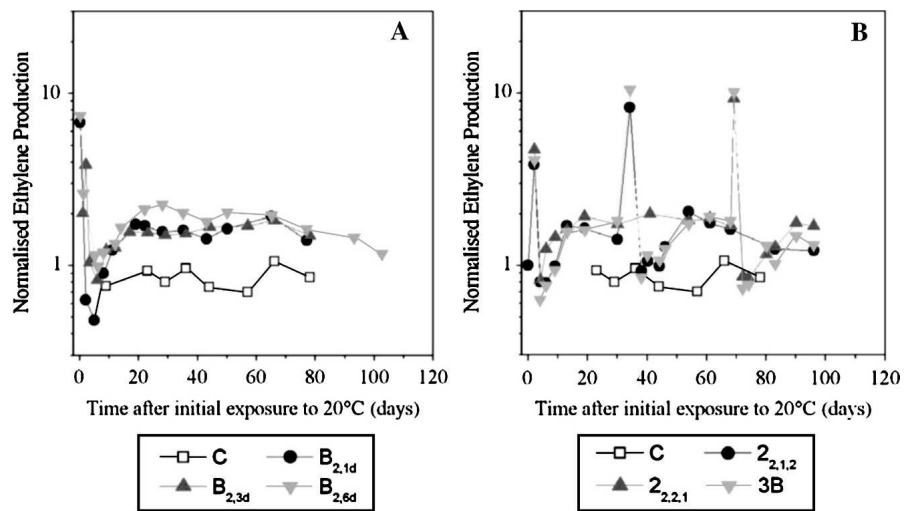


Fig. 4. (A) Influence of the length of exposure to 20 °C and (B) influence of multiple exposures to 20 °C on the normalized ethylene production of ‘Cripps Pink’ apples. Treatments C (open square), B_{2,1d} (solid circle), B_{2,3d} (solid upward triangle), and B_{2,6d} (gray downward triangle) represent exposure time of 0, 1, 3, and 6 d of exposure to 20 °C, respectively. Treatments 2B_{2,1,2} = two exposures (0 and 34 d; solid circle); 2B_{2,2,1} = two exposures (0 and 69 d; solid upward triangle); and 3B = three exposures (0, 34, and 69 d; gray downward triangle).

20 °C and then cooled (treatment B_{2,1d}) initially produced $\approx 50\%$ less ethylene than before the temperature break, whereas fruit exposed to 20 °C for 6 d before cooling (treatment B_{2,6d}) produced ethylene at the same level as before the break. Regardless of the duration of the elevated temperature, at 25 d after initially raising the temperature, all apples produced 50% to 100% more ethylene at 0 °C than before the higher temperature exposure or fruit that remained at 0 °C (treatment C). This altered rate of ethylene production, induced by the previous exposure to 20 °C, was maintained for the remainder of the experiment (80 d at 0 °C; Fig. 4A). This suggests that even a short time (1 d) at 20 °C can trigger an increase in ethylene production in postclimacteric fruit on return to cool storage.

A second or third exposure to 20 °C for 3 d mimicked the response observed for a single exposure (Fig. 4B). On each exposure to 20 °C, the ethylene production increased

by five to six times. Absolute ethylene production was therefore greater during the second and third exposures, possibly as a result of the prevailing higher rate at 0 °C induced by previous temperature exposure. This result supports the findings for the 2003 season in which fruit previously exposed to 20 °C produced more ethylene than control treatments on the initiation of the shelf life period (at 24 d; Table 2; Fig. 3B).

On return to 0 °C storage after the second (or third) exposure (e.g., at 40 d for treatments 2B_{2,1,2} and 3B), ethylene was produced at rates similar to that of fruit not exposed to any period at 20 °C (Fig. 4B). This result is surprising in view of the fact that the fruit were producing approximately double this amount of ethylene before the second (or third) exposure as a result of the first exposure. Five days after the second (or third) exposure, ethylene production once again began to increase and stabilized at 1.5 to

two times that of nonexposed apples ≈ 2 weeks after the 20 °C exposure.

Without exception, exposing postclimacteric 'Cripps Pink' apples to 20 °C resulted in increased rates of ethylene production of the fruit on return to cool storage and (at least initially) on subsequent return to warm storage (Figs. 3B and 4). In general, on return to cool storage, the rate of ethylene production returned to close to that before the initial temperature exposure. The rate remained at this level for ≈ 5 d and then increased to ≈ 1.5 to two times the initial ethylene production rate over the next 10 d. This elevated production was maintained for the remainder of the storage period.

There were no clear influences of harvest maturity (data not shown), time in storage before exposure to 20 °C (assuming that longer stored fruit are postclimacteric), or previous exposures to 20 °C (Fig. 4B) on the magnitude or rate of the response of the induced increase in ethylene production. However, the duration of exposure to 20 °C influenced the initial ethylene production of 'Cripps Pink' apples on return to cool storage (Fig. 4A) with those fruit exposed for 1 d (treatment B_{2,1d}) producing half as much ethylene as that before exposure, whereas those fruit exposed for 6 d (treatment B_{2,6d}) returned to ethylene production levels similar to that before exposure to the warmer temperature.

In this investigation, the increased ethylene production as a response to a break in temperature control of postclimacteric 'Cripps Pink' apples was both delayed (by ≈ 5 d on return to 0 °C cool storage) and sustained (for a period of up to 80 d after the initial break in temperature control; Fig. 4A). Both of these traits suggest that the response is not a result of stress, but rather a shift in metabolic control and altered homeostasis of ethylene production.

Induced increases in ethylene production on return to cool storage have been observed in other investigations that contained variable storage temperature regimes for other apple cultivars (Alwan and Watkins, 1999; Johnston, 2001), peaches (Zhou et al., 2001), and cucumbers (Cabrera and Salveit, 1990). These studies suggest that an increase in ethylene production at low storage temperatures may be a widespread response of fruit exposed to fluctuating temperature regimes.

A burst of ethylene synthesis on transfer of produce from chilled temperatures to warmer temperatures is often seen in apples and is often correlated with increases in 1-aminocyclopropane-1-carboxylic acid (ACC) concentration and ACC oxidase (ACO) activity (Jobling and McGlasson, 1995; Larrigaudiere et al., 1997). The rapid burst of ethylene production on exposure to 20 °C after storage at 0 °C observed in the current work is likely to be facilitated by accumulated ACC, which is used by increased ACO activity on transfer of the fruit to the warmer temperature. Alternatively, it is possible that fluctuations in temperature induce membrane lipid compositional changes and hence ACO function-

ality. The influence of temperature on membrane lipid composition has been confirmed in 'Cox's Orange Pippin' apples (Bartley, 1986). Further investigation is required to determine whether the shift in homeostasis of ethylene production of 'Cripps Pink' apples subjected to a break in temperature control is caused by a change in concentration or activity of ethylene producing enzymes (ACO and ACC synthase), changes in membrane composition that influences ACO functionality, or a combination of both effects.

Despite the influence of fluctuating temperatures on ethylene production, the respiration rate was similar before and after changes in storage temperature (Figs. 2A and 3A), not unlike the response of tomato

respiration rate and ethylene production when exposed to intermittent warming (Artés et al., 1998). This result demonstrates the independence of the two physiological indicators (respiration rate and ethylene production), at least for 'Cripps Pink' apples, and hence suggests that both indicators should be measured to gain a better understanding of fruit physiological status.

Stiffness (acoustic firmness). Exposure to 20 °C resulted in a more rapid decrease in stiffness as measured by acoustic firmness (Figure 5) with those fruit exposed to 20 °C for a longer time showing a greater change in stiffness ($P < 0.001$ at 8 d after initial temperature exposure). On return to cool storage, the subsequent rates of stiffness loss were not different among treatments.

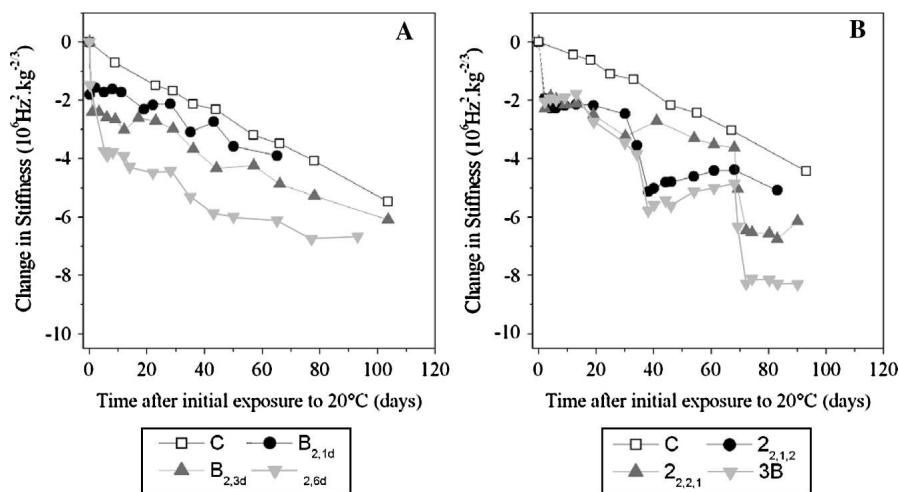


Fig. 5. Effect of (A) length of exposure time after 2 months storage at 0 °C and (B) multiple breaks of 20 °C for 3 d during storage at 0 °C on change in 'Cripps Pink' apple stiffness as measured by an acoustic firmness sensor. Treatments C (open square), B_{2,1d} (solid circle), B_{2,3d} (solid upward triangle), and B_{2,6d} (gray downward triangle) represent exposure time of 0, 1, 3, and 6 d of exposure to 20 °C, respectively. Treatments 2B_{2,1,2} = two exposures (0 and 34 d; circle); 2B_{2,2,1} = two exposures (0 and 69 d; solid upward triangle); and 3B = three exposures (0, 34, and 69 d; gray downward triangle).

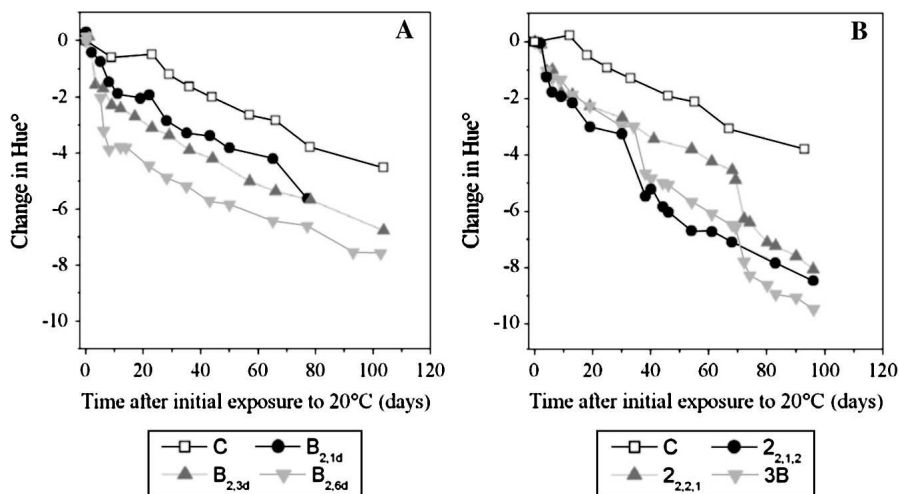


Fig. 6. Effect of (A) length of exposure time after 2 months storage at 0 °C and (B) multiple breaks of 20 °C for 3 d during storage at 0 °C on change in 'Cripps Pink' background hue angle. Treatments C (open square), B_{2,1d} (solid circle), B_{2,3d} (solid upward triangle), and B_{2,6d} (gray downward triangle) represent exposure time of 0, 1, 3, and 6 d of exposure to 20 °C, respectively. Treatments 2B_{2,1,2} = two exposures (0 and 34 d; solid upward triangle); 2B_{2,2,1} = two exposures (0 and 69 d; solid downward triangle); and 3B = three exposures (0, 34 and 69 d; solid diamond).

Elevated temperature exposures later in storage for those treatments exposed on multiple occasions were observed to cause a larger change in stiffness (Figure 5). Whether this increased change in stiffness on secondary exposures was influenced by previous exposure or greater fruit maturity could not be determined. The rate of change of stiffness on subsequent return to cool storage (at 0 °C) was reduced by multiple exposures to 20 °C for 3 d (Figure 5). This reduction in stiffness loss may reflect a positive benefit of what is, in essence, an intermittent warming treatment.

Background hue angle. Exposing 'Cripps Pink' apples to 20 °C after 2 months storage for any length of time caused a reduction in hue angle (i.e., a yellowing of background color; Figure 6). Apples exposed for longer periods of time had a greater reduction in hue angle. Second and third exposures to 20 °C for 3 d had similar effects to the primary exposure on change in hue angle (Figure b). On return to cool storage at 0 °C, the rate of hue angle reduction was ≈ 0.05 °Hue/day and was not different among treatments. These results indicate that the temperature effect on hue angle may be additive, as evidenced by the similar values observed for treatments exposed to a total of 6 d at 20 °C, but by different scenarios (treatments 2B_{2,1,2}, 2B_{2,2,1}, and B_{2,6d}), at the completion of the experiment (comparing Figure a and Figure b).

At 20 °C, the loss of the quality of postclimacteric 'Cripps Pink' apples as measured by stiffness (Figure 5) and hue angle (Figure 6) was more rapid than at the cool storage temperature of 0 °C. This more rapid rate of loss of stiffness and hue angle resulted in differences between exposed and control apples on return to cool storage. In similar studies with apples, Alwan and Watkins (1999) and Johnston (2001) reported that intermittent warming resulted in a lower firmness of the warmed fruit on return to cool storage in contrast with fruit that were not warmed, whereas Artés et al. (1998) found that intermittent warming resulted in greater reductions in hue angle and firmness of tomatoes.

Rates of change of stiffness of 'Cripps Pink' apples at 0 °C were found to be dependent on previous time-temperature history. A single temperature exposure had no influence on the rate of stiffness change on return to cool storage (Figure 5). However, for fruit exposed to two or three exposures to 20 °C, rates of stiffness change in subsequent cool storage were less than control fruit (Figure b). These results may be related in part to the influence of water loss (and resulting product turgor pressure) on the stiffness measurement (Hertog et al., 2004).

Previously published research has established clear links between ethylene production and apple firmness and color changes, including for the 'Cripps Pink' cultivar (Golding et al., 2005; Saftner et al., 2003). Hence, if ethylene has a stimulating effect on fruit softening and chlorophyll degradation

and subsequent skin yellowing, we would expect the rate of change of stiffness and hue angle to increase as a result of increased ethylene production on return to cool storage after a warming period. However, there was no response to increased ethylene production for 'Cripps Pink' in this experiment. Similarly, when Tan and Bangerth (2000) applied exogenous ethylene to 'Golden Delicious' apples (a parent cultivar of 'Cripps Pink') at four different stages of maturity, the first three harvests (preclimacteric) were stimulated to produce more ethylene (through autocatalysis), whereas the late harvest (climacteric) showed no stimulation. These results suggest that subsequent increases of ethylene (either by self-production or exogenous supply) to postclimacteric fruit does not necessarily stimulate more rapid quality losses, because postclimacteric apples may already produce ethylene at saturation levels with respect to the relevant enzyme systems.

The induction of higher ethylene observed in this work suggests that deliberate breaks in temperature control should be avoided, whether the result of measurement protocol or other circumstances, because these breaks may influence the results of postharvest experiments. This, however, provides the researcher with a difficult quandary when, as frequently occurs, fruit stored at different temperatures are to be compared and measured nondestructively. For apples at least, measurement temperature influences firmness (Johnston et al., 2001b) and color (A.R. East, unpublished data) measurement. Furthermore, calibration for the differences in measurement are a function of the maturity of the fruit (Johnston et al., 2001b) making it difficult to fairly compare measurements taken at different temperatures for differently matured fruit. Basic scientific technique suggests that we should measure all treatments at the same temperature. However, this experiment demonstrates that moving fruit to a single temperature may influence the future response of that fruit to their subsequent treatment temperature.

In conclusion, breaks in temperature control that would be considered extreme in commercial situations (20 °C for 1 to 6 d) during storage of postclimacteric 'Cripps Pink' apples at 0 °C in air reduces quality at the time of exposure in comparison with nonexposed fruit. Preclimacteric exposed fruit accelerated toward climacteric development, whereas postclimacteric apples were induced to produce ≈ 1.5 to two times the rate of ethylene production on return to subsequent cool storage. The dynamics of the ethylene production observed in this work demonstrates the complexity of the ethylene production control system. Study of the gene expression and enzyme or plasma membrane compositional changes during the response observed in this work may help to elucidate more of the mechanisms that control ethylene production.

Despite the strong links between ethylene and the rate of change of apple quality characteristics (firmness and background

hue angle), the induced increase in ethylene production on return to cool storage was not observed to increase subsequent rates of quality change at 0 °C. It is suggested that the lack of response of quality characteristics to the increase in ethylene is a result of ethylene levels in nonexposed fruit already exceeding saturation levels for quality loss stimulation.

Literature Cited

- Alwan, T.F. and C.B. Watkins. 1999. Intermittent warming effects on superficial scald development of 'Cortland', 'Delicious' and 'Law Rome' apple fruit. *Postharv. Biol. Technol.* 16:203–212.
- Anon. 2006. Postharvest Technology Research and Information Centre, University of California, Davis home page. 10 July 2006 <<http://postharvest.ucdavis.edu>>.
- Artés, F., F. García, J. Marquina, A. Cano, and J.P. Fernández-Trujillo. 1998. Physiological responses of tomato fruit to cyclic intermittent temperature regimes. *Postharv. Biol. Technol.* 14:283–296.
- Banks, N.H., D.J. Cleland, A.C. Cameron, R.M. Beaudry, and A.A. Kader. 1995. Proposal for a rationalized system of units for postharvest research in gas exchange. *HortScience* 30:1129–1131.
- Bartley, I.M. 1986. Changes in sterol and phospholipids composition of apples during storage at low temperature and low oxygen concentration. *J. Sci. Food Agr.* 37:31–36.
- Cabrera, R.M. and M.E. Salveit. 1990. Physiological response to chilling temperatures of intermittently warmed cucumber fruit. *J. Amer. Soc. Hort. Sci.* 115:256–261.
- DeLong, J.M., R.K. Prange, and P.A. Harrison. 2004. The influence of delayed cooling on quality and disorder incidence in 'Honeycrisp' apple fruit. *Postharv. Biol. Technol.* 33:175–180.
- East, A.R. 2006. The influence of breaks in optimal storage conditions on 'Cripps Pink' apple physiology and quality. Massey University, Palmerston North, New Zealand. PhD Diss.
- Golding, J., S. Satyan, A.C. Rath, J. Jobling, and H. James. 2005. Retain® maintains Pink Lady™ fruit quality during long term storage. *Acta Hort.* 682:119–125.
- Gross, K.C., C.Y. Wang, and M.E. Saltveit. 2004. The commercial storage of fruits, vegetables and florist and nursery stocks. 3rd Ed. *Agri. Hndbk. No. 66.* U.S. Dept. Agr., Agr. Res. Serv., Washington, DC.
- Hertog, M.L.A.T.M., R. Ben-Arie, E. Roth, and B.M. Nicolai. 2004. Humidity and temperature effects on invasive and non-invasive firmness measurements. *Postharvest Biol. Technol.* 33:79–91.
- Jobling, J.J. and W.B. McGlasson. 1995. A comparison of ethylene production, maturity and controlled atmosphere storage life of Gala, Fuji and Lady Williams apples. *Postharv. Biol. Technol.* 6:209–218.
- Johnston, J.W. 2001. Postharvest apple softening: Effects of at-harvest and post-harvest factors. Massey University, Palmerston North, New Zealand. PhD Diss.
- Johnston, J.W., E.W. Hewett, M.L.A.T.M. Hertog, and F.R. Harker. 2001a. Temperature induces differential softening responses in apple cultivars. *Postharv. Biol. Technol.* 23:185–196.
- Johnston, J.W., E.W. Hewett, N.H. Banks, and F.R. Harker. 2001b. Temperature physically affects apple texture. *Acta Hort.* 553:207–209.

- Johnston, J.W., E.W. Hewett, and M.L.A.T.M. Hertog. 2005. Apple (*Malus domestica*) softening in the postharvest coolchain: Effects of delayed cooling and shelf-life temperatures. *New Zeal. J. Crop Hort.* 33:283–292.
- Larrigaudiere, C., J. Graell, J. Salas, and M. Vendrell. 1997. Cultivar differences in the influence of a short period of cold storage on ethylene biosynthesis in apples. *Postharv. Biol. Technol.* 10:21–27.
- Liu, F.W. 1986. Effects of delayed cooling and delayed low-ethylene CA storage on keeping quality of ‘McIntosh’ apples. *J. Amer. Soc. Hort. Sci.* 111:719–723.
- Saftner, R.A., J.A. Abbott, W.S. Conway, and C.L. Barden. 2003. Effects of 1-methylcyclopropane and heat treatments on ripening and post-harvest decay in ‘Golden Delicious’ apples. *J. Amer. Soc. Hort. Sci.* 128:120–127.
- Tan, T. and F. Bangerth. 2000. Regulation of ethylene, ACC, MACC production, and ACC-oxidase activity at various stages of maturity of apple fruit and the effect of exogenous ethylene treatment. *Gartenbauwissenschaft* 65:121–128.
- Watkins, C.B., W.J. Bramlage, P.L. Brookfield, S.J. Reid, S.A. Weis, and T.F. Alwan. 2000. Cultivar and growing region influence efficacy of warming treatments for amelioration of superficial scald development on apples after storage. *Postharv. Biol. Technol.* 19:33–45.
- Zhou, H.-W., S. Lurie, R. Ben-Arie, L. Dong, S. Burd, A. Weksler, and A. Lers. 2001. Intermittent warming of peaches reduces chilling injury by enhancing ethylene production and enzymes mediated by ethylene. *J. Hortic. Sci. Biotechnol.* 76:620–628.